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POSTER ABSTRACTS

The letters **W**, **T**, or **F** of the program/abstract/board number indicate the day (i.e., Wednesday, Thursday or Friday) on which authors display their poster and present their work. The number indicates the board to use for displaying your poster (on the day of your presentation only).

	Abstract/ Poster Board #'s
Wednesday, Poster Session I, 2:00 pm – 4:00 pm	
Cardiovascular Genetics	325W-380W
Complex Traits: Theory and Methods	381W-433W
Evolutionary and Population Genetics	434W-495W
Psychiatric Genetics, Neurogenetics and Neurodegeneration	496W-610W
Statistical Genetics and Genetic Epidemiology	611W-743W
Epigenetics	744W-772W
Gene Structure and Gene Product Function	773W-799W
Genomics	800W-913W
Technology Advancement	914W-943W
Clinical Genetics and Dysmorphology	944W-1037W
Genetic Counseling and Clinical Testing	1038W-1068W
Molecular Basis of Mendelian Disorders	1069W-1158W
Cancer Genetics	1159W-1250W
Cytogenetics	1251W-1287W
Metabolic Disorders	1288W-1338W
Ethical, Legal, Social and Policy Issues in Genetics	1339W-1363W
Prenatal and Perinatal Genetics	1364W-1416W
Reproductive Genetics	1417W-1473W

	Abstract/ Poster Board #'s
Friday, Poster Session III, 2:00 pm – 4:00 pm	
Cardiovascular Genetics	325F-380F
Complex Traits: Theory and Methods	381F-432F
Evolutionary and Population Genetics	433F-493F
Psychiatric Genetics, Neurogenetics and Neurodegeneration	494F-608F
Statistical Genetics and Genetic Epidemiology	609F-742F
Epigenetics	743F-772F
Gene Structure and Gene Product Function	773F-798F
Genomics	799F-912F
Technology Advancement	913F-942F
Clinical Genetics and Dysmorphology	944F-1037F
Genetic Counseling and Clinical Testing	1038F-1069F
Molecular Basis of Mendelian Disorders	1070F-1160F
Cancer Genetics	1161F-1252F
Cytogenetics	1253F-1289F
Metabolic Disorders	1290F-1341F
Ethical, Legal, Social and Policy Issues in Genetics	1342F-1366F
Pharmacogenetics	1367F-1433F
Therapy for Genetic Disorders	1434F-1487F

Thursday, Poster Session II, 2:00 pm – 4:00 pm	
Cardiovascular Genetics	325T-380T
Complex Traits: Theory and Methods	381T-433T
Evolutionary and Population Genetics	434T-495T
Psychiatric Genetics, Neurogenetics and Neurodegeneration	496T-610T
Statistical Genetics and Genetic Epidemiology	611T-743T
Epigenetics	744T-772T
Gene Structure and Gene Product Function	773T-799T
Genomics	800T-913T
Technology Advancement	914T-943T
Clinical Genetics and Dysmorphology	944T-1037T
Genetic Counseling and Clinical Testing	1038T-1068T
Molecular Basis of Mendelian Disorders	1069T-1158T
Cancer Genetics	1159T-1250T
Cytogenetics	1251T-1287T
Metabolic Disorders	1288T-1338T
Ethical, Legal, Social and Policy Issues in Genetics	1339T-1363T
Genetics Education	1364T-1383T
Health Services Research	1384T-1415T
Developmental Biology	1416T-1453T
Cancer Cytogenetics	1454T-1492T

325W

Genotype-phenotype correlation in hypertrophic cardiomyopathy: evaluation of a new slice mutation by minigene functional assay. *E. Maioli[1], M. Marini[2], M.I. Parodi[1], M.L. Galli[1], M. Castagnetta[1], D.A. Coviello[1], R. Ravazzolo[2], P. Spirito[3], L. Perroni[1].* 1) Laboratory of Human Genetics, E.O. Ospedali Galliera, Genova, GE, Italy; 2) Laboratory of Molecular Genetics, IRCCS, Istituto Giannina Gaslini, Genova, Italy; 3) Cardiology Unit, E.O. Ospedali Galliera, Genova, GE, Italy.

Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiac disease affecting 1 in 500 people. The prevalence is greater in males than in females. HCM, defined as unexplained thickening of the left ventricle, represents the most common cause of premature sudden cardiac death (SCD) in the young. Morphologically, HCM can manifest with negligible to extreme hypertrophy, minimal to extensive fibrosis and myocyte disarray, associated or not with left ventricular outflow tract obstruction. The six most common genes involved in HCM are) -myosin heavy chain MYH7, cardiac myosin binding protein C (MYBPC3), cardiac troponin T (TNNT2) , cardiac troponin I (TNNI3), tropomyosins-1 (TPM1), (-actin 1 (ACTC1). Testing for these genes led to identify disease-causing mutations in 30-62% of unrelated HCM patients across the various cohorts tested around the world. One of the major features of HCM is its marked genetic and phenotypic heterogeneity. Carriers of HCM mutations may present clinical course that range from benign forms to malignant forms with a high risk of cardiac failure or sudden cardiac death. This variability in expression is also seen among family members sharing the same mutation. This variable expressivity, which confuses genotype/phenotype correlations, could be partially explained by both environmental influences and genetic modifiers. In case of novel mutations at splice sites or adjacent regions, analysis of mRNA is virtually unfeasible because of the lack of cardiac tissue, where sarcomeric genes are expressed. Here we report on a minigene assay used to overcome this problem in a case of an undescribed putative splicing mutation in the MYBPC3 gene. A HCM affected newborn, deceased at 15 days, was double heterozygous for two mutations of the MYBPC3 gene c.772G>A and c.2414-1G>A. The paternal mutation c.772G>A was reported as causative of HCM. The c.2414-1G>A mutation, involving a splice site was inherited from the unaffected mother and never reported in the scientific literature. It was absent in 500 control chromosomes. The position of the nucleotide substitution and the bioinformatic analysis (Alamut Splicing Predictions) suggested that it could be causative of a splicing abnormality with removal of the functional splice site. As MYBPC3 is selectively expressed in the heart and no cardiac tissue of the proband was available, expression studies could not be performed. To overcome this problem minigene splice assay was performed.

326W

BRAP, encoded by a gene associated with myocardial infarction, binds several key inflammatory molecules. *K. Ozaki[1], Y. Onouchi[1], Y. Nakamura[2], T. Tanaka[1].* 1) Cardiovascular Disease, Center for Genomic Medicine, RIKEN, Yokohama,; 2) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

Myocardial infarction (MI) is a multifactorial disease and among the leading cause of death in the world. The pathogenesis includes chronic formation of plaque inside the vessel wall of the coronary artery and acute rupture of the artery, implicating a number of inflammatory molecules. We have identified a genetic association of a variant in *LGALS2*, encoding galectin-2 that binds an inflammatory cytokine lymphotoxin-alpha (LTA) protein encoded by a gene conferred risk of MI, with the disease. We also showed that a functional variation in *BRAP*, encoding a galectin-2 binding protein, associated with susceptibility to MI in Asian populations. We have found that the BRAP protein seemed to regulate the activity of NF kappa B, a central transcription factor of inflammation. To clarify detailed function of BRAP in the pathogenesis of MI, we searched for its binding partners by using an S-protein tag pull down method and identified several molecules related to cell proliferation and inflammation. TNF receptor-associated factor 5 (TRAF5) was one of them, which plays an important role in the TNF receptor mediated inflammatory systems. Interestingly, we also identified TNF receptor associated protein 1(TRAP1) as a binding partner of BRAP. Combined with previous results that galectin-2 protein binds with lymphotoxin-((LTA; also known as TNF-( ), known as one of the cytokines produced at earliest phase of vascular inflammatory processes, and regulates its extracellular secretion, we hypothesized BRAP-galectin-2 pathway plays a pivotal role in inflammatory signal cascade, specifically TNF receptor mediated inflammation and thereby influence the MI susceptibility.

327W

Hierarchical clustering of genetic variants according to their effect size on multiple lipid phenotypes. *J. Engert[1,2,3], S. Bailey[2], P. Lee[3], N. Rudzicz[3], I. Karp[4,5].* 1) Cardiology Division, Department of Medicine, McGill University Health Centre, Montreal, Quebec, Canada; 2) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 3) The Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada; 4) University of Montreal Hospital Research Centre, University of Montreal, Montreal, PQ, Canada; 5) Department of Social and Preventive Medicine, University of Montreal, Montreal, PQ, Canada.

The results of many genome wide association (GWA) studies have recently been published. However, they frequently analyze only one disease state or phenotype at a time. We reasoned that an analysis of multiple related phenotypes could lead to important insights into their shared genetic etiology. The Global Lipids Genetics Consortium (GLGC) meta-analysis recently made available the p-values for 2.2 million SNPs in their association tests of four lipid traits in >100,000 individuals. Using publicly available allele frequencies, we converted the p-values to effect sizes and then applied hierarchical clustering to the SNPs based on their estimated shared effect sizes across the four lipid traits: total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides. Clustering was performed using Cluster, a package initially developed for clustering of microarray data developed by Michael Eisen. By this naïve adaptation of the original application, SNPs are treated analogously to genes. An eigenvector is calculated from the effect size profile across the traits for each SNP. Resulting eigenvectors are then compared using various similarity metrics. In this analysis we compare correlation and Euclidean metrics with no data normalization or transformation. We hypothesize that clustering of effect sizes may provide a more intuitive interface for the identification of genes that share a similar pathway in the control of lipid metabolism. By incorporating our knowledge of known lipid genes and their cluster assignment, we are able to assign novel genes to known functional pathways. This is important given the multiple tissue types that play a role in plasma lipid traits.

328W

Unraveling the genetics behind lymphedema with whole exome sequencing and near-infrared fluorescence imaging. *M.L. Gonzalez-Garay[1], C.T. Caskey[1], E.M. Sevick[1], O. Hall[1], C.E. Fife[2], E.A. Maus[2].* 1) Institute of Molecular Medicine, University of Texas, 1825 Pressler, Houston, TX; 2) UT Medical School and MHH Wound Care and Lymphedema Clinic, 6410 Fannin, Suite 600, Houston, TX 77030.

Lymphedema is a condition of irreversible tissue swelling caused by a compromised lymphatic system. The disease affects both males and females, sometimes with onset at puberty. Primary lymphedema (PL) has been attributed to genetic causes since the late nineteenth century. There are five known genetic causes of PL (FLT4, FOXC2, SOX18, CCBE1, and CJC2); however the majority of patients with lymphedema do not possess mutations in these genes. When individuals present with lymphedema after trauma or infection, the disease is termed secondary lymphedema (SL). Yet to date, there have been no human genetic studies that utilize next generation sequencing (NGS) for discovery of gene variants across the entire genome or exome that may be causative for either primary or secondary lymphedema. We studied families with multiple affected individuals recruited from the MHH-WCLC. The lymphatic phenotype of family members was non-invasively imaged used NIR fluorescence to directly visualize lymph pumping in the arms and legs and to detect lymphatic vascular anomalies i. Whole exome capture and deep sequencing was used to identify disease gene candidates for individual families. After NGS data analysis, common variants were eliminated by filtering against our control set consisting of samples from 80 non-related lymphedema-free individuals. The remaining variants were annotated using our annotation pipeline and non-synonymous coding variants (ns-cvs) were isolated. We performed co-segregation analysis followed by a triage process in which we evaluated the possible role of the mutated gene on the lymphatic system and the damage of the variant on the protein. Variants were validated using an orthogonal system to detect any false positives. In addition, copy-number variant (CNV) analysis in ongoing to eliminate the possibility that a large insertion or deletion responsible for the observed phenotype. We have identified two rare alleles each in a pair of interactive proteins that participate in the HGF/c-MET and PI3K pathways as the most likely candidates for lymphedema. Two probands, from a single family, inherited the two alleles (one from maternal and the other from paternal origin). Since the mother of the probands developed secondary lymphedema we are hypothesizing that the paternal allele may act as a modifier. Future work in our lab will be directed to experimentally demonstrate the biological significance of this finding.

329W

Genome-Wide Association Study of Transcripts for Genes Implicated in GWAS Studies for Coronary Artery Disease and Blood Phenotypes. *C.J. O'Donnell[1], A.D. Johnson[1], A. Hendricks[1], S. Hwang[1], J.E. Freedman[2], X. Zhang[1].* 1) Division of Intramural Research, National Heart, Lung and Blood Institute; the NHLBI's Framingham Heart Study; 2) Department of Medicine, University of Massachusetts Medical School.

Introduction: Genome-wide association studies (GWAS) have uncovered many loci associated with heart diseases. However, data remain sparse regarding causal/functional genetic variants within implicated loci. We sought to identify regulatory variants (cis-eQTLs) affecting gene expression levels of 96 transcripts selected by their proximity to SNPs discovered in prior GWAS. **Methods**: 1846 participants of the Framingham Heart Study Offspring cohort were included. Total RNA was isolated from leukocytes. High-throughput qRT-PCR TaqMan assays were used to measure gene expression. A total of 96 genes were chosen from top loci in published GWAS studies associated with coronary artery disease (CAD) and blood risk factor traits including haemostatic factors, lipid levels, and RBC count. A GWAS analysis for each of the 96 transcripts was conducted using an additive regression model applied to 2.5 million imputed SNPs adjusting for age, sex and expression level of 3 housekeeping genes. SNPs associated < 100kb of each gene were defined as cis-eQTLs. **Results**: On average, ~150 SNPs are located within 100kb of each gene. Over 1/3 of these genes (N= 35) harbor at least one *cis*-eSNP with P < 0.0005, suggesting trait-associated loci often harbor eQTLs. Notably, the expression level of 10 genes was strongly associated with >1 *cis*-eSNPs (P<5e-08). Among them, most genome-wide significant eSNPs included those for ABO (P<1e-91), FADS2 (P<6e-73), LPL (P<1e-52), APOC2 (P<2e-36), FNTB (P<2e-22), CDKN2B (P<2e-13), FAM117B (P<3e-11), C6orf184 (P<7e-11), ANRIL (P<5e-10), and SCARA5 (P<4e-08). When we examined the NHGRI GWAS catalog, we found these *cis*-eSNPs were significantly associated with CAD risk factors. For example, eSNPs associated with ABO gene expression in our study are also associated with multiple traits including hematological and biochemical traits, plasma E-selectin levels, pancreatic cancer and CAD. Further, significant ABO eSNPs are also reported by other genome-wide cis-eQTL studies in monocyte and CD4+ cells. In *trans*-eQTL analyses we found many SNPs located on different chromosome strongly associated with the expression level of several target genes (P<5e-08). **Conclusion**: Many strong *cis*-acting regulatory variants are localized within loci implicated by GWAS of heart disease/risk factors. Our data also suggest the existence of important *trans*-acting variants. Genetic analysis of gene expression may help identify alleles implicated in disease risk.

330W

GENETIC DETERMINANTS OF MULTIPLE CIRCULATING INFLAMMATORY BIOMARKERS AND THEIR RELATIONSHIP TO INCIDENT HEART FAILURE. *V.K. Topkara[1,2], J. Chu[2], C.C. Gu[2,3].* 1) Department of Medicine, Washington University School of Medicine, Saint Louis, MO; 2) Division of Biostatistics, Washington University School of Medicine, Saint Louis, MO; 3) Department of Genetics, Washington University School of Medicine, Saint Louis, MO.

Background: Activation of inflammatory signaling pathways including TNF-alpha, IL-6, and IL-18 have been implicated in the pathogenesis of heart failure. However, therapies targeting these molecules have not yet proven to be a clinically effective strategy. We sought to profile genetic determinants of six inflammatory biomarkers commonly used in clinical medicine, via genome-wide association (GWAS) analysis of biomarker levels and systematical review of biomarker-associated SNPs for their associations with heart failure incidence and/or mortality. Material and Methods: 2201 patients with complete serum biomarker level data on TNF, TNFR2, IL-6, IL-18, adrenomedullin, and CD40 ligand inflammatory biomarkers from the Framingham Heart Study Offspring Cohort were included in the analysis. Genotyping was performed using Affymetrix 500K Array as detailed in the Framingham SNP Health Association Resource. GWAS was performed using MERLIN to identify variants significantly associated with log-transformed serum biomarker levels. Candidate loci (p-value $<10^{-5}$) were ranked based on number of hits on the 6 biomarkers as endophenotypes. Highly ranked significant genes (score greater than 3) were further investigated for established associations with heart failure through online search. Results: A total of 361 SNPs (only 1 common) were significantly associated with serum inflammatory biomarkers traits at a p-value of $<10^{-5}$. Candidate regions associated with highest rank scores were FIGN-CRB14 for TNF trait, DTWD2-SEMA6A for TNFR2 trait, CDCA7-SP3 for IL-6 trait, SPAST5 for IL-18 trait, C4orf67 for adrenomedullin trait, and AUTS2 for CD40 ligand trait. We identified 4 variants in upstream of LRIG3 gene which was significantly associated with serum IL-6 levels and previously shown to be associated with incident heart failure in African-American population. None of the other candidate gene variants were previously shown to be related to heart failure incidence or mortality. Interestingly, 11 out of 56 candidate genes were shown to be associated with neuropsychological disorders. Conclusion: Serum inflammatory biomarker traits appear to have distinct genetic determinants. Vast majority of the genetic markers associated with serum inflammatory biomarkers have not been associated with heart failure pathogenesis or outcome, however such relationship seems to exist for neuropsychological disorders.

331W

Assessment of a possible relation between osteoporosis and hypertension in spontaneously hypertensive rats and recombinant inbred strains. *R. El Bikai[1], P. Dumas[1], J. Peng[1], S. Koltsova[1], V. Křen[2], M. Pravenec[3], O. Seda[1], J. Tremblay[1], P. Hamet[1].* 1) CHUM Research Center (CRCHUM), Montréal, Québec, Canada; 2) 1st Medical Faculty, Charles University, Prague, Czech Republic; 3) Institute of Physiology, Academy of Sciences of the Czech Republic,.

Aim: Osteoporosis and hypertension are two age related diseases that could be associated with accelerated ageing. Our project focuses on studying the common genetic determinants related with the progression of hypertension and the changes in bone structural parameters associated to osteoporosis. Methods: The spontaneously hypertensive rats (SHR) exhibit a gradual increase in blood pressure during puberty and young adulthood that resembles what is seen in human essential hypertension. In addition to high blood pressure, SHR display many defects related to calcium handling and abnormal bone mineralization. Therefore, the SHR strain and the SHR-derived recombinant inbred strains (RIS), the HXB / BXH set, will serve as our genetic model to study these two phenotypes. RIS are a unique opportunity to study genetically identical animals at different ages. They are considered as a very valuable tool for genetic studies. More then 20,000 single nucleotide polymorphisms are mapped across the HXB/BXH genome and are publicly available. The individual RIS carry fixed different combinations of the parental genotypes which allow the dissection and mapping of the polygenic phenotypes segregating in the RIS. Systolic, diastolic, temperature as well as heart rate were assessed by telemetry measurements at 3, 6, 9 and 12 months of age of 3 RIS and the SHR. The structural parameters of the proximal tibia bone were obtained from in vivo micro-CT scans in the same animals to assess the microarchitectural changes. Results: The results showed that the SHR and the HxB 17 strains displayed the highest significant blood pressure (P<0.0001)compared to the other strains as shown in our telemetry results. The scanning of the proximal tibia bone showed that the HxB 17 and HxB 13 develop an osteoporotic phenotype while ageing since they display a gradual decrease in the bone trabecular volume and in trabecular number (P<0.0001). Conclusion: Our data indicate the existence of strain differences in bone parameters as well as in blood pressure. This phenotypic study allows the assessment of strain distribution pattern of the traits which will then serve to map the genetic loci responsible for the observed differences.

332W

Associations between fibrinolysis pathway genes and ischemic stroke in young adults. *Y. Cheng[1], Y.J. Kim[1], J.W. Cole[2,3], S.J. Kittner[2,3], B.D. Mitchell[1].* 1) Department of Medicine, University of Maryland School of Medicine, Baltimore, MD; 2) Department of Neurology, Veterans Affairs Medical Center, Baltimore, MD; 3) Department of Neurology, University of Maryland School of Medicine, Baltimore, MD.

In healthy individuals, there is a dynamic balance in the processes of clot formation (coagulation) and clot dissolution (fibrinolysis). Ischemic stroke (IS), which can occur when a cerebral vessel is blocked by a fibrin-containing clot, accounts for 87% of all stroke. Therefore, fibrinolysis is an important candidate pathway for IS and its subtypes. We tested 431 SNPs from 12 genes (*PLAT*, *FGG*, *FGA*, *FGB*, *F13A1*, *SERPINE1*, *F2*, *SERPINB2*, *CPB2*, *PLG*, *PLAU*, *F2R*) in the fibrinolysis pathway for association with IS and its subtypes. SNPs were genotyped by Illumina Omni1-Quad array or imputed in 448 cases and 498 controls of European ancestry (EA) and 381 cases and 352 controls of African ancestry (AA), aged between 15-49 years old. SNPs with call rates<95%, HWE P-value<1.0E-7 and minor allele frequency<1% were excluded from analysis. Single SNP association testing was performed using logistic regressions adjusting for age, recruitment stages and population structure in EA and AA, separately. To obtain a gene-set or pathway-set p-value, we used the set-based test implemented in PLINK v1.07 to obtain the set-specific statistic (i.e. the mean of those single SNP statistics that had P<0.05 within the gene or fibrinolysis pathway), and the empirical p-value for the set-specific statistic was calculated by permutation (N=1,000) to maintain the correlation structure among SNPs. SNP-level analyses did not reveal any consistent associations with individual SNPs that replicated across ethnic groups. In gene-based analysis, *PLAU*, a serine protease involved in degradation of extracellular matrix, cell migration, and proliferation and which may affect affinity for fibrin-binding, was significantly associated with overall IS in both EA (P=0.03) and AA (P=0.01), although neither *PLAU* nor any other gene showed consistent associations across ethnic groups in subtype analyses. A test of association with the entire fibrinolysis pathway revealed this pathway to be significantly associated with cardioembolic (CE) stroke in EA (P=0.017) but not with any other stroke subtype or with overall IS. Our data supports a pivotal role for fibrinolysis in the etiology of CE strokes. The fibrinolysis-CE stroke association is particularly interesting in light of the fact that CE stroke is caused by arterial occlusions presumably due to an embolus arising in the heart, and an individual's ability to break down the fibrin clot could impact one's susceptibility to this form of stroke.

333W

FBN1 and _TGFBR2_ mutations in patients with bicuspid aortic valve. *N.R. Abdulkareem, G. Arno, J.A. Aragon-Martin, A.H. Child, M. Jahangiri.* Cardiac and Vascular Sciences, St. George's University of London, London, UK.

Fibrillin-1 expression has been shown to correlate with the differentiation of cushion mesenchymal cells into mature valve cells; therefore its deficiency may account for the development of bicuspid aortic valve (BAV) and the associated more fragile aortic wall which is prone to aneurysmal dilatation. The intrinsic aortic wall properties of decreased fibrillin-1 content have been suggested to be the culprit in aortic aneurysm dilatation associated with BAV. Loss of transforming growth factor-beta receptor 2 (TGFBR2) function can lead to a wide spectrum of connective tissue disorders, and mutations in TGFBR2 have been identified in patients affected by clinically overlapping Marfan-related disorders e.g. thoracic aortic aneurysm and dissection (TAAD2). Fibrillin interacts with TGF-) , collagen and elastin to maintain the mechanical strength and architecture of the aortic wall and so mutations in *FBN1* or *TGFBR2* lead to dysregulated TGF-) signalling causing an increased extracellular matrix degradation and apoptosis resulting in aortic aneurysm. As a result it is important to determine if mutations in *FBN1* or *TGFBR2* are causative in BAV development and related aortic aneurysm formation. A consecutive series of 35 well characterised UK Caucasian BAV patients were included in our study (age, 59.26±12.65 years). BAV was confirmed at surgery. Twenty one patients had concomitant aortic root aneurysm. Patients with Marfan syndrome or other diagnosed connective tissue diseases were excluded. Genomic DNA was extracted from venous blood, and polymerase chain reaction was performed to amplify all 65 *FBN1* and 7 *TGFBR2* exons including the intron/exon boundaries. Targeted gene sequencing was carried out and results were analysed to check for gene mutations and single nucleotide polymorphisms. No pathogenic mutations in either *FBN1* or *TGFBR2* were identified in our series. Other possible candidate genes important in outflow tract development e.g. *GATA5* are currently being studied.

334W

Association study of Fms-related tyrosine kinase 1 (FLT-1) gene polymorphisms with the occurrence of coronary artery disease in Koreans. *p. ahram[1], S. Dong-Jik[1,2], P. Boram[1,3], S. Nak-Hoon[1,3], L. Jin-Woo[1], K. Jisun[1], P. Sungha[1,4], L. Sang-Hak[1,4], J. Yangsoo[1,4].* 1) Cardiovascular Genome Center, Yonsei University College of MedicineYonsei Cardiovascular Genome Center, seoul, Korea; 2) Yonsei University Research Institute of Science for Aging; 3) Department of Biostaistics, Yonsei University College of Medicine; 4) Cardiology Division, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea.

Fms-related tyrosine kinase 1(FLT1) gene encodes a member of the vascular endothelial growth factor receptor(VEGFR) family. The FLT1 protein binds to placental growth factor(PGF) and plays an important role in angiogenesis and vasculogenesis. The role of these factors by angiogenesis in condition of atherogenesis has been an object of active research. Several studies have reported that the levels of FLT1 and VEGF are elevated in patients with cardiovascular risk factors such as hypertension, hyperlipidemia, and atherosclerosis. As far, FLT1 molecule is known to be positively or negatively associated with various cardiovascular diseases, therefore, results of association studies of FLT1 with CAD have been controversial. Thus, we carried out a case-control study to evaluate the presently controversial question of whether FLT1 gene polymorphisms are associated with CAD in the Korean population. This case-control study included 451 CAD patients and 600 controls. Participants were drawn from the patient database of Cardiovascular Genome Center at Yonsei University Health System, Seoul, Republic of Korea. We identified 10 tagging-SNPs in the FLT1 gene by whole gene sequencing, and genotyped using the TaqMan®Assay and single base primer extension assay using SNaPShot Multiplex kit (Applied Biosystems). The allele and genotype frequencies of FLT1 rs664393 polymorphism were significantly differences between case and control groups for multiple testing; Pgenotype=0.008; Pdominant=0.002, OR=0.674(95% confidence interval(CI):0.526-0.864); Pallele=0.004, OR=0.746(CI:0.611-0.912). The SNP rs2296188 was association between the history of MI and without an MI history in dominant(OR=0.674,CI:0.461-0.985;P=0.041) and allele(OR=0.741, CI:0.556-0.988; P=0.041) models, respectively. In addition, the subgroup with a history of MI had significantly difference rs2296188 dominant(OR=0.677,CI:0.486-0.945;P=0.021) and allele(OR=0.738,CI:0.575-0.946;P=0.041) models when compared with controls. Therefore, the major allele (G) of rs664393 was associated with a protective effect for CAD. The differences in rs2296188 genotype distribution between the patient groups according to MI and non-MI status may also reflect the overall atherosclerotic development stage. These findings suggest that FLT1 gene polymorphisms are associated with the occurrence of CAD in Koreans. Further investigations should be performed to evaluate the functional role of these genes as a genetic contributor.

335W

The genetics of dilated cardiomyopathy: a prioritized candidate gene study. *K. Desbiens[1], N. Giannetti[2], I. Ruel[1], J. Genest[1,2], J. Engert[1,2,3].* 1) The Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada; 2) Cardiology Division, Department of Medicine, McGill University Health Centre, Montreal, Quebec, Canada; 3) Department of Human Genetics, McGill University, Montreal, Quebec, Canada.

Dilated cardiomyopathy (DCM) is characterized by left ventricular enlargement and systolic dysfunction resulting in impaired heart function that can lead to heart failure. DCM can broadly be divided into cases for which there is a clear predisposing condition (e.g. hypothyroidism, chemotherapeutic agents, alcoholism, etc.) and those for which the cause is unknown. This condition clusters in certain families (called familial dilated cardiomyopathy or FDC) thus revealing that at least some DCM is determined by genetic predisposition. For these inherited cases, a large number of genes have been implicated and many involve “private” mutations not shared between unrelated families. It has been previously demonstrated that a large number of cases that do not present as familial clusters also have a genetic predisposition and again, a large number of genes have been implicated. The goal of this study was to identify mutations in genes known to be associated with DCM, in a Quebec study sample including both familial and non-familial DCM cases. We sequenced four previously identified genes (Lamin A/C, cardiac troponin T, titin-cap and phospholamban) in DCM patients. These genes were selected based on a previously developed prioritization strategy. In one patient, we discovered a mutation that leads to a stop codon in the Lamin A/C gene. Some of the other coding mutations that we discovered were predicted to be “probably damaging” using PolyPhen. We are currently genotyping those discovered mutations in additional patients, family members and controls as they are recruited. We will also investigate the possible long-term prognosis and response to therapeutic regimes of individuals who have specific mutations.

336W

Knockouts of the murine ortholog of the atypical Williams-Beuren tyrosine-protein kinase gene Baz1b present cardiac and circadian rhythm impairments. *G. Didelot[1], C. Berthonneche[2], A. Sarre[2], Y. Emmenegger[1], P. Franken[1], T. Pedrazzini[2], A. Reymond[1].* 1) Center Integrative Genomics, University of Lausanne, Lausanne, Lausanne, Switzerland; 2) Cardiovascular Assessment Facility, Cardiomet, Centre Hospitalier Universitaire Vaudois, CH-1005 Lausanne, Switzerland.

The Williams-Beuren syndrome (WBS) is a multisystem disorder caused by the hemizygosity of 25 genes, five of which encode transcription factors or chromatin remodeler. Modest perturbations in the dosage of the latter might substantially alter the expression of multiple target genes. Hence these genes are prime candidates to play a role in the phenotype of the patients. To address the physiological and molecular role of one of these genes, the Bromodomain Adjacent to Zinc finger 1b gene (BAZ1B), in vivo, we ablated by transposable element insertion the expression of its ortholog in mice. Almost all Baz1b-/- embryos (97%) die a few hours after birth. The rare survivors do not present any of the craniofacial abnormalities previously identified in knockouts of the murine orthologs of other genes of the WBS interval. They are, however, smaller in size than their control littermates and present a smaller heart with larger ventricular walls. Noticeably, this abnormal heart appears to be associated with an increased cardiac function as measured by the percentage of blood ejected with each contraction. This translates in a significant increase in nighttime activity and sleep perturbation. We are currently characterizing histologically the heart of Baz1b-/- embryos. Potential candidates genes for these multiple phenotypes have been identified by chromatin immunoprecipitation with antibodies raised against the BAZ1B unusual tyrosine-kinase.

337W

Association of PLA2G4A with Myocardial Infarction is Modulated by Dietary Polyunsaturated Fatty Acids. *J. Hartiala[1,2], S. Vikman[1,2], E. Gilliam[1,2], H. Campos[3], H. Allayee[1,2].* 1) Dept Prev Med, USC Keck School of Medicine, Los Angeles, CA 90033; 2) Institute for Genetic Medicine, USC Keck School of Medicine, Los Angeles, CA 90033; 3) Department of Nutrition, Harvard University School of Public Health, Boston, MA 02115.

Background: Leukotrienes are pro-inflammatory molecules derived from dietary polyunsaturated fatty acids (PUFAs) and have been associated with cardiovascular disease (CVD). We previously reported that an A>G variant (rs12746200) of the cytosolic phospholipase A2 group IVA gene (PLA2G4A), which encodes the enzyme that liberates PUFAs from cellular membranes for LT synthesis, decreases the risk of CVD. Objective: We sought to replicate these initial observations with a more clinically relevant phenotype such as myocardial infarction (MI) and determine whether dietary PUFAs mediate this association. Methods: Rs12746200 was genotyped in a Costa Rican case-control dataset (n=3971) and tested for association with MI. Functional experiments were carried out to determine whether rs12746200 led to differences in mRNA expression. Results: Risk of MI was significantly lower in AG/GG subjects compared to AA homozygotes (OR = 0.86, 95% CI 0.75-0.99; p=0.038). This protective effect was observed primarily in AG/GG subjects who were in the upper median for dietary omega-6 PUFAs (OR = 0.73, 95% CI 0.60-0.89; p-interaction=0.04). A similar analysis with dietary omega-3 PUFAs also revealed a trend towards a nutrigenetic association although these results did not achieve statistical significance (p-interaction= 0.12). Functional analysis in human aortic endothelial cells showed that the carriers of the G allele had significantly lower PLA2G4A gene expression (p=0.014), consistent with the athero-protective effect of this variant. Conclusions: These results replicate the association of rs12746200 with CVD phenotypes and provide evidence that the protective effect of this functional PLA2G4A variant is mediated by dietary PUFAs.

338W

Variation at the INSIG2 gene is associated with myocardial infarction in multiple ethnicities of the INTERHEART study. *S. Hooda[1], R. Do[2], G. Pare[3,6], A. Montpetit[3], N. Rudzicz[1], T. Hudson[4], S. Yusuf[5,6], S. Anand[5,6], J. Engert[1,2,7].* 1) The Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada; 2) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 3) McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, Canada; 4) Ontario Institute of Cancer Research, Toronto, Ontario, Canada; 5) Department of Medicine, McMaster University, Hamilton, Ontario, Canada; 6) Population Health Research Institute, McMaster University, Hamilton, Ontario, Canada; 7) Cardiology Division, Department of Medicine, McGill University Health Centre, Montreal, Quebec, Canada.

Previous studies identified a variant of the INSIG2 gene associated with obesity. In addition, the locus harbors variants contributing to allele specific expression. More recently, we have shown that an upstream SNP is associated with ApoB and LDL-C in multiple study samples, a finding that was replicated in the Global Lipids Genetics Consortium and that is consistent with the role of INSIG2 in the regulation of cholesterol levels. Thus, INSIG2 variants may also be associated with myocardial infarction (MI). To address this question, we examined INSIG2 SNPs in 8487 individuals from five ethnicities of the INTERHEART study (Arabs, Europeans, Iranians, Nepalese and South-Asians). A weighted meta-analysis was performed using METAL based on association results of each ethnicity. Two SNPs, rs2113485 and rs889904 were associated with MI (p= 0.008 and 0.05, respectively and MAFs>0.14 in all ethnicities). The most significant SNP, rs2113485, was also significant in the two largest ethnic groups, Europeans and South Asians, when these were analyzed separately (p< 0.03 for both). Consistent with other common genetic susceptibility alleles for MI, we observed greater ORs in individuals <50 years old (OR=1.28, CI=1.11-1.48) in both men (OR=1.24, CI=1.07-1.45) and women (OR=1.89, CI=1.068-3.36). In summary, our results provide evidence that genetic variation at the INSIG2 locus contributes to MI as well as to plasma cholesterol levels in multiple ethnicities.

339W

Vitamin D Association of Sequence Variants in the *SVEP1* Gene and the Prediction of Mortality and Myocardial Infarction. *B.D. Horne[1,2], J.B. Muhlestein[1,3], J.F. Carlquist[1,3], D.J. Rader[4,5], H.T. May[1], M.P. Reilly[4,5], J.L. Anderson[1,3].* 1) Cardiovasc Dept, Intermountain Med Ctr, SLC, UT; 2) Genet Epidemiol, Univ Utah, SLC, UT; 3) Cardiol Div, Univ Utah, SLC, UT; 4) Inst Translational Med & Ther, Univ Penn, Philadelphia, PA; 5) Cardiovasc Inst, Univ Penn, Philadelphia, PA.

Background: In a genome-wide association study for early-onset acute coronary syndromes (unstable angina, myocardial infarction [MI]), two single nucleotide polymorphisms (SNPs) in the Sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1 (*SVEP1*) gene on 9q31.3 had some of the lowest p-values. While those associations did not replicate in validation studies, *SVEP1* is known to interact with calcitriol. Low levels of vitamin D are associated with higher mortality and MI. This study evaluated whether SNPs in *SVEP1* are associated with 25(OH) vitamin D levels and clinical events. Methods: Among patients undergoing coronary angiography (N=1,127), 25(OH) vitamin D level was measured along with rs7035733 (minor allele frequency: 0.08) and rs7863519 (minor allele frequency: 0.20). Patients with 25(OH)D >100 ng/mL were excluded from the study due to potentially toxic levels. Results: Linkage disequilibrium between the two SNPs was low (r2=0.29). In analysis of variance, mean 25(OH)D was 23.0±6.4, 23.1±5.6, and 27.4±4.7 ng/mL (p-trend=0.07) for rs7035733 genotypes GG (n=952), GA (n=168), and AA (n=7), respectively, and was 23.2±6.4, 23.2±6.0, 20.6±7.3 ng/mL (p-trend=0.010) for rs7863519 genotypes TT (n=720), TG (n=363), and GG (n=44), respectively. Together the two SNPs accounted for 0.3% of the variance in 25(OH)D. Patients were followed for clinical events over a mean of 10.5±2.5 years (range 3.8-17.2 years). In bivariable Cox regression, rs7035733 (hazard ratio [HR]=1.39 per allele, 95% CI=1.03, 1.87; p-trend=0.032) and rs7863519 (HR=0.79 per allele, 95% CI=0.63, 0.98; p-trend=0.035) predicted mortality/MI, which remained with adjustment for age, sex, and 25(OH)D: p=0.029 for rs7035733 and p=0.035 for rs7863519 (adjustment for 15 additional clinical variables: HR=1.35, p=0.055 for rs7035733 and, for rs7863519: HR=0.79, p=0.044). Conclusion: Two SNPs in the *SVEP1* gene were associated with vitamin D and with subsequent mortality/MI. *SVEP1* is 215 kilobases in length and produces a 3,571 amino acid product, thus many other potential variants may affect this locus, vitamin D, and outcomes. This gene deserves comprehensive evaluation as a vitamin D-associated locus.

340W

Prediction of Vitamin D Levels Using Three Significant SNPs from a Genome-wide Association Study. *S. Knight, J.L. Anderson, B.D. Horne, H.T. May, J. Huntinghouse, J.F. Carlquist, J.B. Muhlestein.* Intermountain Healthcare, Cardiovascular Department 5121 S Cottonwood Street Murray, Utah 84157.

Background: A recent GWAS found three SNPS, rs2282679, rs12785878, and rs10741657, in or near the genes GC, DHCR7, and CYP2R1, respectively, are associated with vitamin D levels. Here we further evaluate these three SNPs simultaneously in a cardiovascular population. Methods: We studied patients undergoing coronary angiography enrolled in the Intermountain Heart Collaborative Study (n=2213). Genotyping was performed by Taqman real-time PCR for the three variants and additive models were assumed. Vitamin D testing used the enzyme-linked immunoassay (ELISA) by Immunodiagnostics. Univariate and multivariate linear regression analyses using the natural log transformed vitamin D values were done. Results: In univariate analyses, only rs12785878 was significantly associated with vitamin D (p=0.0029) (See table). This SNP, rs12785878, remained the only significant SNP when all three SNPs were considered simultaneously (p= 0.0066) and when other variables (age, sex, BMI and season) were added to the model (p=0.0142). Interactions between rs12785878 and the other two SNPs were not significant. Conclusions: We replicated the association found between the SNP rs12785878 near gene DHCR7, and 25-hydroxyvitamin D concentrations (25-OH vitamin D). DHCR7 codes the enzyme that converts 7-dehydrocholesterol to cholesterol and in de novo synthesis was associated with 25-OH vitamin D concentrations; therefore, these findings have a persuasive biological foundation.

Linear Regression P-Values

	univariate	SNPs only	SNPs+var.
rs2282679	0.4133	0.1194	0.1297
rs12785878	0.0029	0.0066	0.0142
rs10741657	0.0551	0.0803	0.0930

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341W

Rare Recurrent DNA Copy Number Variants Associated with Syndromic Cardiovascular Malformations. *S.R. Lalani[1], C. Shaw[1], X. Wang[1], A. Patel[1], L.W. Patterson[2], K.E. Kolodziejska[1], P. Szafranski[1], Z. Ou[1], Q. Tian[1], S.L. Kang[1], A. Jinnah[1], A.M. Baycroft[2], S. Ali[3], A. Malik[4], P. Hixson[1], L. Potocki[1], J.R. Lupski[1], P. Stankiewicz[1], C.A. Bacino[1], B. Dawson[1], A.L. Beaudet[1], F.M. Boricha[5], R. Whittaker[6], C. Li[7], S.M. Ware[8], S.W. Cheung[1], J.L. Jefferies[9], J.W. Belmont[1].* 1) Dept of Genetics, Baylor College of Medicine, Houston, TX; 2) Section of Pediatric Cardiology, Texas Children's Hospital, Houston, Texas; 3) The University of Texas at Austin, Texas; 4) The Aga Khan University Hospital, Karachi, Pakistan; 5) Department of Pediatrics, University of Texas, Houston, Texas; 6) St John Medical Center, Inc, Tulsa, Oklahoma; 7) Department of Pediatrics, McMaster University, Canada; 8) Division of Molecular Cardiovascular Biology, Cincinnati Children's Hospital and University of Cincinnati College of Medicine, Cincinnati, Ohio; 9) Division of Pediatric Cardiology, Cincinnati Children's Hospital and University of Cincinnati College of Medicine, Cincinnati, Ohio.

Rationale: Clinically significant cardiovascular malformations (CVM) occur in 5-8/1000 live births. Extracardiac malformations are seen in approximately 30% of individuals with CVM, characterized as syndromic CVM. Recurrent copy number variations (CNVs) are an important cause of syndromic CVM, however these account for only a small fraction of described cases. Hypothesis: We hypothesized that many additional rare, highly penetrant CNVs confer susceptibility to CVM and are significantly overrepresented in pediatric populations with syndromic CVM. Methods and Results: Through a genome-wide survey of 203 subjects with syndromic CVM, we identified 55 CNVs greater than 50 kb, that were enriched in the syndromic CVM cohort, and were not present in children with normal cardiovascular studies (n= 872). The enrichment of sixteen of these variants was replicated in an independent CVM cohort (n = 511). In all, 20 variants were overrepresented in the combined 714 cases, and were not present in 2883 unaffected controls (P <0.05); fourteen of these variants were *de novo* in origin (70%). The enriched CNVs included 16q24.3 loss encompassing *ANKRD11* (4/714, P = .001), 5p13.2 gain involving *C50rf42*, *NUP155*, and *WDR70* (3/714, P = .007) and 2q31.3q q32. 1 loss including *PDE1A* (2/714, P = .039). The study also narrowed critical intervals associated with CVM in three well-recognized genomic disorder, such as the Cat-eye syndrome region on 22q11.1 (4/714, P = .001), 8p23.1 loss encompassing *GATA4* and *SOX7* (3/714, P = .007), and 17p13.3-p13.2 loss involving *CAMTA2* (2/714, P = .039). The genes within the enriched CNVs encode proteins that directly interact with proteins such as Nkx2-5, EP300, SMAD5, and TGFBR3. The CNVs disrupt genes involved in filamentous actin/cytoskeleton organization, embryonic development and regulation of Ras protein signal transduction. Conclusions: Our findings implicate novel cardiac specific loci such as 16q24.3 loss, 5p13.2 gain, and 2q31.3-q32.1 loss and delineate highly penetrant regions within established structural variations that are associated with syndromic CVM. The protein interaction network demonstrates that the rare inherited and *de novo* recurrent CNVs within syndromic CVM cohort are enriched for protein encoding genes that directly interact with human cardiac developmental proteins.

342W

Excess of deleterious mutations in genes associated with VEGF-A in Down syndrome-related heart defects. *C.L. Maslen[1], D. Babcock[1], B. Reshey[1], J. Thusberg[2], S. Mooney[2], E. Feingold[3], R.H. Reeves[4], S.L. Sherman[5].* 1) Cardiovascular Med, Oregon Hlth & Sci Univ, Portland, OR; 2) Buck Institute for Research on Aging, Novato, CA; 3) Human Genetics, Univ. Pittsburgh, Pittsburgh, PA; 4) Physiology and Genetic Medicine, Johns Hopkins Univ Schl of Medicine, Baltimore, MD; 5) Human Genetics, Emory Univ. Schl. of Medicine, Atlanta, GA.

Atrioventricular septal defect (AVSD) is a severe congenital heart malformation and a significant health concern because even when the defect is surgically repaired there is increased morbidity and mortality. Our long-term goal is to identify the compilation of modifiers that confer risk for AVSD. We conducted a case-control, candidate gene resequencing study to identify rare variants associated with AVSD using a Down syndrome (DS) cohort that is highly sensitized to developing heart defects. All cases (DS+AVSD) and controls (DS without heart defect) were non-Hispanic white. To date we have resequenced all exons and intron-exon boundaries of 26 genes; 18 were resequenced in 141 cases and 141 controls, and 8 were resequenced in at least 100 of each. More than 3,000 variants were identified. In coding regions the majority of variants detected were single nucleotide variants. Missense variants were analyzed by predictive software (Polyphen, SIFT and MutPred) for the likelihood that the amino acid change would be deleterious. Ten of the 26 genes showed a significant increase of high probability damaging variants in cases compared to controls. Those 10 genes contained 40 case-specific putatively deleterious mutations seen in 57 different cases (>40% of cases examined); 11 case-specific mutations were found in more than one individual. In contrast, only 11 potentially damaging variants were specific to controls (seen in ~9% of controls). This significant skewing toward cases suggests a causal relationship with AVSD for at least some of the mutations. Although the original 26-gene list was not biased in terms of function, the genes showing the excess of case-specific mutations are all related in some way to VEGF-A, suggesting a pathway and rationale for identifying additional genetic modifiers of AVSD. VEGF-A involvement is not surprising given that it is a known regulator of AV canal morphogenesis. However, our study did not find variants in genes from a number of other pathways thought to be equally important in the development of the atrioventricular valves and septa. Overall, these data support our hypothesis that individuals with DS and AVSD carry a significantly higher burden of protein-altering mutations in heart development genes found throughout the genome than individuals with DS but no heart defect, and that genes in the VEGF-A pathway may play a predominant role in the etiology of AVSD.

343W

Defining a new syndrome of arterial calcification with segmental sclerosis: A systems approach to diagnosis. *K. Menghrajani[1], D. Yang[2], C. Shuen[1], T. Markello[1], R. Rupps[3], M. Boehm[2], W.A. Gahl[1], C.F. Boerkoel[1].* 1) NIH Undiagnosed Diseases Program, Office of Rare Disease Research and Medical Genetics Branch, National Human Genome Research Institute and NIH Clinical Center; 2) Laboratory of Cardiovascular Regenerative Medicine, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA; 3) Department of Medical Genetics, University of British Columbia, Vancouver, BC Canada.

Fibromuscular dysplasia (FMD) and similar disorders of segmental arteriosclerosis are estimated to affect 4-5% of the population. FMD, which generally develops between 14 and 50 years of age, is considered an autosomal dominant disorder. However, despite this prevalence and knowledge, the molecular bases of FMD and related disorders remain largely uncharacterized. To address this, we define the molecular pathogenesis of an undescribed syndrome of severe arteriosclerosis through the study of a 17-year-old man with arotation of the intestine, anal atresia and progressive segmental arteriosclerosis of his medium-sized arteries. The arteriosclerosis has caused occlusion of his internal carotid, posterior cerebral, celiac, and superior mesenteric arteries and partial occlusion of his renal arteries. We have used whole-exome sequencing, gene expression analysis, metabolomics, histopathology, cell biology and zebrafish modeling to identify the molecular pathogenesis of his disease. This approach has identified mutations in nine candidate genes, distinctive fragmentation and calcification of the arterial internal elastic lamina, significant changes in the IL6 and WNT signaling pathways, and increased production of TGF-) . We synthesize these observations to characterize this syndrome and to define its potential disease mechanism. In summary, this constitutes one of the first systems approaches to characterization of segmental arteriosclerosis.

344W

Investigation of the role of *PLXNA2* in genetic susceptibility to congenital heart disease. *O. Migita[1], A.C. Lionel[1,2], C.R. Marshall[1,2], S.W. Scherer[1,2], C.K. Silversides[2,3,4], A.S. Bassett[2,3,5].* 1) The Centre for Applied Genomics, Genetics & Genome Biology, The Hospital for Sick Children, Ontario, Canada; 2) University of Toronto, Toronto, Ontario, Canada; 3) Toronto Congenital Cardiac Centre for Adults, University Health Network, Toronto, Ontario, Canada; 4) Obstetric Medicine Program, Mount Sinai Hospital, Toronto, Ontario, Canada; 5) Clinical Genetics Research Program, Centre for Addiction and Mental Health, Toronto, Ontario, Canada.

Tetralogy of Fallot (TOF) is a congenital conotruncal defect that is the most common form of cyanotic congenital heart disease (CHD), affecting 2.5 to 3.5 per 10,000 live births. Family studies show high heritability with sibling recurrence risk of 2-4% for cardiac defects. Although, TOF can be associated with known genetic abnormalities including 22q11.2 microdeletions, trisomy 21 as well as single gene mutations (e.g. *GATA6, JAG1, TBX1*), in the majority of cases the genetic cause of TOF is unknown. In a high resolution genome wide scan for copy number variants (CNVs) in samples from 407 adult TOF patients we detected deletions at the *PLXNA2* gene locus in two unrelated patients. CNVs at this locus were absent in 8,427 population based controls genotyped and analyzed using the same methodology (Fisher's exact test p value= 0.00212). *PLXNA2*, which codes for a plexin protein, plays a role in cardiac neural crest migration during embryonic development. Mice with *Plxna2* mutations exhibit cardiac defects including TOF. Despite the strong functional evidence in support of involvement of *PLXNA2* in TOF, there have been no reports of TOF patients with mutations in this gene. To determine if other mutations in *PLXNA2* are associated with TOF, we re-sequenced the gene in 96 TOF patients of European ancestry that were part of the microarray screen.

In addition to the structural mutations detected, identifying pathogenic sequence variants in *PLXNA2* in TOF cases would support the importance of this gene in cardiac development and enhance our understanding of the genetic causes of major cardiac malformations.

345W

Association of Genomic Loci from a Cardiovascular Gene SNP array with Plasma B-type Natriuretic Peptide Concentration in African Americans: the Jackson Heart Study. *S.K. Musani[1], V.S. Ramachandran[2], A. Bidulescu[3], H. Nagarajarao[1], P. Singh[4], T.E. Samdarshi[1], M.W. Steffes[5], H.A. Taylor[1], E.R. Fox[1].* 1) Department of Medicine, University of Mississippi Medical Center, JACKSON, MS; 2) Boston University School of Medicine, Boston; 3) Morehouse School of Medicine, Atlanta, GA; 4) Texas Tech University Health Science Center, Lubbock TX; 5) University of Minnesota, Minneapolis MN.

B-type Natriuretic Peptide (BNP) maintains salt and water homeostasis as well as regulate vascular function. Abnormalities in the natriuretic peptide system have been implicated in salt-sensitive and obesity-related hypertension across multiple ethnic groups. The genetic determinants of plasma BNP concentration in African Americans however have been poorly characterized despite the higher incidence of hypertension in this population. We hypothesized that variation in plasma BNP among African Americans is due to common and low frequency genetic variants. Methods: Our study population consisted of 2485 Jackson Heart Study participants (54 ± 13 years, 62% women). The median plasma BNP concentration was 7.2 pg/mL. Plasma BNP was measured on a Siemens Advia Centaur machine using a chemiluminescent immunoassay. Genotyping was done using IBC genotyping array, which captures 49320 SNPs across 2,100 candidate genes known to be related to cardiovascular, inflammatory, homeostasis/coagulation and metabolic phenotypes. Pooled residuals of log transformed BNP were generated after adjusting for age, sex and BMI. Participants with MI, CHF, and creatinine levels >2 were excluded. Phenotype-genotype association analyses were performed using PLINK software assuming an additive genetic model. Population stratification was accounted for by incorporating 10 principal components as covariates. To account for multiple testing, we considered only SNPs in approximate linkage equilibrium by pruning based on variance inflation factor. A total of 22811 SNPs were retained, and to maintain an overall type I error of 5%, a statistical threshold of $2.2x10^{-6}$ was employed to declare array-wide significance. Results: We identified 2 common array-wide significant loci in the coagulation factor XII gene F12 ($P=2.8x10^{-9}$) and the natriuretic peptide B gene NPPB ($P=3.14x10^{-7}$). The effective allele frequencies (and genetic effects) were 44% () = 0.20±0.03) and 40% () = -0.17±0.03), and they explained 1.5% and 1.2% of the variance in plasma BNP concentration. The polymorphism in F12 gene was associated with higher plasma BNP concentration, while NPPB was associated with lower levels. Conclusion: Common sequence variants of coagulation factor XII and natriuretic B-type peptide genes are associated with BNP in this community-based cohort of middle aged African Americans. Further studies are necessary to determine potential physiological changes that result from variations in these genes.

346W

Screening for microdeletions and sequencing candidate genes associated with conotruncal heart defects. *K. Osoegawa[1,2], D.M. Iovannisci[1], M.B. Ladner[1], K. Schultz[1], C. Parodi[1], F. Cohen[1], D. Noonan[1], A. Borg[3], G.M. Shaw[4], E. Trachtenberg[1,2], E.J. Lammer[1].* 1) Research Inst, Childrens Hosp Oakland, Oakland, CA; 2) Center for Applied Genomics, Children's Hospital and Research Center Oakland, Oakland, CA; 3) Department of Oncology, Lund University, Sweden; 4) Dept. of Pediatrics, Stanford University School of Medicine, Palo Alto, CA.

Congenital heart defects are the most common anatomical grouping of human birth defects, yet little is known about their etiologies. Conotruncal defects are an important pathogenetic subset of congenital heart defects, comprising nearly 20 percent of the total. DNA samples from a population-based sample of 389 California infants born during 1999-2004 with conotruncal defects were screened for chromosomal microdeletions/duplications as an approach to identify candidate genes for conotruncal defects using array comparative genomic hybridization (array-CGH). Of all cases analyzed, we identified one subject with a novel 3.9 Mb duplication at 8p23.1 and four subjects who had 47,XYY or mosaic 47,XYY karyotypes. We also identified deletion cases at 1q44, 10q23.1, 22q11.21 and 22q11.22. The majority of these chromosomal anomalies were identified from cases with Tetralogy of Fallot. In addition to the genome wide screening with array-CGH, we have developed an efficient strategy for sequencing 10 candidate genes. FGF8, FOXH1, NKX2-5, GATA4, GATA5, GATA6, ISL1, ZFPM2, MEF2C and CRKL are either expressed in secondary heart field cells, for which mutations have been reported in a few infants with conotruncal defects, or identified via array-CGH experiments. Ninety-six DNA fragments or “amplicons” containing exons from these genes were amplified using Fluidigm's Access Array System. The system enabled us to amplify up to 48 samples, each with a unique multiplex identifier, x 48 amplicons (2304 independent PCRs) in a single chip. The amplicons were pooled, and sequenced using the Roche 454 GS FLX system. As of June 1 2011, we have sequenced ~90 DNA samples using this strategy. We developed DNA sequence analysis programs using Perl and Java programming languages for quality control and quality assessment of all DNA sequence data. Initial analyses indicated that we obtained sufficient DNA sequence redundancy (>50 sequence reads per amplicon) from >90% amplicons. The resulting DNA sequences were aligned against reference human DNA sequences, and potential novel mutations or single nucleotide polymorphisms (SNPs) have been identified within protein coding sequences using SeqNext (JSI Medical Systems).

347W

Exome Sequencing and Linkage Filters Identify Novel Candidate Mutations in a Family with Left-Ventricular Non-Compaction. *S. Pan[1], R. Chen[2], M. Perez[1], K. Sallam[1], A. Pavlovic[1], F. Haddad[1], M. Snyder[2], E. Ashley[1].* 1) Division of Cardiovascular Medicine, Stanford Hospital & Clinics, Stanford, CA; 2) Department of Genetics, Stanford University, Stanford, CA.

Background: Left ventricular non-compaction (LVNC) is a rare form of cardiomyopathy that presents as hyper-trabeculation of the myocardium that can lead to heart failure, with a heterogeneous genetic etiology. While previous work has identified several genes that are associated with LVNC, the genetic basis of LVNC in the majority of cases still remains unknown. **Aim:** We sought to use exome sequencing in conjunction with linkage information to identify candidate causative mutations in a family with LVNC. **Methods:** DNA was isolated from the blood mononuclear cells from 6 members of an extended family with LVNC (5 affected, 1 unaffected) as determined by echocardiography. Whole exome capture was performed and libraries were sequenced at a coverage of ~96% of targeted bases and average read depth of ~90x per exome. Coding and splice site variants were called against the hg19 human reference and common SNPs from dbSNP131 were removed from the candidate list. Linkage analysis was also performed using the genotypes called at the positions of common SNPs from the exome data and candidates were further filtered by positions with LOD > 0, using an autosomal dominant model. **Results:** Filtering out common SNPs and using cosegregation, we were initially able to narrow down the list of candidates to 11 missense mutations and 19 indels. Using linkage as a further filter reduced the number of candidate mutations by half, with the majority of excluded variants found to be due to erroneous calls as confirmed by Sanger sequencing. By genotyping 5 further individuals in the family (1 affected, 3 unaffected, and 1 of unclear phenotype), we were able to narrow the current list of candidates to two missense mutations, one in *amphiphysin* which encodes a bridging integrator protein implicated in clathrin-mediated endocytosis and actin polymerization, and another in *versican* which encodes a proteoglycan that has a vital role in early myocardial development. **Conclusion:** We used linkage analysis on exome sequencing data as an effective filter for candidate mutations and to control for sequencing error. These filters along with cosegregation data identified mutations in *amphiphysin* and *versican* as possible causative mutations in LVNC. Further analysis of the biological role of these mutations, as well as an analysis of structural variants, are currently underway.

348W

Novel associations of SNPs and transcripts with paraoxonase activity in Mexican-American families. *E.E. Quillen, D.L. Rainwater, T.D. Dyer, M.A. Carless, J.E. Curran, M.P. Johnson, H.H.H. Goring, J.W. MacCluer, E.K. Moses, J. Blangero, L. Almasy, M.C. Mahaney.* Department of Genetics, Texas Biomedical Research Institute, San Antonio, TX.

Low serum activity of the paraoxonase 1 (PON1) protein is associated with decreased antioxidant and anti-inflammatory activity by high-density lipoprotein (HDL) cholesterol leading to atherosclerosis and heart disease. Mutations in the gene *PON1* have been associated with the majority of the observed variation in serum paraoxonase activity; however, there remains substantial unexplained variation. To identify additional genes contributing to PON1 activity, 990 Mexican-American participants in the San Antonio Family Heart Study were genotyped for approximately one million SNPs on a series of Illumina microarrays. PON1 activity was assessed on four substrates: paraoxon (+/- high levels of salt), phenyl acetate (+/- high levels of salt), dihydrocoumarin, and diazoxon. Genome-wide association analysis was performed conditioning on variation in the *PON1* region on chromosome 7 and adjusting for stratification in the sample using principal components. The phenotypic variation explained by age, sex, *PON1* alleles, and newly identified loci differs among the substrates. On average, age explains 2.9% of phenotypic variance, sex explains 0.5%, and allelic variation in and around *PON1* explains 42% with a range of 22% for phenyl acetate - salt to 66% for paraoxon + salt. Three SNPs (rs1158731, chr 1; rs6985222, chr 8; and rs7245370, chr 18) are significantly associated ($p < 5e-8$) with one of the measurements of paraoxonase activity. rs6985222 is adjacent to *CYP7B1*, one of a family of cytochromes involved in cholesterol catabolism. An additional seven SNPs are suggestively associated ($p < 5e-7$) with one of the measurements. Using the Illumina Sentrix Human Whole Genome (WG-6) Series I BeadChips, gene expression levels for 47,289 transcripts were characterized in the sample. The ten SNPs significantly or suggestively associated with PON1 activity were found to be significantly associated ($p < 1e-5$) with variation in the expression of 38 transcripts. Among these are lipin-1 (*LPIN1*), a candidate for adipose development and triglyceride metabolism; sterol 27-hydroxylase (*CYP27A1*), which is involved in the degradation of cholesterol and the production of 27-hydrocholesterol, a protein elevated in individuals with hypercholesterolemia and found in atherosclerotic lesions; and macrophage scavenger receptor 1 (*MSR1/CD204*), a macrophage-expressed receptor previously implicated in atherosclerosis.

349W

Hunting for Novel Dilated Cardiomyopathy Genes using Haplotype Sharing and Exome Sequencing. *R.J. Sinke[1], A. Pósafalvi[1], K.Y. van Spaendonck-Zwarts[1], J.P. van Tintelen[1], P.A. van der Zwaag[1], L.G. Boven[1], J.J. Bergsma[1], W.B. Koetsier[1], D.J. van Veldhuisen[2], M.P. van den Berg[2], R.M.W. Hofstra[1], J.D.H. Jongbloed[1].* 1) Dept Genetics, UMC Groningen, Groningen, Netherlands; 2) Dept Cardiology, UMC Groningen, Groningen, Netherlands.

Background: Idiopathic dilated cardiomyopathy (DCM) is a clinically highly heterogeneous disorder, characterized by dilation and impaired contraction of the left ventricle. Monogenic inheritance is observed in one-third of the idiopathic cases and mutations in the >40 known genes explain only ~25% of familial cases. In order to identify novel DCM disease genes, we investigated two DCM families by Haplotype Sharing Test (HST) combined with exome sequencing. Methods: To localise novel DCM genes, 250K SNP genotyping was performed to identify regions shared between patients from a single pedigree. The HST was applied to (I) a family with 4 DCM and 2 peripartum cardiomyopathy patients and (II) a family with 3 DCM patients and 1 patient with reduced left ventricular function. To subsequently identify the corresponding disease genes, DNA of the index-patient and an affected relative from each family were analysed by exome sequencing. Results: The HST revealed largest shared haplotypes of 71 cM on chromosome 15 containing ~600 genes (family I) and 46 cM on chromosome 9 containing ~475 genes (family II). Exome sequencing revealed potentially pathogenic variants shared by the two affected family members in both families. Confirmation by Sanger sequencing and carriership analysis in affected family members and healthy controls is in progress. Conclusions: We identified potentially pathogenic variants in the largest shared haplotypes in two DCM families. Our results show that a combination of the HST and exome sequencing is a promising approach to identify novel genes underlying familial cardiomyopathies.

350W

Massively parallel sequencing of the mitochondrial genome in patients with pediatric cardiomyopathies. *M. Tariq[1], V.W. Zhang[2], F. Scaglia[2], L.J. Wong[2], S.M. Ware[1,3].* 1) Division of Molecular Cardiovascular Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Mitochondrial diseases are clinically and genetically heterogeneous disorders caused by alterations in either nuclear or mitochondrial genomes. They are associated with a variety of clinical symptoms including cardiomyopathy, in which they have classically been characterized by infantile onset, rapid progression, and fatal outcome. The underlying gene defects have been identified in only a minority of cases. We hypothesized that mitochondrial disorders are an under-recognized cause of pediatric cardiomyopathy and occur in patients with isolated cardiomyopathy without additional systemic symptoms. To investigate this hypothesis, we used massively parallel sequencing to analyze the mitochondrial genome (mtDNA) and 9 nuclear genes known to cause mitochondrial disease in 84 pediatric cardiomyopathy patients, including 11 patients (13% of cohort) who met modified Walker criteria for mitochondrial disease based on their biochemical and clinical evaluations. We identified 15/84 patients (18%) homoplasmic for previously described mtDNA mutations. Variants previously associated with myopathy and cardiomyopathy, m.5567T>C in $tRNA^{Trp}$ gene, m.5783G>A in $tRNA^{Cys}$ gene, and m.8348A>G in $tRNA^{Lys}$ gene, were identified in five patients. Ten patients had variants in genes known as LHON secondary mutations, including eight patients with haplogroup T and m.4917A>G (p.N150D in *ND2* gene), one patient with m.3394T>C (p.Y30H in *ND1* gene) and one patient with m.15257G>A (p.D171N in *cytochrome b* gene). Altogether, these mutations were found in 22% patients (5/23) with left ventricular noncompaction, 17% (3/18) with hypertrophic, 21% (4/19) with restrictive, and 16% (3/19) with dilated cardiomyopathy. On average, each patient has 3 novel changes which were not reported in mitomap and mtDB, suggesting that additional candidates exist within the cohort. No homozygous or compound heterozygous mutations were identified in 9 nuclear genes screened (*SCO2, SOD2, TSFM, TK2, COX17, SURF1, DGUOK, NDUFA11, and NDUFS8*). Overall, 18% of patients (2/11) meeting modified Walker criteria had mtDNA mutations (m.5567T>C in $tRNA^{Trp}$ and m.15257G>A; p.D171N in *cytochrome b* gene). The remaining mutations were identified in patients that were not previously suspected to have a mitochondrial etiology for their cardiomyopathy (13/73 patients). This study shows the importance of mtDNA genomic analysis and demonstrates that mtDNA variants may play a role in pediatric cardiomyopathy.

351W

Mutation in MYOF: a new cardiomyopathy gene? *M. Wallace[1], M. Burch[2].* 1) Prof, Molec Gen & Microbiol, Univ Florida, Gainesville, FL; 2) Molec Gen & Microbol, Univ Florida, Gainesville, FL.

Increasingly it is being found that idiopathic cardiomyopathy, especially in children, is caused by autosomal dominant mutations in genes such as those involved in sarcomere function. Mutations in such patients/families have now been found in dozens of genes. A family with dilated cardiomyopathy (DCM) was investigated for mutations in 8 candidate genes using single-strand conformational analysis (SSCP) analysis of exons containing functional domains or exons previously reported with mutations. A gene that had not previously been reported mutated in cardiomyopathy (or any other condition), but was a logical candidate, myoferlin (MYOF), was included in the analysis. Myoferlin is a very large type II membrane protein, associating with both the plasma and nuclear membranes, related to dysferlin (a muscular dystrophy gene). It binds both calcium and phospholipids, and is highly expressed in the heart and skeletal muscle. The gene spans 175kb on chromosome 10q24, with 53 exons encoding 2061 amino acids. __ MYOF exons were analyzed, and an abnormal gel pattern was seen in the proband of this family (UF574) in exon 13. DNA sequencing of the PCR product identified a single-base deletion in the open reading frame, c.1216delG, on one allele. This deletion causes a frameshift at codon 405 (normally Gly), resulting in a premature stop 7 codons into the aberrant frame. This exon is in the region containing a C2 domain and region necessary for interaction with EDH2. This mutation was found in the proband's mother, who is asymptomatic for signs of cardiomyopathy. However, the mutation was found in an affected brother but not an unaffected sib. While NCBI shows several isoforms of MYOF, including some as short as 437 amino acids, this specific DNA change has not previously been reported. Its identification in a family with DCM implicates it in the etiology of this condition, although the mother would be considered currently non-penetrant based on symptoms. This is the first report of a MYOF mutation in cardiomyopathy. Further analysis is needed to validate the involvement of this gene/mutation in the pathogenesis of DCM.

352W

Replication study of prior GWAS candidate genes/loci for coronary artery diseases in the genetic isolated Newfoundland and Labrador population. *Y. Xie[1,2,4], J. Cui[1], E. Randell[1], J. Renouf[2], S. Li[1], A. Pope[3], S. Sun[4], W. Gullive[4], F. Han[1].* 1) Dept Lab Med, Faculty of Medicine, Memorial University, St John's, NF, Canada; 2) Laboratory Medicine Program, Eastern Health, St. John's, NL, Canada; 3) Dept. of Molecular Genetics, Newfound Genomics, St. John's, NL, Canada; 4) Disciplines of Medicine, Faculty of Medicine, Memorial University, St. John's, NL, Canada.

Genome-wide association studies (GWAS), have provided some promising achievements for a number of multifactor diseases including coronary artery disease (CAD). However, many of these GWAS candidate genes failed to been replicated in studies using different ethnic populations which indicates the variety of genetic modifiers among different ethnic populations. Additionally, the genetic heterogeneity in the studied population can reduce the sensitivity in detection of weak genetic effects and leads to false negative results in replication studies. The population of Newfoundland and Labrador (NL) is a well known genetic isolated population, and this population has a high prevalence of CAD in Canada. As a part of our ongoing study, 15 genetic variants from 12 selected prior GWAS candidate genes/loci have been genotyped in 500 patients with myocardial infarction (MI) and 500 age and sex matched controls from the NL population to determine the disease risk impact in the studied population. Genotyping was carried out by using the Sequenom's MassARRAY system. Among the 12 studied genes/loci, only the 9p21 locus (rs 133049, rs 10757274, rs238306 and rs238307) was successfully associated to the patients (P<0.000 in all SNPs). This association was further confirmed in another study with enlarged sample size (1,000 MI patients and 1,000 controls) from the same population (P<0.000 in all SNPs). We, therefore, conclude that the 9p21 locus is a genetic susceptibility for CAD in the NL population. The failure of replicating other11 GWAS candidate genes for CAD in NL patients strongly suggests the diversity of genetic modifiers for CAD in NL population.

353W

SNP-based genome-wide analysis identifies a novel EFEMP2 mutation in four unrelated families with dilatation of the ascending aorta. *Z. Al-Hassnan[1,6], S. Tulbah[2], A. Hakami[1], S. Mohammed[3], A. Al-Omrani[4], A. Almesned[5], M. Al-Fayyadh[4,7].* 1) Dept. of Medical Genetics; 2) Dept. of Genetics; 3) Dept. of Pathology & Laboratory Medicine; 4) King Faisal Heart Institute, King Faisal Specialist Hospital & Resaerch Center, Riyadh; 5) Prince Sultan Cardiac Center, Qassim, Saudi Arabia; 6) Alfaisal University, Riyadh; 7) King Saud University, Chair of Sudden Death Research, Riyadh, Saudi Arabia.

Dilatation of the ascending aorta (AA) is rarely inherited in an autosomal recessive pattern that results from mutated EFEMP2 gene in patients with cutis laxa (type 1A). Here we describe the identification of a novel EFEMP2 mutation in 9 patients, from 4 unrelated Saudi families, with dilatation of the AA in the absence of the skin and joint manifestations of cutis laxa. The clinical presentation ranged from episodes of cyanosis in a 5-month-old infant to incidental finding in an asymptomatic adult. Echocardiogram measurements revealed a wide-spectrum of severity of the dilatation of AA, varying from a Z-score of 33.9 in an asymptomatic 8-year-old child to a Z-score of 5 in a 20-year-old lady. Intrafamilial variability was also evident; 2 unrelated individuals were detected at the age of 17 and 20 years through family screening after the diagnosis in their severely affected siblings. Eight patients underwent replacement of the AA. Pathological examination showed myxoid and cystic medial degeneration. In one non-consanguineous family, SNP-based analysis using Affymetrix® Axiom™ Genome-Wide Array detected a homozygous block, shared by 2 affected siblings, on chromosome 11q13 where EFEMP2 gene is located. Sequencing EFEMP2 identified a novel homozygous mutation c.481G>A (p.E161K) in the two affected children as well as in all the other 7 patients. The parents in all the four families were heterozygous carriers and the unaffected children were either homozygous normal or heterozygous carriers. To our knowledge, this is the largest cohort of patients reported with mutated EFEMP2. Our work describes the wide phenotypic expression and the inrafamilial variability of the AA dilatation as a result of a novel EFEMP2 mutation that is probably a founder allele in the Saudi population. In addition, we also illustrate that in highly inbred populations, genome-wide homozygosity analysis can be powerful in identifying haplotypes carrying founder homozygous mutations for rare recessive disorders even in non- consanguineous families.

354W

A study of the adiponectin Q as a susceptibility gene for atherosclerosis. *M. Al-Najai, M. Vigilla, E. Andres, S. Elhawari, D. Gueco, P. Muiya, N. Mazher, B.F. Meyer, M. Alshahid, N. Dzimiri.* King Fisal Specialist Hospital, Riyadh, Riyadh, Saudi Arabia.

Adiponectin (ADIPOQ) is a protein that plays an important role in the regulation of various processes that may lead to atherosclerosis. In a preliminary genome-wide linkage study using the Affymetrix Gene Chip 250 sty1 mapping array, we identified several loci, among others, in chromosome 3 region which harbours the ADIPOQ gene linked to early onset coronary artery disease (CAD) in a Saudi family of 11 individuals with predominant heterozygous familial hypercholesterolemia. We then sequenced the gene in 200 individuals to identify variants of interest, followed by a population-based association study for 8 SNPs with myocardial infarction (MI)/CAD in 4638 Saudi individuals (2301 CAD cases versus 2338 angiographed controls) using the Applied Biosystems real-time PCR procedure. Two of the studied variants, the rs2082940C>T[Odds ratio(95% Confidence Interval = 1.13(1.02-1.25); p=0.022) and the rs1063537C>T [1.16(1.04-1.30; p=0.008) conferred risk for MI. These associations followed a recessive mode of inheritance. Interestingly, the rs1063539 (p=0.079) was only weakly associated with CAD [1.29(0.99-1.68); p=0.058) and hypertension [0.74(0.55-1.03); p=0.073]. Our results demonstrate that the ADIPOQ gene harbours susceptibility variants for MI in the Saudi population.

355W

Thrombospondin 4 gene harbours a common susceptibility locus for atherosclerosis and its risk traits. *M.M. Alrasheed[1], M.G. Vigilla[1], D. Gueco[1], P. Muiya[1], S. Elhawari[1], M. Al-Najai[1], E. Andres[1], B.F. Meyer[1], M. Alshahid[2], N. Dzimiri[1].* 1) Genetics, King Faisal Specialist Hospital and Research Centr, Riyadh, Saudi Arabia; 2) King Faisal Heart Institute, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

Thrombospondin 4 (THBS4) is a member of adhesive glycoprotein family that mediates cell-to-cell and cell-to-matrix interactions, and may be involved in localized signaling in the developing and adult nervous system. By virtue of its function, genetic changes in THBS4 gene are likely to constitute cardiovascular risk. In present study, we first identified a linkage of early onset of coronary artery disease (CAD) to chromosome 5, which encompasses the THBS4 gene locus, and sequenced the gene to identify variants of interest. We then pursued a population-based association study for CAD and its risk factors involving 4 SNPs (rs17885704G>A, rs438042A>T, rs17882513A>G, rs1866389C>G) in 4650 Saudi individuals (2307 CAD cases versus 2343 controls) using the Applied Biosystems real-time PCR procedure. The rs17885704 conferred risk for CAD [Odds ratio(95% Confidence Interval = 1.53(1.34-1.76); p<0.00001], myocardial infarction [1.40(1.22-1.61; p<0.00001], hyperlipidaemia [1.89(1.61-2.21; p<0.00001], hypertension [1.6740(1.4422-1.9261; p<0.00001] and exhibited borderline association with type 2 diabetes mellitus [1.14(0.99-1.30; p<0.060]. The associations followed a dominant mode of inheritance for all four variables. Another SNP, the rs1866389, was also weakly associated with CAD. These results clearly demonstrate that the rs1788704G variant of the THBS4 gene constitutes a locus for independently acquiring atherosclerosis and its important risk traits.

356W

Identification of Additional Genetic Factors Contributing to LQTS in the First Nations Population in Northern BC. *L. Arbour[1,2], S. Asuri[1,2], S. McIntosh[1], L. Fields[1].* 1) Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Division of Medical Sciences, University of Victoria,Victoria, BC, Canada.

Introduction: Congenital Long QT syndrome (LQTS) is a potentially fatal condition predisposing to ventricular tachyarrythmias characterized by a prolonged QT interval on electrocardiogram (QTc >460). Although usually rare, LQTS is common in First Nations people of Northern British Columbia. Inheritance for LQTS is considered of single gene origin (twelve known genes accounting for 80% of cases) but frequently involves additional genes and interactions with exogenous triggers characteristic of a complex disease. We have previously identified a novel missense mutation (V205M) in KCNQ1in this FN population and to date 328 participants have enrolled, 58 carry the mutation. Mechanistic studies and epidemiological evidence for a prolonged QT interval amongst carriers support pathogenesis. However, some individuals with the V205M mutation are more severely affected than others, and some with a prolonged QT interval without the V205M mutation have been identified, indicating the possibility of other genetic factors altering the phenotype or accounting for LQTS in this community. Such a founder population allows the opportunity to carry out studies which might provide insight. Family based linkage analysis was undertaken to identify additional contributing loci. **Methods:** Of the 328 community participants, those with QT interval >470 ms were included as potential “cases” (n=61), 27 of those with the V205M mutation (OR 5.07 95% CI 2.64-9.7). Medical records were reviewed for co-existing morbidities that might increase the QT interval and those cases were excluded for this analysis. Ultimately after genotyping remaining cases with one array of the Affymetrix mapping 500K 2 array sets, 22 cases were considered informative. An “affecteds” only, multipoint linkage analysis was carried out using Merlin software. Linkage disequilibrium was controlled for to prevent inflation of LOD scores and parametric and nonparametric modeling was utilized. **Results:** Maximum LOD scores of >3.0 were seen at an 8 cM region on chromosome 10q and a narrow region on chromosome 2p. LOD scores above 2.5 were seen at tighter regions on chromosomes 4, 15 and 21. Several potential candidate genes contributing to the LQTS phenotype in this population were identified in these regions, including those previously implicated in the regulation of the cardiomyocyte cytoskeleton, ion channels and other cardiac conditions. Further exploration is underway.

357W

Genome-Wide Association Study of Hypertension in a Diverse Population. *G. Beecham[1], A. Patel[2], N. Sikka[1], M. Nakamura[2], P. Goldschmidt-Clermont[2], M. Pericak-Vance[1], D. Seo[2].* 1) Hussman Institute for Human Genomics, University of Miami, Miami, FL; 2) University of Miami, Miami, FL.

BACKGROUND Coronary heart disease is the leading cause of death in the United States, with hypertension being one of the major underlying etiologies. Patients with hypertension are more likely to suffer a myocardial infarction or sudden cardiac death. Though the role of genetics on hypertension has been studied in European ancestry populations, its role in Hispanic populations is less clear. To further determine the genetic etiology of hypertension, we have performed a genome-wide association study of hypertension using a diverse set of cardiac catheterization patients. METHODS This study utilizes the Miami Cardiovascular Registry of the University of Miami Miller School of Medicine. A diverse set of patients were ascertained through cardiac catheterization labs; the dataset consists of over 65% Hispanic and 15% African American. All 2,000 patients were phenotyped for both systolic and diastolic blood pressure. Genotype data were generated using the Affymetrix SNP array 6.0 platform for 900,000 SNPs. Extensive QC tests were performed to ensure the integrity of the data, including sample filters (gender consistencies, efficiency thresholds, etc), SNP filters (HWE, genotyping efficiency, MAF, etc), and EIGENSTRAT methods to correct for population substructure. We tested for association using linear regression using the PLINK software package, and included smoking, cholesterol, age, sex, and three vectors describing the population substructure of the sample. RESULTS For the systolic phenotype we detected strong association ($p < 0.00001$) at 12 SNPs on 7 different chromosomes, in 6 different genes. Included in these is one SNP with p-value < 1x10-6 (rs2240491, p-value= 9.04x10-7) near the OTOG gene. For the diastolic phenotype we detected strong association ($p < 0.00001$) at 8 SNPs on 6 different chromosomes, in 3 different genes. Included in these are variants in the DAB1 gene, a gene previously associated with schizophrenia and one SNP with p-value < 1x10-6 (rs1105266, p-value=2.45x10-7). This SNP is in the PRMT10 gene, a putative protein arginine methyltransferase. CONCLUSION These signals and genes are being further investigated in our full dataset. And additional analyses are underway. Additional analyses include a gene-based pathway analysis and a gene-gene interaction analysis. These data provide promising results that may lead to further insights into the genetics of anatomic coronary artery disease.

358W

A genome wide association study of carotid intima-medial thickness: a surrogate marker for cardiovascular disease. *S.H. Blanton[1], A.H. Beecham[1], L. Wang[1], C. Dong[1], D. Della-Morte[1], D. Cabral[1], M. Elkind[2], R. Sacco[1], T. Rundek[1].* 1) Human Genetics, Univ Miami, Miami, FL; 2) Columbia Univ, New York, NY.

Carotid intima-medial thickness (CIMT) is a recognized risk factor for cardiovascular disease. The genetic underpinnings of CIMT are not known. The aim of this study was to identify risk loci for CIMT by performing a genome-wide association study. CIMT of 991 individuals from the population-based Northern Manhattan Study was measured by high-resolution B-mode ultrasound and expressed as the total IMT defined as mean of the maximum (IMTm). Other carotid segment-specific IMT phenotypes (including bifurcation-BIF, common-CCA and internal carotid artery-CCA) were also examined. Genotyping was done using the Affymetrix SNP array 6.0. Linear regression was used to look for association between the SNPs and our phenotypes while controlling for age, sex, date of ultrasound, pack years of smoking, hypertension, diabetes, dyslipidemia, BMI, and WHR. Additionally, two vectors from Eigenstrat were used to control for substructure. While no SNPs met genome-wide significance, we observed strong associations ($p < 1.0\times10\text{-}5$) with IMTm on 5 chromosomes (1, 5, 9, 11, 20) and 4 genes including in Astroactin 1 (ASTN1), Astroactin 2 (ASTN2), and Rho GTPase activating protein 26 (ARHGAP26). Most notable were associations in ASTN1 and ASTN2 on chromosomes 1 and 9, respectively, because of the association of ASTN1 with other carotid segments. Nine ASTN1 SNPs were associated with BIF ($p < 1.0\times10\text{-}6$) and to a lesser degree with ICA (p from $9.0\times10\text{-}3$ to $1.2\times10\text{-}1$) and CCA (p from $6.9\times10\text{-}5$ to $1.2\times10\text{-}2$). Other strong associations ($p < 1.0\times10\text{-}5$) were seen for BIF in WDR66 and ARHGAP26; for CCA in SBF2, ASTN2, and USP6NL; and for ICA in SETX, TLL1, PRND, and NOS1AP. The genes identified in our study do not play an obvious role in atherosclerosis. Fine mapping and pathway analysis are being undertaken to further investigate these genes/regions in CIMT.

359W

Renin gene polymorphism modulates extent of hypertrophy in hypertrophic cardiomyopathy, independent of blood pressure. *N. Carstens[1], L. Van der Merwe[2], M. Revera[3], M. Heradien[4], A. Goosen[4], P.A. Brink[4], J.C. Moolman-Smook[1].* 1) US/MRC Centre for Molecular and Cellular Biology, Stellenbosch University, Tygerberg, Western Cape, South Africa; 2) Biostatistics Unit, Medical Research Council of South Africa, Tygerberg, South Africa; 3) Department of Cardiology, IRCCS San Matteo Hospital, Pavia, Italy; 4) Department of Medicine, University of Stellenbosch Health Sciences Faculty, Tygerberg, South Africa.

Hypertrophic cardiomyopathy (HCM), an inherited primary cardiac disorder mostly caused by defective sarcomeric proteins, serves as a model to investigate left ventricular hypertrophy (LVH). The disease manifests extreme variability in the degree and pattern of LVH, even in HCM patients with the same causal mutation. Previous studies identified renin-angiotensin-aldosterone system (RAAS) components as hypertrophy modifiers in HCM; however, investigations of the renin section of the RAAS pathway in HCM have not previously been reported. We investigated six single nucleotide polymorphisms (SNPs) within the renin (REN) gene for association with heritable cardiac hypertrophy traits in a cohort of families that each harbour one of three HCM founder mutations. After adjustment for known hypertrophy confounders and the primary HCM causal mutation, we report a significant association between rs1464816 and left ventricular mass (LVM) ($p = 0.021$) as well as a composite hypertrophy score that best described the variability in hypertrophy in the present cohort ($p = 0.013$). We demonstrate that variation in REN plays a role in modulating hypertrophy in HCM, independent of blood pressure. Given the efficacy of the direct renin inhibitor in promoting LVH regression in hypertensive patients, this study informs on potential anti-hypertrophic therapy targets for HCM.

360W

Blood pressure and retinal arterial narrowing: assessing causality through Mendelian randomization. *C.Y. Cheng[1,2,3], X. Sim[4], M.K. Ikram[2,3,5], B. Cornes[3], J.J. Wang[6,7], P. Mitchell[6], Y.Y. Teo[2,4,8,9], S.M. Saw[2,3], T. Aung[1,3,10], E.S. Tai[2,5], T.Y. Wong[1,3,7,10].* 1) Department of Ophthalmology, National University of Singapore, Singapore; 2) Department of Epidemiology and Public Health, National University of Singapore; 3) Singapore Eye Research Institute, Singapore; 4) Centre for Molecular Epidemiology, National University of Singapore, Singapore; 5) Department of Medicine, National University of Singapore; 6) Centre for Vision Research, Department of Ophthalmology, University of Sydney, New South Wales, Australia; 7) Center for Eye Research Australia, University of Melbourne, Melbourne, Australia; 8) Department of Statistics and Applied Probability, National University of Singapore, Singapore; 9) Graduate School for Integrative Science and Engineering, National University of Singapore, Singapore; 10) Singapore National Eye Centre, Singapore.

Purpose: Retinal arteriolar narrowing is strongly associated with higher blood pressure (BP) and independently predicts clinical hypertension. However, previous cross-sectional observational studies prevent direct inference of causality between BP and development of retinal arteriolar narrowing. We aimed to generate estimates of the association between BP and retinal arteriolar caliber that were free from confounding and reverse causation. **Methods:** We examined the association between BP and retinal arteriolar caliber using conventional linear regression among 1976 participants from the Singapore Malay Eye Study. We compared these estimates with those derived from a Mendelian randomization framework with variants in four recently-discovered loci for BP in East Asians. We used a genetic risk score as the instrument variable, by combining four SNPs (rs17030613 [*ST7L-CAPZA1*], rs6825911 [*ENPEP*], rs35444 [*TBX3*], and rs17249754 [*ATP2B1*]) and weighting each SNP by its strength of association with systolic (SBP) and mean arterial BP (MABP). **Results:** The genetic risk score was significantly associated with both SBP (F = 13.5, P <0.001) and MABP (F = 19.0, P <0.001), but not associated with potential confounders (body mass index, lipid, blood glucose, diabetes, smoking, alcohol drinking, and C-reactive protein). On controlling for age, gender, genetic ancestry and potential confounders, each 10 mmHg increase in SBP and MABP was associated with a 1.4 μm (95% confidence interval [CI]: 1.1, 1.7) and 2.8 μm (95% CI: 2.3, 3.2) narrowing of retinal arterioles (both P <0.001), respectively. In the Mendelian randomization analysis using the genetic risk score as an instrument, the estimated causal effect was a decrease in arteriolar caliber of 1.3 μm (95% CI: -2.2, 4.8) and 1.7 μm (95% CI: -3.2, 6.6) for each 10 mmHg increase in SBP and MABP, respectively. The difference between the linear regression and instrumental variables estimates was not significant for both SBP and MABP (both P >0.05, by the Durbin-Wu-Hausman tests). **Conclusion:** The point estimates from the instrumental variable analysis did not substantially differ from those obtained from conventional regression analysis, but were not significant. Based on this Mendelian randomization study, we could not precisely estimate the causal association between BP and retinal arteriolar caliber. A larger sample size is needed to determine the causal effects.

361W

A Genome-wide Gene Expression Association Study of Young-Onset Hypertension in Han Chinese Population of Taiwan. *K.M. Chiang[1,4], H.C. Yang[2], J.W. Chen[3], W.H. Pan[1].* 1) Institute of Biomedical sciences, Academia Sinica, Taipei, Taiwan; 2) Institute of Statistical Science, Academia Sinica, Taipei, Taiwan; 3) National Yang-Ming University School of Medical and Taipei Veterans General Hospital, Taipei, Taiwan; 4) Institute of Life Science, National Defense Medical Center, Taipei, Taiwan.

Hypertension is a common and complex disorder. Although many large-scale genome-wide association studies have been performed, only a few studies have successfully identified the loci that are related to the hypertension, not to mention the scanty Asian studies. Young-onset hypertension (YOH) may be a more feasible target disorder to investigate than the late-onset one due to its stronger genetic component. We performed a two stage genome-wide gene expression association study to map YOH susceptibility genes. In the first stage, we analyzed 126 YOH cases and 149 normotensive controls with age, sex and BMI adjusted in all the analyses. In the second stage, an independent set of 127 YOH cases and 150 normotensive controls were used to validate the findings from the first stage. Phalanx Human OneArray v5.1 (30,275 transcripts) was used to measure the whole genome gene expression profiles. Several researches have been shown that CNVs may cause the gene dosage effect. According to this issue, we also check the CNVs status in the genes that we found. About 4,300 CNV SNP probes on the Illumina HumanHap 550K Genotyping BeadChip were used for CNV calling. Two softwares, PennCNV and QuantiSNP which implements a hidden Markov model (HMM) that integrates multiple sources of information to infer CNV calls for individual genotyped samples were used to identify the CNV regions. In addition to the single gene tests, we also perform the gene set analyses based on 4277 Gene Ontology categories, 299 BioCarta pathways and 128 KEGG pathways. Several gene sets have been identified in our study (p-value < 10^{-5}). We will also use WTCCC data to verify those gene sets by the SNP-based Gene set analysis.

362W

Cardiomyopathy and sudden cardiac death in Newfoundland. *F. Curtis[1], S. Connors[2], A. Williams[2], J. Houston[3], T. Young[3], K. Hodgkinson[4].* 1) Provincial Medical Genetics, Eastern Health, St. John's, Newfoundland, Canada; 2) Cardiology, Memorial University, St. John's Newfoundland, Canada; 3) Molecular Genetics, Memorial University, St. John's, Newfoundland, Canada; 4) Clinical Epidemiology and Genetics, Memorial University, St. John's, Newfoundland, Canada.

Introduction: Newfoundland is a genetic isolate, known for its founder population. Research into cardiomyopathies (CM) and sudden cardiac death (SCD) began in 1996 with a team of academics and clinicians. Aim: To ascertain all families with SCD and cardiomyopathy in Newfoundland, and determine their genetic etiology. Method: All families consnting to the research were originally referred for clinical work-up, firstly to the Provincial Medical Genetics Program, then to a Genetics/Cardiac clinic formed in 2004. Results: Since 1996, 369 families (the largest family comprising 1200 subjects over 10 generations) were ascertained. There were 17 different referral diagnoses, 50% of which were cardiomyopathies (dilated CM (DCM), hypertrophic CM (HCM) and CM not specified), 28% with a family history of SCD, 8% for arrhythmogenic right ventricular CM (ARVC), and 2% for Long QT syndrome (LQTS). The remaining 12% comprised 11 other referral diagnoses. To date 15 genes associated with inherited heart conditions with varying mutations have been found in74 families (from 135 families with DNA available (57%)). Of these 74, 30% (22 families) have the *TMEM43* mutation discovered by our team in 2008 and 32% (23 families) have either a *MYBH3* or *MYH7* mutation. Twelve families have mutations in other ARVC genes, and 4 families have *LMNA* mutations. At least three families have more than one mutation segregating, and ongoing genotype phenotype studies are taking place. Since the start of the clinic, referral numbers continue to increase with 47% of the referrals falling into the last three years. Discussion. We have determined the genetic etiology of over 50% of families with available DNA to date. Families with large histories and negative for known mutations will be clinically ascertained and genetic research will continue. Our combined team of clinicians, epidemiologists, geneticists, bioethicists, genetic counsellors, nurses and molecular geneticists have an unparalleled opportunity to determine the genetic and clinical epidemiology of all Newfoundlanders.

363W

Marfan Syndrome - A comparison of the Ghent nosologies. *J. Dean[1], J. Crow[2], W. Lam[2].* 1) North of Scotland Regional Genetics Service, Ashgrove House, Aberdeen, United Kingdom; 2) South East Scotland Regional Genetics Service, Western General Hospital, Edinburgh, United Kingdom.

Marfan syndrome is a variable, autosomal dominant connective tissue disorder whose cardinal features include ascending thoracic aortic aneurysm, lens subluxation, and skeletal abnormalities. Some of the milder features may be seen in unaffected people, which complicates the clinical diagnosis. The Ghent 1996 nosology was devised to assist diagnostic consistency, but is complex requiring evaluation of 30 clinical features. It is unreliable in children because of age-dependent penetrance of some features. The revised Ghent 2010 nosology is intended to address some of these issues. We reviewed findings in Marfan index patients from the East of Scotland using both nosologies to compare effectiveness and ease of use of each. 71 patients fulfilled Ghent 1996. Using Ghent 2010, 58 of these were diagnosed as Marfan syndrome, 13 were diagnosed unaffected. A fibrillin 1 mutation was detected in 79% of cases diagnosed using Ghent 1996, and 87% of cases diagnosed using Ghent 2010. 3 cases diagnosed Marfan by Ghent 1996 were scored unaffected by Ghent 2010, yet had fibrillin 1 mutations. Ghent 2010 is more specific but less sensitive for diagnosing fibrillin 1 mutation Marfan syndrome than Ghent 1996. Current cardiac findings have more weight, other findings less. Some Marfan patients, who may develop aortic aneurysm in the future, will be missed by Ghent 2010. Patients who fulfil Ghent 1996 should have fibrillin 1 testing and appropriate aortic follow-up.

364W

The Complex Genetics of ARVC/D in Maritime Canada. *S. Dyack[1 2 3], J. Hathaway[2], A. Crowley[2], C. Gray[3 4], M. Gardner[3 4].* 1) Department of Paediatrics, Dalhousie University, Halifax, NS, Canada; 2) Maritime Medical Genetics Service, IWK Health Centre, Halifax, NS, Canada; 3) Department of Medicine, Dalhousie University, Halifax, NS, Canada; 4) Division of Cardiology, QEII Health Sciences Centre, Halifax, NS, Canada.

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is an inherited condition that can lead to fatal arrhythmias and shows great clinical variability and genetic heterogeneity. Here we describe difficult genetic and cardiac management issues faced as a result of complex genetic test results and the expression of clinical phenotypes in these families. A retrospective chart review was undertaken of probands and their assessed family members referred to Genetics for a personal or family history of ARVC/D. Our Genetic/Cardiology team sees all ARVC families in our region. A total of thirty-three probands were assessed between 2008-2011. Twelve (36.4%) were found to carry a mutation(s) felt to be definite or likely pathogenic. Six (18%) probands were found to carry a VUS. Twelve probands (34.4%) were found to carry neither a mutation nor a VUS. In 4 probands (12.1%), testing could not be completed. We have had difficult counseling and management issues in this population including: Sex specific penetrance for a presumed pathogenetic change in a large family with a DSP mutation, apparent autosomal recessive inheritance for severe ARVC with minimal expression in “carriers”, overlap of features of long QT syndrome and ARVC in several families, and cases where there is a clear pathogenic mutation, but gene negative individuals experience life threatening events. These cases illustrate that the clinical evaluation of family members by expert Cardiologists in concert with a Medical Genetics assessment and genetic testing is optimal to ensure that families receive accurate risk management. Especially in smaller families, it can be difficult to provide accurate counselling and management based on genetic test results alone.

365W

Clinical, Cytogenetic and Molecular studies in Egyptian Children with Congenital Heart Disease (CHD). *M.O. EL RUBY[1], S. El-Seaady[2], N. Helmy[1], H. Hussein[1], A. El-Gerzawy[1], A. Fayez[1], G. Noreldin[1], A. Abdel-Rahman[1], N. Ismaeil[1], M. Kobeisy[1], R. Lotfy[1], A. El Darsh[1], I. Hussein[1].* 1) National Research Centre, , El Bohooth st., Cairo ,Egypt; 2) Faculty of Medicine -Cairo University - Cairo - Egypt.

The cardiovascular anomalies responsible for congenital heart disease (CHD) are common birth defects and are a leading cause of infant mortality, morbidity. CHD is a primary feature of many genetic syndromes, but genetic causes of non-syndromic CHD are increasingly being recognized. CHD affects at least one in 100 live births. It ranges widely in severity, from tiny holes between heart chambers that close naturally, to life-threatening abnormal structures such as hypoplastic left-heart syndrome. Recently identified disease genes for syndromes associated with CHD are also increasing. To date, very few studies were performed in Egypt for studying the genetic basis of CHD. This study included 500 cases with CHD out of total 8000 cases referred as genetic disorder to the Clinical Genetic Department of the NRC during a period of 6 years (2004-2010). It aims to identify at risk families and types of syndromes with CHD, identify types of chromosomal anomalies causing these diseases & study the spectrum of mutations in known candidate genes. Chromosomal analysis was performed on 200 cases. Molecular studies were carried out for 189 cases. Mutation detection was done using PCR/ SSCP and DNA sequencing. Associated malformations as known syndromes included Russell Silver, Jacobsen, Noonan, Costello, Apert, Kamptomelic dysplasia, McKusik Kaufman, Rubinstien-Taybi, William, Holt-Oram & Roberts. Chromosomal anomalies reported are trisomy 21, trisomy 13, trisomy 18, 46, XY, t (14;18), 46, XYt (8;9), 47, XX, + mar ; 46, XX del 11q ,46, XX, dup13q, 47, XXX, 45, X, 47XXY, 46, XX, inv(18). DNA sequencing of NKX2.5 gene revealed two different mutations in two unrelated patients. Sequencing of GATA4 gene in patients with TOF has shown polymorphism in exon 6 at nucleotide 53423 (A-G) in 5 patients. The genetic investigation results of Nkx2.5 gene concluded the presence of missense mutation in one case with TOF, two missense mutation in two cases of ASD and one silent mutation in one ASD case. Results of GATA4 gene concluded the presence of two silent mutations in two TOF cases ,and 3 SNPs in 9 TOF cases .Results of 22q11.2 critical DiGeorge region concluded the presence of microdeletion in 6 DiGeorge/ VCF syndrome cases .Results of PTPN11 gene concluded the presence of Polymorphism -21 (C ◇ T) in one case with Noonan syndrome.

366W

Motivation to pursue genetic testing in individuals with a personal or family history of cardiac events or sudden death. *K.E. Erskine[1], L. Cohen[2], M. Stolerman[3], E. Paljevic[4], C.A. Walsh[5], T.V. McDonald[6], R.W. Marion[7], S.M. Dolan[1].* 1) Department of Obstetrics & Gynecology and Women's Health, Montefiore Medical Center, Bronx, NY; 2) Montefiore Medical Center, Bronx, NY; 3) Ferkauf Graduate School of Psychology, Yeshiva University, Bronx, NY; 4) Pediatric Heart Center, Children's Hospital at Montefiore, Bronx, NY; 5) Pediatric Dysrhythmia Center, Children's Hospital at Montefiore, Bronx, NY; 6) Departments of Molecular Pharmacology and Medicine/Cardiology, Albert Einstein College of Medicine, Bronx, NY; 7) Divisions of Developmental Medicine & Genetics, Children's Hospital at Montefiore, Bronx, NY.

In the era of personalized medicine, genetic testing is becoming increasingly more available for diagnostic and predictive testing for a variety of genetic conditions. Previous studies have assessed the motivations of individuals pursuing this genetic testing, particularly in the area of hereditary cancer syndromes and have found that individuals cite a variety of motivations for pursuing testing. In the area of sudden cardiac death, genetic testing exists to detect mutations responsible for cardiac channelopathies, such as Long QT syndrome and Brugada syndrome, and results of this testing can be used to shape medical management and lifestyle changes for the proband as well as to screen at-risk family members. An interdisciplinary team at the Montefiore-Einstein Center for Cardiogenetics (MECC) has interviewed a total of 49 individuals, who were recruited from the MECC, Sudden Unexplained Death in Childhood Program (SUDC), and Sudden Arrhythmic Death Syndromes Foundation (SADS). The participants were engaged in open-ended interviews or focus groups, and these sessions were recorded and transcribed verbatim. Of the 49 individuals interviewed, 36 described their motivation to pursue genetic testing. Key motivations identified include: personal medical management, concern about children or future generations, wanting to relieve uncertainty or to obtain peace of mind, and in response to the recommendation of a physician. Through data from previous studies as well as the responses of these participants, it is clear that individuals have varying motivations for pursuing genetic testing that must be recognized in order to provide effective genetic counseling and obtain informed consent.

367W

Identification of EGFR polymorphisms that interact with body weight and systolic blood pressure in young Finnish adults and the prevalence of hypertension in elderly Canadians. *M. Fan[1], T. Lehtimaki[2], O. Raitakari[2], M. Kähönen[2], R.W. Davies[1], L. Chen[1], G.A. Wells[1], R. McPherson[1], R. Roberts[1], A.F.R. Stewart[1].* 1) University of Ottawa Heart Institute, Ottawa, Ontario, Canada; 2) Department of Clinical Chemistry, University of Tampere, Tampere, Finland.

Background: Hypertension is more frequent in obese subjects and substances released from adipocytes may link obesity to increased blood pressure. Methods and Results: We performed a genome-wide interaction analysis for single nucleotide polymorphisms that associate with hypertension through interaction with body weight. A discovery population consisted of the control group from the Ottawa Heart Genomics Study, elderly European-ancestry Canadian men aged >65 and women aged >70 years without symptoms of coronary artery disease (N=2392). A threshold for association was set at $p<0.01$ and polymorphisms were selected by biological plausibility. We identified a polymorphism, rs7795743 (MAF=0.37) in the epidermal growth factor receptor (EGFR) gene where body weight showed an interaction with hypertension ($P=2\times10^{-4}$). The interaction was tested in the Cardiovascular Risk in Young Finns cohort comprised of 2103 young adults aged 24-39 years. In the Young Finns cohort, the interaction of rs7795743 with body weight was also related to systolic blood pressure (P=0.009), but not to diastolic blood pressure. rs7795743 directly influenced the body mass index (BMI) either among young Finnish adults (P=0.009) or elderly Canadians (P=0.019). No influence was found on blood pressure. In a stratified analysis according to body weight, the prevalence of hypertension was significantly increased among carriers of the minor allele in the over-weight group (BMI>27.8, p=0.006). Conclusion: Polymorphisms in EGFR gene influence the risk of hypertension differently among obese and non-obese individuals. Given the complexity of hypertension, our study reveals the need to refine hypertensive phenotypes to identify genetic loci that confer risk through phenotypic interaction.

368W

Prioritized-GWAS based on linkage information identifies novel putative loci influencing coagulation. *F. Gagnon[1], A. Dimitromanolakis[1], G. Antoni[1,2], A. Martinez[3], N. Greliche[2], A. de Buil[3], J.M. Soria[3], P.E. Morange[4], P.S. Wells[5], D.A. Tregouet[2], L. Sun[1,6].* 1) Dalla Lana School of Public Health, University of Toronto, Toronto, Canada; 2) INSERM, UMR_S 937, Paris, France; 3) Unitat de Genomica de Malalties Complexes, Institut de Recerca Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; 4) INSERM, UMR_S 626; Université de la Méditerranée, Marseille, France; 5) Ottawa Health Research Institute, Ottawa, Canada; 6) Department of Statistics, University of Toronto, Toronto, Canada.

Better understanding of the modulation of coagulation Factor XII (FXII) will provide critical insight into thrombi formation and repair, and inflammatory processes. The identification of new molecular pathways underlying some complex traits has been possible with genome-wide association studies (GWAS). However, most often, GWAS do not provide overwhelming evidence for association and results are difficult to replicate due the inherent challenges associated with multiple hypothesis testing. An alternative strategy is to use oligogenic genome-wide linkage studies (GWLS), which can be powerful to identify loci, but these regions are imprecise. We hypothesized that coupling the information brought by both strategies can increase the efficiency for identifying loci. We applied such combined approach to study coagulation FXII levels using 5 multigenerational French-Canadian families (n=253) ascertained through single probands with venous thrombosis and carrier of Factor V Leiden variant. We conducted a combined analysis involving: 1) GWLS using 6522 informative and independent GWAS SNPs (MAF>0.2; r^2<0.8); 2) family-based GWAS using 528438 autosomal SNPs after QC; 3) combined analysis of GWLS and GWAS results using the novel stratified false discovery rate (SFDR) approach (Yoo et al. 2010), allowing for SNPs with some evidence of linkage to have higher weight in the prioritized-GWAS. In addition to the known structural gene encoding FXII protein, regions on chr 1 and 11 showed promising evidence for both linkage (LOD>1.5) and association ($p=10^{-5}$). When SFDR was applied to these results, several SNPs in chr 1 and 11 loci reached genome-wide significance. The strongest evidence for association was observed at the SLC35F3 locus (q-value=0.0003). Interestingly, interaction between the SLC35F3 locus and the structural gene encoding FXII protein was detected (p=0.005) in the French-Canadian families. This SLC35F3 locus was then validated in the GAIT study for the) 2-glycoprotein 1 trait and in a case-control study of venous thrombosis, suggesting a pleiotropic effect of the SLC35F3 locus. The biological plausibility of such pleiotropic effect is supported by) 2-glycoprotein 1 being known to inhibit coagulation factor FXI activation by FXIIa. Our prioritized-GWAS incorporating linkage information allowed the identification of novel loci implicated in coagulation, despite the initial absence of genome-wide significance based on association or linkage alone.

369W

Association of e2, e4 alleles of polymorphism Apo E in Mexican pre-eclampsia patients. *M. Gallegos Jr[1], A. Acevedo[1], LE. Figuera[3], AM. Puebla[2], GM. Zuñga[4].* 1) Dept Med Molec, Guadalajara, CIBO, IMSS, Jalisco, Mexico; 2) Laboratorio de Inmunifarmacologia, CUCEI, UdeG; 3) Division de Genetica, CIBO, IMSS; 4) Laboratorio de Mutagenesis, CIBO, IMSS.

The etiology of preeclampsia is unknown, but different studies have been observed that the endothelial cell injury and altered endothelial cell function play a role in this syndrome and also observed the increased values of triglycerides and cholesterol in patients with preeclampsia. The allele e4 is a genetic marker for dyslipidaemia, the e2 allele has been suggested they might increase the risk of developing preeclampsia and the e3 allele is most common in the population. In the present study we describe to frequency of e3, e2 and e4 alleles of polymorphism Apo E in 51 patients and 49 woman health controls from Mexico. The frequency observed of 18.8% (19/102) and 5% (5/98) of e2 allele; 64.7% (66/102) and 90.8% (89/98) for allele e3; 16.7% (17/102) and 4.1% (4/98) for allele 4 in cases and controls respectively. Statistically different (p<0.05) were observed when to compared each one alleles in the studied groups. These results suggest that e2 and e4 allele is risk allele for Mexican preeclampsia patients.

370W

Does genetics add value? Our experience of cardiac genetics referrals at Loma Linda University Medical Center Center (LLUMC) in the USA, highlighting the need for a protocol for cardiac genetic work-ups. *J. Gold[1], S. Ramanathan[1], J. Bartley[1], L. Bailey[2], R. Clark[1].* 1) Pediatrics,Department Genetics and Metabolism Loma Linda University Medical Center, Loma Linda, CA; 2) Department Cardiothoracic Surgery, Loma Linda University Medical Center, Loma Linda, CA.

LLUMC is internationally recognized in infant transplants, pioneering the baboon to infant heart xenotransplant in 1984 and an allotransplant program since 1985. The study is from a review of 102 patients seen during the year April 2010-May 2011 with cardiac disease by geneticists at LLUMC. A total of 532 patients were seen in the genetics department over the same period. Individuals were seen as consults on the PICU, NICU or genetics clinic. The patients were referred to the genetics clinic pre or post cardiac transplant or when cardiac anomalies were isolated or part of a syndrome e.g. Williams. This has initiated the need for a genetics protocol prior to cardiac transplant. Methods: Review of all cardiac cases that were evaluated. Patients were seen due to isolated cardiac disease or as part of a work-up for multiple congenital anomalies. The data is from the department data base. A total of 19.2% (n102) of all cases referred to the genetics department had a cardiac component. I will present the data and individual cases, for example: 6 year old female, known to have a restrictive cardiomyopathy phenotype. Post cardiac-transplant, on histological examination hypertrophic cardiomyopathy was seen on the explanted heart. Genetic testing demonstrated a pathogenic mutation in TNN 13 and a variant of unknown significance in MYH7. We still await the test results for the parents and younger sibling. All, except the subject, had negative ECG and echocardiograms. Results: Total number of individuals with a cardiac anomaly is 102. 18.6 % (n19) have a syndrome diagnosis. 16.6% (n17) have a micro-deletion or duplication. Of these the commonest is the micro deletion syndrome 22q11 29.4% (n5).The total with chromosomal anomalies including aneuploidy and micro- deletion / duplications is 25.5% (n26). The commonest aneuploidy was trisomy 21 (n6). Conclusion From this review of data presented the value of genetics input is clear. It is difficult to evaluate value. It was recently defined as outcome achieved relative to cost incurred. But what cost can be attributed to a diagnosis? Because of the wide variation in cases it is important to have a protocol in place to be uniform in the treatment of all individuals with cardiac anomalies. I will present a protocol for pre-transplant patients. It is difficult to evaluate value. It was recently defined as outcome achieved relative to cost incurred.

371W

Genome-Wide Association Study for Resting Heart Rate: Variants Detection and Replication. *B. Gombojav[1], D.H. Lee[1], Y.S. Ju[2], J.I. Kim[2,3], J.S. Seo[2,3], M. Lee[1], Y.M. Song[4], K. Lee[5], J. Sung[1].* 1) Complex Disease and Genetic Epidemiology Branch, Department of Epidemiology and Institute of Environment and Health, School of Public Health, Seoul National University; 2) Genomic Medicine Institute Medical Research Center Seoul National University; 3) Department of Biochemistry and Molecular Biology Seoul National University College of Medicine; 4) Department of Family Medicine, Samsung Medical Center, and Center for Clinical Research, Samsung Biomedical Research Institute, SungKyunKwan University School of Medicine, Seoul, Korea; 5) Department of Family Medicine, Busan Paik Hospital, Inje University College of Medicine, Korea.

Background: Previous studies have identified increased resting heart rate as an independent risk factor for cardiovascular morbidity and mortality. Although heritable factors play a substantial role in regulating heart rate, evidence about specific genetic determinants is limited. A recent genome-wide association study that enrolled individuals of Asian population has revealed a locus (6q22.31) associated with pulse rate. Objective: To identify common genetic variants associated with heart rate. Methods: A genome - wide association analyses was performed among 3,029 individuals from 661 families of the Healthy Twin Study Korea, and 1026 individuals from 73 extended families of the GENDISCAN study Mongolia. The association between heart rate (adjusted for age and sex) and genetic markers analyzed by Affymetrix GeneChip v6 and Illumina 610K was estimated. A total of 516,452 markers for Korean and 586,875 markers for Mongolian samples remained after cleaning for conventional quality control. Heart rate was measured by electrocardiogram in resting state. Individuals were excluded if they had atrial fibrillation (n = 4), atrioventricular block (n = 13), right bundle branch block (n=6), left anterior fascicular block (n=8) and myocardial infarction (n = 12) as well as ischemia (n = 7). Results: The estimated heritability of heart rate was 0.44 (P <.0001) for Korean, and 0.32 (P <.0001) for Mongolian samples. We have identified six novel loci, which associated with heart rate: 6q22.31 near AL713649 (P = 1.02 x 10-6); 22q13.1 near CACNA1I (P < .00001); 7q33 near CHRM2 (P < .00002); 3p21.31 near LTF (P < .00001); 1p31.1 near KIAA0786 (P < .00002); and 4q22.3 near LIM (P = 5.94 x 10-7). Among the six candidate loci, 6q22.31 has been reported to associate with heart rate and CACNA1I controls the rapid entry of Ca 2 + into a cell. The genotypic effect of AL713649, individuals with the GG genotype had a significantly higher heart rate than those who had TG and TT, the mean heart rate was 67.4 bpm, 65.9 bpm and 65.4 bpm, respectively (P < .0013). Individuals with the CC genotype of CACNA1I had a significantly higher heart rate than those who had TC and TT, the mean heart rate was 66.6 bpm, 64.8 bpm, and 60.3 bpm, respectively (P < .0164). Conclusion: We have identified six candidate genetic loci harboring common genetic variants that were associated with resting heart rate. Further studies are required to replicate these findings and biological validation.

372W

Salt-sensitivity of blood pressure is strongly associated with polymorphisms in the sodium-bicarbonate symporter. *L. Gordon[1], R. Carey[2], C. Schoeffel[2], J. Gildea[2], J. Jones[3], H. McGrath[2], M. Park[1], S. Williams[1], P. Jose[3], R. Felder[2].* 1) Center for Human Genetics Research, Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN; 2) Departments of Medicine and Pathology, University of Virginia School of Medicine, Charlottesville, VA; 3) Center for Molecular Physiology Research, Children's National Medical Center, Department of Pediatrics and Medicine, George Washington University School of Medicine and Health Sciences, Washington, DC.

The $NaHCO_3$ cotransporter gene (SLC4A5) on chromosome 2 encodes a protein that transports Na^+ and HCO_3^- electrogenically across the basolateral membrane of many cell types, including renal tubule cells, to the interstitial fluid and ultimately into the circulation. Single nucleotide polymorphisms (SNPs) of SLC4A5 have previously been associated with baseline and 10-year follow-up blood pressure (BP). We tested the hypothesis that SNPs in SLC4A5 and other candidate genes are associated with salt-sensitivity of BP (SS) in Caucasians. Tests for genetic association for BP traits were performed in 185 subjects of European ancestry ages 18-70 y with body mass index (BMI) 18-30, consuming an isocaloric constant diet containing 1g protein/kg body weight /d and 60 meq/d K^+ with a randomized order of 7 days low Na^+; (10 mEq/d) and 7 days high Na^+ (300 mEq/d) intake. SS was defined as a⁄ 7mm Hg increase in mean arterial pressure (MAP) with the subject on high as compared to low Na^+ intake. A total of 41 polymorphisms in 17 candidate genes were assayed. Association with hypertension at baseline revealed only one marginally significant association, rs3830634 in caveolin 1 (CAV1) (P= 0.027). Association analyses with SS revealed several variants that marginally associated with SS in SLC4A5, the dopamine D2 receptor (DRD2), and G protein-related kinase-4 (GRK4) (P < 0.05). However, two SNPs in SLC4A5 (rs10177833 and rs7571842) demonstrated significant results using logistic regression. These two SNPs had P values of $3.5X10^{-4}$ and $8.0X10^{-4}$ and odds ratios of 3.7 and 2.9 in unadjusted regression models, respectively. After adjusting for BMI and age, the associations became stronger (P values $8.9X10^{-5}$ and $2.6X10^{-4}$ and ORs 4.7 and 3.5, respectively). These two SLC4A5 SNPs were in linkage disequilibrium (LD) in both our SS and salt resistant (SR) subjects, but the strength of the LD was greater in SR subjects, (r^2= 0.87 and 0.98, respectively), further supporting the conclusion of an association of SLC4A5 with SS. Lastly, we assessed whether there were any associations with a negative response to salt loading (i.e., a decrease of MAP with high sodium intake). One SNP in DRD2 (rs6276) revealed a highly significant association (P=$8.9X10^{-4}$, OR= 2.5 in an unadjusted model and P= $7.5X10^{-4}$, OR= 2.6 in a model adjusting for BMI and age). Therefore, SLC4A5 variants are strongly associated with SS and DRD2 is negatively associated with SS in Caucasians.

373W

ENPP1 polymorphisms influence the risk of developing calcific aortic valve stenosis. *S. Guauque-Olarte[1], V. Ducharme[1], N. Gaudreault[1], P. Pibarot[1], P. Mathieu[1], Y. Bossé[1,2].* 1) Centre de recherche Institut universitaire de cardiologie et de pneumologie de Québec, Laval University, Quebec, Canada; 2) Department of Molecular Medicine, Laval University, Quebec, Canada.

Calcific aortic valve stenosis (AS) affects 2% of people older than 65 years. AS is a life threatening disease that is increasing in prevalence with the aging population and for which there is currently no medical treatment. Calcification of the aortic valve is a defining pathological feature of AS. Loss-of-function mutations in the ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) gene are known to cause bone diseases and arterial calcification. ENPP1 is also known to generate inorganic pyrophosphates, which are inhibitors of calcification. ENPP1 is thus a biological candidate gene for AS. The goal of the present study was to perform a genetic association study testing ENPP1 polymorphisms in a case-control series of patients with AS. The study involved 457 patients who underwent surgical aortic valve replacement for severe AS. Tagging SNPs were selected using Haploview and the European-derived (CEU) HapMap genotyping dataset. SNPs in ENPP1 previously associated with risk factors for the progression of AS were forced into the SNP set. A total of 30 SNPs were genotyped using the Illumina® BeadXpress platform. The controls were 3,294 white individuals from the Illumina® iControlDB, genotyped on the HumanHap550 genotyping BeadChip. The MACH program was used to infer the genotypes of 5 SNPs not genotyped in controls using the phased haplotypes of 60 unrelated individuals from the HapMap CEU as a reference set. The association tests were performed on 20 SNPs that passed genotyping quality control filters. Six SNPs were significantly associated with AS (p < 0.05). Following correction for multiple testing, three SNPs were significantly associated with AS including rs1800949 located in the promoter (p = 2.4E-03), rs9402349 in intron 9 (p = 1.2E-03) and rs7754586 in the 3'UTR region (p = 6.0E-04). The pairwise linkage disequilibrium for these three SNPs measured by r^2 range from 0 to 0.14. The results of this study suggest that a number of independent SNPs in the ENPP1 gene modulate the risk of AS. Further analyses are needed to understand how these associated SNPs lead to abnormal function of the ENPP1 gene that in turn promotes the development of AS.

374W

ABCA1 gene promoter DNA methylation is associated with a deterioration of HDL-cholesterol metabolism in familial hypercholesterolemia. *S. Guay[1,2], D. Brisson[2,3], J. Munger[1,2], D. Gaudet[2,3], L. Bouchard[1,2].* 1) Deparment of biochemistry, Université de Sherbrooke, Sherbrooke, Québec, Canada; 2) ECOGENE-21 and Lipid Clinic, Chicoutimi Hospital, Saguenay, Québec, Canada; 3) Department of medicine, Université de Montréal, Montréal, Québec, Canada.

Background: The cholesterol contained in high-density lipoproteins (HDL-C) is known as a negative clinical biomarker of cardiovascular disease (CVD), especially coronary artery disease (CAD). Significant interindividual variations in circulating HDL-C levels is common, even in subjects at high risk of CVD such as those with familial hypercholesterolemia (FH). Although heritability estimates for circulating HDL-C levels are high (up to 60%), the related genes and molecular mechanisms remain to be identified. The ATP-binding cassette A1 (ABCA1) is a membrane protein involved in cholesterol and phospholipid transfer from peripheral cells to nascent HDL particles in circulation, a key step of reverse cholesterol transport. ABCA1 deficiency is associated with decreased HDL-C levels and an increased CVD risk. Recently, DNA methylation profile perturbations at the *ABCA1* gene promoter have been observed in adults exposed in utero to famine. Our hypothesis is that DNA methylation variation at the *ABCA1* gene promoter explains interindividual differences in circulating HDL-C levels among FH patients. **Methods/Results:** DNA from 51 FH patients (74.5% men), all heterozygous for the same mutation in the *LDLR* gene (p.W66G), was purified from whole blood samples. Twenty-five of these patients were under hypolipidemic treatment before the beginning of the study (mean therapeutic wash-out of six days), whereas the remaining 26 were naïve of treatment. *ABCA1* DNA methylation levels were measured using bisulfite-converted DNA pyrosequencing. Cholesterol and phospholipids levels were measured enzymatically in HDL particles isolated by ultracentrifugation. The *ABCA1* gene promoter DNA methylation was negatively correlated with circulating HDL-C (r=-0.39; p=0.007) and HDL2-phospholipid levels (r=-0.43; p=0.044) when controlled for age, gender, waist circumference and fasting triglyceridemia. Although not significant, FH patients with CAD (n=11) had higher *ABCA1* DNA methylation levels compared to those without CAD (41.3% vs. 35.9%; p=0.076). This trend was confirmed in an independent sample of 223 heterozygous p.W66G FH patients (36.7% vs. 34.7%; p=0.077), especially in women (n=133; 39.3% vs. 34.7%; p=0.022). **Conclusion:** According to these results, epigenetic modification of the *ABCA1* gene promoter could explain a significant proportion of the interindividual circulating HDL-C level variation and is associated with CAD expression among FH women.

375W

LQTS in Northern BC:A more severe cardiac phenotype but minimal impact on auditory function with homozygosity for KCNQ1 V205M rules out Jervell and Lange Nielson syndrome. *H. Jackson[1], S. Lauson[1], R. Rupps[2], S. McIntosh[1], B. Casey[2], A. Tang[1], L. Arbour[1].* 1) University of British Columbia, Victoria, British Columbia , Canada; 2) University of British Columbia, Vancouver, British Columbia , Canada.

Background: Two First Nations communities in northern British Columbia are affected disproportionately with LQTS due to two different KCNQ1 mutations (V205M and R591H). The current known mutation rate within these two communities (1/125) is predicted to be one of the highest in the world at 20 times that which is expected for the general population. The question remains, however, as to whether carrying more than one of these mutations is associated with a more severe clinical phenotype and/or with deafness (Jervell and Lange Nielsen syndrome (JLNS)). Methods: All individuals identified have been tested for both mutations. Clinical information was recorded. QT intervals were determined in all leads by the tangent method and the longest QT interval in any available ECG recording was used in the determination of the `peak' QTc (± SD). QTc values were calculated using the Bazett correction method and an average RR interval for the ECG. Results: More than 70 individuals were identified to have at least one mutation. While no compound heterozygotes have been identified, four individuals were homozygous for the KCNQ1 V205M mutation. Homozygous V205M carriers had a significantly higher `peak' QTc (570.3 ± 91.12 ms) when compared to 41 heterozygous V205M carriers (472.9 ± 37.40 ms, p<0.0001) and the first to third degree relatives (n=58) without mutations (437.5 ± 27.82 ms). Two of four homozygous cases report mild childhood onset hearing loss. The other two reported no hearing loss, one confirmed with normal audiology. Three are treated with beta blockers, one with an implanted defibrillator. Conclusion:The lack of pre-lingual hearing loss categorizes the phenotype of our patients as RWS even though their genotypes confirm homozygosity of V205M. Furthermore, heterozygous carriers in this community also present with cardiac symptoms, supporting the autosomal dominant nature of LQTS caused by the KCNQ1 V205M mutation. The double-hit on normal cardiac repolarization and the greater reduction of IKs current experienced by homozygous mutation carriers is presumed to lead to a more severe clinical cardiac phenotype. Meanwhile, the resultant current must be sufficient to maintain homeostasis in the auditory pathway. However, because two of the cases have reported childhood onset hearing loss, milder, post-lingual hearing loss due to KCNQ1 mutations cannot entirely be ruled out and a further audiology study is planned for this population.

376W

Assumption-free heritability estimates of electrocardiographic measures in the TwinsUK study. *Y. Jamshidi[1], I.M. Nolte[2], H. Riese[2,3], P. van der Harst[4], F.W. Asselbergs[6], T.D. Spector[5], H. Snieder[2].* 1) Biomedical Sciences Division, St Georges University of London, London, United Kingdom; 2) Department of Epidemiology, Unit of Genetic Epidemiology, and Bioinformatics, University Medical Center Groningen (UMCG)/University of Groningen (RuG), Groningen, The Netherlands; 3) Interdisciplinary Centre for Psychiatric Epidemiology, UMCG/RuG, The Netherlands; 4) Department of Cardiology, UMCG/RuG, Groningen, The Netherlands; 5) Department of Twin Research and Genetic Epidemiology, King's College London, United Kingdom; 6) Department of Cardiology, Division of Heart and Lungs, University Medical Center Utrecht, Utrecht, The Netherlands.

The standard 12-lead electrocardiogram (ECG) measurements are effective markers both for predicting the outcomes of patients with heart disease as well as for predicting cardiovascular mortality in healthy subjects. A number of genome-wide association studies have recently investigated the genes associated with ECG traits. The gene variants discovered thus far explain <10% of the total variability in population ECG characteristics, when heritability (h^2) estimates suggest that up to 50% of this variability is genetic. Classical twin analysis allows estimation of the effects of genetic factors on the variance of a trait. The power to estimate these variance components is derived by assuming that MZ-twins have inherited identical genomes and DZ-twins share on average 50% of their genes. A higher MZ than DZ-twin correlation suggests that the trait under study is heritable. Some have argued that assumptions of the twin model may be invalid resulting in inflated h^2 estimates, thus explaining part of the missing h^2. In order to help characterize more reliably than previously possible the heritability for ECG traits, we obtained estimates from 3,313 twins from the TwinsUK cohort, using both the classical twin method and an assumption-free method which makes use of genome-wide coverage of genetic markers to directly estimate degree of resemblance between relatives in a model free from confounding with non-genetic factors. Univariate structural equation models were fitted for assessing the genetic and environmental contributions to the ECG traits. The AE model combining additive and unique environmental factors produced the best fit for all traits except the Cornell product, which could be predominantly explained by common and unique environmental factors. Modest to high heritability estimates (h^2>0.5) were obtained for all ECG traits (Heart rate, PR interval, QRS duration, RV5SV1, QT interval, Sokolow-Lyon product, Cornell product) using both methods. Our study is one of the largest to date looking at the heritability of ECG measures and one of the first to compare the two methods of estimating heritability in a comprehensively phenotyped twin cohort. The two different methods give comparable but also unique information. Reasons for these different findings will be discussed. Accurate h^2 estimates are crucial as they provide a yardstick against which the success of the h^2 explained by GWAS can be gauged.

377W

Genes and viruses in idiopathic (dilated) cardiomyopathy; a pilot study. *I.PC. Krapels[1], Y.H.J.M. Arens[1], A.T.J.M. Helderman-van den Enden[1], C. Eurlings[2], R. Jongbloed[1], A. van den Wijngaard[1], S.R.B. Heymans[2].* 1) Clinical Genetics, Maastricht University Medical Center, Maastricht, Netherlands; 2) Cardiology, Maastricht University Medical Center, Maastricht, the Netherlands.

Background: dilated cardiomyopathy (DCM) is characterized by impaired contractility and dilatation of the ventricles. Mutations in the currently known DCM-genes only explain a small number of DCM patients. The clinical phenotype in families with a known DCM mutation varies considerably. Cardiotropic viruses are involved in DCM and could explain the differences in clinical phenotype among individuals. We assessed the interaction between viral en genetic components in a group of idiopathic DCM patients. Methods: genetic analysis was performed through CardioCHIP analysis, a CHIP-based high throughput resequencing platform, simultaneously analysing 34 genes involved in inherited cardiomyopathies. The presence of cardiotropic viruses was assessed on the cardiac biopsies. Results: cardiac biopsy data was available for 177 patient; 59 (33%) of them had viral presence on cardiac biopsy (Parvo virus, >250 copies per mcg isolated DNA). One patient had an active myocarditis. Cardiochip data was available for 55 patients; 34 of them (62%) had genetic variations (either polymorphism, unclassified variant of mutation). The combined data of the Cardiochip analysis and cardiac biopsy was available for 30 patients. Of these 30 patients, 22 patients had no virus presence on biopsy. 12 of these 22 had genetic variants (55%), compared to 50% (4/8) in the group of patients with virus presence. Conclusion: Although based on small numbers, these data demonstrate that the presence of a viral infection as a cause for dilated cardiomyopathy does not exclude a genetic component. Further studies will address whether environmental factors such as a viral infections explain the difference in phenotype between patients and families with a known DCM mutation.

378W

Genetic Testing for Dilated Cardiomyopathy (DCM) in the United States: A Health Technology Assessment. *A. Lea, S. Levine, D. Allingham-Hawkins.* Hayes, Inc, Lansdale, PA.

Objectives: DCM may be caused by either genetic or nongenetic factors. The genetics of DCM are complex, with many genes associated with it, but each gene accounting for only a small proportion of cases. Approximately 90% of DCM is inherited in an autosomal dominant fashion, although recessive and X-linked inheritance patterns have also been observed. Identification of asymptomatic relatives of individuals with a genetic cause of familial DCM is likely to be clinically useful by allowing preventative interventions. This health technology assessment focused on reviewing the evidence that linked individual genes with DCM for the purpose of preventative screening. Methods: Laboratories that provided clinical genetic testing for DCM in the United States (US) were surveyed to identify genes for which screening is available. For each of these genes, a literature search was performed to identify data linking sequence variants in the gene with DCM. This evidence base was reviewed to characterize the strength of the association of genes with DCM through 4 characteristics: pathogenicity in 2 or more reports; severity of phenotype; penetrance; and inheritance pattern. Results: Seven laboratories were identified that provided genetic testing for DCM in the US. Included in the DCM testing panels were 41 genes. There was a low degree of consistency of genes tested by different laboratories; there were only 8 genes (ACTC1, LAMP2, LDB3, LMNA, MYBPC3, MYH7, TNNT2, and TPM1) in common for 6 of the 7 laboratories. Review of the evidence indicated that the relationship between sequence variants and phenotype was well defined for only 3 genes (LMNA, MYH7, and TNNT2). For 10 genes (EMD, LAMP2, MT-ND6, MT-TD, MT-TH, MT-TM, MT-TQ, MT-TS1, MT-TS2, and TTR), no evidence was identified that linked them with DCM. The relationship with DCM was incompletely defined for the remaining 28 genes. Conclusions: Clinical genetic testing for DCM is available for 41 genes in the US; however, a review of available evidence indicates that for only 3 of these genes is the genotype-phenotype relationship clearly defined. While research in the genetic underpinnings of DCM is finding more genes reported to be associated with DCM, the evidence base supporting the associations with DCM is variable. Additional research to define the genotype-phenotype relationship may be needed before clinical genetic testing for sequence variants in the majority of genes associated with DCM has clinical utility.

379W

MYLK and INK4/ARF genes are the susceptibility genes of cerebral small vessel disease in Chinese population. *W. Li[1,2], G. Li[3], X. Zhao[2], B. Xin[1], J. Lin[2], Y. Shen[2], X. Liang[2], H. Gao[2], X. Liao[2], Z. Liang[4], Y. Wang[2].* 1) DDC Clinic for Special Needs Children, Middlefield, OH; 2) Department of Neurology, Beijing Tiantan Hospital, Beijing, China; 3) Department of Neuropathology, Beijing Neurosurgical Institute, Beijing, China; 4) Department of Neurobiology, Capital Medical University, Beijing, China.

Backgroud and purpose: Cerebral small vessel disease (CSVD) is a common insidious progressive cerebrovascular disease in middle-aged and elderly. Previous studies showed that CSVD might have a genetic background .The project aims to investigate susceptibility loci and candidate genes associated with CSVD. **Methods and materials:** All study subjects admitted Beijing Tiantan Hospital from June 2009 to September 2010. The total study population is 792 Han Chinese, including 197 cerebral small vessel disease patients(S), 198 large artery atherosclerosis (stenosis greater than 50%) patients (L), 200 hypertensive cerebral hemorrhage patients (H) and 197 stroke-free control individuals(C). Genomic DNA was extracted from venous blood sample. Genotyping of the 15 SNPs in 4 genes (MYLK, AQP4, NINJ2 and INK4/ARF) were done using Multiplex Snapshot assay. Binary logistic regression analysis was performed for each SNP based on five models, including codominant model, dominant model, recessive model, overdominant model and log-additive model. The significant SNP loci were selected for further analysis of allelic and genotypic frequency in C, S, H, and L. Allelic and genotypic frequencies of cerebral small vessel disease at significant SNP loci were analysis in gender, diabetes, hyperlipidemia, smoking and drinking. **Result:** Evidence of association with CSVD was observed for 2 tagSNPs using permutation multitest: rs2222823 SNP locus (MYLK gene) in overdominant model, P = 0.0019, adjust p value was 0.03, T/A genotype, OR was 0.52 (95% CI, 0.35-0.79); rs2811712 SNP locus (INK4/ARF gene) in codominant model, P = 0.0035, adjust p value was 0.05, C/C genotype, OR was 0.47 (95% CI, 0.20-1.15). Further, the evidence of significant difference was found at rs2222823 in overdominant model between hypertensive intracerebral hemorrhage (IH) and CSVD, P value was 0.035, T/A genotype, OR was 0.65 (95% CI, 0.43-0.97). The prevalence rate of risk factors in CSVD, IH and large artery atherosclerosis (LAA) were 29.5%, 19.4% and 41.0% respectively in diabetes; 54.6%,47.2% and 76.3% respectively in hyperlipidemia; 42.5%,48.2% and 59.1% respectively in smoking; 37.3%,41.7% and 44.2% respectively in drinking. **Conclusion:** Our study found the evidence of significant association with CSVD at tagSNP loci, rs2222823 (MYLK) and rs2811712 (INK4/ARF), suggesting MYLK and INK4/ARF gene are likely susceptibility genes of CSVD.

380W

Novel *ACTA2* mutation in a large family with familial thoracic aortic aneurysms and dissections exhibiting reduced penetrance in a female carrier. *M.A. McGinniss[1], H. Wang[2], J.N. Young[3], R. Calhoun[3], S. Mayberg[3], J. Martinezmoles[4], A.W. Grix[4], B.A. Westerfield[2], L.S. Pena[2], D.J. Penny[2], Y. Fan[2], S.A. Boyadjiev[1].* 1) Section of Genetics, Department of Pediatrics, University of California, Davis Medical Center, Sacramento, CA; 2) The John Welsh Cardiovascular Diagnostic Laboratory, Section of Cardiology, Department of Pediatrics, Baylor College of Medicine, Houston, TX; 3) Division of Cardiothoracic Surgery, University of California, Davis Medical Center, Sacramento, CA; 4) The Permanente Medical Group, Department of Medical Genetics, Sacramento, CA.

Familial thoracic aortic aneurysm and/or aortic dissection (TAAD) is a genetically heterogeneous disorder that is inherited in an autosomal dominant fashion. Mutations in *ACTA2*, encoding smooth muscle-specific alpha-actin, account for approximately 14% of familial TAAD. Only 19 *ACTA2* mutations have been described to date. In addition to the risk for TAADs, mutations in the *ACTA2* gene have been associated with increased risk for coronary artery disease and premature stroke. We report a large Italian-American family with a history of acute ascending and descending aortic dissections segregating in an autosomal dominant manner. The age of the first acute cardiac event is between 30 and 53 years of age. The mother and a brother of the proband died prior to surgical intervention at 53 and 45 years of age, respectively. In addition, two of the proband's brothers presented with acute aortic dissections and survived emergency surgical repair of the ascending aorta. Direct sequencing of the *ACTA2* gene in the proband revealed a novel heterozygous missense mutation (c.193T>C; p.Met49Thr) that was not detected in 190 control chromosomes. Polyphen-2 analysis indicates that this mutation is probably deleterious. This mutation was present in a symptomatic brother and in one clinically unaffected sister who had normal size of the aortic root at age 37. No affected family members had a history of premature stroke, coronary artery disease, iris flocculi and/or livedo reticularis on clinical examination. Results from this family expand the spectrum of *ACTA2* mutations causing TAAD by identifying a novel mutation and provides further phenotypic characterization. It also provides further evidence of reduced penetrance of the mutant allele and/or variable expressivity in female carriers. Functional studies of the Met49Thr mutation are ongoing.

381W

Single nucleotide polymorphisms analysis of INSL3 and RXFP2 genes in Mexican patients with idiopathic cryptorchidism. *M.D. Chávez-Saldaña[1], J.C. Gutiérrez[1], I. Zamora[1], O. Cuevas[2], J. Rojas[1], O. Gutiérrez[1], E. Yokoyama[3], R.M. Vigueras[1].* 1) Laboratorio de Biología de la Reprod., Instituto Nacional de Pediatría S.S, México, Distrito Federal, Coyoacan, Mexico; 2) Servicio de Urología;Instituto Nacional de Pediatría S.S México, D.F; 3) Departamento de genética Humana, Instituto Nacional de Pediatría S.S México, D.F.

Criptorchidism (CO) or not testicular descent is considered the most common congenital birth defect in male children and could present itself like an isolated manifestation (idiopatica) or associated with a congenital malformation syndrome. CO is an important factor for male infertility and testicular malignancy on adulthood, its etiology is, in most cases, remain unknown, itself might be considered a complex disease in which multiple genes are involved in the testicular descent like INSL3, RXFP2 and HOXA10. In this study we analyzed some single nucleotide polymorphisms (SNPs) in the INSL3 and RXFP2 genes, that could contribute as risk factors or susceptibility for the idiopatic CO in Mexican patients. 85 patients were included with idiopatic CO and one hundred kids on the control group, where 3 and 9 SNPs were analyzed from previously present genes respectly. The allelic discrimination analysis was realized through the TAQ-MAN method. The SNPs association with the CO type was analyzed with the SPSS® statistic program. In the case of the INSL3 gene, after the allelic discrimination assays, the patients that were out normal for the three analyzed polymorphisms were put through the automatic sequencing analysis from exon 2 of this gene. 100% from patients but also from the control group, were out normal homozygous for the p.R102C and p.R105H from the gene INSL3 variables, while the p.R102H variable was detected in only one patient in heterozygous state, with bilateral CO and without any family antecedents. With the search of unknown alterations in the INSL3 gene, two alterations were detected: p.R105R and the p.T86M, on both cases patients presented bilateral CO. On the other hand the frequency of the analyzed variables in the RXFP2 gene is very similar to other populations, although, comparing the frequency of these variables with the patients and the control group, the c.51869A-G variable showed significative differences, since the GG genotype or at risk, is more common on idiopatic CO patients, on the other hand realizing the genotype-fenotype correlation, it shows that the c. 30704C-T variable, the TT genotype or at risk is more common on bilateral CO patients than those with unilateral CO. In the future knowing the type and distribution from the allelic variables in our population, will allow the improvement of risk prevention methods and susceptibility for this malformation and in the future the infertility and testicular cancer preventive care.

382W

Molecular Markers for Effective prognosis in Systemic Lupus Erythematosus Patients at risk of Renal and Cardiovascular Diseases. *R. Chandirasekar[1], K. Sasikala[1], D. Nedumaran[2], K. Suresh[1], R. Jayakumar[1], R. Venkatesan[1], B. Laksmankumar[4], G. Karthik[3], S. Arun[1].* 1) Zoology, Bharathiar University, Coimbatore, India; 2) Government Medical College and Hospital, Coimbatore, India; 3) Department of Bioinformatics, Bharathiar University, Coimbatore, India; 4) Kongunadu arts and science college Coimbatore, India.

Systemic Lupus Erythematosus (SLE) is a complex, prototypic autoimmune disease that predominantly affects women population. The hallmark of SLE is the generation of auto antibodies that react with self nuclear and cytoplasmic antigens, culminating in immunologic attacks to body organs. Recent evidences suggest that SLE is a complex genetic disease, contributes with environmental and genetic factors. Angiotensin-converting enzyme (ACE) plays an important role in the development of SLE, expressed in a wide range of tissues including lung, vascular endothelium, kidney, Cardiovascular and testis. ACE catalyses the conversion of Angiotensin I to Angiotensin II by its metalloproteinase enzymatic activity and plays a major role in the renin-angiotensin and kallikrein-kininogen systems. ACE gene I/D polymorphisms, particularly DD genotype has been associated with lupus susceptibility and with risk of Renal Disease and cardiovascular disease. angiotensin II, plays an integral role in the regulatory system responsible for endothelial control and vascular tone. The study was analysed and evaluated the ACE gene polymorphic variants among the recruited 48 SLE patients associated with the Cardiovascular and renal disease (SLE was confirmed by Anti Nuclear antibody (ANA) and dsDNA tests) and 48 controls, on the basis of biochemical parameters (total serum cholesterol (HDL, LDL, VLDL, TGL) and C-reactive protein (CRP)) and molecular investigation by Polymerase chain reaction (PCR) and Restriction fragment length polymorphism (RFLP). Results: the biochemical parameter of this study showed that SLE patients had higher levels of cholesterol (36%) and CRP levels higher than normal reference range (<5 mg/L) compared to the controls. The genotype frequency of ACE gene polymorphism determined in SLE patients DD 46%, ID 30% and II 24% compared to controls showed the variance of higher the risk in SLE patients with DD polymorphism P = 0.0034. Hence our findings confirmed the ACE gene polymorphic variant has a major part in leading to cardiovascular disease. At present we focused the research toward RAAS pathway and kallikrein-kininogen systems associated gene for identifying the cause of cardiovascular disease.

383W

Dynamic modularity beyond “date” and “party” hubs in protein-protein interaction networks. *X. Chang, K. Wang.* Zilkha Neurogenetic Institute, University of Southern California, 1501 San Pablo Street, Los Angeles, CA, USA 90089.

The concept of ``date" and ``party" hubs has been widely accepted in the area of protein-protein interaction networks. Party hubs interact with most of their partners simultaneously, while date hubs bind different partners at different locations and times. In theory, date hubs preferentially connect functional modules to each other, whereas party hubs preferentially act inside functional modules. The two types of hubs also display profound differences regarding topological roles and evolutionary constraint. Despite the preponderance of the evidence in its favor, there is still debate about the concept of date and party hubs owing to the potential biases of statistical model or datasets. We divided the network into modules and assigned roles to hubs according to network topology rather than on the basis of expression data. We applied our approaches on two datasets of yeast from different studies, one of which supported the “date” and “party” hubs concept and the other did not. Both datasets were generated on high-throughput yeast two-hybrid system with different filter thresholds and criterions for curation. The first dataset contains 2,561 proteins and 5,992 interactions, the second dataset includes 4,011 proteins and 10,055 interactions. We identified 23 and 16 modules from the two datasets respectively. Most modules have corresponding modules in the other dataset which shares a statistically significant number of nodes after Fisher's exact test. Noticeably, most genes involved in “ribosome biogenesis and their assembly” are enriched in one single module that displays high co-expression with their neighbors. Hubs of this module also contributed largely to the formation of the bimodal distribution of hub co-expression, which motivated the date/party distinction. We also found that co-expression of the nodes in functional modules was highly related to specific conditions under which the expression data was obtained. Therefore, party hubs in these modules could be mistakenly regarded as date hubs due to low co-expression levels calculated from the integration of datasets from various conditions. Our results confirmed the widespread existence of party hubs related to functional modules. As for date hubs, they may exist theoretically; however, using merely the co-expression measurements, we cannot define the date/party distinction.

384W

Testing for a mixture of effects on either disease or quantitative traits across a set of rare variants. *G. Clarke, A. Morris.* Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford, OX3 7BN, United Kingdom.

Multiple rare variants either within or across genes are believed to collectively influence phenotypic expression. The increasing availability of high throughput sequencing technologies offers the opportunity to study the effect of rare variants on complex traits but appropriate analytical methods are required. Methods must be able to account for a collection of rare variants displaying a combination of protective, deleterious and null effects. Recently Neale et al.1 proposed a C-alpha score test to test for the presence of a mixture of effects on a disease phenotype across a set of rare variants. Here we propose an alternative C-alpha score test for the analysis of sequence level data that can be applied to both disease and qualitative phenotypes and which can adjust for the presence of covariates. For qualitative traits and variants with an allele frequency greater than 0.5%, our method has comparable or slightly better power than tests based on collapsing sets of variants when all effects are in the same direction and much greater power when a mixture of protective and deleterious variants are studied. For binary traits and variants with an allele frequency greater than 0.5%, our method has comparable or slightly better power than the method proposed by Neale et al. Our test is a simple, fast and effective method, which can account for covariates and which can be applied directly to both quantitative and binary traits in the analysis of sequence level data 1. Neale BM, Rivas MA, etc. Testing for an unusual distribution of rare variants. PLoS Genet 2011;7(3).

385W

Pathway association analysis using an overall test for the most promising SNPs. *M. Mattheisen, W. Yip, P. Lipman, C. Lange*. Department of Biostatistics, Harvard School of Public Health, Boston, MA.

Pathway-based and set-based approaches (PAA) have been developed to facilitate the analysis of GWA study data sets. While exploiting existing biological knowledge of gene function/pathways, these approaches are capable to identify genes with large genetic effects, but also new genes/ pathways in which each single SNP (or even gene) confers only a small degree of disease risk. The combined effects of these SNPs/genes can then be implicated in the development of disease. Several methods using a wide range of algorithms have been proposed in the context of pathway association analyses. These approaches typically examine whether a group of related genes in the same functional pathway (or functional sets) are jointly associated with a trait of interest. For these methods the power to detect true disease association depends on the underlying genetic model and the phenotype under study. Nevertheless, under simple, realistic disease models, PAA can strongly outperform the GWAS single-marker approach. Here we present a new powerful approach, which is based on a recently published method for the follow-up of GWAS results (Lipman et al. 2011). Similar to the aforementioned study we partition the P-values for the SNPs in the gene-sets or pathways into a predetermined number of bins (J+1). While one bin collects all P-values of the SNPs that will be of less interest (i.e., all SNPs below a predefined threshold theta) the other J bins partition the P-values falling between 0 and theta. We then count how many P-values fall into each bin. If the bins contain more P-values than expected under the null-distribution, there is evidence to reject the null hypothesis and conclude that the gene-set/ pathway is of interest for the disease under study. We discuss the properties of the test statistic and compare the results for our method and several available approaches (among others ALIGATOR and GenGen) using both, a simulation study and a large scale GWAS data set. Finally we evaluate the usage of Kolmogorov-Smirnov tests in the context of pathway association analyses. Lipman PJ, Cho MH, Bakke P, Gulsvik A, Kong X, Lomas D, Anderson W, Silverman E, Lange C. On the Follow-Up of Genome-wide Association Studies: An Overall Test for the Most Promising SNPs. Genet Epidemiol 2011 (in press).

386W

Modeling of the Interaction between the Serotonin Regulation Pathway and the Stress Response, mediated by the Hypothalamic-Pituitary-Adrenal Axis, using a Boolean Approximation: a Novel Study for Depression. *O.A. Moreno-Ramos[1,2], M.C. Lattig[1], A.F. Gonzalez[2].* 1) Biological Sciences, Laboratorio de Genetica Humana, Bogota, Colombia; 2) Grupo de Diseño de Productos y Procesos, Departamento de Ingeniería Química, Universidad de los Andes, Bogota, Colombia.

Depression shows a high prevalence in the overall population and is a major public health problem worldwide (WHO, 2004). According to different studies performed in twins, a heritability of approximately 40%; is observed and it increases if another member of the family suffers from other affective disorders. Thus, it has been hypothesized that depression has a strong genetic background, and genes in the serotonin metabolic pathway have been implicated in the occurrence of the disease. Environmental factors such as stressor events are also strongly associated with depression. The principal aim of this work was to model the interaction between the serotoninergic pathway and the stress response pathway mediated by the hypothalamic-pituitary-adrenal axis using a Boolean approximation. We also determined which genes, when knocked out in-silico, cause the highest impact on the model stability. Furthermore, in this model we simulate the impact antidepressants have on the biological pathway. The results obtained indicate that the network is stable with or without stress, but that stability is reached faster when the pathway is run without stress. Also, we observed that the biological model generated predicts steady states (attractors) for each of the different runs performed. Those attractors change in shape, especially when antidepressants were also included in the simulation. We also found that when the model involves both the serotoninergic pathway and stress, antidepressants, like the SSRIs, do not have the expected effect. This work also predicted that the genes that cause the highest impact on this model stability were CREB, BDNF (which in different association studies, has been associated to major depression disorder) and TR2) ; followed by the genes and metabolites that are related to the serotonin synthesis.

387W

Large-scale transcriptome study in the Sardinian founder population. *M. Pala[1,5], M. Marongiu[1], A. Mulas[1], R. Cusano[1], F. Crobu[1], F. Reinier[2], R. Berutti[2,5], M.G. Piras[1], C. Jones[2], D. Schlessinger[4], G. Abecasis[3], A. Angius[1], S. Sanna[1], F. Cucca[1,5].* 1) Istituto di Ricerca Genetica e Biomedica (IRGB), CNR, Monserrato, 09042, Italy; 2) CRS4, Advanced Genomic Computing Technology, Pula, Italy; 3) Center for Statistical Genetics, University of Michigan, Ann Arbor, MI; 4) Laboratory of Genetics, National Institute on Aging, Baltimore, Maryland, USA; 5) Dipartimento di Scienze Biomediche, Università di Sassari, Sassari, Italy.

With the advent of next-generation sequencing technologies, high throughput dissection of complex traits and diseases can be extended to underlying biological mechanisms by assessing changes in the transcriptome or methylome. Studies conducted so far were limited by the small sample size, were restricted to the correlation of only one clinical condition (or none), or relied on polyA enrichment protocols that detect only a small fraction of total RNA species. We are sequencing the RNA of 1,000 individuals from the Sardinian founder population enrolled in the SardiNIA project and hence characterized for >200 quantitative traits, with measurements repeated in follow-up visits. To survey both coding and non-coding RNA species, we used an rRNA-depletion protocol and focused on peripheral blood mononuclear cells (PBMC) to catalogue transcription variation in a critical tissue implicated in the wide range of immune-affected phenotypes. The augmented scale facilitates the discovery of novel splicing isoforms and novel species from regulatory regions that extend to “inter-genic” regions as well as within known genes, which can be directly tested for correlations with clinical conditions. In addition, because this RNA sequencing effort is coupled with DNA sequencing of the same samples, eQTLs and RNA-editing sites can be assessed as well. Preliminary analysis of a pilot run of 12 samples, with an average of 50 million reads (51+51 nucleotides at paired ends) sequenced using the Illumina HiSeq2000, shows the relative enrichment of different RNA subclasses. Reads were aligned to the reference genome with TopHat, and an average of 91% were properly mapped. Of those, less than half (45%) fall, on average, within known exons; the others define inferred novel transcripts. Among all the aligned reads, those in unequivocally known transcript classes include protein coding (14%), mitochondrial rRNA (8%), pseudogene (1%), miscRNA (0.6%), lincRNA (0.5%), snoRNA (0.3%), snRNA (0.2%) and miRNA (0.05%) species. The remaining reads map within transcripts annotated in multiple categories; with low abundance in rRNAs (as expected by the chosen protocol); outside annotated transcripts or to multiple genomic sites. This relatively unbiased representation of the transcriptome provides the basics for the ongoing integration with corresponding DNA sequencing and phenotypic trait values.

388W

Development and use of a pipeline to generate strand and position information for common genotyping chips. *N.W. Rayner[1,2], M.I. McCarthy[1,2,3].* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 2) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Churchill Hospital, Oxford, United Kingdom; 3) Oxford National Institute for Health Research Biomedical Research Centre, Churchill Hospital, Oxford, UK.

With the increasing use of ever larger meta-analyses the number of data sets being combined has increased dramatically. This can lead to a number of problems, not least in ensuring all data sets are aligned to the same genomic build and strand. Traditionally data sets in meta-analyses were likely to have been imputed, thereby aligning the SNPs to the reference panel, using their alleles and Minor Allele Frequency (MAF). Imputation can still encounter problems, for example with A/T and G/C SNPs having a MAF close to 50% or those SNPs that do not appear in the reference panel. When utilising directly genotyped data, an ever increasing occurrence with the new large genotyping arrays, the problem becomes even more acute. Options do already exist to update the genome build such as the lift-over tool at UCSC however this does not provide the orientation to the reference strand which can change. The situation is further complicated by a profusion of genotype calling algorithms, leading to the final calls being reported to a number of different orientations, something we have observed in ~20% of cohorts studied to date. To address these issues we have created a pipeline to map SNPs on the genotyping platforms to the genome using flanking or probe sequences from the annotation files. The core of the pipeline is a local installation of a BLAT server, to map the sequences to the genome. This is wrapped in a set of Perl programs which automate the running of the server and the processing of the input and output files. The final output is a set of files that give, the position and orientation of each SNP to the genome build used, the SNPs that do not map to the genome and those SNPs that show mappings to more than one position. The strand and position files have been generated for a number of ongoing meta-analyses such as the DIAGRAM replication effort where 26 cohorts comprising ~85,000 samples, all utilising the same Illumina iSelect Metabochip genotyping platform, have successfully combined directly genotyped data on 196,725 SNPs to follow up top signals from the earlier DIAGRAM meta-analyses. We have expanded the number of files available by running the most common Illumina and Affymetrix chips for genome builds >35, the resulting files have all been uploaded to a web site where they are freely available. This page is constantly updated to keep pace with new chips, the differing orientations of the genotype calling algorithms and changes in the genome build.

389W

Association of Endothelial nitric oxide synthase gene haplotypes with systemic lupus erythematosus in Kuwaiti Arabs. *M. Haider[1], S. Alfadhli[2], B. Altamimy[2], K. Alsaeid[1].* 1) Dept Pediatrics, Fac Med, Kuwait Univ, Safat, Kuwait; 2) Dept Medical Lab Sci, Fac Allied Health, Kuwait Univ, Safat, Kuwait.

Endothelial nitric oxide synthase (eNOS) catalyses the production of nitric oxide, which has been shown to participate in the pathogenesis of systemic lupus erythematosus (SLE). eNOS gene polymorphism may have an effect on eNOS gene expression, eNOS protein synthesis and enzymatic activity. We investigated the influence of eNOS gene polymorphisms on susceptibility to SLE. eNOS T-786C, G894T and intron 4 27-base pair tandem repeat (VNTR4) polymorphisms were investigated in 152 Kuwaiti SLE patients and 184 controls using RFLP-PCR, direct sequencing and fragment analysis. Allele, genotype and haplotype frequency comparisons, Hardy-Weinberg equilibrium and linkage disequilibrium (LD) analysis were performed. No significant association was detected between SLE and single-nucleotide polymorphisms (SNPs) T-786C and G894T. VNTR4 allele 4b was associated with susceptibility to SLE (OR 1.89, p = 0.023), as was the genotype 4bb (OR 2.41, p = 0.007). However, allele 4a was protective (OR 0.53, p = 0.023), as was genotype 4ab (OR 0.41, p = 0.007). T-786C and VNTR4 were in high LD (r (2)= 0.34). Haplotypes T4bC and C4aG of the three tested polymorphisms had a susceptibility effect on SLE (OR 1.89 and 4.23 at p = 0.005 and 0.001, respectively), while haplotypes T4aG and C4bG had a protective effect (OR 0.06 and 0.11 at p = 0.000001 and 0.0005, respectively). The novel finding in our study is that individual eNOS polymorphisms most likely do not exert a major influence on susceptibility to SLE, but they have significant effects when combined within a specific haplotype.

390W

***FRIZZLED* mutations in patients with Neural Tube Defects.** *V. Capra[1], P. De Marco[1], E. Merello[1], G. Piatelli[1], A. Cama[1], Z. Kibar[2].* 1) Lab U.O. Neurochirurgia, Istituto Scientifico G Gaslini, Genova, Italy; 2) Department of Obstetrics and Gynecology, CHU Sainte Justine Research Center and University of Montreal, Montreal, Québec, H3T 1C5, Canada.

Neural Tube Defects (NTDs) are severe congenital malformations caused by failure of the neural tube to close during neurulation. Their etiology is complex involving both environmental and genetic factors. During embryogenesis of CNS, the major driving force essential to the shaping of the neural plate is a process which is referred to as convergent extension (CE): a process by which cells elongate medio-laterally and intercalate with other neighboring cells forming a longer, narrower array. This changes lead to the conversion of an initially wide and short neural plate into a narrow and elongated one. A crucial role in CE has been assigned to the Planar Cell Polarity (PCP) pathway, a highly conserved, non-canonical Wnt-Frizzled-Dishevelled signaling cascade, that involves a number of additional core genes including *Stbm/Vangl*, *Flamingo (Fmi)*, *Prickle (Pk)*, and *Diego (Dgo)*. The identification of mutations of PCP genes in NTD mouse models, such as the *Loop-tail* mouse carrying mutations in the *Ltap (Vangl2)* gene, suggests that such genes may be good candidates also for human NTDs. Recently, we identified mutations in two human PCP genes, *VANGL1* and *VANGL2*, providing evidence for a pathogenic role of PCP signaling in human NTDs. Other PCP genes have a potential role in convergent extension movements and neural tube closure, and hence need to be studied in large human NTDs cohorts. Frizzled are seven-pass transmembrane receptors that transduce PCP signalling. It is widely accepted that binding of the phosphoprotein Dishevelled (Dvl/Dsh) and its membrane recruitment by Frizzled is the critical event for PCP signaling. Double mutants *Fzd3* $^{-/-}$/ *Fzd6* $^{-/-}$ embryos exhibit craniorachischisis and curled-tail with a fully penetrance, a partially penetrant failure of eyelid closure, and misorientated auditory and vestibular sensory hair cells, providing the most direct evidence for a functional connection between PCP components and mammalian tissues fusion processes. Expression studies in humans have shown that *FZD3* and *FZD6* are widely expressed in both embryonic and adult tissues, including brain and central nervous system. Based on the highly penetrant occurrence of NTDs in double *Fzd3* $^{-/-}$/*Fzd6* $^{-/-}$ mutant mice, we have investigating the role of the human orthologues, *FZD3* and *FZD6*, by resequencing a cohort of 473 unrelated NTDs patients and 639 ethnically-matched controls.

391W

Replication of a FOXP2 association with motor sequencing during an oral task in families with familial speech sound disorder. *B. Peter[1], M. Matsushita[2], K. Oda[2], W. Raskind[2].* 1) Speech & Hearing Sci, Univ of Washington, Seattle, WA; 2) Dpt of Medicine, Univ Washington, Seattle, WA.

PURPOSE: This study was conducted with the approval of the University of Washington Human Subjects Division. Its purpose was to replicate a FOXP2 association with measures of motor sequencing ability in a dyslexia family sample (Peter et al., 2010) in a new sample of families with familial speech sound disorder (SSD). SSD is a disorder affecting the acquisition of intelligible speech in the absence of known causes. There is evidence that SSD has a genetic etiology, but causal genes have not yet been identified. FOXP2 is causal in a rare syndromic form of disordered speech and language. It was not implicated in a sample of individuals with a motor-based form of SSD but its role in component phenotypes of common, nonsyndromic SSD is unknown. METHOD: Five multigenerational families with evidence of familial SSD (N = 39) participated in a variety of behavioral tasks including diadochokinetic testing. Standard scores were computed for the speed of producing monosyllabic strings of /pa/ (“papapa ...”) and disyllabic strings of /pata/ (“patapatapata ...”). Higher standard scores in the monosyllabic task, compared to the disyllabic task, were interpreted as a relative deficit in motor sequencing. This standard score difference proved to be largely robust against age effects across the lifespan. As in our 2010 study, we typed six FOXP2 markers tagging relevant genomic elements and tested linear association models using the qfam algorithm in PLINK. RESULTS: A significant association between a SNP in the promoter and CpG island region, rs923875, and the motor sequencing measure was found (empirical p = .0067). No other SNP markers reached nominal or adjusted statistical significance. CONCLUSIONS: Results from this SSD family study replicate our 2010 finding that SNP markers in the promoter region and transcription start site of FOXP2 were associated with measures of motor sequencing in oral and finger tasks in dyslexia families. This outcome is consistent with the hypothesis that a locus in the FOXP2 promoter and start site region influences motor sequencing in both dyslexia and SSD and that this trait may be an endophenotype in these disorders.

392W

Relative Impact of Genetic and Epigenetic Factors on Gene Expression in Tumor Tissue Samples. *H. Dong[1], L. Jin[1], M. Xiong[2,1].* 1) Sch Life Sci, Fudan Univ, Shanghai, China; 2) Human Genetics Center, University of Texas School of Public Health, Houston, TX 77030, USA.

Gene expression is influenced by genetic factors such as mutation, SNPs, CNVs and epigenetic factors such as methylation, histone modifications and miRNA. An essential issue in understanding how genetic and epigenetic variation regulates the gene expression is to estimate the proportion of gene expression variation explained by SNPs, CNVs, methylation and miRNA variation. Traditional single marker analysis will miss many variants which individually have small genetic effects, but collectively make a large contribution to the phenotypic variation and miss potential linear and nonlinear interaction among genomic and epigenomic variants. To overcome this limitation, we propose to change quantitative trait analysis platform from single-variant regression (SVR) to joint genomic and epigenomic analysis. The dimension of all available genomic and epigenomic variation is extremely high. Ultrahigh dimensional genomic and epigenomic data analysis poses great challenges. To meet the challenge, we propose to use a recently developed sparse locally linear embedding (LLE), a parse manifold learning algorithm as a powerful high dimensional data reduction tool to project high dimensional genomic and epigenomic data into a low dimensional space. Then, we use lasso regression on the reduced data in low dimensional space to estimate the impact of genomic and epigenomic variants on gene expression. The combined sparse LLE and lasso regression were applied to the two cancer tumor tissue datasets: TCGA Glioblastoma Multiform (198 tumor tissue samples) and Ovarian Cancer (512 tumor tissue samples). We have made several remarkable findings. First, we showed that on average, expression variance was explained mainly by miRNA, methylation, CNVs, rather than SNPs. Especially, the contribution of miRNA and methylation on gene expression variation is larger and more direct than CNVs and SNPs. Second, the contribution of SNPs to miRNA and methylation variation is small. The contribution of CNVs to miRNA is small, but their contribution to methylation cannot be ignored. The above observations could be replicated in GBM and ovarian cancer studies. Our study demonstrates the feasibility and power of sparse manifold learning and lasso regression for evaluating the contribution of genetic and epigenetic variation to gene expression valuation.

393W

***eNOS* (27bp deletion/insertion in intron 4) and *Mn-SOD* (p.Val16Ala) polymorphisms analysis in type 2 diabetic retinopathy (DR) patients from north India.** *P. Badhan, V. Vanita.* Department of Human Genetics, Guru Nanak Dev University, Amritsar, India.

Purpose: Present study aimed to elucidate role of 27bp deletion/insertion polymorphism in intron 4 of *eNOS* and p.Val16Ala in *Mn-SOD* with DR in north Indian patients. Material and Methods: In this cross-sectional case-control association study 256 patients were enrolled (150 with DR and 106 with type 2 diabetes (T2D) without any sign of retinopathy as controls). All these patients were recruited from Dr. Daljit Singh Eye Hospital, Amritsar. *eNOS* (27bp deletion/insertion in intron 4) polymorphism was analyzed by PCR and agarose gel electrophoresis and *Mn-SOD* (p.Val16Ala) polymorphism by PCR-RFLP. Chi-square test was performed to analyze the genotype distribution and allele frequencies using statistical calculator (StatPac) V.3.0. Using SPSS V.16.0, one way ANOVA test was performed to find significant association of various clinical parameters (age of patients, age of onset of diabetes, duration of diabetes, BMI, systolic and diastolic blood pressure) with diabetic retinopathy. Results: Among the two polymorphisms screened, *eNOS* (27bp deletion/insertion in intron 4) showed no statistically significant association in allele frequency (p=0.4288) and genotype distribution (p=0.3379) with DR. However, *Mn-SOD* (p.Val16Ala) polymorphism showed statistically significant association in genotype distribution (p= 0.028), and not in allele frequency (p=0.2233). Calculated odds ratios (OR) for the genotypes of *Mn-SOD* (p.Val16Ala) polymorphism were 1.05 (95% CI: 0.5976-1.8374, p=0.8705), 0.4265 (95% CI: 0.2650-0.6865, p=0.0004) and 1.78 (95% CI: 0.9807-3.2384, p=0.058) for CC (mutant allele)-, TC- and TT- (wild type) genotypes respectively and in present study CC-genotype is found to be associated with diabetic retinopathy. Also association was found in age of onset of diabetes (p=0.021), duration of diabetes (p=0.001), BMI (p= 0.032) and systolic blood pressure (p=0.00). Conclusion: In present study, no association has been observed in *eNOS* (27bp deletion/insertion in intron 4) polymorphism with type 2 diabetic retinopathy in patients from north India. Thus this polymorphism in *eNOS* does not seem to be a good biomarker for DR. However, a statistical significance has been observed in *Mn-SOD* (p.Val16Ala) polymorphism with type 2 diabetic retinopathy indicating that it might be a risk factor for the development of diabetic retinopathy. Therefore, p.Val16Ala polymorphism in *Mn-SOD* appears to be a good biomarker in determining genetic susceptibility to DR.

394W

NTF4 mutations in primary open-angle glaucoma. *L. Chen[1], T. Ng[1], P. Tam[1], A. Fan[1], M. Zhang[2], C. Tham[1], N. Wang[3], D. Lam[1], C. Pang[1].* 1) Ophthalmology & Visual Sci, Chinese Univ Hong, Hong Kong, China; 2) Joint Shantou International Eye Center of Shantou University and the Chinese University of Hong Kong, Shantou, China; 3) Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing, China.

Purpose: The neurotrophin-4 (NTF4; GLC1O) gene has recently been implicated in primary open-angle glaucoma (POAG). This study was to determine the contribution of NTF4 mutations in POAG in the Chinese populations. Methods: 950 unrelated Chinese Han subjects, consisting of a Hong Kong cohort of 390 POAG patients and 230 controls, a Shantou case-only cohort of 130 patients and a Beijing case-only cohort of 200 patients, were screened for mutations in the coding region and exon-intron boundaries of NTF4 by using direct DNA sequencing. Constructs carrying the missense variants detected were generated using site-directed mutagenesis and transfected into HeLa cells for subsequent solubility and migration analyses. Results: Three variants, p.Pro151Pro, p.Gly157Ala and p.Ala182-Val, were identified. p.Pro151Pro was detected in 3 POAG and 1 control subject. The missense variants p.Gly157Ala and p.Ala182Val were detected in one patient each, but not in the control subjects. The p.Gly157Ala variant protein was less soluble in Triton X-100. HeLa cells transfected with either mutant construct showed less migration as compared to those transfected with the wildtype construct. Conclusions: The two missense NTF4 variants detected in our study are likely to be functional mutations. NTF4 mutations may thus contribute to a small proportion (~0.3%) of Chinese POAG.

395W

Integrating gene expression data with genome-wide association studies to identify novel asthma susceptibility candidate genes. *D.A. Cusanovich, C. Billstrand, C. Chavarria, K. Michelini, A.A. Pai, C. Ober, Y. Gilad.* Human Genetics, University of Chicago, Chicago, IL.

Despite recent success identifying novel genotype-phenotype associations in human populations, a large proportion of inter-individual variation in susceptibility to many complex diseases remains unexplained. Results from genome-wide association studies (GWAS), a common approach for mapping disease-associated loci, rarely account for a major proportion of the heritability of complex traits, a phenomenon that has been termed “the missing heritability problem.” Several models have been proposed to account for missing heritability in GWAS, including the suggestion that the heritability of complex traits can be explained by a large number of common alleles with small effects. Typical GWAS, however, lack the power to distinguish true genetic associations with small-effect alleles from the large number of false positives. To address this gap, we combined gene expression data with a GWAS for lymphocyte count, an intermediate phenotype associated with susceptibility to asthma. Specifically, we integrated results from a GWAS for lymphocyte count in 462 Hutterites and genome-wide gene expression profiles from lymphoblastoid cell lines derived from 96 Hutterites. Using this approach, we found that eQTLs for genes whose expression levels are correlated with lymphocyte counts in the 96 individuals also have significantly stronger association signals with lymphocyte counts in the 462 individuals. Importantly, a subset of eQTLs that were associated with lymphocyte count also exhibits significantly stronger association signals with asthma in an independent meta-analysis of GWAS in European populations (the GABRIEL study). In addition, we found that those genes with expression patterns correlated with lymphocyte counts are 2.5 times more likely to be associated with asthma than genes with uncorrelated expression patterns in the HuGE Navigator. Moreover, these genes are enriched for “NK cell mediated cytotoxicity,” “hematopoietic cell lineage,” and “T cell receptor signaling” KEGG pathway annotations. All of this substantiates these genes as promising asthma susceptibility candidates. More generally, our observations suggest that a multitude of small-effect loci may contribute to inter-individual variation in lymphocyte count, and to variation in susceptibility to asthma.

396W

Genome-wide Linkage and Association Analyses in Uterine Leiomyomata Reveal *FASN* as predisposition gene. *S.L. Eggert[1], R. Kavalla[2], K.L. Huyck[4], P. Somasundaram[2], E.A. Stewart[5], A.T. Lu[6], J.N. Painter[7], G.W. Montgomery[7], S.E. Medland[7], S.A. Treloar[7], L. Rose[8], P.M. Ridker[8,9], D.I. Chasman[8,9], N.G. Martin[7], R.M. Cantor[6], C.C. Morton[2,3,9].* 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Departments of Obstetrics, Gynecology and Reproductive Biology and; 3) of Pathology, Brigham and Women's Hospital, Boston, MA; 4) Department of Medicine, Dartmouth-Hitchcock Medical Center, Lebanon, NH; 5) Mayo Clinic, Rochester, MN; 6) Department of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA; 7) Queensland Institute of Medical Research, Brisbane, Australia; 8) Division of Preventive Medicine, Brigham and Women's Hospital, Boston, MA; 9) Harvard Medical School, Boston, MA.

Uterine leiomyomata (UL) are the most common pelvic tumors in women. UL pose a major public health problem given their prevalence (>70%) and rate of symptoms (~25%) in women of reproductive age and their indication for >200,000 hysterectomies in the U.S. annually. A genetic basis for tumor development is supported by recurrent chromosomal aberrations and a genetic component to UL predisposition is supported by analyses of ethnic predisposition, twin studies, and familial aggregation. A genome-wide SNP linkage panel was applied to 261 white UL sister pair families from the Finding Genes for Fibroids study. Two significant linkage regions were detected in 10p11 (LOD=4.15) and 3p21 (LOD=3.73) while five additional linkage regions were identified with LOD scores >2.00 in 2q37, 5p13, 11p15, 12q14, and 17q25. Fine mapping was performed with an independent sample of white women from the Women's Genome Health Study (WGHS) and 39,115 SNPs positioned under the linkage regions. Cases were defined as women who reported being diagnosed with fibroids under age 40 years, and/or had a hysterectomy, and have a mother or sister also diagnosed with fibroids. A standard case/control analysis was performed with 746 cases and 4487 controls; 44 SNPs were identified with suggestive p-values $<10^{-3}$. Replication was performed with the 44 suggestive SNPs in a third independent sample of white women from an Australian twin cohort in which cases are defined as women who have been diagnosed with fibroids. A standard case/control analysis was performed with 484 cases and 610 controls; seven SNPs were identified with p-values <0.05. Of the seven candidate SNPs, six are in a block of linkage disequilibrium in 17q25.3 which spans the genes fatty acid synthase (*FASN*) and coiled-coil domain containing 57 (*CCDC57*). The p-values of the six candidate SNPs reach genome-wide significance after meta-analysis of the WGHS and Australian samples. By tissue microarray immunohistochemistry, we found FAS protein expression elevated (3-fold) in UL when compared to matched myometrial tissue. Preliminary qPCR results show the same trend for mRNA expression of *FASN*. FAS transcripts and protein levels are up-regulated in many cancers (e.g. colon, prostate, breast, ovary, endometrium) and seem to play a role in tumor cell survival. *FASN* represents the first UL predisposition allele identified in white women by a genome-wide, unbiased approach and opens a new field of UL research concerning cell metabolism.

397W

Association of Gene Variants and Biochemical Markers with Chronic Obstructive Pulmonary Disease. *A. Jyothy[1], B. Ashrafunnisa[1], A. Venkateshwari[1], A. Munshi[1], S.V. Prasad[2].* 1) Institute of Genetics & Hospital for Genetic Diseases, Hyderabad-16, A.P. India; 2) Govt.Chest Hospital ,Hyderabad ,Andhra Pradesh, India.

Chronic Obstructive Pulmonary Disease (COPD), a chronic inflammatory disorder of lungs, is characterized by an inability to normally move air in and out of lungs . Airflow limitation in patients with COPD is not fully reversible. The pathophysiology of COPD encompasses multiple injurious processes including inflammation, cellular apoptosis, altered cellular and molecular alveolar maintenance, abnormal cell repair, extracellular matrix destruction and oxidative stress. Twin studies, family studies and case-control association studies have suggested that genetic factors are important in COPD risk. A number of genes like (1- Antitrypsin, Matrix Metalloproteinase-1&3 (MMP-1&3), Tissue Inhibitory Metalloproteinase-2(TIMP-2), Hemoxygenase-1 (HMOX-1), Microsomal Epoxide Hydrolase (EPHX1), Tumor necrosis factor-((TNF- ( ) and Surfactant Protein B (SFTPB) have been implicated in the pathogenesis of COPD. The genes related to nitric oxide synthesis on lung airway are also been studied because of their important role in airway remodeling. The gene variants of endothelial nitric oxide synthase (eNOS) , matrix mtallopoteinase-3(MMP-3) , cytochrome 450A1 (CYPA1) and tumor necrosis factor alpha (TNF-( ) and biochemical markers associated with COPD in a South Indian Population from Andhra Pradesh are being evaluated. The results of the study will be presented. Research into genetics of COPD, may be helpful in developing new treatment strategies for better management.

398W

A family-based association study between vitiligo and positional candidate genes EDNRA, PTPN12, PTK2B and ADAM9. *L. Nascimento[1], C. Silva de Castro[1,2], R. Werneck[1], M. Mira[1].* 1) PPGCS-CCBS, PUCPR, Curitiba, Parana, Brazil; 2) Department of Dermatology, Santa Casa de Misericordia Hospital, PUCPR, Curitiba, Parana, Brazil.

Vitiligo is an acquired systemic, chronic, multifactorial, polygenic disease characterized by macules devoid of melanic pigment and identifiable melanocytes. A previous study detected evidence for linkage between vitiligo and loci on chromosomes 7q21 an 8p21 in a collection of multigerational families presenting generalized vitiligo associated with other autoimmune diseases. Moreover, a genome-wide linkage scan conducted in Chinese families affected with generalized vitiligo localized a new susceptibility locus on chromosomal region 4q13-q21. To date, no strong candidate gene has emerged from these linkage susceptibility loci to date. We selected four biological candidate genes located at these three chromosomal regions for a comprehensive association analysis involving 35 tag SNPs completely capturing the information of genes endothelin receptor type A - EDNRA (Cr 4), protein tyrosine phosphatase, non-receptor type 12 - PTPN12 (Cr7), protein tyrosine kinase 2 beta - PTK2B (Cr8) and disintegrin and metalloproteinase domain 9 - ADAM9 (Cr8). All SNPs were genotyped in 596 individuals distributed in 212 trios composed by an affected child and both parents. Markers were genotyped using the mass spectrometry-based SEQUENOM MassARRAY platform. Family-based association analysis was performed as implemented in the FBAT software. Logistic multivariate analysis was performed as implemented in the SAS software. No evidence of allelic or genotypic association ($p<0.05$) was detected between the gene variants and vitiligo, even when co-variables age-of-onset and presence/absence of additional autoimmune diseases were included in the analysis. The exact nature of the linkage observed between vitiligo and chromosomal regions 7q21, 8p21 and 4q13-q21 remains elusive.

399W

CNV screening identifies chromosomal regions of interest and candidate genes in patients with orofacial clefts. *M. Simioni, T.K. Araujo, C.V. Maurer-Morelli, I. Lopes-Cendes, V.L. Gil-da-Silva-Lopes.* Medical Genetics, FCM/UNICAMP, Campinas, Brazil.

Cleft lip and palate (CL/P) is one of the most common craniofacial defects with birth prevalence ranging from 1:500 to 1:2500. Different etiological factors are involved on the genesis of clefts, including gene-gene interactions and environmental influences. To date, a number of genes have been suggested as playing a role in CL/P, but they account for only a small proportion of patients. The screening for copy number variation (CNV) in the human genome has proven to be a very powerful strategy to narrow down candidate disease gene and/or regions involved in various disorders, as well as in clefts. The purpose of this study was to analyze the role of CNVs and the genes included within this variations in a group of patients with CL/P. We studied a total of 23 trios (proband-parents) and 20 control individuals without orofacial clefts in at least 3 generations. All probands were clinically classified according to the International Clearinghouse for Birth Defects and Surveillance and Ressearch (ICBDSR 2007). CNV screening was performed using the Genome-Wide Human SNP Array 6.0 (Affymetrix) and analyzed by the Affymetrix Genotyping Console software. We identified six candidate genes, possibly associated with CL/P, among the CNVs detected: two duplications on 5q13.2 and 9p24.3regions (GTF2H2 and FOXD4 genes - transcription factors); a deletion on 19p13.3 region (FGF22 gene - fibrobrast grow factor); a 3.8Mb duplication on 8p23.1 region (SOX7 and GATA4 genes - transcription factor and binding protein, respectively); and a deletion on 1p36.11 region involving TCEB3 gene. This gene encodes a type of elongin protein that makes part of a complex with the SPRY protein family in which SPRY1 gene is a member, SPRY1 has been previously associated with CL/P. All of these CNVs are de novo (not identified in the parents) and, except for 8p23.1 duplication, they were not present in the control group genotyped. To validate the CNVs found in these patients, an independent method is underway. Financial support: FAPESP, CNPq.

400W

Analysis of *VEGF* (-634 C/G and 936 C/T) polymorphisms in T2D retinopathy patients from North India. *H. Singh, V. Vanita.* Department of Human Genetics, Guru Nanak Dev University, Amritsar, India.

Purpose: The aim of present study was to analyze the -634 C/G polymorphism of the 5' untranslated region (5'UTR) and 936 C/T polymorphism of the 3' untranslated region (3'UTR) of *VEGF* with diabetic retinopathy (DR) in T2D patients from north India. Material and methods: In this case-control association study, 820 unrelated patients with T2D were collected from Dr. Daljit Singh Eye Hospital, Amritsar. Out of these, 471 T2D patients had different types of retinopathies. Of these 275 patients were diagnosed with Proliferative Diabetic Retinopathy (PDR), 84 with Pre-proliferative Diabetic Retinopathy (PPDR), 112 with Back-ground/Non proliferative Diabetic Retinopathy (BDR/NPDR) and 349 patients having T2D but no any sign of retinopathy were analyzed as controls. Both *VEGF* -634 C/G (5'UTR) and 936C/T (3'UTR) polymorphism analyses were undertaken by PCR-RFLP. One way analysis (ANOVA) test was undertaken with the help of SPSS V 16.0 on all the clinical parameters of DR patients and controls. Genotypes and allele frequencies were compared for Chi-square test with the help of statistical calculator (StatPac) V.3.0. Results: The present study revealed statistically significant difference between age, age of onset, and duration of diabetes when different DR groups (PDR, PPDR, BDR) were compared with controls. However, the genotypes (p=0.7988) and allele frequencies (p=0.7959) with *VEGF*-634 C/G (5'UTR) polymorphism in DR patients showed no statistically significant difference as compared to controls. In case of *VEGF* 936 C/T (3'UTR) polymorphism, we observed statistically significant difference between age of onset, and duration of diabetes when different DR groups were compared with controls. The genotypes (p=0.967) and allele frequencies (p=0.910) with VEGF 936 C/T polymorphism in DR patients however, showed no statistically significance as compared to controls. Further according to present findings, when PDR, PPDR, and BDR groups were compared individually with controls for *VEGF*-634 C/G and 936 C/T polymorphism, no statistically significant differences were observed. Conclusion: We observed no any association of *VEGF* -634 C/G and 936 C/T polymorphisms with DR in the analyzed north Indian patients. Therefore, we conclude that these polymorphisms are not useful markers for the diabetic retinopathy.

401W

Angiotensin converting enzyme (ACE) gene I/D polymorphism analysis in type 2 diabetic retinopathy patients from North India. *V. Vanita[1], H. Singh[1], I.R. Singh[2], J.R. Singh[3].* 1) Department of Human Genetics, Guru Nanak Dev University, Amritsar, India; 2) Dr. Daljit Singh Eye Hospital, Sheranwala Gate, Amritsar, India; 3) Central University of Punjab, Bathinda, India.

Purpose: The aim of present study was to analyze the ACE I/D (insertion of 287bp DNA fragment in intron 16) polymorphism with diabetic retinopathy (DR) in type 2 diabetic (T2D) patients from north India. Materials and methods: In this case-control association study, 857 unrelated patients with T2D were collected from Dr. Daljit Singh Eye Hospital, Amritsar. Out of these, 507 diabetic patients had different types of retinopathies. Of these 290 patients were diagnosed with Proliferative Diabetic Retinopathy (PDR), 94 with Pre-proliferative Diabetic Retinopathy (PPDR), 121 with Back-ground/Non proliferative Diabetic Retinopathy (BDR/NPDR) and 350 patients having T2D but no signs of retinopathy were also analyzed as controls. ACE I/D polymorphism analysis was undertaken by polymerase chain reaction (PCR) followed by agarose gel electrophoresis. Chi-square test was performed to analyze the genotypes distribution and allele frequencies using statistical calculator (StatPac) V.3.0. Using SPSS V.16.0, one way ANOVA test was performed to find significant association of various clinical parameters (age of patients, age of onset of diabetes, duration of diabetes, BMI, systolic and diastolic blood pressure) with diabetic retinopathy. Also the ACE I/D genotype distribution of DR (PDR, PPDR, BDR) patients was analyzed with controls for any modifications on severity of the retinopathy. Results: The present study revealed statistically significant difference between age, age of onset, and duration of diabetes when DR patients were compared with controls. The genotypes (p=0.1790) and allele frequencies (p=0.6115) with ACE I/D polymorphism however showed no statistically significance with DR as compared to controls. However, patients having PPDR showed higher frequency of ID genotype (p=0.0327), when compared with controls. Conclusion: Present study reveals no association of ACE I/D polymorphism with diabetic retinopathy in the analyzed north Indian patients. Hence, we conclude that this polymorphism does not seem to be a useful marker for the diabetic retinopathy. However, the association of ID genotype with PPDR when compared with controls indicates that it may have a role in proliferation.

402W

Genetically determined metabotypes in the context of complex diseases. *C. Gieger[1], S-Y. Shin[2], A-K. Petersen[1], G. Zhai[3,4], T.D. Spector[3], N. Soranzo[2], K. Suhre[5,6,7].* 1) Institute of Genetic Epidemiology, Helmholtz Zentrum Munich, German Research Center for Environmental Health, Neuherberg, German; 2) The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton UK; 3) Department of Twin Research & Genetic Epidemiology, King's College London, UK; 4) Discipline of Genetics, Faculty of Medicine, Memorial University of Newfoundland, Canada; 5) Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum Munich, German Research Center for Environmental Health, Neuherberg, Germany; 6) Faculty of Biology, Ludwig-Maximilians-Universität, Planegg-Martinsried, Germany; 7) Department of Physiology and Biophysics, Weill Cornell Medical College in Qatar, Education City - Qatar Foundation, Doha, Qatar.

The better understanding of mechanisms controlling human health and disease, in particular the role of genetic predispositions and their interaction with environmental factors, is a prerequisite for the development of safe and efficient therapies for complex disorders, such as type 2 diabetes and cardiovascular disease. Genome wide association studies (GWAS) have identified many risk loci for complex diseases, but effect sizes are generally small and information on the underlying biological processes is often lacking. Associations with metabolic traits as functional intermediates can overcome many of these problems and potentially inform individualized therapy. Advances in analytical biochemistry have made it possible to obtain global snapshots of metabolism allowing for GWAS with broad panels of metabolite concentrations in different body fluids, like blood serum, plasma or urine. In a series of GWAS we have identified more than 40 genetic loci, typically explaining a large fraction of the variation of the associated metabolic trait. In the majority of cases a protein biochemically related to the associated metabolic traits is encoded at these loci which we call genetically determined metabotypes. We present results from our recent study in the German KORA study and the British TwinsUK study (N = 2,800), that provided us a wide spectrum of disease related and pharmaceutically relevant genetic associations. For instance, the *AHR* locus associates with coffee consumption habits in a meta-analysis of over 35,000 individuals - we find an association of the same locus with serum caffeine concentration in our study of 2,800 individuals (P-value = 4.8×10^{-15}). Furthermore, a locus at 19q13, including *RASIP1* and *FUT2*, showed an association with retinal vascular caliber in 15,000 Europeans. The original paper proposes *RASIP1* as the causative gene due to its involvement in endothelial cell motility, angiogenesis and vessel formation. However, our data show at the same locus ($r^2 = 0.61$) an association with metabolites related to blood coagulation, indicating that these properties and rather the functional related *FUT2* gene are at the basis of the association with this microvascular disorder. These examples show the high power and the ability of genetically determined metabotypes to better characterize risk factors. To make this resource available for the scientific community, we have implemented a freely available database to query newly discovered risk loci.

403W

Elucidating Gene Pathways Underlying Insulin Resistance Using Integrated Approaches of Genome-Wide Association Study, Expression QTLs and Pathway Analysis. *K. Hao[1,4], X. Yang[3,4], J.W. Knowles[2], T.L Assimes[2], H.J. Cordell[5], W. Xie[6], M.N. Weedon[6], H. Zhong[3,4], T.M. Frayling[6], L-T. Ho[7], M. Keller[10], D.M. Greenawalt[1], D.M. Kemp[1], F. Abbasi[2], E. Ferrannini[8], J. Paananen[9], E. Ingelsson[11], L.M. Kaplan[12], P.S. Tsao[2], A. Attie[10], P. Lum[4], E.E. Schadt[3,4], M. Laakso[9], M. Walker[5], A. Hsiung[13], T. Quertermous[2], GENESIS, EUGENE2, SAPPHIRe, ULSAM, RISC.* 1) Merck Research Labs, 33 Ave. Louis Pasteur, Boston, MA 02115; 2) Stanford University, Division of Cardiovascular Medicine, Falk CVRC, 300 Pasteur Dr., Stanford, CA 94304; 3) Sage Bionetworks, 1100 Fairview Ave N., Seattle, WA 98109; 4) Rosetta Inpharmatics LLC, a Wholly Owned Subsidiary of Merck & Co. 401 Terry Ave. N, Seattle, Washington; 5) Newcastle University, Institute for Cell and Molecular Bioscience, and Institute of Human Genetics, Newcastle upon Tyne, UK; 6) Institute of Biomedical and Clinical Science, Peninsula Medical School, University of Exeter, Magdalen Rd., Exeter, EX1 2LU, U.K; 7) Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei, Taiwan. Section of Endocrinology and Metabolism, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, Faculty of Medicine, School of Medicine, Nat; 8) Department of Internal Medicine, Pisa School of Medicine, Pisa, Italy; 9) Department of Medicine, Univesrity of Eastern Finland, Kuopio, 70210 Kuopio, Finland; 10) Department of Biochemistry, University of Wisconsin, 433 Babcock Drive Madison, WI 53706-1544; 11) Department of Medical Epidemiology and Biostatistics, Karolinska Institute, SE-171 77 Stockholm, Sweden; 12) Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts; 13) Division of Biostatistics and Bioinformatics, Institute of Population Health Sciences National Health Research Institutes, Zhunan, Taiwan.

Objective: Impaired insulin sensitivity (IS) represents a risk factor for cardiovascular disease and type 2 diabetes. Genome-wide association studies (GWAS) of type 2 diabetes and IS surrogates have primarily identified genes related to) cell action rather than IS. We conducted a GWAS for IS as defined by quantitative measures of insulin sensitivity in the GENESIS consortium to identify genetic factors that modulate IS.

Research Design and Methods: The GENESIS consortium is comprised of three studies of Caucasian subjects, namely, RISC (n = 1,042), Stanford (n = 381), and EUGENE2 (n=595), and one of East Asian subjects (SAPPHIRE, n = 455). Standard single marker association analyses within each study were performed along with a meta-analysis of all Caucasian subjects (n = 2018). We augmented these analyses with the integrative SNP set enrichment test (SSET) that leverages GWAS association, genetics of gene expression, and knowledge-driven pathways or data-driven gene networks.

Results: Single-marker association testing did not identify SNPs with genome-wide significant associations, likely due to the moderate sample size. However, in the integrative analyses, pathways or gene sets relevant to obesity, fat cell differentiation, calcium homeostasis, mitochondrial dysfunction, lipid metabolism, and cell cycle were significantly enriched for IS polymorphisms at Bonferroni-corrected p <0.05 using SSET.

Conclusions: Multiple gene sets were found to be associated with IS, suggesting a complex genetic architecture. The systems biology approach we applied may be especially useful where study size is limited.

404W

Genome-wide association study on the variation of quantitative trait may help identify important genetic variants for complex diseases, an example of CHD risk in T2D patients. *Z. Liu[1], Q. Qi[2], F. Hu[2], L. Qi[2], L. Liang[1].* 1) Department of Epidemiology, Harvard School of Public Health, Boston, MA; 2) Department of Nutrition, Harvard School of Public Health, Boston, MA.

Extreme levels of quantitative metabolic traits may be important to complex diseases, for example low HDL is associated with higher risk of Type-2 diabetes (T2D) and coronary heart disease (CHD). Genome-wide association studies for quantitative traits have been focusing on the comparison of the mean levels of the traits among individual with different genotypes. While this approach successfully identified many genetic variants associated with extreme level of quantitative traits, it might miss the genetic variants related to the increased perturbation or variation of the trait, which is another source leading to extreme level of the quantitative trait. In this study, we show that novel genetic variants may be identified by testing association with the different levels of the variation of quantitative traits instead of the mean values. In particular, we carried out a genome-wide association scan for the variation of HDL using the 700,000 SNPs on Affymetrix 6.0 array typed on 1461 (NHS: 660; HPFS: 801) T2D cases (505 with CHD and 745 without CHD, 66 stroke and 145 unknown) from a T2D study in the Nurses Health Study (NHS) and the Health Professionals Follow up Study (HPFS). After adjusting for age, BMI and eigenvectors obtained from genotype, we found a locus, SLC2A9, on chr4p16.1 significantly associated with the variation of HDL in both NHS and the HPFS samples (meta-analysis pvalue=4.2x10-9). The SLC2A9 locus we identified has been shown to be associated with uric acid level which is a risk factor for T2D and CHD and might imply important gene-environment interaction or other mechanism underlying the genetics of HDL. We further tested the association with CHD risk in T2D and found that the SNP rs1996335 (in perfect LD with rs1401441, r2=1) was associated with CHD risk in diabetic men (OR [95%CI]: 0.52 [0.29-0.94], P=0.029) but not in diabetic women (1.24 [0.74-2.08], P=0.40). In summary, we show that genome-wide association study on the variation of quantitative trait provides a promising approach to identify new loci and improve the understanding of the genetic bases of complex diseases.

405W

Sub-phenotyping via Metropolis-coupled MCMC to improve the power in association studies. *Z. Geng[1], S. Z&oumlllner[1,2].* 1) Biostatistics, Univ Michigan, Ann Arbor, MI; 2) Psychiatry, Univ Michigan, Ann Arbor, MI.

Genetic heterogeneity in complex traits is a major challenge for identifying risk genes. Such heterogeneity may be the result of broad, descriptive definitions of a disease and many common diseases may have unidentified genetically more homogeneous subtypes. Under this hypothesis, genetic heterogeneity is reflected in clinical heterogeneity of a disease and clinical traits or sub-phenotypes (covariates) may be correlated with specific risk variants. However, single covariates may not provide enough information to identify such subtypes. Thus, we propose a method to identify a cluster of covariates that are co-inherited with affection status compatible with a single underlying risk variant. Focusing on related individuals, we identify clusters of covariates that are heritable, rather than just correlated due to reasons unrelated to genetics. We consider a dataset of related affected individuals with a large number of measured covariates but no genotype data. We fit the covariate data to a model where a subset of covariates is affected by an unobserved risk variant, while the remaining covariates are independent of the genotype. Using a Metropolis-coupled MCMC, we identify the subset of informative covariates and the posterior probabilities of all model parameters. Based on these parameters, we obtain a logistic model of carrying a specific risk variant conditional on the covariates. Using this model, we can then calculate a posterior probability of carrying the risk variant to each individual in a population sample. Using simulated data of affected sibpairs, we illustrate the ability of our algorithm to correctly estimate model parameters. Even when assuming only weak effects of the underlying risk variants on each individual covariate, we are able to correctly identify the underlying model and identify carriers of the underlying risk variant. Moreover, we show the gain of power obtained in a case control study from up-weighting cases that have a higher probability of carrying the risk variant. We emphasize a model of rare risk alleles with intermediate effect sizes and show that our method can be used to select a subset of individuals for sequencing. In summary, we have developed a novel approach to mine rich phenotypic data for disentangling underlying genetic heterogeneity. This algorithm can be used to improve the power of single marker tests in GWAS and to prioritize individuals in resequencing studies.

406W

Balanced Translocations and Inversions in the Genome of Finns. *T. Varilo[1], T. Luukkonen[2,3], M. Pöyhönen[4], L. Peltonen[2,3,7,10], A. Palotie[2,7], K. Aittomäki[4], J. Ignatius[9], K.O.J. Simola[5], J.D. Terwilliger[2,8], R. Salonen-Cajander[6].* 1) Dept of Medical Genetics, University of Helsinki, Helsinki, Finland; 2) Inst for Molecular Medicine Finland; 3) Nat Inst for Health and Welfare, Finland; 4) Dept of Clinical Genetics, Helsinki University Central Hospital; 5) Dept of Pediatrics, Tampere University Hospital; 6) Dept of Medical Genetics, Väestöliitto, Helsinki; 7) Welcome Trust Sanger Inst, Hinxton, Cambridge, UK; 8) Dept of Genetics and Development, Columbia University, NYC, NY, USA; 9) Dept of Clinical Genetics, Turku University Central Hospital; 10) Deceased.

Finland is acknowledged of its high standard of clinical medicine and in the disease gene hunt of its founder populations. What is perhaps not so well recognized in the field of genetics is that Finland has probably the most comprehensive health registers and records: hospitalizations, surgeries, chronic diseases, cancers and prescriptions etc. have been filed for decades. Relying on this infrastructure we have gathered clinical information from all distinct hospitals and laboratories on all reciprocal balanced translocations and inversions identified in Finland, and are constructing a national database (www.fintransloc.org). By analyses of the medical records and by searches of additional national registers, we are obtaining novel information of not only monogenic traits with unidentified mutations, but also of multifactorial traits associated with any given chromosomal abnormality. Moreover, we believe that such a database will greatly assist genetic counseling efforts and scientific collaborations. To date, we have surveyed all the 2575 hospital or laboratory contacts involving translocations or inversions consisting of 572 families plus singletons. The largest family consists of some 40 carriers who have common ancestors in the 1600s. We are breakpoint mapping two interesting pilot families (phenotypes: aortic aneurysm and height)--with significant linkage signal exactly at the breakpoint--to provide shortcut in the identification of their predisposing disease genes. A nationwide systematic sample collection of DNA, RNA and cells with so far over 80% coverage of participants was launched in Aug 2010.

407W

A chromosome 21 association study suggests DSCAM as a susceptibility locus for the Hirschsprung disease endophenotype in Down syndrome. *A.S. Jannot[1,2,3,4], A. Pelet[2,4], A. Henrion-Caude[2,4], M. Masse-Morel[2,4], S. Arnold[5], I. Ceccherini[6], S. Borrego[7,8], R.M.W. Hofstra[9], A. Munnich[2,4], A. Chakravarti[5], J. Amiel[2,4], F. Clerget-Darpoux[1,3], S. Lyonnet[2,4].* 1) Unite 669, INSERM, Villejuif, France; 2) INSERM U-781, AP-HP Hôpital Necker-Enfants Malades, Paris 78743, France; 3) Université Paris Sud, Faculté de médecine, Le Kremlin-Bicêtre 94275, France; 4) Département de Génétique, Université Paris Descartes, Faculté de Médecine, Paris 75006, France; 5) Center for Complex Disease Genomics, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; 6) Laboratorio di Genetica Molecolare, Istituto Gaslini, Genova 16147, Italy; 7) Unidad de Gestión Clínica de Genética, Reproducción y Medicina Fetal. Instituto de Biomedicina de Sevilla (IBIS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Sevilla, 41013 Spain; 8) CIBER de Enfermedades Raras (CIBERER), ISCIII, Sevilla, 41013, Spain; 9) Department of Genetics, University Medical Center Groningen, Groningen 9700, The Netherlands.

Hirschsprung disease (HSCR), or aganglionic megacolon, is a congenital disorder characterized by the absence of enteric ganglia along a variable length of the distal intestine. The RET gene is a well-established susceptibility locus in all isolated forms cases of HSCR, and some syndromic variants. Predisposition to HSCR in Down syndrome patients strongly suggests that additional factor(s) contributing to HSCR map to chromosome 21. To identify these additional factors, we carried out a GWAS focused on chromosome 21 in a series of 28 cases with the association of Down syndrome and HSCR, and their parents. We successfully genotyped 7,691 SNPs on chromosome 21. After correction for multiple testing, the only significant result was obtained for 2 SNPs in complete linkage disequilibrium (rs2837770 and rs8134673) located in intron 3 of the DSCAM gene (nominal p-value p= 2.2x10-5, and p-value after correction for multiple testing=0.018). Importantly, DSCAM is expressed in the developing enteric nervous system. Association with these SNPs was replicated (p=0.01) using an independent sample of 220 non-syndromic HSCR patients and their parents. Our association findings are supported by two previous independent studies mapping an HSCR susceptibility factor in a region including the DSCAM gene locus. Furthermore, the methodology proposed in order to perform chromosome 21 association analyses in Down syndrome patients could also help to deconstruct the genetic bases of other endophenotypes of trisomy 21 syndrome.

408W

Prediction of complex multifactorial disease: comparing family history and genetics. *C.B. Do[1], J.M. Macpherson[1], D.A. Hinds[1], B. Naughton[1], U. Francke[1,2], N. Eriksson[1].* 1) 23andMe, Inc, Mountain View, CA; 2) Stanford University School of Medicine, Stanford, CA.

In clinical practice, family history is generally considered an important risk factor for a wide variety of complex diseases. Over the last several years, however, commercial SNP-based tests for low penetrance common variants associated with complex diseases have become increasingly available. Although the use of family history and genetic risk prediction is well understood for simple Mendelian disorders, to date, little is known regarding the relative performance of these methods for diseases with complex multifactorial inheritance.

Using quantitative genetic theory, we analyze the influence of disease architecture on the predictive capacity of family history and genetic models with the goal of understanding the range of scenarios in which one of the approaches may be advantageous compared to the other. We find that for extremely common multifactorial disorders (>10% prevalence, e.g., type 2 diabetes in the United States), an idealized family history-based algorithm can achieve predictive accuracies on par with SNP-based models that account for 20-30% of the heritability of the disease, meeting or exceeding what SNP-based models have achieved thus far. However, many diseases of important public health consequence are neither extremely common nor extremely rare, occurring in one out of every 100 to 1,000 individuals (e.g., Crohn's disease). For these diseases of moderate frequency, the point of equivalency occurs at only 1-4% of the variance explained, well within the detection limits of GWAS today. These findings suggest that for less common diseases, currently known genetic factors may in fact already be better discriminators of risk than their family history-based counterparts, despite the large fraction of missing heritability that remains to be explained.

Our results provide insight into the influence of disease architecture on risk prediction performance and its implications for clinical practice. In particular, our findings highlight the potential pitfalls of drawing conclusions regarding the relative merits of family history and genetics without considering the broad spectrum of human disorders, and illustrate the importance of considering both family history and genetics in combination when evaluating an individual's predisposition to disease.

409W

Assessment of Psoriatic Quality of Life and Qunatity of Disease by using PDI and PASI. *R.K. Golee, R. Singla, R. Kaur.* Human Biology, Punjabi University, Patiala, Patiala, Punjab, India.

Psoriasis is an ancient and universal inflammatory longest known disease of human beings. It is an autoimmune disease which is more prone to peripheral portion and joints. The main objectives of the present study was to access the quality of life, affected body surface area, inheritance pattern and epidemiological study in punjabi population of India. These parmeters were analysed by using the Psoriasis area and sevrity index (PASI) developed by Fredrikkson and Patterson in 1978 and Psoriasis disability index (PDI) developed by Finlay in 1999 (revised form), family tree and statistical calculations. Epidemiological study was done in the Mansa district from the Civil Hospital from the last five years skin OPD's record and it was observed that a total of 82500 skin patients were registered for routine check up and only 300 patients were clinically diagnosed as suffering from psoriatic disease ranging from chronic to mild form. Percentage analysis shows that 0.3636% population of this area suffered from psoriasis. Family tree analysis shows that more males(20.82%) suffered from the disease as compared to females(7.39%). Male female ratio comes out to be 5:1 with mean age of patient 42.33 years. Mean chronicity age of the psoriatic patients comes out to be 1.95 years. The total PASI score ranges between 4% to 19.9% in males where as in females it lie between 10.6% to 13.5%. The average value of Head and Neck score was 3.3%, Upper Limb 8.64%, Trunk 12.6%, Lower Limb 10.2%. When the PDI administered for understanding the effect of psoriasis on the daily life of patients, the result shows trmendous high score as highest PDI score is 29 out of total 30. The average value of PDI for females was 25.2 while the males stand out at 19.65. The higher range of PDI showed that the disease effects greatly to the quality of daily life and hinders their routine activities. So, it concluded that psoriasis causes major discomfort in their daily life which is difficult to handle as the pain and itching some times lead to suicidal tendencies, psychosocial stress, unbearable behaviour and addiction to drugs, smoking and alcoholism.

410W

Assessing Pleiotropic Influences on Comorbid Disease Phenotypes. *R.C. McEachin, A. Karnovsky, J.D. Cavalcoli, M.A. Sartor.* CCMB, Univ Michigan, Ann Arbor, MI.

Complex diseases are predisposed, in part, by genetic variation, and are often overrepresented for comorbid phenotypes (e.g. diabetes and hypertension are frequently comorbid). This comorbidity is consistent with the existence of pleiotropic genetic variation influencing both disease phenotypes. A Genome Wide Association Study (GWAS) assesses evidence of association between individual SNPs and a single disease phenotype, and GWAS datasets for related phenotypes are being generated. We consider how GWA data from studies of multiple comorbid disease phenotypes may be effectively merged to reveal pleiotropic influences. Fisher meta-analysis is an approach for combining summary statistics (i.e., p-values), which can be used to merge GWA data derived from multiple studies, each testing for association between SNPs and a single disease phenotype. If all necessary assumptions are met, the distribution of the Fisher statistic is known and significance of association can be assessed simply. We recently showed that a nonparametric adaptation of Fisher meta-analysis, where we assess significance by permutation, can be useful in identifying pleiotropic variation underlying comorbidity, based on GWAS summary data from related phenotypes (McEachin, et al., ISCB GLBIO 2011). Other groups have developed approaches to this problem, based on assumed distributions of statistics derived from combining primary data from GWA studies for related phenotypes. While the various approaches appear to have utility, in this work we compare methods based on standardized input. Notably, with observed data, the true genetic effect is not known, so both positive and negative controls are generally unavailable. To overcome this obstacle, we compare methods using simulated genotype data, where pertinent details of the population, SNP minor allele frequency, and the strength of the genetic influence are controlled, yielding true positive and negative controls. We show that the overall results of testing for pleiotropic effects may vary, depending on the approach used. However, for pleiotropic variation that strongly influences the comorbidity, differences between the methods may be modest and we may be well on our way to identifying these effects.

411W

Using protein-protein interactions to long QT syndrome proteins reveals networks underlying genetic association to QT interval variation. *E.J. Rossin[1,2,3], A. Lundby[4], A.B. Steffensen[4], S. Pulit[1,3], P. DeBakker[1,3], A. Pfeufer[6], C. Newton-Cheh[1,2,3], K. Lage[5], J. Olsen[4].* 1) The Broad Institute, Cambridge, MA; 2) Massachusetts General Hospital, Boston, MA; 3) Harvard Medical School, Boston, MA; 4) University of Copenhagen, NNF Center for Protein Research, Copenhagen, Denmark; 5) Center for Biological Sequence Analysis, Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark; 6) Institute of Human Genetics, Helmholtz Center Munich, Germany.

Variation of the QT interval duration in the heart's electric cycle is a risk factor for sudden cardiac death and drug-induced arrhythmia. Genome-wide association studies (GWAS) have identified 11 genomic loci associated with QT interval variation. We have previously shown that the leveraging protein-protein interaction (PPI) data to identify physical connections between proteins encoded in associated loci can help propose underlying mechanisms, pinpoint critical genes and predict new associations (Rossin et al. 2011). We hypothesized that looking for physical interactions between proteins in QT-interval variation associated loci and 12 proteins known to be important in cardiac ion channel function and disrupted in Mendelian long-QT syndromes (LQTS) may be similarly fruitful. To test this, we first applied a published *in silico* network analysis tool (called DAPPLE) to the 11 loci and 12 LQTS genes. We found more connections between these input proteins than chance expectation (p=1e-4), suggesting a function common to interacting proteins. To see if this network could predict new genetic associations, we tested the 497 yet-unassociated proteins in the network in a new QT-interval duration meta-analysis consisting of 100,000 individuals. Using a composite test for association, we found that the genes encoding these proteins were enriched for association (p=2e-4) and that 5 of the genes were in newly genome-wide significant regions, more than chance expectation. To expand our search experimentally and in the relevant tissue, we immuno-precipitated proteins encoded by 5 LQTS genes (*CACNA1C*, *CAV3*, *KCNH2*, *KCNQ1* and *SNTA1*) in mouse cardiac tissue and resolved their associated complexes by mass-spectrometry. Similar to what we observed *in silico*, we found that proteins that bind to the 5 Mendelian baits were encoded for by genes enriched for association (p=1.1e-6) and contained proteins encoded in 8 genome-wide significant loci, suggesting the likely candidate in those regions. One particular interaction had not be previously described in the literature, so we followed up with electrophysiological measurements and confirmed a functional effect on ion channel current mediation of one of the LQTS channels by a QT-interval associated protein. We therefore show that common genetic variation associated to the QT-interval is spread across a network of proteins that interact with each other and known LQTS proteins.

412W

optiCall: A robust genotype calling algorithm for rare, low-frequency and common variants. *T. Shah, C.A. Anderson.* Human Genetics, Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, United Kingdom.

Burgeoning whole-genome and whole-exome sequencing projects are likely to require large-scale microarray based follow-up studies. Already, custom arrays such as Metabochip and Immunochip utilize SNPs identified through population-based sequencing efforts such as 1000 genomes to better survey loci known to underpin variation across related phenotypes. Typically, the allelic probes on these custom arrays have undergone less stringent quality control compared to those that make it onto mass-produced GWAS arrays. This drop in probe quality, in addition to a greater focus on low-frequency and rare variants, presents many problems for existing genotype-calling algorithms. To tackle this problem we have developed a novel genotype calling algorithm, optiCall, which can accurately call SNP genotypes across the entire minor allele frequency spectrum (including SNPs with atypical cluster positions) for both small and large sample sizes. Existing methods typically either call genotypes on a SNP-wise or sample-wise basis, whereas optiCall uses both sources of information to make accurate genotype calls. First, a random subset of the intensity data, both within and across samples, is clustered to create a data-derived prior for genotype-class probabilities across the X and Y intensity range. Second, a mixture model is fitted on a SNP-wise basis, guided by the data-derived prior. The result is that rare variants are accurately called because they fall in regions of high probability defined by the prior, and common variants are well called by the SNP-wise guided mixture model in the second step. optiCall internally estimates clustering quality using Hardy-Weinberg equilibrium (HWE), with any SNPs showing significant deviation from HWE subjected to a series of rescue steps designed to accurately call SNPs with atypical cluster positions. Using Immunochip data we compare genotype calls from optiCall to those from alternative algorithms, and to a set of manually-derived calls. We show that existing algorithms have unfavoured areas of the minor allele frequency spectrum or were unable to accurately call SNPs with atypical cluster positions. optiCall performs well across the minor allele frequency range and deals well with atypical SNPs that existing algorithms consistently call incorrectly. optiCall is available for download at https://bitbucket.org/tss101/opticall/.

413W

Assessment of methylation in combination with computational assessments of allelic imbalance in human fibroblasts. *J.R. Wagner[1], B. Ge[2], S. Busche[2], T. Pastinen[2,3], M. Blanchette[1].* 1) School of Computer Science McGill University, Montreal, Quebec, Canada; 2) McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, Canada; 3) Department of Human and Medical Genetics, McGill University Health Centre, McGill University, Montreal, Quebec, Canada.

Allelic expression (AE) occurs when the two alleles of a given genomic locus are transcribed at different levels in a given cell, either because of epigenetic inactivation of one of the two alleles, or because of genetic variation in regulatory regions. We have developed a computational approach to analyze high throughput data measuring levels of AE and identify human genomic regions with AE in an unbiased and robust statistical manner. In brief, this approach consists of applying a Hidden Markov Model (HMM) with the added property of having specific transition probabilities for each locus in the array. We have combined AE measurements at ~750k single nucleotide polymorphisms (SNPs) dispersed across genic and intergenic regions throughout human autosomes, and measured in fibroblast cell lines from 58 individuals, with a high-throughput methylation experiment measuring methylation levels at ~450k probes measured in fibroblasts from the same individuals. We found that SNPs whose phased haplotype values correlate with methylation levels at nearby probes (mSNPs) are also frequently associated with genomic regions exhibiting AE levels, as determined from our HMM approach. Interestingly, of the correlations between mSNPS and AE found, a substantial percentage were in regions not previously annotated as genes. The majority of highly correlated pairings occur when the mSNP is 5-10kb from the AE region. We find overall that methylation is an important contributor to allelic expression and also to regulation in particular categories of genomic regions.

414W

Evidence of genetic interaction between SNPs in candidate genes that may confer increased risk for oral clefts. *M.A. Mansilla[1], M.E. Cooper[2], K. Christensen[3], R.G. Munger[4], T.H. Beaty[5], M.L. Marazita[2,6], J.C. Murray[1].* 1) Dept Pediatrics, Univ of Iowa, Iowa City, IA; 2) School of Dental Medicine, Univ of Pittsburgh, Pittsburgh, PA; 3) Univ of Southern Denmark, Odense, Denmark; 4) Utah State Univ, Logan, UT; 5) Johns Hopkins Univ, School of Public Health, Baltimore, MD; 6) Department of Psychiatry, Univ of Pittsburgh, PA.

Cleft lip with or without cleft palate (CL/P) is a common congenital anomaly of complex etiology that affects about 1/700 births worldwide and imposes substantial medical, social and economic burden on families. Advances in genetic counseling and prevention for CL/P rely on identification of genes controlling risk. In the past two years five genes have been identified as influencing occurrence of CL/P thhrough linkage and/or association, including *IRF6, FOXE1, MAFB, ABCA4-ARGHAP29* and the gene desert region, on chr. 8q24. In an effort to determine the impact of apparent risk alleles when they are present together, we calculated population attributable risks (PAR) and odds ratios (OR) for the most significant SNPs in the above mentioned candidate genes and pairwise combinations across genes in case-control cohorts from Denmark (290 cases and 223 controls) and Utah, USA (233 cases and 493 controls). The two most significant PARs were found for rs13041247 in *MAFB*, PAR 26 % with an accompanying OR=1.8 and for rs987525 in 8q24, PAR 16 % with an estimated OR=3.1, among individuals who are homozygous for these risk alleles in the Denmark study (rs13041427 risk allele T, rs987525 risk allele A). Moreover, a combined effect for these two SNPs showed an increased risk for those homozygous individuals at both variants with a PAR 32 % and a estimated OR increase from 1.8 (*MAFB* - rs13041427 - TT) and 3.1 (8q - rs987525 - AA) to 10.7 (*MAFB* - rs13041427 - TT X 8q - rs987525 - AA). For the Utah study, when considered individually the markers rs987525 (in 8q24) gave an estimated PAR 16 % (with an OR=1.7), rs642961 (in *IRF6*) gave a PAR 15 % (with an OR=1.6) and rs 3758249 (in *FOXE1*) gave a PAR 13 % (with an OR= 1.7) presented an increase in attributable risk when one risk allele was present at each of the three markers, PAR 27 % (with an OR =2.2) (rs987525 risk allele A, rs642961 risk allele A, rs3758249 risk allele G). These findings should improve our understanding gene by gene interactions and could be applied to genetic counseling and possible prevention programs. (Support: DE016148 and DE008559).

415W

Genome-wide copy number analysis uncovers a new HSCR gene: *NRG3*. *M. GARCIA-BARCELO[1], C.S.M. TANG[1,2], M.T. SO[1], C.R. MARSHALL[3], S. SCHERER[4], S. CHERNY[2], P. SHAM[2], P. TAM[1].* 1) Department of Surgery, Li Ka Shing Faculty of Medicine, The University of Hong Kong; Hong Kong, China; 2) Department of Psychiatry, Li Ka Shing Faculty of Medicine, The University of Hong Kong; Hong Kong, China; 3) Program in Genetics and Genome Biology and The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Ontario, Canada; 4) The McLaughlin Centre and the Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.

Hirschsprung disease (HSCR) is a congenital disorder characterized by aganglionosis of the distal intestine. To assess the contribution of Copy Number Variants (CNVs) to HSCR, we analysed the data generated from our previous genome-wide association study on HSCR patients whereby we identified NRG1 as a new HSCR susceptibility locus. Analysis of 129 Chinese patients and 331 ethnically matched controls showed that HSCR patients have a greater burden of rare CNVs (p=1.50x10-5), particularly for those encompassing genes (p=5.00x10-6). We identified 246 rare-genic CNVs exclusive to patients. Among those, we detected a *NRG3* deletion (p=1.64x10-3). Subsequent NRG3 follow-up (96 additional patients and 105 controls) revealed 9 deletions and 2 de novo duplications among patients and two deletions among controls (p=1.67x10-6). Importantly, *NRG3* is a paralog of *NRG1*. Stratification of patients by presence/absence of HSCR-associated syndromes showed that while syndromic-HSCR patients carried significantly longer CNVs than the non-syndromic or controls (p=1.50x10-5), non-syndromic patients were enriched in CNV number when compared to controls (p=4.00x10-6) or the syndromic counterpart. Our results suggest a role for *NRG3* in HSCR etiology and provide insights into the relative contribution of structural variants in both syndromic and non-syndromic HSCR. This would be the first genome-wide catalog of copy number variants identified in HSCR.

416W

Finding structural variants in individual human genomes with Random Forests. *J.J. Michaelson[1,2], J. Sebat[1,2,3].* 1) Beyster Center for Molecular Genomics of Neuropsychiatric Diseases,University of California, San Diego, La Jolla, CA; 2) Department of Psychiatry, University of California, San Diego, La Jolla, CA; 3) Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA.

The detection of structural variants (SVs) from high-throughput short read sequencing data is a complex and unsolved problem. Most SV detection methods available today utilize information derived from a small number of metrics such as depth of coverage, read pair signals, or a combination of the two. These approaches have proven useful for identifying some SVs, but problems of sensitivity and specificity remain.

To address these issues, we have derived additional discriminating metrics from aligned sequencing data, and have brought the discovery problem into a supervised learning paradigm. We trained a Random Forest classifier to discriminate known SVs from invariant regions and false positives called as SVs by other methods. When presented with new data, the classifier was able to identify SVs at high sensitivity and specificity.

The development of this method has led to several contributions. First, the classifier can be viewed as a single method that embodies the strengths of the various calling methods that provided training calls; users need not expend the effort of merging callsets from disparate methods to obtain reliable results. Second, the method is adaptive. As calls are experimentally validated or invalidated, they may be added to the training data, leading to a new classifier that has learned from its previous successes and failures. In addition, as new discriminating features are proposed (e.g. as a means to identify a new class of SV), they may be incorporated into the classifier without a fundamental redevelopment of the method.

417W

Identification of a novel clefting locus through genome-wide CNV analysis. *A.L. Petrin[1], J.M. Dierdorff[2], B.A. Lentz[1], X. Hong[2], G. de la Garza[3,4], R.A. Cornell[4], J.C. Murray[1], J.R. Manak[1,2].* 1) Department of Pediatrics, University of Iowa, Iowa City, IA; 2) Department of Biology, University of Iowa, Iowa City, IA; 3) Department of Otolaryngology-Head and Neck Surgery, University of Iowa, Iowa City, IA; 4) Department of Anatomy & Cell Biology - University of Iowa, Iowa City, IA.

Orofacial clefts are one of the most common birth defects affecting 1-2 per 1000 births and have a complex etiology involving both genetic and environmental factors. The recent use of high resolution array-based comparative genomic hybridization (aCGH) has increased the ability to detect sub-microscopic deletions and amplifications that can be causative for complex diseases such as cleft lip and palate (CLP). We report progress on an ongoing study to search for causal or contributory copy number variants (CNVs) using whole-genome tiling arrays that offer comprehensive high resolution analysis of copy number changes across the genome. Analysis of 120 samples with cleft lip and palate (CLP) and 43 samples with cleft palate only (CPO) identified several rare CNVs ranging in size from 1 to 110kb. In one family with CPO we found a deletion of *MSX2*, a homeobox gene that is a good candidate gene for clefting as prior studies have identified point mutations in cases with isolated cleft and craniosynostosis. Additionally, we identified deletions of *ISM1* in 2 independent families with CLP. *ISM1* is a strong candidate for clefting as it is in the same synexpression group as *FGF8*, mutations in which cause Kallman syndrome which has clefting as a feature. Morpholino knockdown of ism1 in zebrafish resulted in severely malformed or absent jaw and pharyngeal cartilage consistent with *ism1* playing a role in craniofacial development. Our study demonstrates a successful high throughput pipeline from CNV identification to gene knockdown in a vertebrate model system and reveals *ISM1* as a new clefting locus in humans. NIH R01 DE021071.

418W

Multi-allelic and complex copy number variations (CNVs) of contiguous genes for Fc. receptors *FCGR2* and *FCGR3* and heat-shock protein *HSP70* in healthy subjects and systemic lupus erythematosus (SLE). *Y.L. Wu[1,2], B. Zhou[1], D.J. Birmingham[2], G.C. Higgins[1,2], L.A. Hebert[2], B.H. Rovin[2], C.Y. Yu[1,2].* 1) Center for Molecular and Human Genetics, the Research Institute at Nationwide Children's Hospital, Columbus Ohio 43205; 2) College of Medicine and Public Health, The Ohio State University, Columbus, Ohio.

Receptors for immunoglobulin Fc., Fc. RIIA and IIB, Fc. RIIIA and IIIB are important effectors of immune response. CNV of *FCGR3B* has been suggested to be a risk factor for human SLE but the published data are controversial because of a lack of definitive methodologies to accurately determine the multi-allelic CNVs of *FCGR3A* and *3B*, their neighboring genes *FCGR2A*, *2B* and *2C*, and heat-shock protein *HSP70* genes *HSPA6* and *A7*. The objectives of this study are to create specific techniques to elucidate the patterns of *FCGR2-HSP70-FCGR3* segmental duplications on chromosome 1q23, and to determine the CNVs of *FCGR3A* and *FCGR3B* in healthy subjects and SLE patients. The study population included 281 SLE patients and 485 unrelated healthy controls of European ancestry. Pulsed-field gel electrophoresis (PFGE) using genomic DNA samples digested with *Pme*I enzyme was applied to determine segmental duplications. *Taq*I restriction fragment length polymorphisms (RFLPs) were used to elucidate copy numbers of *FCGR2A*, *2B* and *2C*; *FCGR3A* and *3B*; and *HSP70* genes *HSPA7* and *HSPA6*. *Pme*I-PFGE and *Taq*I-RFLPs revealed discrete 85-kb segmental duplications for contiguous genes *FCGR2, HSP70* and *FCGR3*, which are termed an FRH module. One to five FRH modules on a haplotype were detectable by PFGE. Monomodular FRH usually had the structure *FCGR2A-HSPA6-FCGR3A-FCGR2B*. Each additional FRH module was inserted between *FCGR3A* and *FCGR2B*, and mostly included *FCGR2C-HSPA7-FCGR3B*. However, *HSPA6* instead of *HSPA7*, and *FCGR3A* instead of *FCGR3B*, could be present in a duplicated module because of gene conversion-like events. A deficiency of *FCGR3B* was caused either by the presence of a monomodular structure with one copy of *FCGR3A*, or a bimodular structure with two copies of *FCGR3A* but no *FCGR3B*. Homozygous and heterozygous deficiencies of *FCGR3B* had a frequency of 12.5% in SLE and 6.2% in controls (odds ratio: 2.16, 95% CI: 1.29-3.60; p=0.0044). By contrast, high copy numbers of *FCGR3B* had a frequency of 8.2% in SLE and 12.0% in controls. Similar CNV phenomena were observable for *FCGR2C* and *HSPA7*. In conclusion, we have defined 85-kb segmental duplications of FRH that contribute to the multi-allelic and complex CNVs of *FCGR3B* and *FCGR3A*, and showed that homozygous and heterozygous deficiency of *FCGR3B* is a common risk factor with medium effect size for human SLE. Notably, the frequency of *FCGR3B* deficiency in SLE is three to four times lower than those reported in the literature.

419W

Eight novel susceptibility genes associated with type 2 diabetes and glycaemic traits in a gene rich linkage region on chromosome 3q for T2D and metabolic traits - what can be learnt? *T. Andrew[1], W. Lau[2], K. Direk[1], H. Elding[2], N. Maniatis[2].* 1) Department of Twin Research & Genetic Epidemiology, Kings College London, London, United Kingdom; 2) Genetics, Evolution & Environment, University College London, Gower Street, UCL, London WC1E 6BT.

The chromosome 3q quantitative trait locus (QTL) that influences metabolic traits (including insulin resistance) is one of the most replicated human quantitative trait linkages. There is also meta-analytic evidence for linkage to this region for type 2 diabetes (T2D). Given that T2D is defined in relation to elevated fasting glucose serum levels (>7mmol/l), it is to be expected that any evidence of association with T2D disease should also be observed for glycaemic traits, but genome-wide association studies to date have failed to show such a relationship. Using prior evidence of linkage to chromosome 3q, we set out to test this hypothesis as part of a candidate 3q locus study. Using the WTCCC1 type 2 diabetes data (Affymetrix 500K, n=4864) and two TwinsUK population-based samples measured for insulin and glucose fasting serum levels (Illumina 610k, n=1611 and 317k, n=801), we confirm association to *IGF2BP2* and the *SLC2A2* gene region at 3q and identify and replicate four novel disease susceptibility loci - *MFSD1*, *PLD1*, *NAA-LADL2* and *MCCC1*. In addition, upon meta-analysis, we identify a further 4 novel genes *VEPH1*, *BCHE*, *KCNMB2* and *ABCC5* of interest ((=< 1E-05). For the ten genes showing evidence of association, eight are associated with both T2D and glycaemic traits and two show association with T2D only (*IGF2BP2*, *ABCC5*). This is the first time that association results for glucose homeostasis have been consistently shown for both disease and healthy populations relating to the same underlying trait, which suggests that previous inability to detect association for both phenotypes to the same genes is likely to reflect a lack of study power rather than any distinct aetiology that separates T2D and normal fasting glucose levels. This talk will focus upon the importance of high-resolution genetic maps for fine mapping (e.g. based upon international projects such as the HapMap consortium) and why it may be premature to abandon the CDCV hypothesis.

420W

Leveraging genome-wide association data to detect genetic pleiotropy at loci associated with glycmic and skeletal phenotypes. *L.K. Billings[1,2,3], Y.H. Hsu[4,5,6], R.J. Ackerman[1], J. Dupuis[7,8], B.F. Voight[1,2,9], ARIC Study[10], ASCOT Study[11], SUVIMAX Study[12], Fenland Study[13], BHS Group[14], AMISH Study[15], DESIR Study[16], French Obese[16], FamHS Study[17], Swedish Twins[18], GEMINAKAR[18], Finnish Twins[18], D. Karasik[4,6], J.B. Meigs[2,19], D.P. Kiel[4,6], J.C. Florez[1,2,3,9], the DIAGRAM Consortium, the GEFOS Consortium, the MAGIC investigators.* 1) Center for Human Genetic Research, Simches Research Building - MGH, Boston, MA, USA; 2) Department of Medicine, Harvard Medical School, Boston, MA, USA; 3) Diabetes Research Center (Diabetes Unit), Department of Medicine, Massachusetts General Hospital, Boston, MA, USA; 4) Hebrew SeniorLife Institute for Aging Research and Harvard Medical School, Boston, MA, USA; 5) Molecular and Integrative Physiological Sciences Program, Harvard School of Public Health, Boston, MA, USA; 6) Framingham Heart Study, Framingham, MA, USA; 7) Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA; 8) National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, MA, USA; 9) Broad Institute of Harvard and Massachusetts Institute of Technology (MIT), Cambridge, MA, USA; 10) Department of Biostatics, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, USA; 11) International Centr for Circulatory Health National Heart & Lung Institute, Imperial College London, London, UK; 12) Institut National de la Santé et de la Recherche Médicale, Centre de Recherchedes Cordeliers, Paris, France; 13) Medical Research Council Epidemiology Unit, Cambridge, UK; 14) School of Population Health, University of Western Australia, Crawley, WA, Australia; 15) University of Maryland, Lancaster, PA, USA; 16) CNRS UMR 8199, Institut Pasteur de Lille, F-59019, France; 17) Division of Statistical Genomics, Washington University School of Medicine, St. Louis, MO, USA; 18) Finnish Genome Center in Helsinki & Uppsala University, Sweden; 19) General Medicine Division, Massachusetts General Hospital, Boston, MA, USA.

Prior studies have provided compelling evidence linking skeletal and glucose metabolism. Despite normal to high bone mineral density (BMD), people with type 2 diabetes (T2D) have a higher risk of fracture, and laboratory mouse models have linked glucose regulation to bone-derived hormones. We leveraged data from genome-wide association studies (GWAS) to detect genetic pleiotropy linking BMD and glycemic traits to further explain the epidemiological and experimental observations connecting bone disease and T2D. We examined SNPs previously associated with BMD or glycemia-related traits at levels of genome-wide significance (P<5 x 10-8), the contribution of common variation in their annotated genes, and the correlation with nearby variants induced by linkage disequilibrium for association with the alternate phenotype in two existing GWAS meta-analysis datasets: 1) up to 46,186 participants in the Meta-Analyses of Glucose and Insulin-Related Traits Consortium (MAGIC) and 2) up to 19,195 participants in the Genetic Factors for Osteoporosis (GEFOS) consortium. BMD SNPs found to be associated with glycemic phenotypes and glycemic SNPs associated with bone phenotypes at test-wide levels of significance were followed-up in either ~19,000 participants with in silico genotype and glycemia data and a large case-control T2D meta-analysis or eQTL analysis in human osteoblasts, respectively. A BMD-associated locus, ITGA1, was associated with fasting glucose in the MAGIC discovery cohort, with one SNP (rs6867040) nominally significant in an in silico replication. A glycemia-related SNP (rs287716) in locus, ADCY5, was associated with femoral neck BMD in GEFOS. Another glycemia- and height-related locus, JAZF1, was suggestively associated with BMD. Selected SNPs at these loci appear to underlie BMD and glycemic traits irrespective of body mass index (BMI), as they were not shown to be highly associated with BMI in about 123,000 genotyped participants from the GIANT consortium. We highlighted the genes ITGA1, ADCY5, and JAZF1 as potential new candidates influencing both BMD and glycemia-related traits. New insights into genetic pleiotropy of both conditions may further underscore the link between skeletal and glycemic phenotypes, improve our understanding of their pathogenesis, and reveal potential targets for bone disease and T2D therapies.

421W

Searching for Low Frequency Functional Variants in a Region Linked to Type 2 Diabetes. *S.F Field[1], I. Prokopenko[2,3], S. Tuna[1], C. Groves[3], P. Akin[1], J. Broxholme[2], L.J. Baier[4], C. Bogardus[4], J. Chan[5], S.K. Das[6], P. Froguel[7,8], R. Hanson[4], C. Hu[9], W. Jia[9], R.C. Ma[5], B. Mitchell[10], N.W. Rayner[2,3], A.R. Shuldiner[10], L. Stein[11], M.I. McCarthy[2,3], P. Deloukas[1], International Type 2 Diabetes 1q Consortium.* 1) Human Genetics, Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, United Kingdom; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX3 7BN, UK; 3) OCDEM, University of Oxford, UK; 4) PERCB, Diabetes Epidemiology and Clinical Research Section, NIDDK, NIH, Phoenix, AZ; 5) Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong; 6) Wake Forest University School of Medicine, Department of Internal Medicine, Section on Endocrinology and Metabolism, Winston-Salem, NC; 7) CNRS UMR 8090, Institute of Biology and Lille 2 University, Pasteur Institute, 59019 Lille, France; 8) Genomic Medicine, Hammersmith Hospital, Imperial College, London, UK; 9) Shanghai Diabetes Institute, Shanghai Jiaotong University, No6 Peoples Hospital, Shanghai, China; 10) Faculty of Medicine, University of Maryland, Baltimore, MD; 11) Cold Spring Harbour Laboratory, New York, NY.

Genome-wide association (GWA) analysis has proved a powerful tool for the discovery of common variants influencing type 2 diabetes (T2D) risk. To date more than 40 susceptibility loci have been identified. However, the known variants account for no more than 10% of the inherited risk. It is likely that a substantial proportion of the remaining risk is contributed by low frequency or rare variants, including structural variants (SVs), which are not covered by GWA SNP arrays. A 23Mb region on chromosome 1q21-25 has shown evidence of linkage to T2D in 8 ethnically diverse populations. Association studies have failed to identify any common variant associations underlying this signal. We are performing two studies to identify rare and low frequency variants causal for the linkage signal, one focusing on SVs, the other and one on rare SNPs in coding regions. SVs were investigated using a Nimblegen tiling array spanning the complete 1q region. The SV assay was carried out on 360 case and control samples from the various populations in which a linkage signal had been found. The samples were all compared to a single control and signal ratios were used to determine the dose variation for each probe. Results were filtered for variants which appeared enriched in cases or controls, and these SVs are being validated using multiplex amplicon quantification (MAQ). The second resequencing study involves 1872 case and control samples from 8 populations in a pooled format. These samples are being run on Nimblegen and Agilent pulldown assays targeted at the exons of all 1q genes. In a pilot analysis of the SV experiment, we identified 21 variants of which 8 were novel. Of the novel SVs, two were validated by MAQ. The two novel SVs identified in the interim analysis are a deletion in intron 1 of GABRB3 and a duplication in an intergenic region between SPRR2G and LELP1. The SV in GABRB3 was identified in 5% of the samples and the SV in SPRR2G-LELP1 in 20%. Analysis of the full data set identified 68 putative SVs (of which 43 are novel) which are undergoing validation. To date, the resequencing assay has identified 19,478 putative novel variants, of which 11,869 have a MAF of <0.01, and APOA2 (P = 0.003) and THEM4 (P = 6X10-6) have shown an enrichment of rare variants in cases in multiple populations. Validation of these novel, rare variants is a key step in explaining the molecular basis of the linkage signal in the 1q region and in unravelling the aetiology of T2D.

422W

Examination of rare variants in HNF4(in European Americans with Type 2 Diabetes. *J.N. Hellwege[1, 4, 5], P.J. Hicks[2], N.D. Palmer[2, 4, 5], B.I. Freedman[3], D.W. Bowden[2, 4, 5].* 1) Program in Molecular Genetics & Genomics, Wake Forest School of Medicine, Winston Salem, NC; 2) Department of Biochemistry, Wake Forest School of Medicine, Winston Salem, NC; 3) Department of Internal Medicine-Nephrology, Wake Forest School of Medicine, Winston Salem, NC; 4) Center for Diabetes Research, Wake Forest School of Medicine, Winston Salem, NC; 5) Center for Genomics and Personalized Medicine, Wake Forest School of Medicine, Winston Salem, NC.

The hepatocyte nuclear factor 4-((HNF4( ) gene codes for a transcription factor which is responsible for regulating gene transcription in pancreatic beta cells, in addition to its primary role in regulation of hepatic genes. Mutations in this gene can lead to maturity-onset diabetes of the young (MODY), an uncommon, autosomal dominant, non-insulin dependent form of diabetes. Mutations in HNF4(have only been found in a handful of individuals, and only occasionally have they segregated completely with MODY. In addition, due to a similarity of phenotypes, it is unclear what proportion of type 2 diabetes (T2D) in the general population is due to MODY. In this study, 27 rare (MAF <0.05) and common variants were chosen for genotyping in a European American population of 1270 T2D cases and 1017 controls from review of databases and literature implicating HNF4(variants in MODY and T2D. Of the 27 variants there were 16 missense mutations four nonsense mutations, three splice site mutations, two small deletions and one 6-bp deletion in the P2 promoter region. Seventeen variants were found to be monomorphic in this population. Two cases and one control subject had one copy of the 6-bp P2 promoter deletion. The common intron 1 variant (rs6103716; MAF = 0.31) was not significantly associated with disease status (p>0.8). The missense variant Thr130Ile (rs1800961; MAF = 0.027) was also not significantly different between cases and controls (p>0.2). Of the six variants that were found to be rare and polymorphic, Val393Ile (n=1), IVS-4-4G<A (n=1), and IVS-5+1G>A (n=2), were found as heterozygotes in cases only; Glu276Gln (n=1), was found as a heterozygote in controls only and Arg244Gln (n=2 cases, 2 controls) and Ile454Val (n=2 cases, 1 control) were found as heterozygotes in both cases and controls. Due to the small numbers, a pooled chi-squared analysis of rare mutations was used to assess the overall burden of variants between T2D cases (92 rare alleles) and controls (59 rare alleles). This analysis revealed no significant difference (p=0.18).We conclude there is no evidence to suggest that HNF4(variants contribute significantly to risk of T2D. In addition, the observation of some mutations in controls suggests they are not highly penetrant MODY-causing variants.

423W

Using time-to-event analysis for genome-wide association study in a long-term cohort of diabetic retinopathy. *S.M. Hosseini[1,2], K. Howard[3], A.P. Boright[4], L. Sun[5,6], A.D. Canty[7], S.B. Bull[5,8], B.E. Klein[3], R. Klein[3], A.D. Paterson[1,2,5].* 1) Genetics & Genome Biology Program, The Hospital for Sick Children, Toronto, Canada; 2) Institute of Medical Science, Univ Toronto, Toronto, Canada; 3) Dept of Ophthalmology & Visual Sciences, Univ Wisconsin, Madison, WI, USA; 4) Dept of Medicine & University Health Network, Univ Toronto, Toronto, Canada; 5) Dalla Lana School of Public Health, Univ Toronto, Toronto, Canada; 6) Dept of Statistics, Univ Toronto, Toronto, Canada; 7) Dept of Mathematics & Statistics, McMaster Univ, Hamilton, Canada; 8) Prosserman Centre for Health Research, Samuel Lunenfeld Research Institute of Mount Sinai Hospital, Toronto, Canada.

Diabetic retinopathy (DR) affects up to 82% of the patients with type 1 diabetes (T1D) and is largely determined by diabetes duration and poor glycemic control. However, consistent with a genetic contribution, the severity of DR clusters in families. So far, few genetic variants have been identified with convincing and consistent association with DR.

White T1D participants (n=627) in the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) were genotyped by Illumina HumanOmni1-Quad assay. A logistic regression association analysis of DR [level 35/<35 or worse (step⁄ 4) on ETDRS scale] at the first visit [293 cases vs 334 controls (ETDRS step<4)] does not make full use of the cohort nature of the study. We used repeated retinal photographs taken at 5 study examinations over 25 years to define a time-to-DR outcome which was analyzed as a survival trait using parametric models to address both left and interval censoring. We built models to adjust for the effect of glycemia and other relevant covariates in the analyses.

The results from the two GWAS analyses are largely consistent (rank correlation of 0.73 for SNP-specific test statistics, p<0.001). Survival analysis, however, seems to improve the power compared to the logistic regression (14 vs 2 SNPs with p<1E-5; genomic control 3 1.01 vs 0.99). No SNP in either analysis met the genome-wide significance threshold. The strongest association was observed for a group of SNPs on 7q36.1 near *AGAP3*. The results will be replicated in independent cohorts.

The use of time-to-event analysis and explanatory covariates improves our ability to identify loci for DR. These findings have implications for future genetic studies of DR.

424W

Fine-mapping haplotype analysis of established type 2 diabetes loci using the "Metabochip". *A. Kumar[1,6], A.P. Morris[1], R. Magi[1], P. Deloukas[2], P. Donnelly[1], T. Frayling[3], A. Hattersley[3], C.N.A. Palmer[4], A.D. Morris[4], M.I. McCarthy[1,5].* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, Oxfordshire, United Kingdom; 2) Wellcome Trust Sanger Institute, Hinxton, United Kingdom; 3) Institute of Biomedical and Clinical Science, Peninsula Medical School, Exeter, United Kingdom; 4) Biomedical Research Institute, University of Dundee, Ninewells Hospital & Medical School, Dundee, United Kingdom; 5) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, United Kingdom; 6) Swiss Tropical and Public Health Institute, University of Basel, Switzerland.

The number of established loci influencing susceptibility to type 2 diabetes (T2D) is now in excess of 40. Most of these have been identified by single-SNP analysis of genome-wide association studies, but together they explain less than 10% of the disease heritability. We have thus undertaken haplotype-based analysis within established T2D loci to identify multi-SNP signals of association that might better explain the genetic architecture underlying the disease. We have considered UK subjects with 5,938 T2D cases and 9,356 controls. The samples were genotyped using the "Metabochip" (a custom iSELECT array with ~195,000 SNPs), designed to support large-scale follow-up of putative novel associations for T2D and other cardiometabolic traits, as well as fine-scale mapping of established loci. We followed two parallel haplotype (phasing by Plink's E-M algorithm) analysis strategies: (i)forward selection, adding SNPs in turn to a haplotype in order to maximise the association signal; and (ii) a complete evaluation of all possible 2- and 3-SNP haplotype combinations, in four T2D fine-mapping loci (CDKN2A/B, KCNQ1, FTO, TCF7L2). Meta-analysis of "omnibus" haplotype association p-values across the two cohorts was performed via Fisher's method. Our analyses revealed stronger signals of association of T2D with haplotypes than single SNPs in two loci: KCNQ1 and CDKN2A/B. In KCNQ1, an omnibus haplotype test of rs163184, rs2237896 and rs10400376 (all r2<0.04) yielded the strongest signal of association with p=2.0x10-14. The specific GGG haplotype was the most strongly associated, with p=5.65x10-13, OR=1.20 [95%CI 1.14-1.26], while the strongest single SNP association was for rs163184 (p=1.1x10-9). In CDKN2A/B, the strongest association signal was observed for a 3 SNP omnibus haplotype test of rs10757283, rs10811660 and rs73652848 (p=6.7x10-16). The best individual association was with haplotype CGT with p=5.63x10-14, OR=1.26 [95%CI 1.16-1.29]. The 3-SNP omnibus haplotype test was not markedly more significant than a 2-SNP omnibus haplotype test of rs10757283 and rs10811660, p=2.7x10-15. In comparison, the best single SNP rs7018475 was associated with p=6x10-9. rs10757283 and rs10811660 are correlated (r2=0.26) and define three clades of haplotypes that have been previously demonstrated to be associated with T2D. Our results highlight the potential for haplotype-based analysis to elucidate the complex underlying genetic architecture of the T2D susceptibility loci.

425W

Relationship between Diabetes Risk and Admixture in Post-Menopausal African American and Hispanic American Women. *L. Qi[1], R. Nassir[2], R. Kosoy[2], L. Garcia[1], J. Curb[3], L. Tinker[4], B. Howard[5], J. Robbins[2], M. Seldin[2].* 1) Dept of Public Health Sciences, Univ California, Davis, Davis, CA; 2) Univ California, Davis, Davis, CA; 3) University of Hawaii, Honolulu, HI; 4) Fred Hutchinson Cancer Research Center, Seattle, WA; 5) MedStar Health Research Institute/Georgetown University, Washington, DC.

In the United States, type 2 diabetes is more prevalent in African Americans and Hispanic Americans than in European Americans. To assess whether continental admixture is correlated with diabetes risk in these admixed groups, we conducted an admixture analysis using 16,476 women from the Women's Health Initiative (WHI), who self identified as African American (AFA) or Hispanic American (HA). Self-reported diabetes treated with pills or insulin shots at baseline and during following up were ascertained based on the WHI questionnaire. We genotyped 92 ancestry-informative markers and used STRUCTURE analysis to estimate the proportion of sub-Saharan African (AFR), Amerindian (AMI) and European (EUR) admixture. Cox regression models were used to examine the association between genetic admixture and diabetes risk, with and without accounting for socioeconomic status (SES), and adiposity measures including body mass index (BMI) and waist hip ratio (WHR). Neighborhood SES was obtained using a standardized geocoding protocol and a summary measure of each participant's neighborhood SES was estimated from tract-level data. AFR admixture was significantly associated with diabetes risk in AFA women when adjusting for entry age alone (HR = 3.08, $p < 0.0001$), and after further adjusting for neighborhood SES (HR = 2.36, $p < 0.0001$), and adiposity measures (BMI or WHR) (HR = 2.02 or 2.23, $p < 0.0001$). In HA women, AMI admixture had significant associations with diabetes risk when adjusting for entry age alone (HR = 2.50, $p < 0.0001$), and this association remained significant after further adjustment for SES (HR = 1.97, $p = 0.0003$), and BMI (HR = 2.03, $p = 0.0002$). In HA women, AFR admixture was not correlated with diabetes risk ($p > 0.5$) while EUR admixture appeared to have a protective effect against diabetes. In addition, in both AFA and HA women, SES showed significant negative association with diabetes risk while BMI or WHR had significant positive associations with diabetes risk, with and without adjusting for genetic admixture. Thus, admixture, SES and BMI/WHR each independently contribute to diabetes risk after accounting for each of the other factors, emphasizing the importance of considering both genetic and environmental causes in the etiology of type 2 diabetes. The data provide additional support to the hypothesis that differences in ethnic origins may be critical to etiologic and therapeutic studies.

426W

Genetic role on sleep disorder. *M. Falah[1], M. Asghari[1], M. Houshmand[2], M. Farhadi[1].* 1) 1Department & Res Centr, Gen, Tehran, Iran; 2) National Institute for Genetic Engineering and Biotechnology, Tehran, Islamic Republic of Iran.

Sleep is one of the great mysteries of science. In the adult population, about one third is suffering from temporary sleep disturbances. About one in ten is suffering from a chronic sleep disorder which is also affecting the persons mood and efficiency daytime. Sleep disorder commonly involve genetic susceptibility, environmental effect, and interactions between these factors. These days, the hereditary of sleep patterns has been shown in studies of monozygotic twins, and electroencephalogram patterns offer a unique genetic fingerprint which may assist in the identification of genes involved in the regulation of sleep. Genetic factors are also thought to play a role in sleep disorder for instance: - Narcolepsy is a disabling sleep condition and research has revealed it has a complex hereditary pattern. Researchers discovered that a specific gene causes symptoms in mice and dogs that looked like the symptoms that were associated with narcolepsy in humans. The narcolepsy gene produces a protein in the brain (called orexin, or hypocretin) that promotes wakefulness. A reduction in this wakefulness-promoting protein or in the receptors for this protein may underlie narcolepsy in humans. - Another sleep disorder is Sleep Apnea, an extreme form of snoring in which an individual's airway becomes partially or completely blocked, leading to repetitive awakenings. This is a complex pattern because there are likely to be multiple genes involved. Genes influence how fast or slow our internal clock runs and, as a result, how closely it—and our body's functions—align with the 24-hour day. Changes in these genes, were known as mutations, from one generation to the next can affect the clock's timing, and the results of this change were shown as in advanced sleep phase syndrome (ASPS) and delayed sleep phase syndrome (DSPS). Gene profiles of sleep impairment are consisted of: Bmal1, NPAS2/Clock, Dec2, 5-HT1a, Orexin /Hcrtrt2 and Ube3A...etc Those who carry the mutation sleep on average two hours less a night — but still function perfectly well. While the mutation is probably rare, it offers the strongest evidence yet of the role genes play in our need for sleep. We expect a long road still ahead before scientists solidly understand sleep because it's a complex phenotype. If we can understand this biological pathway, perhaps we can come up with a therapeutic compound. Maybe we can help people sleep less in a safe way.

427W

Sequencing of Whole Blood Cell RNA: Duplex Specific Nuclease Treatment for Removal of Abundant RNA Species. *K. Fitch[1], S. Luo[2], P. Beineke[1], H. Tao[1], M. Wu[1], S. Rosenberg[1], J. Wingrove[1].* 1) CardioDx, Inc, Palo Alto, CA; 2) Illumina, Inc, Hayward, CA.

Background: RNA sequencing using Next Generation Sequencing methods (NGS) is rapidly replacing microarrays to measure changes in gene expression. However, sequencing of whole blood RNA can be confounded by the high abundance of RNAs such as hemoglobin. We describe the use of Duplex-Specific Nuclease (DSN) to remove high abundance RNAs, and compare RNA sequence data from treated RNA to that obtained by microarray and RT-qPCR. Methods: RNA was purified from whole blood collected in Paxgene tubes from consented donors. RNA from multiple individuals was combined into 5 independent pools. Total RNA was fragmented, converted to cDNA and then subjected to adaptor ligation and PCR. Amplified cDNA from the first pool was either treated with 2 units of DSN for 5 hours, or left untreated; the four remaining pools were treated with DSN as described above. cDNA was then sequenced on the Illumina GAII platform using standard Illumina protocols. Results: Sequencing of untreated cDNA resulted in 257,709 reads per kilobase of exon model (RPKM) for HBA1 sequence, 25% of the total reads sequenced. Treatment with DSN reduced this to 926 RPKM for HBA1, effectively reducing the amount of HBA1 by 99.6%. Comparable results were seen for HBA2 and HBB (99.7 and 99.8% reductions respectively). Comparison of the four treated pools to microarray and RT-qPCR data derived from the same samples showed good concordance (median r2 value of 0.547, 17,069 genes; median r2 value of 0.841, 176 genes respectively). DSN treatment also reduced the levels of other high abundance genes such as histone, ribosomal and ubiquitin components as compared to microarray and sequence data by linear regression. Conclusions: Treatment of whole blood total RNA with DSN appears to be an effective way to reduce high abundance transcripts, prior to RNA sequencing, improving the depth of the transcriptome revealed. In addition, better correlation was observed between RNA sequencing and RT-qPCR data than between microarray and RT-qPCR data.

428W

Increased African Ancestry and Decreased Lung Function in Puerto Rican and African American Children with Asthma and Mediation by Environmental Risk Factors. *K.A. Drake[1], C.R. Gignoux[1], L.A. Roth[1], S. Sen[1], S. Thyne[1], P. Avila[2], L. Borrell[3], H.J. Farber[4], R. Kumar[5], W. Rodriguez-Cintron[6], J. Rodriguez-Santana[7], D. Serebrisky[8], M. Lenoir[9], K. Meade[10], E. Brigino-Buenaventura[11], E.G. Burchard[1].* 1) Univ CA, San Francisco, San Francisco, CA; 2) Northwestern Univ, Chicago, IL; 3) City Univ of New York, NY, NY; 4) Texas Children's Hospital, Houston, TX; 5) Children's Memorial Hospital, Chicago, IL; 6) VA Medical Center, San Juan, PR; 7) Centro de Neumologica Pediatrica, San Juan, PR; 8) Jacobi Medical Center, NY, NY; 9) Bay Area Pediatrics, Oakland, CA; 10) Children's Hospital Research Center Oakland, Oakland, CA; 11) Kaiser Foundation Research Institute, Oakland, CA.

Genetic ancestry has been shown to be associated with many phenotypes including lung function, and may be a useful clinical proxy in some cases. However, the underlying causes of these associations are unidentified population-specific genetic and environmental variables. An increase in African ancestry was previously reported to be associated with a decrease in lung function as measured by forced expiratory volume in one second (FEV_1) in several healthy African American adult populations. FEV_1 decreased by anywhere between 2.39 and 8.14 mL per percentage increase in African ancestry depending on the population. This finding demonstrated that African American individuals were being misclassified based on predictive lung functions that did not account for ancestry, but it was unclear whether the association was coming from an increase in African ancestry or a decrease in European ancestry. We replicated this association in 540 Puerto Rican children with asthma and 437 African American children with asthma. Global ancestry in these populations was determined from 400 ancestry informative markers using the program ADMIXTURE. The Puerto Rican children and African American children had 35% and 77% African ancestry, respectively, although there was wide variation in both populations. FEV_1 decreased 6.03 mL for every percentage increase in African ancestry ($p=0.0001$) in our Puerto Rican population and 6.79 mL ($p<0.00001$) for every percentage increase in our African American population, adjusting for age, age^2, height^2, BMI, recruiting site, sex, and Native American ancestry if appropriate. The fact that Puerto Ricans are admixed with three ancestral populations allowed us to determine that an increase in African ancestry specifically is associated with a decrease in FEV_1. We demonstrated the use of graphical causal inference models to estimate the mediating effect of environmental variables on this ancestry-lung function association. We estimated the proportion of the total effect of African ancestry on FEV_1 that was due to a child's second hand smoke exposure; in Puerto Ricans this proportion was 0.5% (95% CI: -3.1%-5.6%) and in African Americans it was 0.8% (95% CI: -4.7%-4.5%). Although these data indicate that the association between African ancestry and FEV_1 is not being driven by second hand smoke exposure, we discuss how the proportion of the association owed to other causal environmental and genetic risk factors may be identified using this methodology.

429W

Genetic vectors - consolidated genotyping data, in association study of rheumatoid arthritis. *I. Sandalov[1], L. Padyukov[2].* 1) Condensed Matter Physics, Royal Institute of Technology, Kista-Stockholm, Sweden; 2) Rheumatology Unit, Department of Medicine, Karolinska Institutet, Stockholm, Sweden.

The highlight of modern genetics is an exponential growth of data and increasing complexity of relations between found genetic variations and the phenotype. We suggested to look on genotyping data as on an ensemble of spin chains and to analyze the data as a quantum system. In this approach SNP is considered as a spin with three states corresponding to possible genotypes. Each person is characterized by single genetic vector (GV), which is a combination of genotypes or a multi-spin state. This consolidation of genotypes in GVs integrates multiple genetic variants in a single statistical test and excludes ambiguity of biological interpretation for allele and haplotype associations. To examine the validity of the approach, we analyzed 1,820 rheumatoid arthritis (RA) cases and 947 healthy controls from the Swedish EIRA study. GVs were constructed from 8 SNPs in HTR2A locus and HLA-DRB1 shared epitope (SE) alleles. When empirical GVs were used for analysis of the structure of the study population we observed stratification for 338 GV subgroups. Among those, 36 GVs were represented only in control group (5.81% of all healthy individuals), while 127 GVs were found only in RA group (14.9% of all RA cases). Two- and three-point statistical correlations for genotypes, chosen from contrast GVs, were evaluated and used for a detection of the genotype-genotype interactions. We replicated our findings in independent study of RA (NARAC) and found that 131 GVs coincide and contain vast majority of individuals in both study populations. The correlators for both cohorts show that interaction between one set of the genotypes (rs2070040, AG; rs977003, AA or CC) favors the development of RA, whereas for another set of genotypes (rs2070040, TT and rs977003, AC), as well as for rs977003, AC genotype at the absence of SE, it plays the protective role ($p<10^{-10}$). This method can be applied for the analysis of moderately associated loci from GWAS for selection individuals with either contrasting GVs or for optimization of putative “functional” combinations of the genotypes. Additionally, by using our approach, the interactions along the locus (including LD measures) or more extended interactions for combined sets from different loci are possible to assess.

430W

Novel approaches for discovery and replication in large scale association studies. *D.V. Zaykin, C-L. Kuo.* Dept Biostatistics, NIEHS/NIH, Res Triangle Park, NC.

We report methods for estimating the proportion of false discoveries among top R association results in large-scale association studies. Our approach gives an estimate of such proportion (R-FDR) for any given study, as well as its expected value. R-FDR estimate can be used to prioritize association results for replication, while the expected R-FDR can be used for sample size calculation in planning studies. Our approach incorporates effect size distribution as well as linkage disequilibrium through accurate approximations for probabilities of ranks of true associations. Genes can be weighted differentially or organized into “tiers”, depending on whether they belong to a candidate pathway. One important difference with conventional approaches is that we directly control FDR for a given number of top hits, rather than controlling FDR for a fixed significance threshold.

431W

Interpreting familial whole-genome sequencing data for the identification of genetic variants influencing epilepsy susceptibility. *E.K. Ruzzo[1, 3], E.L. Heinzen[1, 3], R. Wedel[2], K.V. Shianna[1], D. Ge[1], R. Ottman[2, 3], D.B. Goldstein[1, 3].* 1) Center for Human Genome Variation, Duke University School of Medicine, Durham, North Carolina 27708, USA; 2) G. H. Sergievsky Center and Departments of Epidemiology and Neurology, Columbia University, and Division of Epidemiology, New York State Psychiatric Institute, New York, NY, USA; 3) Epigen Consortium.

Epilepsy is highly heritable, yet the genetic contribution to the vast majority of cases remains elusive. Due to extreme locus heterogeneity, classic case-control sequencing study designs may fail to uncover epilepsy susceptibility variants in small, heterogeneous samples. In this study, we exploited multiplex epilepsy families to reduce heterogeneity and sequenced multiple affected related individuals to reduce the number of candidate variants. Our familial study design enables multiple levels of evaluation for each of the millions of identified variants, prior to detailed candidate gene analyses or follow-up genotyping: i) comparison to sequenced control genomes (>300) ii) identification of shared familial variants iii) prioritization of variants falling in linkage peaks and iv) overlap of variants or affected genes across families or in sporadic epilepsy cases. This multilevel approach shrinks the pool of candidate variants to be genotyped to assess familial cosegregation and enrichment in large case-control cohorts. In this study, we performed whole-genome or exome sequencing of 56 epilepsy patients from 29 multiplex families, all sequenced to high coverage (>30x). Following alignment of the short-sequence reads, variant calling, quality control filtering, and the multi-level evaluation outlined above, we identified a set of 367 candidate single nucleotide variants (SNVs) that were exceedingly rare, or completely absent outside affected family members, and likely to impact the encoded protein product. Importantly, candidate variants were genotyped in a much larger cohort of 1,000 epilepsy patients and 2,500 controls using a custom-genotyping chip. Variants still enriched or exclusively found in cases vs controls were evaluated for familial cosegregation. We found that no single variant explained a large number of families, indicating that distinct genetic etiologies exist. Several variants were shared within a family, rare or absent in controls, and seen in additional sporadic cases. Of particular interest are: a stop gain in USP45 (emerging evidence supports the importance of ubiquitination in neuronal function), and nonsynonymous variants in RBM15 (CNS expressed RNA binding protein) and PKD1 (seizures observed in patients with microdeletions encompassing PKD1). These variants are currently being tested for cosegregation. We continue to utilize this familial approach for the discovery of novel genetic variants that increase epilepsy susceptibility.

432W

Chronic Rhinosinusitis in Cystic Fibrosis Heterozygotes. *A. Powers[1], W. Regelmann[2], J. Phillips[2], C. Milla[3], X. Wang[4,5].* 1) Fairview Health Services, University of Minnesota Medical Center Fairview, Minneapolis, MN; 2) Dept. of Pediatrics, University of Minnesota Medical Center, Fairview, Minneapolis, MN; 3) Dept. of Pediatrics, Stanford University, Palo Alto, CA; 4) Dept. of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN; 5) National Eye Institute, NIH, Bethesda, MD.

Cystic fibrosis (CF) is the most common life-threatening autosomal recessive disorder in the Caucasian population, with an incidence of one in 2000-4000 live births and a prevalence of approximately 30,000 affected individuals in the US. CF clinical presentation includes sino-pulmonary symptoms. Chronic inflammation of sinuses and nasal mucosa starts in early stages of the disease. Chronic rhinosinusitis (CRS), defined as persistent inflammation involving nasal and paranasal mucosa, is an almost invariable feature of CF. However, CRS is the most prevalent chronic condition in the United States and can be seen in isolate or other systemic conditions besides CF. Genetics play a very important role in many of these systemic conditions. While these systemic conditions, and specifically CF, may not be accountable for the majority of CRS patients, the genetic contributing factors of these conditions may contribute significantly to the etiology of CRS in general. Previously, a higher frequency of CF carriers was found in the general CRS patients and a higher CRS incidence was found in CF obligate carriers in the greater Maryland area. To verify these findings in a different geographical area, we conducted a similar survey study with parents of CF patients visiting University of Minnesota Medical Center, Fairview (UMMC). Biological parents of any patient visiting the CF Clinic at UMMC were invited to participate by the genetic counselor. A study invitation letter, IRB approved consent form, and study questionnaire were provided if interested. Detailed questions regarding sinus conditions were adopted from previously used surveys. One hundred fifty four parents completed the survey. Forty three individuals met the criteria for CRS, therefore having self-reported CRS. This accounts for about 28% of the participating obligate CF carriers. It was estimated that 13% to 14% of US population had self-reported CRS. Incidence of CRS in the CF carrier population was increased in Minnesota, which is consistent with the previously demonstrated results. The CF carrier frequency is approximately one in 28 in the N. American Caucasian population. All states in the US now have CF newborn screening and carrier testing is frequently offered as part of routine prenatal care. A controlled comparison of CRS frequency in appropriate controls is warranted to help determine if therapeutic strategies used in CF patients may aid CRS treatment in parents and other CF carriers.

433W

A Note on Robust TDT-Type Test under Informative Parental Missingness. *J. Chen[1], K. Cheng[1,2,3].* 1) Biostatistics Center and Graduate Institute of Biostatistics, China Medical University, Taipei, Taiwan; 2) Biostatistics Center and College of Public Health, China Medical University, Taichung, Taiwan; 3) Graduate Institute of Statistics, National Central University, Chungli, Taiwan.

Many family-based association tests rely on the random transmission of alleles from parents to offspring. Among them, the transmission/disequilibrium test (TDT) may be considered to be the most popular statistical test. The TDT statistic was proposed to evaluate nonrandom transmission of alleles from parents to the diseased children. However, in family studies, parental genotypes are not always available. Quite often, the offspring genotype affects the severity of offspring phenotype or/and the age at onset and in turn affects the parental missingness. In such case, the nonrandom transmission of alleles may also occur even when the gene and disease are not associated. As a consequence, the usual TDT or its variations would produce excessive false positive conclusions in association studies. In this note, we propose a TDT-type association test which is not only simple in computation but also robust to the joint effect of population stratification and informative parental missingness. The test statistic does not rely on any model and also allows for having different mechanisms of parental missingness across subpopulations. We use a simulation study to compare the performance of new test and the TDT and point out the advantage of the new method.

434W

Bioinformatic analysis of protein turnover of intronless gene products. *T.O. Onabanjo, R.J. Slater, E.A. Bryson, N.C. Madu, R. te Boekhorst.* School of Life Sciences, University of Hertfordshire, Hatfield, United Kingdom.

Most Eukaryotic genes are interrupted by introns, while prokaryotic genes are entirely intronless. This seems to support the so called `introns-late hypothesis' that introns emerged in the course of evolution in Eukaryotes. However, many eukaryotic histone and GPCR genes are predominantly intronless and there are intronless genes among those coding for proteins associated with transcription regulation, DNA replication and DNA repair. Thus the overall efficiency of such processes may be significantly influenced by rapid transcription of these genes and the translation and turnover of the resulting proteins. Given the importance of DNA repair proteins in preserving the integrity of the genome and the likely need for this process to be rapid and efficient, we investigated whether there was any difference between the half-life of DNA repair proteins encoded by intronless genes and genes with introns. Our study identified 170 DNA repair proteins in the human genome, out of which only 4.1% were encoded by intronless genes. TermiNator was used to predict the N-terminal residue after posttranslational modifications and the half-life of the proteins from the N-end rule. There was no significant difference between the half-life of proteins resulting from intronless and intron containing DNA repair genes. A similar result was obtained using Protparam when predicted protein stabilites were compared. We also analyzed the entire human genome using TermiNator and found no significant difference in the half-life of proteins resulting from intronless and intron containing genes. These findings indicate that the evolutional advantage that introns bring by enabling alternative splicing is so great that it justifies the extra time and energy spent on transcribing them, even when the resulting proteins are only required for a short time.

435W

The Influence of Real-Life Topologies on Boolean Models of Gene Regulatory Networks. *C. Darabos[1], F. DiCunto[2], M. Tomassini[3], P. Provero[2], M. Giacobini[2], J.H. Moore[1].* 1) Dartmouth Medical School, Lebanon, NH, USA; 2) University of Torino, Torino, Italy; 3) University of Lausanne, Lausanne, Switzerland.

Gene-on-gene regulations are key components of every living organism. Dynamical abstract models of genetic regulatory networks (GRNs), such as Kauffman's random Boolean networks (RBN), help explain the genome's evolvability and robustness by the structural topology of the network formed by genes, as vertices, and regulatory interactions, as edges. In the original RBN model, the network was a random structure, and extensions studied other synthetic topologies, such as scale-free network. In this work, we use two regulatory-networks modules of biological organisms, yeast and mouse embryonic stem cell, as support for Boolean network models to study how real topology compares to artificial ones. Additionally, we propose a threshold-based dynamic Boolean update function (replacing the original RBN's random function) in which the promoting and repressing effect of each gene product interaction is taken into consideration. In order to investigate the dynamical behavior of these two study case models, we visualized the phase transition between order and chaos into the critical regime using Derrida plots. We also proposed a complementary measure, the criticality distance that allows us to discriminate between different regimes in a quantitative manner. Moreover, we portray the entire state spaces of the two real-life GRNs using RBN-specific statistical measurements (attractor state space). Simulation results on two real-life genetic regulatory networks show that there exist a set of parameters in both update functions that allow “organic” systems to operate in the critical regime, and that these values are comparable in both case studies. The analyze of the attractor space hints at “cell specialization” in the case of ES cells, and cell cycle in yeast. Finally, we use a third real-life regulatory network, along with its inferred Boolean update functions to validate the results obtained, and we show a 91% accuracy of our threshold-based update function. The update function confers additional realism to the model, while actually reducing the complexity and solution space, thus making it easier to investigate.

436W

Compensatory amino acid changes as a signal of human disease. *D. Jordan[1,2], I. Adzhubey[2], S. Sunyaev[2].* 1) Biophysics Program, Harvard University, Cambridge, MA; 2) Division of Genetics, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School.

Human disease mutations of high penetrance sometimes correspond to wild type amino acid variants in vertebrate orthologs. This observation has previously been interpreted as a result of compensatory amino acid changes. We analyzed a large dataset of well-annotated mutations involved in Mendelian diseases using new comparative genomics data on 46 vertebrate species. We observed that over 8% of mutations correspond to wild type amino acids in vertebrate species. We tested whether these cases can be explained by compensatory effects of other amino acid changes in these species. For a large fraction of observations, the presence of a human disease variant in vertebrate orthologs is accompanied by a synchronous change at several other amino acid sites. This observation is limited to disease mutations and is in sharp contrast to human neutral sequence variants. We show that a simple statistic based on co-evolving sites can differentiate between neutral and pathogenic mutations that are observed as wild-type amino acids in vertebrate orthologs. We analyze this result to distinguish true compensatory changes from fast-evolving sequence regions and alignment or mapping errors. This analysis quantifies the error produced by models of evolution and population genetics that treat multiple sites as evolving independently in a constant evolutionary landscape. We also conclude that using a co-evolution statistic like that reported here can greatly increase accuracy of computational predictions of the pathogenic effect of human missense mutations based on comparative genomics data.

437W

Impact of genetic variants on coding genes: genomic sequence and protein structure conservation. *E. Khurana[1, 2], L. Habegger[2], A. Abyzov[1, 2], M. Gerstein[1, 2, 3].* 1) Department of Molecular Biophyics & Biochemistry, Yale University, New Haven, CT; 2) Program in Computational Biology and Bioinformatics, Yale University, New Haven, CT; 3) Department of Computer Science, Yale University, New Haven, CT.

Several genomic variants are known to be associated with diseases, while many variants occur in healthy humans. In order to understand the underlying differences between these two kinds of variants, we computed and compared several properties of the two sets of variants: disease-causing variants from Human Gene Mutation Database (HGMD) and those reported in three different populations of healthy individuals in pilot phase of the 1000 Genomes Project. Firstly, in order to check which coding sites are most prone to mutation in healthy individuals, we computed the selection pressure at each coding site. This was done by using a maximum likelihood approach and a phylogenetic tree with gene alignments from multiple species. We find that most missense single nucleotide polymorphisms (SNPs) with high-allele frequencies occur at sites evolving neutrally. Moreover, we are also able to identify ~200 sites that are evolving under positive selection and are polymorphic amongst humans. Secondly, it has been reported before that evolutionary conservation is an essential feature that helps to identify damaging SNPs - in order to test the hypothesis that the same is true for small insertions and deletions (indels), we computed evolutionary conservation scores for small in-frame deletions associated with diseases as well as for those observed in healthy humans. We find that disease-associated deletions are significantly more conserved amongst species and hence evolutionary conservation promises to be an essential marker for identification of damaging in-frame deletions. Finally, we extended our analysis from genomic sequence to protein structure. Protein structures are assigned to a specific SCOP family (Structural Classification of Proteins) if they share similar structure and function, even though they might not share a similar genomic sequence. Structural alignment of all proteins within each SCOP family reveals that SNPs associated with diseases occur at residues which show significantly more structural conservation, i.e. regions on three-dimensional protein structure which are less plastic amongst the family. Thus, our results provide insights about human genomic variants that are polymorphic in healthy individuals vs those that cause diseases, and hence should further aid studies which try to identify and understand genetic variants causing diseases.

438W

Genetic structure of Jewish populations on the basis of genome-wide single nucleotide polymorphisms. *N.M. Kopelman[1], L. Stone[1], D.G. Hernandez[2], D. Gefel[3], A.B. Singleton[2], E. Heyer[4], M.W. Feldman[5], J. Hillel[6], N.A. Rosenberg[7].* 1) Porter School of Environmental Studies, Tel Aviv University, Ramat Aviv, Israel; 2) Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, Maryland, USA; 3) Department of Medicine, Barzilai Hospital, Ashkelon, Israel; 4) Muséum National d'Histoire Naturelle - Centre National de la Recherche Scientifique-Université Paris 7, Paris, France; 5) Department of Biology, Stanford University, Stanford, California, USA; 6) Robert H Smith Institute of Plant Sciences and Genetics, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel; 7) Department of Human Genetics, University of Michigan, Ann Arbor, Michigan, USA.

The Jewish population forms a genetically structured population, due to historical migrations and diverse histories of the various Jewish communities. Discerning the ancestry and population structure of different Jewish populations is important for understanding the complex history of the Jewish communities as well as for research on the genetic basis of disease. Using >500,000 genome-wide single-nucleotide polymorphisms, we investigated patterns of population structure in 438 samples from 30 Jewish populations in the context of additional samples from non-Jewish populations. The collection of Jewish populations studied incorporates a variety of populations not previously included in other genomic population structure studies of Jewish groups (e.g. NM Kopelman et al. 2009 BMC Genet 10:80; G Atzmon et al. 2010 AJHG 86:850-859; DM Behar et al. 2010 Nature 466:238-242; SM Bray et al. 2010 PNAS 107:16222-16227; JB Listman et al. 2010 BMC Genet 11:48). We identify fine-scale population structure within the Jewish samples, including notable distinctions separating Ashkenazi, Mizrahi, Sephardi, and North African populations. Additionally, we identify distinctions within major regional groups, including a separation among the North African populations of Libyan, Moroccan, and Tunisian Jewish samples and a separation among the Mizrahi populations of Bukharan, Georgian, Iranian, and Iraqi Jewish samples. These results supply enhanced information regarding Jewish population structure, providing a basis for further detailed analysis of the genetic history of Jewish populations.

439W

Estimating a date of mixture of ancestral South Asian populations. *P. Moorjani[1,2], N. Patterson[2], P. Govindaraj[3], L. Singh[3,4,5], K. Thangaraj[3,5], D. Reich[1,2,5].* 1) Dept Gen, Harvard Medical School, Boston, MA; 2) Broad Institute, Cambridge, MA; 3) Centre for Cellular and Molecular Biology, Hyderabad, India; 4) Genome Foundation, Hyderabad, India; 5) These authors co-directed the project.

Linguistic and genetic studies have shown that most Indian groups have ancestry from two genetically divergent populations, Ancestral North Indians (ANI) and Ancestral South Indians (ASI). However, the date of mixture still remains unknown. We analyze genome-wide data from about 60 South Asian groups using a newly developed method that utilizes information related to admixture linkage disequilibrium to estimate mixture dates. Our analyses suggest that major ANI-ASI mixture occurred in the ancestors of both northern and southern Indians 1,200-3,500 years ago, overlapping the time when Indo-European languages first began to be spoken in the subcontinent. These results suggest that this formative period of Indian history was accompanied by mixtures between two highly diverged populations, although our results do not rule other, older ANI-ASI admixture events. A cultural shift subsequently led to widespread endogamy, which decreased the rate of additional population mixtures.

440W

The Influence of Whole Genome Duplication and Subsequent Diversification on Environmental Robustness and Evolutionary Innovation in Gene Regulatory Networks. *Q. Pan, C. Darabos, A. Tyler, J. Payne, J.H. Moore.* Dartmouth Medical School, Lebanon, NH, USA.

Whole genome duplication and subsequent diversification in biological systems constitute powerful mechanisms for the discovery of new phenotypes and for the protection of these phenotypes against environmental perturbation. Here, we use Random Boolean Networks to investigate the influence of these genetic mechanisms on the relationship between evolutionary innovation and environmental robustness in gene regulatory networks. We find that whole genome duplication is highly deleterious in ancestral environments, but provides fitness advantages in novel environments, which come at the cost of reduced environmental robustness. We then show that the subsequent diversification of duplicated networks, via the loss of regulatory interactions, can partly negotiate this tradeoff, improving evolutionary innovation and environmental robustness. The results of this study provide a foundation for understanding the role of robust gene regulatory networks in the genotype-phenotype mapping relationship.

441W

The Effect of Assortative Mixing on the Robustness of Gene Regulatory Networks. *D. Pechenick, J. Payne, J.H. Moore.* Dartmouth Medical School, Lebanon, NH, USA.

Gene Regulatory Networks (GRNs) comprise collections of genes and regulatory rules that describe gene expression in cells. GRNs are robust in that perturbation, for example knocking out a gene, doesn't necessarily yield a different expression profile. GRNs are also evolvable in that new phenotypes can be realized through mutation. Populations of GRNs can achieve both robustness and evolvability by occupying large neutral spaces, which consist of all the different genotypes that are accessible by point mutations and lead to the same phenotype. Here we investigate how assortativity, a structural property of networks, influences neutral space size. Assortativity describes the extent to which nodes that possess a certain number of connections are themselves connected to nodes with the same number of connections. We use Boolean Networks to model GRNs, where nodes represent genes and edges represent regulatory interactions. The expression states of genes are either on or off, and gene expression for all genes is updated simultaneously at discrete time points according to deterministic regulatory rules, which we define as a network's genotype. As gene expression states are updated they eventually repeat to form a cycle, called an attractor, which we define as a phenotype. We use random walks to estimate the size of a network's neutral space, which we use as a proxy for robustness. We build Boolean Networks with multiple average degrees and degree distributions, with a range of different assortativity values. We find that positively assortative networks have higher mutational robustness than negatively assortative networks, and that this is most noticeable in chaotic networks with a constant input degree. In ordered and critical networks with varying degree distributions, which more closely resemble real-world biological GRNs, the effect of assortativity on mutational robustness is negligible. To explain these observations, we identify a simple mechanism that involves the dense core of interconnected genes that appears in networks with high but not low assortativity. While assortativity can influence mutational robustness for certain types of networks, it is unlikely that real GRNs evolve toward higher robustness by means of altering assortativity.

442W

Bioinformatics tools for population genetics and genetic epidemiology: automatic data format conversion for population genetics and genetic epidemiology through a dynamic pipeline. *M.R. Rodrigues, W. Magalhaes, M. Machado, E. Tarazona-Santos.* Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

Population genetics and genetic epidemiology require data analyses using different software. Since most of these software are not compatible in terms of accepted input and output formats, solutions to allow interoperability are required. Manual modification of files is intractable when handling information of hundreds or thousands of individuals and SNPs. One solution to guarantee the interoperability of different software is to create conversion-specific scripts that automatically map the output format of one software into the input format of another. A more comprehensive approach is to combine these conversion-specific scripts through a pipeline to increased the number of possible conversions that are available to the user. For example, having a script (S_{AB}) that converts format A into format B and another script (S_{BC}) that converts format B into format C, when a conversion from format A into C is required we can combine scripts S_{AB} and S_{BC} in a pipeline to provide this conversion, instead of having to create a third conversion script. Therefore, here we propose a conversion pipeline to solve the interoperability problem. Currently, our format conversion pipeline handles data formats compatible with the following software: PolyPhred (for polymorphism identification from aligned row sequences reads), PHASE (to infer chromosome phase), DnaSP (population genetics analysis), Structure (population structure inferences), Sweep (natural selection inferences), Haploview (linkage disequilibrium analysis) and R-based tools for population genetics/genetic epidemiology such as HierFstat. The pipeline handles general file formats such as SDAT, NEXUS and PrettyBase. It is composed of 15 conversion tools implemented in Perl, which allow for 26 format conversions. Our pipeline extends an existing conversion pipeline (Machado et al. 2011, Investigative Genetics) with the addition of new conversion tools, and is implemented using a graph-based approach that allows us to generate conversion pipelines dynamically. The advantage of this dynamic approach is that it guarantees the extensibility of the pipeline, making it easy to add new conversion scripts (i.e. conversion scripts compatible with new formats created by the 1000Genomes Project, such as the SAM format, and with publically available script repositories such as BioPerl). Our format conversion pipeline is interactive and available online through a web interface. Supported by: CAPES, CNPq, NIH, FINEP-Epigen.

443W

Genome of the Netherlands: family-based whole genome sequencing of 250 Dutch trio families. *C. Wijmenga[1], M. Swertz[1], H. Cao[2], L. Francioli[1,3], F. van Dijk[1], K. Ye[4], R. Chen[2], L. Karssen[5], J. Laros[4], B. van Schaik[6], J. Bovenberg[4], P.E. Slagboom[4], D.I. Boomsma[7], C.M. van Duijn[5], G.J. van Ommen[4], Y. Du[2], P.I.W. de Bakker[3] on behalf of the GoNL consortium.* 1) University Medical Center Groningen, the Netherlands; 2) BGI, Shenzhen, China; 3) University Medical Center Utrecht, the Netherlands; 4) Leiden University Medical Center, the Netherlands; 5) Erasmus University Rotterdam, the Netherlands; 6) Amsterdam Medical Center, the Netherlands; 7) Free University Amsterdam, the Netherlands.

The Genome of the Netherlands (GoNL) is a national collaboration aimed at establishing a map of Dutch genetic variation by whole genome-sequencing of 250 trio families (i.e. 1000 independent Dutch genomes) including 20 families with 2 offspring (10 families with monozygotic and 10 with dizygotic twin pairs). The trio families consist of unselected individuals of Caucasian origin. The families participate in prospective population-based cohorts and are equally distributed across 11 provinces of the Netherlands. This project is expected to reveal most of the genetic variation within the Dutch population with frequencies greater than 0.5%. This family-based design represents a unique resource to accurately reconstruct haplotypes as we can infer phase, to better characterize structural variation, and to establish the rate of *de novo* mutational events. This resource will also serve as a reference panel for imputation in the available genome-wide association studies in Dutch cohorts (>100,000 samples) to refine association signals and to test for association to rare population-specific variants. In collaboration with BGI, GoNL has completed sequencing of genomic DNA of all 250 Dutch trio families (770 individuals) on Illumina HiSeq at an average coverage depth of 12x. We observed >99.0% SNP concordance when we compared the GoNL data with immunochip data comprising some 200,000 SNPs including ~50,000 rare variants (MAF<5%). Principal component analysis of the immunochip data showed a clear North-South gradient within the Netherlands. We have performed in-depth analysis on chromosome 20 using 20 trio's (60 samples). We observed 16,700 non-overlapping sites that appear unique to the Netherlands when compared to the 1000 Genomes Project phase 1 data. More than 90% of these sites have allele count <3. Extrapolating from these data, we expect to reveal many more low-frequency variants that were unobserved in the European 1000 Genomes data. The resource will be made available to the community, and are expected to help in the interpretation of medical sequencing projects.

444W

Automated determination of phased genotypes from family HLA typing data. *N. Leahy, A. Madbouly, L. Gragert, C. Malmberg, J. Paulson, M. Maiers.* Bioinformatics, National Marrow Donor Program, Minneapolis, MN.

Assigning HLA haplotype phase through family typing is helpful in the search process of selecting donors for hematopoietic stem cell transplants and is useful for population genetic studies. Manual segregation of informative pedigrees to assign phase is laborious and error-prone, but the absence of high resolution HLA typing presents challenges to automation. We developed an algorithm that will identify haplotypes within a group of related individuals in the presence of ambiguous data. Family data was used to generate phase information for all available family members at HLA-A, -B, -C, -DRB1 and -DQB1 loci. All alleles were considered for broad HLA typings. The algorithm performed all possible pair-wise comparisons within a single family and scored comparisons based on completeness, agreement with other comparisons and probability the pair shares at least one haplotype. Resolved haplotypes were reported for all family members along with occurrences of homozygosity, missing data and both or neither alleles shared at a locus. The results were compared to phased genotypes determined by manual segregation. Our algorithm was evaluated with three datasets consisting of 1) 401 and 2) 2566 mother-child pairs (802 and 5132 individuals, respectively) and 3) a dataset of 419 families (1398 individuals) where family size ranged from 2 to 10 with a mean family size of 3.34 and median of 3. Individuals in the first set were typed at up to three loci: HLA-A, -B and -DRB1. Individuals in the other two sets were typed at up to all five loci. The automated pedigree analysis was fully consistent with manual segregation. In the first dataset phased genotype information was obtained in 76.6% of individuals for three loci, 18.3% for two and 3.7% for one while 1.5% could not be resolved. In the second, phase information was obtained in 0.2% for five loci, 10.4% for four, 40.2% for three, 39.9% for two and 8.0% for one while 1.3% remained unresolved. In the third, information was obtained in 16.9% for five loci, 13.9% for four, 37.7% for three, 19.0% for two, 8.4% for one and 4.1% unresolved. Our tool for automated pedigree analysis is significantly faster compared to the manual segregation method. Direct observation of genotype phase information would be useful in the validation of haplotype inference methodologies. Future developments will combine information from family HLA typing with population HLA frequencies to infer the most likely HLA alleles for untyped loci.

445W

A web application for calculating allelic killer immunoglobulin-like receptor (KIR) haplotype frequencies and linkage disequilibrium statistics. *D. Roe, C. Vierra-Green, M. Maiers.* Bioinformatics Research, National Marrow Donor Program, Minneapolis, MN., U.S.A.

Killer immunoglobulin-like receptors (KIRs) are expressed on natural killer cells and some T cells. They are ligands for Class I human leukocyte antigen (HLA) proteins and help regulate the immune response to them. As such, they have been associated with the outcomes of some types of hematopoietic stem cell transplants and the occurrence or progression of several diseases. Its ~200 Kb genetic region is part of the Leukocyte Receptor complex on chromosome 19 (19q13.4). It is very polymorphic allelically and structurally, as its haplotypes have undergone numerous insertions, deletions, and rearrangements. Most of its genotypes are structurally ambiguous; even some structural haplotypes are ambiguous if gene copy number is not considered. Haplotypes are categorized in two ways: into centromeric/telomeric regions (designated by a `c' or `t') depending on chromosome position and A/B (designated by a `A' or `B') depending on the presence of `activating' versions of the KIR genes. For example, a haplotype consisting of the first A centromeric region and the second telomeric region is indicated by cA01~tB02. This polymorphism complicates the imputation of haplotype frequencies from unphased genotypes, which can be valuable for understanding the inheritance and diversity of these gene regions. Haplotype frequencies are often a prerequisite for many techniques in population genetics. We have developed a web tool that uses an expectation maximization algorithm to predict structural and allelic haplotype frequencies and their corresponding linkage disequilibrium statistics. Input consists of a collection of unphased genotypes and a set of structural centromeric and telomeric reference regions into which the genotypes must fit. The algorithm utilizes a decision matrix for resolving paralogous genes and generates predictions for the centromeric, telomeric, and combined regions. We demonstrate its use on a set of 506 samples of Northern European ancestry, each with three-digit allele-level typings for 14 KIR genes. Five haplotypes constitute 90% of the predictions: cA01~tA01(46%), cA01~tB01(13%), cB01~tA01(8%), cB01~tB01(10%), cB01~tA01(13%). These results may be used to impute allelic haplotypes from structural ones, thereby potentially increasing the power and resolution of association studies.

446W

Haplotype Diversity and Linkage Disequilibrium of the Dyslexia Candidate Gene *DCDC2* in 90 Populations: Patterns for Alphabetic and Logographic Languages. *B.M.P. Bowen[1], S. Kosmaczewski[1], N. Powers[1], P. Paschou[2], W.C. Speed[1], J.R. Gruen[1], K.K. Kidd[1].* 1) Genetics, Yale University, New Haven, CT; 2) Department of Molecular Biology and Genetics, Democritus University of Thrace, Kimmeria, Greece.

Doublecortin Domain-Containing 2 (*DCDC2*) has been associated with dyslexia in English, German, and Italian-speaking populations. A comparison of allele frequencies across HapMap populations for SNPs in the LD blocks spanning *DCDC2* showed a high failure rate for a SNP in East Asian populations. This led us to hypothesize that the high failure rate was due to a deletion in intron 2 that is associated with dyslexia in European populations. We genotyped 2800 individuals from diverse populations and found that the deletion frequency was five times higher in Asian populations than in European populations. Given the association of *DCDC2* with reading ability in populations with alphabetic languages, we decided to investigate whether there are differences in the genetic architecture of *DCDC2* in populations with alphabetic versus logographic languages (English compared to Chinese, for example). We combined genotype data from the HapMap, HGDP, and Kidd lab on 90 populations including more than 3,000 individuals originating from Africa, Europe, Asia, the Pacific, and the Americas. In total we used 43 SNPs across *DCDC2* to build haplotypes using fastPHASE and estimated pairwise linkage disequilibrium across the gene at varying intensities using Haplot to identify core haplotypes. We then focused on 15 SNPs in intron 2 of *DCDC2* and found a haplotype in East Asian populations that accounts for a considerable proportion of the genetic variation that exists in this population. These results suggest that underlying genetic variation might have contributed to the differential development of alphabetic versus logographic written language in some populations.

447W

***PLIN4* variant associated with muscle strength in young females.** *F. Orkunoglu-Suer[1], H. Gordish-Dressman[1], P. Clarkson[2], P. Thompson[3], T. Angelopoulos[4], P. Gordon[5], N. Moyna[6], K. Panchapakesan[1], M. Abdel-Ghani[1], L. Pescatello[7], P. Visich[8], R. Zoeller[9], E. Hoffman[1], J. Devaney[1].* 1) Research Cntr for Genetic Med, CNMC, Washington, DC; 2) Dep of Kinesiology, U.of Massachusetts, Amherst, MA; 3) Division of Cardiology, Henry Low Heart Center, Hartford Hospital, Hartford, CT; 4) Center for Lifestyle Medicine and Department of Health Professions, University of Central Florida, Orlando, FL; 5) Department of Physical Medicine and Rehabilitation, School of Medicine, University of Michigan, Ann Arbor, MI; 6) School of Health and Human Performance, Dublin City University, Dublin 9, Ireland; 7) Department of Kinesiology & Human Performance Laboratory, University of Connecticut, Storrs, CT; 8) Human Performance Laboratory, Central Michigan University, Mount Pleasant, MI; 9) Department of Exercise Science and Health Promotion, Florida Atlantic University, Davie, FL.

Background: Prelipin 4 (PLIN4) is a protein that coats intracellular lipid storage droplets and plays a key role in the cellular deposition and mobilization of triacylglycerol facilitating free fatty acid uptake. *PLIN4* expression in humans is the highest in adipose tissue but also shows expression in skeletal muscle. Human (*PLIN4*) has been localized to 19p13.3, within a region previously linked to urinary albumin excretion. **Methods:** In our current study, we hypothesize that a variant, located in the 3'UTR (rs8887) of the *PLIN4* gene that has been shown to modulate obesity phenotypes, would be associated with subcutaneous fat volumes, skeletal muscle volume and muscular strength phenotypes in young adults. In addition, we hypothesized that rs8887 genotype would be linked with fat, muscle and strength response to a 12-week resistance training intervention in these young individuals. To explore these hypotheses, we genotyped the rs8887 variant in our cohort of young individuals (FAMUSS; n= 755) that have undergone a mentored 12-week strength training protocol on the non-dominant arm. Genotype/phenotype associations prior to and following the 12-week exercise intervention were tested using an analysis of covariance model with age and weight as co-variates. **Results:** Females with a copy of rare allele (A allele) for rs8887 had significantly lower pre-intervention measures of isometric strength for the upper arm (GG: n=125; 69.7 ± 2.1 kgs; GA/AA: N=313; 63.2 ± 1.3 kgs; p = 0.011). We did not see any other associations with any of our measured phenotypes in females or males. **Conclusion:** Our data suggests that the PLIN4 variant rs8887 modules muscle strength in females but does not have an effect on the response of subcutaneous fat, muscle, and strength response to a resistance training intervention. The rare allele (A allele) for rs8887 affects the binding of miR-522, which has been shown to have importance in the development of fetal tissue. We need to explore the role of miR-522 in skeletal muscle plasticity and confirm our results in additional populations with similar phenotypes.

448W

Role of Haplotypes on Chromosome 6p in the Susceptibility to Psoriatic Arthritis (PsA). *F.J. Pellett[1], F. Siannis[3], V.T. Farewell[3], P. Rahman[2], V. Chandran[1], D.D. Gladman[1].* 1) Rheumatology, Toronto Western Research Institute, Toronto, Ontario, Canada; 2) Memorial University, St. John's, Newfoundland, Canada; 3) MRC Biostatistics Unit, Cambridge, United Kingdom.

Introduction: PsA, an inflammatory arthritis associated with psoriasis (Ps), is associated with alleles of the HLA-B and -C loci on 6p. The Tumour Necrosis Factor (TNF)-(gene is implicated in PsA while corneodesmosin (CDSN) is a proposed candidate gene for Ps. Both genes are in the HLA region. We aimed to investigate whether the association with PsA was related to linkage with HLA alleles. Methods: Genomic DNA from 204 PsA patients and 101 healthy controls from Toronto (TO), an admixed population, and 235 patients and 102 controls from Newfoundland (NF), a founder population, was tested. Molecular HLA typing of the HLA-B and C loci was performed using the reverse line blot technique (RELI SSO). SNPs at positions 238,308, 857, 863 and 1031 of the TNF-(gene promotor and 619, 1215, 1236 and 1243 of the CDSN gene were genotyped by time of flight mass spectrometry using the Sequenom platform. Chi- square analysis was used to test for association of any SNP with either PsA susceptibility or specific HLA alleles. Haplotype analysis was performed using the haplo.stats program. Results: No association with any of the SNPs was seen in either patient group compared to their respective controls. However, associations were seen with specific HLA-B and/or -C alleles. At TNF-238, the minor A allele was strongly associated with HLA-B*57 ($p<0.000001$); at CDSN-1215, the minor G allele with HLA-B*13 and C*06 ($p<0.000001$) and at TNF-308, the minor A allele with HLA-B*08 ($p<0.000001$). There were distinct SNP haplotypes extending from the TNF locus to the CDSN locus associated with specific HLA-B and -C alleles. These haplotypes were seen in both control and patient groups. The TNF/CDSN SNP haplotype associated with HLA-B*57 and HLA-C*06 was increased in both patient groups while that with HLA-B*38 and HLA-C*12 was increased in the Toronto patients compared to controls. The TNF/CDSN haplotype associated with HLA-B*07 and HLA-C*07 was decreased in both patient groups. Conclusion: The results suggest that the SNP haplotypes are conserved across this chromosome region. The previously reported TNF/CDSN SNP association with Ps/PsA is probably due to association between the HLA-B*57 HLA-C*06 haplotype with Ps/PsA. The effect of these SNP haplotypes on disease susceptibility and progression in PsA requires further investigation.

449W

ASSOCIATION ANALYSIS OF THE 5-HTTLPR POLYMORPHISM OF THE SEROTONIN TRANSPORTER GENE WITH TYPE 2 DIABETES MELLITUS AND INCREASED BODY MASS INDEX. *V. Peralta Leal[1], E. Leal Ugarte[1], JP. Meza Espinoza[1], IP. Davalos Rodriguez[3,4], A. Bocanegra Alonso[5], RI. Acosta Gonzalez[5], E. Gonzales[2], S. Nair[2], J. Duran Gonzalez[2].* 1) Facultad de Medicina e Ingeniería en Sistemas Computacionales, Universidad Autónoma de Tamaulipas, H. Matamoros Tamaulipas, México; 2) Department of Biological Sciences and Center of Biomedical Studies, University of Texas at Brownsville, Brownsville, Texas 78520; 3) División de Genética, CIBO, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México; 4) Doctorado en Genética Humana, CUCS, Universidad de Guadalajara, Guadalajara, Jalisco, México; 5) Clinical Analyses Department, Unidad Académica Multidisciplinaria Reynosa-Aztlán, Universidad Autónoma de Tamaulipas, México.

Background: The 5HTTLPR Serotonin transporter polymorphism has been associated recently with changes in eat behavior, body weight and diabetes development and Mexico is one of the countries with the highest number of obese and diabetic individuals. The aim of this study is to identify an association of the 5-HTTLPR polymorphism of the SLC6A4 gene with type 2 diabetes and/or higher body mass index. Methods: From a Mexican-Mestizo population, 138 individuals diagnosed with type 2 diabetes and 172 unrelated controls were included in this study, for a subsequent analysis, all subjects were grouped according their body mass index values in overweight/obese (>25) and non-obese (<25). Polymerase Chain Reaction was performed for identification of the 5-HTTLPR alleles L and S as previously described. Conclusions: In the present study we didn't found an statistical association between the 5-HTTLPR polymorphism and type 2 diabetes in Mexican population (P= 0.12); interestingly, analysis of L allele and increased body mass index shows an association in the analyzed individuals after adjusting for age, sex and type 2 diabetes (P=0.02, OR 1.74).

450W

Genetic variant of autophagy related 5 homolog (*ATG5*) is associated with asthma in the French Canadian founder population. *A.H Poon[1,2], S.M Tse[3], A.A Litonjua[3,4], S.T Weiss[3,5,6], Q. Hamid[1], C. Laprise[1,2].* 1) Meakins-Christie Laboratories, McGill University Health Centre, Montreal, Quebec, Canada; 2) Department of Sciences fondamentales, Université du Québec à Chicoutimi, Chicoutimi, PQ, Canada; 3) Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA; 4) Division of Pulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA; 5) The Partners Center for Personalized Genetic Medicine, Partners Health Care; Massachusetts, USA; 6) Department of Medicine, Harvard Medical School, Boston, MA, USA.

RATIONALE: We hypothesized that variants of genes in the autophagy pathway are associated with asthma. We conducted a family-based association study to investigate whether single nucleotide polymorphisms (SNPs) in the unc-51-like kinase 1 (*ULK1*), sequestosome 1 (*SQSTM1*), microtubule-associated protein 1 light chain 3 beta (*MAP1LC3B*), beclin 1 (*BECN1*) and autophagy related 5 homolog (*ATG5*) genes are associated with asthma. **METHODS**: We investigated 51 SNPs in 5 candidate genes in an asthma family-based study from northeastern Quebec, Canada (Saguenay-Lac-Saint-Jean familial collection (SLSJ)). A total of 1338 individuals (483 nuclear families) with known asthma status were included in the analysis. Probands were recruited if they fulfilled at least 2 of the following 3 criteria: (1) a minimum of 3 clinic visits for acute asthma within 1 year, (2) 2 or more asthma-related hospital admissions within 1 year, or (3) steroid dependency, as defined by either 6 months of oral or 1 year of inhaled corticosteroid use. SNPs which spanned±10 kilobase (kb) of the candidate genes were chosen. Association between SNPs and asthma was assessed using the family-based association test statistic (FBAT) under an additive model, with $p<0.05$ as evidence of statistical significance. Replication of association was carried out in non-Hispanic Caucasian participants of the Childhood Asthma Management Program (CAMP). **RESULTS**: Of 1338 individuals, 336 were either probands or affected siblings. Of this group, the mean age was 16.45 (standard deviation (SD) +/-9.43) years. 77.1% was atopic. The mean (SD) forced expiratory volume in 1 second (FEV_1) % predicted was 94.1(20.1)%. After Bonferroni correction, SNP rs12212740 of ATG5 was statistically significant ($p = 0.0002$). SNP rs12212740 was not associated with asthma in CAMP, however, it was associated with FEV_1 (adjusted for age, sex and height^2) ($p<0.05$). In the SLSJ cohort, rs12212740 was associated with adjusted FEV_1($p=0.007$), and the direction of association was consistent with that observed for CAMP. **CONCLUSION**:SNP rs12212740 of *ATG5*, is associated with asthma and FEV_1 in the SLSJ study and with FEV_1 in the CAMP cohort. **Funding sources**: Richard and Edith Strauss Foundation, Canada Research Chair on Genetic Determinants of Asthma; and the CAMP Genetics Ancillary Study is supported by U01 HL075419, U01 HL65899, P01 HL083069, R01 HL086601, and T32 HL07427 from the NHLBI, National Institutes of Health (NIH).

451W

Tumor necrosis factor-alpha and IL4 genes polymorphism is not associated with smoking-related COPD in Northern India population. *R.K. Shukla[1], S. Kant[1], S. Bhattacharya[2], B. Mittal[3].* 1) Pulmonary Medicine, CSMMEDICAL UNIVERSITY, (ERSTWHILE KGMC), LUCKNOW, INDIA, India; 2) Physiology, CSMMEDICAL UNIVERSITY, (ERSTWHILE KGMC), LUCKNOW, INDIA, India; 3) Depratment of Genetics, Sanjay Gandhi Post Graduate of Medical Sciences, Raebarely Road, LUCKNOW, INDIA, India.

Background: Chronic Obstructive Pulmonary Disease (COPD) is driven by abnormal inflammatory reactions in response to inhaled particles and fumes. Therefore, inflammatory mediators are postulated to be of distinct importance. Susceptibility to COPD is, in part genetically determined. A single nucleotide polymorphism (SNP) in tumor necrosis factor- alpha (TNF-(308) has been inconsistently associated with COPD and its phenotypes interleukin- IL- 4 (70bp VNTR) has also been reported to be associated with COPD. In the present study we have explore the association with northern Indian population. Material & Methods: We analyzed the genetic variants TNF-(-308 G>A and IL4 (70bp VNTR) in 412 subjects, all the study subject were diagnosed by pulmonary function test (FEV1 /FVC ratio <70%). 204 COPD patients (148 patients with tobacco smoke related COPD and 56 patients with non tobacco smoke related COPD), and 208 healthy controls. Genomic DNA from peripheral blood lymphocytes was used for genotypic analysis by polymerase chain reaction. Results: TNF-(-308 A allele frequency was not significantly different between the population control subjects and the smoking-related (9.0 % vs.12.5%, P= 0.47), or non smoking-related COPD patients (6.6% vs. 4.5 %, p= 0.38) However, significant differences were observed between smoker COPD vs non smoker population controls (12.5% vs.4.55%, P= 0.025).In case of IL4 (70bp VNTR), allele 1 and allele 2 were also not significantly different among population control subjects, smoking related COPD and non smoker COPD patients. Conclusion: We found that the TNF-(-308 A allele may play a role in the susceptibility to smoking-related COPD, and IL4 (70bp VNTR) alleles were not associated with Smoking related COPD, although the determinants between smokers susceptible to the development of COPD and those resistant to it cannot be explained by a single gene polymorphism.

452W

A unified model of Meiosis combining Recombination, Nondisjunction, Interference and Fertility. *H.R. Johnston IV[1], D.J. Cutler[2].* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205; 2) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA 30322.

Male and female recombination rates differ greatly across the broad scale of human chromosomes. The cause of this observation has yet to be identified. To this end, we have created a unified model of meiosis that combines knowledge of recombination, non-disjunction, interference and fertility. The model we create is based on the observation that chiasmata are the mechanism that enables the normal segregation of chromosomes during meiosis. Non-disjunction is the result of a failed segregation event. In our model, non-disjunction occurs both when no chiasmata are present between pairs of non-sister chromatids as well as when multiple chiasmata are present close together between pairs of non-sister chromatids. Other elements of our model include having no chiasmata occur between sister chromatids as well as concluding male meiosis immediately while arresting female meiosis between birth and the mother's age at conception. Using this framework, we assume female chiasmata are evenly distributed across a chromosome. Male chiasmata, however, follow a beta-distribution with a higher density of events near the telomeres. We also allow female chiasmata to dissolve over time at a rate which is Laplace distributed with a mean dissolving age of 49 years and a variance of 72 years. The number of chiasmata in any female meiosis is Poisson distributed with mean 80 chiasmata. Finally, the mean distance apart at which closely spaced recombination events cause a non-disjunction event is exponentially distributed with mean 350 Kb for two-strand double recombination events, 99 Kb for three-strand doubles and 58 Kb for four-strand doubles. Our model correctly predicts the rate of fertility, trisomy 21 occurrences and trisomy 13 occurrences for each maternal age. The recombination rates of each simulated chromosome match closely to the available deCODE recombination maps. In addition, we exactly match the observed 40 recombination events per female meiosis as shown in Broman, at al. As a result, we can conclude that the rates of known trisomies are simply the result of the size of their respective chromosomes. Our model will enable us to address additional questions, such as the potential cause of the beta-distribution of male recombination events, in the future.

453W

New deletions missed by current PCR techniques could lead to errors in UGT2B17 genotyping for doping tests in sports and association studies. *L. Wang[1, 2], G. Bademci[1], A.L. Torres[1], C. Jauregui[1], S. Zuchner[1,2], W.K. Scott[1,2], G.W. Beecham[1,2], E.R. Martin[1,2], J.M. Vance[1,2].* 1) Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine.

BACKGROUND: UDP glucuronosyltranserases (UGTs) are a family of proteins that catalyze the glucuronidation of endogenous and exogenous lipophilic compounds, including steroids, bile acids, xenobiotic drugs, and environmental pollutants. The glucuronidated derivatives are more easily excreted via the urine or bile. Among UGTs, UGT2B17 is the most important player in steroid hormone metabolism. The genomic location of UGT2B17 spans 31 Kb on chromosome 4, within a 117 kb region that is frequently deleted in humans. CNV of UGT2B17 has been associated with prostate cancer and osteoporosis, and has an impact on the doping tests in sports. Furthermore, CNV of UGT2B17 generates minor histocompatibility antigen and is associated with the risk of graft-versus-host disease in stem cell transplantation. Therefore, accurately genotyping the UGT2B17 CNV is of broad clinical interest. Previously, a PCR-based method has been developed to genotype UGT2B17 deletion. We sought to compare this method with CNV analysis based on SNP array and quantitative PCR (qPCR). METHODS: For the PCR analysis, the primers C (amplifying a 316 bp region within exon 1 of UGT2B17) and J (amplifying an 884 bp region spanning the deletion breakpoints) were used as described before. For the SNP array analysis, the Illumina Infinium 610-quad BeadChip and PennCNV were used to determine CNV using an existing GWAS study on Parkinson disease. For the qPCR analysis, the TaqMan® Copy Number Assay (Hs03185327_cn) targeting the intron 1 - exon 1 border of UGT2B17 and the CopyCaller™ Software were used to estimate copy number for UGT2B17. RESULTS: In total, 530 independent non-Hispanic white samples were analyzed using all three different methods. Discordant UGT2B17 CNV genotypes were found for 21 (PCR vs SNP array), 15 (PCR vs qPCR), and 11 (SNP array vs qPCR) individuals. Interestingly, there are 13 individuals with concordant UGT2B17 CNV genotypes based on qPCR and SNP array CNV analysis, but were assigned a different CNV genotype by the PCR-based method. CONCLUSION: The qPCR seems to be the most robust CNV test for the UGT2B17 deletion. Our data suggest that genetic variations around breakpoints of the 117 Kb deletion encompassing the UGT2B17 gene interfere with the PCR-based genotyping method. Although the frequency is low, the PCR-based method could lead to misinterpretation of doping test results and mismatch for minor histocompatibility antigen.

454W

Signatures of selection in human transcription factor binding sites and prediction of functional consequences of noncoding mutations. *A. Siepel, L. Arbiza, B.A. Aksoy, M.J. Hubisz, B. Gulko, I. Gronau, A. Keinan.* Biol Stats & Comput Biol, Cornell University, Ithaca, NY.

By comparing patterns of polymorphism within a species with patterns of divergence between species, it is possible to characterize the extent and modes by which natural selection has shaped present-day genomes. This approach has been used for decades to study human protein-coding sequences, but applications in noncoding regions have so far been limited. We have combined the complete genome sequences of 45 unrelated individuals recently produced by Complete Genomics with chromatin immunoprecipitation and sequencing (ChIP-seq) data for 47 transcription factors from the Encyclopedia of DNA Elements (ENCODE) project, to enable the first systematic genome-wide study of polymorphism and divergence of human regulatory elements. We have developed a new probabilistic modeling approach that allows us to contrast patterns of polymorphism and divergence in short noncoding functional elements with those in neutrally evolving flanking regions. Simulation experiments demonstrate that our methods effectively distinguish positive and negative selection, are robust to population structure, bottlenecks, and population growth, and are much less strongly biased by the presence of weak negative selection than are standard McDonald-Kreitman methods. We show that the binding sites of most transcription factors have experienced significant levels of selection, in some cases at least as strong as with nonsynonymous sites in protein-coding regions. Negative selection dominates, in general, but the binding sites of certain transcription factors (notably, FOXP2 and GATA-1) have also been strongly influenced by positive selection. In many cases, binding sites are strongly enriched for signatures of selection (both positive and negative) near genes whose Gene Ontology (GO) categories are compatible with the functions of the transcription factor of interest. We also examine the relationships between signatures of selection and various local features of interest, such as the expression levels of nearby genes, position relative to the proximal promoter region, and histone modifications. Finally, we show how our methods can be adapted to predict the functional consequences of mutations in noncoding regions across the genome. We expect these methods to be useful in interpreting the potential function of noncoding loci identified in genome-wide association studies.

455W

Locally Linear Embedding (LLE) for Human Population Structure Studies. *H. Siu[1], L. Jin[1], M. Xiong[2].* 1) MOE Key Laboratory of Contemporary Anthropology, School of Life Sciences, Fudan University, Shanghai, 200433, China; 2) Human Genetics Center, University of Texas School of Public Health, Houston, TX 77030, USA.

The dimension of the population genetics data produced by next-generation sequencing platforms is extremely high. It is increasingly recognized that the high dimension reduction that can reduce the noises and effectively extract useful population genetics information from the data holds a key to the success of population structure and historical inference from next-generation sequencing data. This motives us to use locally linear embedding (LLE) that projects high dimensional genomic data into low dimensional, neighborhood preserving embedding, as a general framework for population structure and historical inference. To facilitate application of the LLE to population genetic analysis, we systematically investigate several nice properties of the LLE and reveal the connection between the LLE and principal component analysis (PCA). Identifying a set of markers and genomic regions which could be used for population structure analysis will provide invaluable information for population genetics and association studies. In addition to identifying the LLE-correlated or PCA-correlated structure informative markers and genomic regions enriched with these markers, we have developed a new statistic that integrates genomic information content in a genomic region for collectively studying its association with the population structure and lasso algorithm to search such regions across the genomes. We applied the developed methodologies to low coverage pilot dataset in 1000 Genomes Project and PHASE III Mexico dataset of the HapMap released in May, 2010 and demonstrated that the LLE has higher power to separate populations than PCA. We observed that 25.09%, 44.95% and 21.41% of common variants and 89.2%, 92.4% and 75.1% of rare variants were the LLE-correlated markers in CEU, YRI and ASI, respectively. We also identified 3,575 genomic regions enriched with the LLE-correlated SNPs, 3,675 genomic regions enriched with the PCA-correlated SNPs and 1,898 structure informative genomic regions that were search by lasso algorithm. We characterize the genotype frequency and linkage disequilibrium (LD) patterns of the structure informative genomic regions and interestingly observed that 24.65%, 53.38% and 57.55% of identified selection regions by Tajima D test were overlapped with the structure informative genomic regions detected by the lasso, LLE and PCA enrichment analysis, respectively.

456W

Integrative SNP Genotyping for Parallel Sequencing in Population Genomics Studies. *Y. Wang[1], J. Yu[1], A. Sabo[1], R. Gibbs[1], O. Thousand Genomes Consortium[2], F. Yu[1].* 1) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX; 2) 1000 Genomes Consortium.

Parallel sequencing is highly promising for population genomics studies. We analysis 1000 Genomes Project Phase I dataset by an integrative SNP Genotyping Pipeline (SNPTools) which consists the following components and innovations: Effective Base Depth (EBD) statistics summarizes sequencing details and enables statistical modeling on sequencing data; Variance ratio based site scoring statistics discovers SNP sites with high sensitivity and specificity; BAM-specific Binomial Mixture Modeling (BBMM) captures the heterogeneity of sequencing data and generates robust and raw genotype likelihoods from sequences; Novel imputation engine refines raw genotype likelihoods by utilizing LD information and produces high quality genotype and haplotype calls; An integration component that combines multiple existing data sources. We found the 1000 Genomes Project Phase I (1) low-pass (~5X) data has >98%; sensitivity and specificity in SNP site discovery measured by corresponding HapMap, OMNI and Axiom datasets; (2) low-pass only data achieves ~99.5%; overall genotyping accuracy (3) the variation of genotyping error rates across chromosomes are largely explained by recombination rate (4) Integration of multiple data sources (e.g. exome sequences and microarrays) in to low-pass data shows comparable accuracy against traditional microarray technologies. (5) Although the entire low-pass dataset is large (~25T), it can be processed in reasonable time (<1 month). These evidences support the utility of parallel sequencing for large scale population genomics studies at reasonable cost.

457W

Amylase gene copy numbers and salivary amylase activity in ethnically diverse populations from Ethiopia. *C.C. Elbers[1], S.R. Thompson[1], A. Ranciaro[1], D.W. Meskel[2], G. Belay[2], S.A. Tishkoff[1].* 1) Department of Genetics, School of Medicine, University of Pennsylvania, Philadelphia, USA; 2) Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia.

Starch is an important carbohydrate in the human diet, especially among agricultural societies. The catabolism of starch into simple sugars is initiated by the salivary enzyme amylase. It is known that the enzymatic activity of salivary amylase shows significant variation among individuals, which is influenced by both environmental and genetic factors. Salivary amylase (*AMY1*) gene copy numbers are correlated with amylase activity. It has been suggested that *AMY1* copy numbers have been under positive natural selection based on the observation that high-starch consuming populations have more *AMY1* copy numbers compared to low-starch consuming populations.

DNA, saliva and phenotype data were collected from 760 unrelated individuals from 17 diverse Ethiopian ethnic populations. Together, the samples represent high genetic, phenotypic and dietary diversity, and include populations practicing agriculture, pastoralism and hunting-gathering. We assessed the association between copy numbers in the *AMY1* gene and salivary amylase activity using linear regression. Analyses were adjusted for the potential confounders of age, sex, smoking and fasting status.

The mean *AMY1* copy number was 6.6 with a range of 2 to 17. *AMY1* gene copy numbers were positively correlated with salivary amylase enzyme activity ($r = 0.45$; $p = 2.83 \times 10^{-32}$) among all Ethiopians. The correlation differed per ethnic group, ranging from $r = 0.34$ in the Agaw population to $r = 0.74$ in the Sheko population. Compared to agriculturalists, pastoralists had fewer *AMY1* copy numbers (6.4 ± 0.1 vs 6.0 ± 0.2; $p = 0.007$) and lower salivary amylase enzyme activity (77.4 ± 3.4 U/ml vs 59.3 ± 4.5 U/ml; $p = 0.01$). Populations that traditionally have a high-starch diet have more *AMY1* gene copy numbers and higher amylase activity compared to low-starch consuming populations. However, *AMY1* copy numbers cannot entirely explain amylase activity in all populations and, therefore, follow-up studies on additional genetic and environmental factors influencing amylase activity are needed.

458W

Characterizing Genes Associated with both Complex and Mendelian Diseases. *W. Jin[1], S. Xu[1], P. Qin[1], L. Jin[1,2].* 1) Chinese Academy of Sciences Key Laboratory of Computational Biology, Chinese Academy of Sciences and Max Planck Society (CAS-MPG) Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shangh; 2) Ministry of Education (MOE) Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai 200433, China.

Genetic disorders have been traditionally classified as either Mendelian or complex diseases. This nosology has greatly benefited genetic counseling and development of gene mapping strategies. However, we found that 54.1% (524 of 968) of the Mendelian diseases genes in hand-curated OMIM database (hOMIM) also involved in complex diseases according to genetic association database (GAD), and those genes underlying both Mendelian and complex disease have never been systematically analyzed. In this study, we classified human genes into five categories: Mendelian and complex diseases (MC) genes, Mendelian but not complex diseases (MNC) genes, complex but not Mendelian diseases (CNM) genes, essential genes and OTHER genes. Our analysis showed that MC genes on average associated with 1.77 Mendelian diseases and 5.83 complex diseases, which are significantly higher than other disease genes (P<2.8×10-6). Analysis showed that MC genes involved in more complex networks than MNC and CNM genes, but less than essential genes. We also found that MC genes encode the longest proteins, and have the most transcripts among all gene categories. Interestingly, although expression level of MC genes is similar to that of essential genes, tissue specificity of MC genes is significantly higher than that of any other gene categories (P< 7.5×10-5). Further analysis showed that MC genes were under stronger long-lasting purifying selection than CNM genes. However, compared with the other gene categories, MC genes were also under stronger recent positive selection similar with CNM genes. And over-representing of CNVs in CNM genes indicates the important role of CNVs in genetic basis of complex disease. Comparative analysis of the representing pattern of CNVs in different gene categories also suggested that MC genes seemly have been under both purifying and positive selection. Analysis showed that disease genes exhibited different relative evolutionary rate in different timescales. Based on this elaborate classification, we not only characterized the genes underlying both Mendelian and complex diseases, but also gained some new insights on the other four kinds of genes.

459W

A Map of Copy Number Variations in Chinese Populations. *H. Lou[1], S. Li[2], Y. Yang[2], X. Zhang[2], B. Wu[2,3], L. Jin[1,2,3], S. Xu[1].* 1) Chinese Academy of Sciences Key Laboratory of Computational Biology, Chinese Academy of Sciences and Max Planck Society (CAS-MPG) Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shangh; 2) Ministry of Education (MOE) Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai, China; 3) Children's Hospital Boston, Harvard Medical School, Boston, MA, USA.

It has been shown that the human genome contains extensive copy number variations (CNVs). Investigating the medical and evolutionary impacts of CNVs requires the knowledge of locations, sizes and frequency distribution of CNVs within and between populations. However, CNV study of Chinese minorities, which harbor the majority of the genetic diversity of Chinese populations, has been underrepresented considering the same efforts in other populations. Here we constructed, to our knowledge, a first CNV map in 7 Chinese populations representing the major linguistic groups in China with 1,440 CNV regions identified using Affymetrix SNP 6 Array. Considerable differences in distributions of CNV regions between populations and substantial population structure were observed. We showed ~35% of CNV regions in minority ethnic groups are not shared by any two populations under comparison and suggested that the contribution of minorities to genetic architecture of Chinese population cannot be ignored. We further identified highly differentiated CNV regions between populations and genes enriched in these regions. For example, a common deletion in Dong and Zhuang (44.4% and 50%) was not observed in Han Chinese, which overlaps two keratin-associated protein genes contributing to the structure of hair fibers. Interestingly, the most differentiated CNV deletion between CEU and YRI reported in previous studies containing CCL3L1 gene, was also the highest differentiated regions between Tibetan and other populations. Besides, by joint analyzing CNVs and SNPs we found a CNV region containing gene CTDSPL with near perfect LD between flanking SNPs in Tibetan while not in other populations except CHD. Furthermore, we found the SNP taggability of CNVs in Chinese populations was much lower than that in European population. Our results suggested the necessity of a full characterization of CNVs in Chinese populations, and the CNV map we constructed serves as a useful resource in further evolutionary and medical studies.

460W

Human amylase gene copy number variation in Korea and Mongolia population. *H.Y. Son[1], C.H. Park[1], J.S. Seo[1,2], J.I. Kim[1,2].* 1) Dept. of Biochemistry and Molecular Biology, Seoul National University College of Medicine, Seoul, Korea; 2) Genomic Medicine Institute, Medical Reseach Center, Seoul National University, Seoul, Korea.

Copy number variations (CNVs) have recently been considered as important human genomic variant, which associated with phenotypic difference and disorder. The copy number of salivary amylase genes (AMY1) have shown to be related with ethnic difference of starch diet. Here, we compared AMY1 copy number in 2 groups of 1039 Koreans(531 Ansan, 508 Sihwa) and 2 groups of 811 Mongolians(400 Dashvalvar, 411 Orkhontuul). We estimated copy number by TapMan real-time PCR method. The average of copy number was 6.27 and 6.39 in Korean and 5.83 and 6.49 Mongolian populations, respectively. The low copy group(Mongol Dashvalvar, 5.83) show significant difference than others. In addition, comparison of AMY1 copy number with body mass index(BMI) and waist hip ratio revealed significant association in Mongol population. These results suggest that lower copy number of AMY1 may be related with obesity in low starch diet population.

461W

The population genetics of native Peruvian populations: evolutionary inferences and biomedical implications. *E. Tarazona-Santos[1], L. Pereira[1], M. Scliar[1], G.B. Soares-Souza[1], R. Zamudio[1], L.W. Zuccherato[1], F. Kehdy[1], W. Magalhaes[1], M.R. Rodrigues[1], G. Bertorelle[2], E. Hollox[3], S.J. Chanock[4], R.H. Gilman[5, 6, 7].* 1) Dept Biol Geral, CP 486, Univ Federal Minas Gerais, Belo Horizonte, MG, Brazil; 2) Universita degli studi di Ferrara, Ferrara, Itália; 3) Univeristy of Leicester, Leicester, UK; 4) National Cancer Institute, US; 5) Johns Hopkins School of Public Health, US; 6) Asociación Benéfica PRISMA, Peru; 7) Universidad Cayetano Heredia, Peru.

Peru has the largest Native American population in South America. In the Andes, populations derive from a unique complex of societies that evolved during the last 10000 years along the Andes and its adjacent coast. During PreColumbian times, they reached a level of socioeconomic development unmatched elsewhere in South America. Smaller populations are settled in the Amazonian region, and in the hereafter called transition area between the Andes and the Amazonas. A mestizo population of millions is also settled in current Peruvian cities. We are studying the population genetics of Peruvians, addressing evolutionary inferences and biomedical implications of their diversity. We summarize three results: (1) Using 20kb resequenced from neutral inter-genic regions, we made inferences about the history of Andeans (Quechua) and a population from the transition area (Shimaa). These populations derived from an ancestral population with an effective size (N_e) of ~6000 individuals that split about 1025 years ago. At this time the N_e of Quechuas (N_e=5000) was five times larger than the N_e of the Shimaas, the former showing an evidence of a posterior demographic expansion, and the latter of a bottleneck. Thus, Andean and transition-area populations, even if they speak languages from different families and being culturally differentiated, share very recent ancestral populations. (2) Populations considered as mestizo have very high Native American ancestry. A sample (n=297) from Lima, genotyped for 106 AIMs, has 78.4% of Native American ancestry, suggesting that the Peruvian population is a potential target for large epidemiological studies in Native Americans. (3) We identified genes with variants that are common in Native Americans and rare elsewhere. The FCGR3B CNV locus has an idiosyncratic high frequency of deletions in our native samples. This variant has been elsewhere associated with lupus erythematosus and rheumatoid arthritis, two diseases associated with Native American ancestry. On the basis of the genotyping of 1442 SNPs on 411 genes, we determined that the following genes, that on the basis of recent GWAS are associated with complex diseases, show a differentiated haplotype structure in Native Americans: CASR, CAV1, CYP19A1, DRD2, FUT2, IL13, IL15, IL6R, IL7R, KRT23 and SCARB1 are highly differentiated from Africans/Europeans; ADH1C, APOB, CASR, CCND1, CDKN2A, CDK5, CYP19A1, ESR1, IGF2, IL1RN, LEPR and SCARB1 are highly differentiated from Asians.

462W

Development of Human Variation Database in Japanese Integrated Database Project. *A. Koike[1], N. Nishida[2], M. Kawashima[2], M. Yoshida[1], I. Inoue[3], S. Tsuji[4], K. Tokunaga[2].* 1) Cent Res Lab, Hitachi, Ltd, Kokubunji, Japan; 2) Dept Human Genetics, Univ. Tokyo; 3) Dept. of Mol. Life Sci. and Mol. Med., Nat. Inst. of Genet; 4) Dept Neurology, Univ. Tokyo.

Recent progresses in SNP typing and massively parallel sequencing technologies enabled us to explore disease related loci/variations on a genome-wide scale in a cost-effective high-throughput manner and have been successful for identifying disease related loci/variations. To date, our organization has created a repository database for SNP-based genome-wide association studies (GWAS) (https://gwas.lifesciencedb.jp/) to achieve continuous and intensive management of GWAS data and data-sharing among researchers and has widely called for data submission. In this study, we have extended the GWAS database to a Human Variation Database to accumulate variations including short/long insertions/deletions and structural variations mainly in Japanese or Asian population, which were detected by various measurements including massively parallel sequencers. In the database, variations related to disease susceptibility, virus resistance, and drug response are registered along with statistical genetic results and simple clinical characteristics to clarify the locus specific characteristics. Since some genes are widely known to be related to multiple diseases, variations and diseases are made to be cross-searchable to shed light on relationships among various diseases. Given that high-throughput data can suffer from relatively high error rates caused by multiple factors, quality control is carried out and reliability scores are assigned to variations. Variations in healthy Japanese individuals are also systematized so as to be useful for exploitation of disease-related variations. In the database, variation positions and statistical results along with other information such as genes, ncRNAs, and conservation scores are presented by a graphic viewer to facilitate understanding of biological functions of disease-related variations. Comparison between various study results obtained by different institutions with different populations and their meta-analysis are also available. The same data are also managed as a distributed annotation system (DAS) to invoke useful data from other databases. In this presentation, we introduce the overview of database structure and methods for variation detection, quality filtering, and statistical genetic analysis and data management policies. Acknowledgement: This work was supported by the contract research fund “Database Integration program” from the Japan Science and Technology Agency.

463W

Establishing the contribution of candidate genes to the development of ESKD in Puerto Rico. *Y.M. Afanador[1], A.V. Washington[1, 2], J.C. Martínez-Cruzado[1], T.K. Oleksyk[1].* 1) Department of Biology, University of Puerto Rico, Mayaguez Campus, Mayaguez, PR 00680; 2) Department of Anatomy, Universidad Central del Caribe School of Medicine, Bayamon, PR 00960.

Kidney disease is the ninth leading cause of death in Puerto Rico. Dialysis treatment for End Stage Kidney Disease (ESKD) in the island is estimated to be approximately 4,000 patients annually, and close to 24% of them die every year. Focal Segmental Glumerulosclerosis (FSGS) and Diabetic Nephropathy are among primary causes of ESKD, accounting for 30 and 40% of disease occurrence, respectively. FSGS is reported to be the most common primary glomerulopathy in the United States where it is most common among individuals of African ancestry, while Type 2 Diabetes Mellitus (T2DM) is more widespread among Hispanic populations. Both contribute to ESKD among Puerto Ricans.

The purpose of this study was to establish the relative contribution of the genetic factors associated with T2DM, FSGS, or ancestry to ESKD in Puerto Rico. We created a panel of 16 SNPs found in 12 candidate genes and a panel of Ancestry Informative Markers (AIMs), and used them to genotype 144 ESKD patients and 2x set of matched controls using TaqMan Open Array System (ABI). We report associations of these candidate genes as well as the relative ancestry contribution of European, African and Native American ancestries to ESKD. Hispanics are the highest growing minority population in the US. As a highly admixed population, Puerto Ricans may prove to be a useful model for studying the genetic basis for kidney disease, especially in Hispanic populations, which carry a greater risk of acquiring the disease compared to non-Hispanic whites.

464W

A Genome Wide Search for Type 2 Diabetes Susceptibility Genes in Arab Families. *H. Alsafar[1], H. Cordell[2], O. Jafer[3], S. Jamieson[4], K. Khazanehdari[3], J. Blackwell[4,5], G. Tay[6].* 1) Khalifa University of Science, Technology & Resear, Abu Dhabi, United Arab Emirates; 2) Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, United Kingdom; 3) Molecular Biology and Genetics Laboratory, Central Veterinary Research Laboratory, Dubai, United Arab Emirates; 4) Telethon Institute for Child Health Research, Centre for Child Health Research, The University of Western Australia, Subiaco, Western Australia; 5) Cambridge Institute for Medical Research and Department of Medicine, School of Clinical, Medicine University of Cambridge, Cambridge, United Kingdom; 6) Centre for Forensic Science, The University of Western Australia, Crawley Western Australia.

Type 2 Diabetes (T2D) is currently the fastest growing debilitating disease in the world. In the United Arab Emirates (UAE), it has been estimated that one out of five people between the ages of 20 to 79 lives with this disease. Due to an increasing prevalence of T2D in the region, lifestyle management strategies with an emphasis on prevention are required. Determining genetic risk factors can also make an important contribution to understanding the processes leading to disease. A genome wide association study (GWAS) using a family based association test (FBAT) in an extended family of 178 members from the UAE (66 diabetic and 112 healthy individuals) were genotyped using the Illumina Human 660 Quad chip array was undertaken in order to identify gene(s) and mechanisms associated with disease. The study revealed 21 new association signals from single nucleotide polymorphisms (SNPs) within five genes (RBM47, KCTD8, GABRB1, SCD5 and PRKD1). Six SNPs within PRKD1 on chromosome 14 were found to be most strongly associated with T2D in this Arab population. It has been suggested that PRKD1, a serine/threonine kinase, plays an important role in insulin secretion. The strongest statistical evidence for this new association signal was from rs10144903 in intron 1 of PRKD1, with the overall estimate of effect returning an odds ratio of 3.72 (95% confidence interval, 1.28-10.82; p-value = 3.92E-06). This study is the first GWAS for T2D in families of Arab descent, and these findings may provide important insights into the pathogenesis of T2D in Middle Eastern populations. Comparative analysis with other ethnic groups could assist in dissecting the mechanisms that cause the disease.

465W

Influence of Ethnicity in Association with UCP2 -866G/A, PGC1((Gly 482 Ser) and mtDNA 10398G/A Polymorphisms in Orissa Population Groups. *A. Behura[1,2], R.N.K. Bamezai[2], G.B.N. Chainy[1].* 1) Department of Biotechnology, Utkal University, BHUBANESWAR, Orissa, India; 2) National Center of Applied Human Genetics, School of Life Sciences, JNU, New Delhi India.

Type 2 Diabetes (T2D) involves complex interplay of both genetic and environmental factors leading to two distinct defects in insulin secretion and insulin action. In this study, we explored a total of 1205 individuals (508 cases and 697 controls) belonging to Orissa population, to elucidate the role of some of the polymorphisms of the candidate genes: UCP2 -866G/A, mtDNA 10398G/A and PGC1((Gly 482 Ser). These polymorphisms are suggested to be associated with T2D susceptibility in literature and are mainly involved with the activity of pancreatic beta cells, affecting glucose-stimulated insulin secretion. India exhibits different ethnic population structure comprising various caste groups existing from ages which have mostly remained endogamous. Thus to assess the impact of ethnicity, these individuals were further stratified on the basis of respective caste groups i.e. (Brahmins, Kshatriya, OBC and SCs). Genomic DNA was extracted by phenol-chloroform method and PCR-RFLP procedure was used for genotyping. Binary logistic regression was applied to test the association of risk factors with T2D after adjusting for age, sex and BMI. In total population UCP2-866 AA+GA vs GG (p=0.003), mtDNA 10398 A/G (p=1.8E-9) and PGC1(GG+GA vs AA (p=7.86E-12) significantly provided risk towards T2D. Caste based stratification revealed UCP2-866 AA+GA vs GG significantly provided risk only in Kshatriyas (p=0.005) and mtDNA 10398A/G in Brahmins (p=0.002), Kshatriyas (p=0.00005) and OBC (p=0.003). While in PGC1(GG+GA vs AA, all the caste groups: Brahmins (p=0.0002), Kshatriyas (p=0.0003), OBC (p=0.006) and SCs (p=0.01) are significantly associated with T2D. Thus the present investigation concludes that differential pattern of association of polymorphisms is observed for different caste groups, suggesting the putative role of ethnicity. Thus, for risk calculation and proper medical intervention, knowledge of the ethnicity and nature of variation in risk factors need serious attention.

466W

Exceptions to the "One Drop Rule"? DNA evidence of African ancestry in European Americans. *J.L. Mountain[1], J.M. Macpherson[1], C.B. Do[1], B.T. Naughton[1], R.A. Kittles[2], N. Eriksson[1].* 1) 23andMe, Inc, Mountain View, CA; 2) Institute of Human Genetics, University of Illinois at Chicago, Chicago, IL.

Genetic studies have revealed that most African Americans trace the majority (75-80%, on average) of their ancestry to western Africa. Most of the remaining ancestry traces to Europe, and paternal lines trace to Europe more often than maternal lines. This genetic pattern is consistent with the "One Drop Rule," a social history wherein children born with at least one ancestor of African descent were considered Black in the United States. The question of how many European Americans have DNA evidence of African ancestry has been studied far less. We examined genetic ancestry for over 77,000 customers of 23andMe who had consented to participate in research. Most live in the United States. A subset of about 60,000 shows genetic evidence of fewer than one in 16 great-great-grandparents tracing ancestry to a continental region other than Europe. They are likely to consider themselves to be entirely of European descent. We conducted two analyses to understand what fraction of this group has genetic evidence of some ancestry tracing recently to Africa. We first identified individuals whose autosomal DNA indicates that they are predominantly of European ancestry, but who carry either a mitochondrial (mt) DNA or Y chromosome haplogroup that is highly likely to have originated in sub-Saharan Africa. Of the 60,000 individuals with 95% or greater European ancestry, close to 1% carry an mtDNA haplogroup indicating African ancestry. Of approximately 33,000 males, about one in 300 trace their paternal line to Africa. We then identified the subset of these European Americans who have estimates of between 0.5% and 5.0% of ancestry tracing to Africa. This subset constitutes about 2% of this set of individuals likely to be aware only of their European ancestry. The majority (75%) of that group has a very small estimated fraction of African ancestry (about 0.5%), likely to reflect African ancestry over seven generations (about 200 years) ago. We estimate that, overall, at least 2-3% of individuals with predominantly European ancestry have genetic patterns suggesting relatively deep ancestry tracing to Africa. This fraction is far lower than the genetic estimates of European ancestry of African Americans, consistent with the social history of the United States, but reveals that a small percentage of "mixed race" individuals were integrating into the European American community (passing for White) over 200 years ago, during the era of slavery in the United States.

467W

African genome sequencing reveals diversity and origin of the "European" 17q21.31 inversion polymorphism. *K. Meltz Steinberg[1,2], F. Antonacci[1,2], J.M. Kidd[1,5], C.D. Campbell[1,2], P. Sudmant[1,2], L. Vives[1,2], M. Malig[1,2], L. Scheinfeldt[3], W. Beggs[3], M.P. Donnelly[4], K.K. Kidd[4], S.A. Tishkoff[3], E.E. Eichler[1,2].* 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) Howard Hughes Medical Institute, University of Washington, Seattle, WA; 3) Department of Genetics and Biology, University of Pennsylvania, Philadelphia, PA; 4) Department of Genetics, Yale University, New Haven, CT; 5) Department of Genetics, Stanford University, Stanford, CA.

The 17q21.31 inversion polymorphism represents one of the most dynamic and evolutionarily complex regions of the human genome. The two haplotypes exist in direct (H1) and inverted (H2) orientation and show no recombination over nearly 2 Mb resulting in extended linkage disequilibrium. The two haplotypes are among the most ancient in the human species diverging 2.3 million years ago. Interestingly, the H2 is found primarily in individuals of European/Mediterranean descent where changes in the flanking duplication architecture predispose carriers to the 17q21.31 microdeletion syndrome. We recently identified a 205 kb duplication associated with 30% of European H1 haplotypes while a smaller 155 kb duplication in the same region is fixed in European H2 haplotypes. We sought to study the architecture and genetic diversity of this region in more detail using next-generation sequencing, arrayCGH and fluorescence *in situ* hybridization. We sequenced a Massai human genome, analyzed copy number variation from 542 African genomes for this region, and genotyped 821 unrelated African individuals from 23 geographically diverse ethnic groups with variable modes of subsistence. We identify eight new structural haplotypes (3 related to H2 and 5 related to H1). These vary dramatically in size from 1.08 to 1.5 Mb and differ based on orientation, gene organization and duplication architecture. We find that the H2 haplotype is absent from the Western African populations but is enriched in hunter-gatherer groups from Eastern and Central Africa (San Bushman, Pygmies, Hadza and Sandawe). In addition, the H2-specific duplication is polymorphic in Africans. SNP genotyping and copy number estimates from over 1500 unrelated individuals from worldwide populations support the hypothesis that the nonduplicated H2 represents the ancestral state and demonstrate that the H1 duplication is specific to Europe. We estimate that the evolutionary age of the H2 duplication is significantly older (850,000 years) when compared to the H1 duplication (320,000 years old). Remarkably, most Europeans (60%) carry either the H1 or H2 duplication, which is virtually absent (<5%) in most other continental groups. Our results support the hypothesis that the H2 haplotype without extensive duplication is the ancestral haplotype, the H1- and H2-specific duplications emerged independently, and the duplications arose prior to the human migration out of Africa.

468W

Positive and balancing selection acting on the lectin-like oxidized low density lipoprotein receptor 1 (*OLR1*) intronic regions. *I.M. Predazzi[1], A. Rokas[2], N. Schnetz-Boutaud[1], N.D. Williams[1], A. Tacconelli[3], A. Deinard[4], J.L. Haines[1], G. Novelli[5], G. Sirugo[3], S.M. Williams[1].* 1) CHGR, Vanderbilt University, Nashville TN 37232, USA; 2) Department of Biological Sciences, Vanderbilt University, Nashville, TN 37235, USA; 3) Unità di Genetica Medica, Ospedale San Pietro FBF, Rome, Italy; 4) Department of Anthropology, University of Minnesota, Minneapolis, MN; 5) Department of Biopathology, Tor Vergata University of Rome, Italy.

The lectin-like oxidized low density lipoprotein receptor 1 (*OLR1*) is the principal endothelial scavenger receptor for oxidized low density lipoproteins (ox-LDLs). *OLR1* also plays a role in innate immunity through its function as an endothelial receptor for the outer membrane protein peptide A (OmpA) of *Klebsiella* and *Chlamydia pneumoniae*. Several associations have been reported between OLR1 and phenotypes related to atherosclerosis. A non-synonymous variant in exon 4 alters the function of the receptor, changing the positive isopotential surrounding the active site; in addition, *OLR1* introns (especially intron 4) and 3'UTR contain SNPs that associate with alternative splicing of the mRNA, leading to an isoform, lacking part of the functional domain. Despite these interesting functional data, the importance of *OLR1* variations in relation to disease remains unclear. Under the hypothesis that regions that confer functional differences and hence affect disease risk are non-neutral, we tested for evidence of both ancient (inter-specific) and recent selection (intra-specific) across the whole gene as well as focusing in regions that have been previously associated with function. We sequenced the entire coding region and crucial intronic regions in a sample of 48 non-human primates from 12 species, combined them with 7 reference primate sequences and performed interspecies tests for ancient selection in coding and non-coding regions (dN/dS and McDonald-Kreitman tests). We also tested for recent selection by analyzing genetic structure in haplotypes from the four principal HapMap populations (CEU, YRI, JPT and CHD) using Analysis on Molecular Variance (AMOVA) and Tajima's D. We found evidence of ancient selection in *OLR1* intron 4 but not in the coding region. Intra-specific analyses indicated evidence of balancing selection (particularly in the CEU sample, Tajima's D = 4.04) and with significant genetic structuring (Fst = 0.117; $p < 0.001$). Analyses only including an LD block containing intron 4, indicated more population differentiation (Fst = 0.270; $p < 0.001$) and comparable levels of balancing selection (Tajima's D = 3.50). Intron 4's involvement in alternative splicing, suggests that this may have been the target of both ancient and recent selection. These data suggest that selection is operating on a non-coding region within a disease risk locus, and may be taking place at different evolutionary time points, both ancient and recent.

469W

A likelihood framework to model gene expression evolution through a phylogeny. *R.V. Rohlfs[1], P. Harrigan[1], D. Brawand[2,3], M. Soumillon[2,3], A. Necsulea[2,3], H. Kaessmann[2,3], R. Nielsen[1,4].* 1) Department of Integrative Biology, University of California, Berkeley, Berkeley, CA; 2) Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland; 3) Swiss Institute for Bioinformatics, Switzerland; 4) Bioinformatics Center, University of Kopenhagen, Kopenhagen, Denmark.

Despite fascinating theoretical results about the important role of gene expression changes in recent primate and human evolution, experimental exploration of these hypotheses has been limited by the unavailability of robust gene expression data across species. Recent technological innovations, specifically the development of RNA-Seq, have facilitated the collection of reliable gene expression data across tissues and species. These data enable comparative evolutionary analysis of gene expression, allowing exploration of long-standing hypotheses regarding the role of gene expression in adaptation. We have implemented a statistical model for the evolution of gene expression through a phylogeny based on an Ornstein-Uhlenbeck process. This model accounts for phylogenetic relationships between species, constraints on gene expression, and the possibility of directional selection on gene expression level. Observed gene expression data can be fit to various models, including unconstrained neutral expression evolution, constrained expression evolution, and constrained expression evolution with lineage-specific directional selection. The likelihood of the data under different models in this framework can be compared to test hypotheses regarding gene expression evolution and selection. We have demonstrated the validity of these methods through simulation studies and applied the framework to study an RNA-Seq dataset quantifying expression in six tissues and in a panel of mammalian species, with an emphasis on primates and humans. Our analyses confirm that expression evolution is better modeled by accounting for phylogenetic structure between species. We show that a large portion of the genes considered appear to be under evolving with constraints on expression level, implying widespread negative selection. Further, our analysis suggests that the expression levels of some genes may have been subject to direction selection, specifically along the human and primate lineages. For instance, the gene showing the most evidence for selection in the primate lineage, COL25A1, is down-regulated in primates and has previously been found to be involved with Alzheimer's disease and to cause atypical behavior when over-expressed in animal models.

470W

Evidence for extensive ancient admixture in different human populations. *J. Wall[1], S. Kim[1], C. Gignoux[3], A. Woerner[2], F.L. Mendez[4], K. Veeramah[2], M.F. Hammer[2, 4].* 1) Inst Human Gen and Dept Epidemiology and Biostatistics, Univ California, San Francisco, San Francisco, CA; 2) ARL Division of Biotechnology, University of Arizona, Tucson, AZ; 3) Department of Bioengineering and Therapeutic Sciences, Univ California, San Francisco, San Francisco, CA; 4) Dept of Ecology and Evolutionary Biology, Univ Arizona, Tucson, AZ.

We generated whole-genome sequences from four Biaka pygmies and analyzed them along with the publicly available genomes of 69 individuals from a range of different ethnicities. We scanned each of the 73 genomes for regions with unusual patterns of genetic variation that might have arisen due to ancient admixture with an `archaic' human group. While a majority of the most extreme regions were really misalignment errors, we did find hundreds of regions that likely introgressed in from archaic human ancestors, and we estimate the amount and the timing of these ancient admixture events. These regions were found in the genomes of both sub-Saharan African and non-African populations. While Neandertals are a natural source population for ancient admixture into non-Africans, the source for ancient admixture into sub-Saharan African populations is less obvious.

471W

Positive Selection, Purifying Selection, and Neo-Functionalization in Human Disease-Related Zinc-Finger Proteins. *G.J. Wyckoff[1], J.H. Laity[2], L.S. Feng[2], A. Solidar[3].* 1) Div Molec Biol & Biochem, Univ Missouri-Kansas City, Kansas City, MO; 2) Div. Cell Bio. and Biophysics, Univ Missouri-Kansas City, Kansas City, MO; 3) Vassa Informatics, Kansas City, MO.

Neofunctionalization, defined as the creation of novel functions from existing genetic functionality, is one of the cornerstones of molecular evolutionary genetics. Without this process, the genetic toolkit would be limited to a small set of functions derived early in evolutionary time, or to those functions that arose via completely independent, random mutation of silenced genes (functional recapture) or from changes to non-functional segments that have become functional due to random changes. However, while neo-functionalization often involves the processes of random mutation, duplication, and positive selection, many different scenarios have been examined. Here, we examine a set of Cys2-His2 (C2H2) zinc-finger proteins. The zinc finger containing family of proteins is a large and relatively diverse group; over 1200 members can be found in Genbank. C2H2 Zinc finger proteins are well represented in this group, and are usually found functioning in traditional roles of mRNA- or DNA-binding . However, a portion of C2H2 zinc fingers have been found that act in tandem to form metal-responsive elements, and these zinc fingers operate only in tandem where one finger stabilizes the other finger so it can perform DNA or RNA-binding functions. These “two-finger” zinc fingers, far from being disparate beads on a string, are functionally interconnected. This represents a novel, regulatory function after a random mutation produced what appears to be a neutral mutation; the novel, selected function apparently only came into being after an internal duplication of a zinc-finer domain within an already functional protein. Further, we show that this new function is under a large degree of purifying selection, and the position of the initial random mutation appears to be “locked in” over evolutionary time, both within and between zinc-finger proteins. These genes are especially relevant, as members within this family have essential roles in development, and many have been implicated in genetic disorders and cancers.

472W

A population genetics analysis of MetaboChip data comparing African-American and European-descent populations. *W.S. Bush[1], K.E. North[2], S. Buyske[3], B. Voight[4], D.C. Crawford[1], the PAGE study.* 1) Ctr Human Gen, Vanderbilt Univ, Nashville, TN; 2) Department of Epidemiology and Carolina, Center for Genome Sciences, University of North Carolina Chapel Hill Chapel Hill, NC; 3) Department of Statistics & Biostatistics, Rutgers University, Piscataway, NJ; 4) The Broad Institute of Harvard and MIT, Boston, MA.

Studying the genetic differentiation of human sub-populations can provide valuable clues about the underlying causes of disease and differences in their prevalence. To date, most population genetic studies have been conducted using reference samples from the International HapMap Project or 1000 Genomes Project (1KGP), each of which provide extensive coverage of either common or complete variation, but both of which have limited sample sizes. The Population Architecture using Genomics and Epidemiology (PAGE) study is a network of several population-based studies of diverse populations, including the Atherosclerosis Risk in Communities (ARIC), the Multiethnic Cohort, and the Women's Health Initiative. The goals of PAGE are several-fold including the generalization and characterization of genome-wide association study (GWAS)-identified variants and genomic regions in African Americans. To this end, we typed the Illumina MetaboChip, a custom BeadChip of ~200K variants that targets GWAS-identified variants as of 2010, rare variants from the pilot 1KGP, and fine-mapping variants for ~250 regions associated with metabolic diseases in 5,897 African American PAGE participants. These PAGE MetaboChip data provide an unprecedented opportunity to compare the allele frequencies of common and less frequent SNPs in thousands of individuals. In this work, we explore differences between African Americans and European-descent populations to identify variants that show evidence of population divergence and evolutionary pressure. Using Wright's fixation index (Fst), 3632 SNPs with Fst > 0.15 indicating moderate population differentiation. Of these, 12 SNPs have been related to phenotypes in previous GWAS studies, including Crohn's disease, height, freckles and skin pigmentation, coronary heart disease and hypertension, rheumatoid arthritis, celiac disease, and colorectal cancer. This work provides further insight into the genetic architecture of metabolic traits, and highlights loci that may have arose due to environmental adaptations.

473W

A comparative study of genetic and epigenetic regulatory mechanisms in primates. *C.E. Cain[1], A.A. Pai[1], R. Pique-Regi[1], J.F. Degner[1], M. Myrthil[1], N. Lewellen[1], J.K. Pritchard[1,2], Y. Gilad[1].* 1) Department of Human Genetics, University of Chicago, 920 E. 58th Street, Chicago IL 60637; 2) Howard Hughes Medical Institute, University of Chicago, 920 E. 58th Street, Chicago IL 60637.

Changes in gene regulation are thought to play an important role in adaptation and speciation, especially in primates. However, the extent to which changes in different regulatory mechanisms underlie gene expression evolution is not yet known. To address this gap, we investigated the contribution of changes in different genetic and epigenetic regulatory mechanisms to inter-species differences in gene expression levels. Specifically, we collected gene expression data and obtained genome-wide profiles of epigenetic markers, transcription factor binding, and markers of chromatin state in lymphoblastoid cell lines (LCLs) from humans, chimpanzees, and rhesus macaques. We found strong evidence for the conservation of histone modification localization and DNA methylation patterns. Regardless of species, highly expressed genes are more likely than lowly expressed genes to have epigenetic profiles characterized by presence of the histone modification H3K4me3 and absence of DNA methylation at their proximal promoters. Moreover, we observed an enrichment of inter-species differences in epigenetic marks in genes that are differentially expressed between species. We found evidence for regulatory changes due to turnover in transcription factor binding due to inter-species differences in the transcription factor protein (trans mechanism) or to the binding sites (cis mechanism). By obtaining profiles of multiple epigenetic modifications and markers of chromatin state in the same samples, we are able to achieve a more comprehensive understanding of the combinatorial patterns that underlie differences in gene expression levels between primates.

474W

RANTES gene polymorphism in HIV-infected South African population. *N. Chin'ombe, M. Skelton, C. Dandara.* IIDMM, Human Genetics, University of Cape Town, Observatory 7925, Cape Town, South Africa.

Introduction Several host restriction gene polymorphisms have been linked to susceptibility to HIV infection. Limited information on these polymorphisms is available in the African population. The RANTES (regulated upon activation normal T cell expressed and secreted), a well-characterized AIDS restriction gene, encodes a ligand for CCR5. A single nucleotide polymorphism (In1.1C) in the intron of RANTES has previously been shown to reduce the expression of the gene, thereby increasing the chance of HIV infection and accelerated disease progression. Objective To investigate frequency of RANTES In1.1C gene polymorphism in the South African population infected with HIV. Methods DNA was isolated from blood of HIV-infected patients and health control subjects. Polymerase chain reaction was used to amplify the RANTES allele. Restriction fragment length polymorphism method was used to determine the genotypes (In1.1 T/T, T/C and C/C) of the RANTES. Results Out of the 281 South African individuals infected with HIV, 7 (2.5%) were homozygous to the RANTES In1.1C allele, 92(32.7%) were heterozygote carriers (In1.1T/C) and 182 (64.8%) had the normal allele (In1.1T/T). The allele frequency for the RANTES In1.1C was 0.19 in this population. In the control population (n = 95), 2.2% carried the In1.1C/C genotype and the allele frequency was 0.16. Conclusion The RANTES In1.1C allele is present in the South African population.

475W

Analysis of the genetic basis of disease in the context of human migration. *E.C. Corona[1], R.C. Chen[1], C.P. Patel[1], A.M. Morgan[1], M.S. Sikora[2], C.B. Bustamante[2], A.B. Butte[1].* 1) Biomedical Informatics, Stanford University, Stanford, CA; 2) Genetics, Stanford University, Stanford, CA.

Genetic diversity across different human populations can inform on our current understanding of the genetic-basis of disease. The effects of migration on the emergence or regression of the genetic contribution to complex disease susceptibility have never been comprehensively studied and contrasted. We estimate the genetic contribution to disease susceptibility of 173 diseases in 1043 unrelated individuals across 51 populations of the Human Genome Diversity Panel. The genetic risk of each disease is estimated for all populations by combining all known genome-wide association studies published with each population's genetic substructure. By incorporating a phylogenetic tree establishing worldwide relationships across the 51 studied populations, we estimate when and where genetic risk of each disease underwent differentiation exceeding what is expected by genetic drift. We observe that genetic risk of Type 2 Diabetes underwent strong differentiation towards decreased risk in the ancestral population common to Native Americans and East Asians. Membranous Nephropathy underwent differentiation towards decreased risk gradually as populations migrated Eastward. Alzheimer's disease underwent differentiation towards decreased risk solely in two East Asian populations (Dai and Cambodia). This analysis represents the first comprehensive characterization of disease susceptibility differentiation associated with migration events. We anticipate that by highlighting groups of populations that show differences in disease susceptibility along migration trajectories, our findings will enable detailed analysis pertaining to the driving forces behind population differences in the genetics-basis of disease susceptibility.

476W

Conserved SNPs are enriched for disease-susceptability loci and eQTLs in LCLs. *P. Evans, E. Gamazon, N. Cox.* Gen Med, Univ Chicago, Matteson, IL.

Finding functional variation in the human genome has the potential to increase our ability to find the genes responsible for a number of complex diseases. Much work has already been done on annotating the genome but little is still known about functional elements outside of genes. However, if we can restrict our searches of genomic variation to only variants that are functional then we can greatly reduce the penalty for multiple testing as most variation should play no role in disease. This has been done for many new gene-based tests. One example is using eQTL information from a given tissue to link functional variation to specific genes. This has the limitation that the information garnered may not be informative unless you are looking in the relevant tissue-type for a given disease. One solution to this problem is to use nontissue-dependent types of information to find variation that is likely to be functional. One possible alternative are SNPs that reside within evolutionarily conserved elements within the genome. Over long enough time lines, non-functional sites in two related species should vary between genomes, while elements that are functional will remain constant as purifying selection removes any variants that arise in these elements. Therefore by looking between sequences of two or more species that are suitably divergent, we can find elements that are likely to be functional. This would allow us to find functional SNPs that would not be dependent on tissue type, although some of these conserved elements may have regulatory functions that are tissue-specific. We have used the conserved elements defined by Pollard et al, Genome Research 2010, to find SNPs in HapMap in these conserved elements. SNPs in conserved elements are highly enriched for eQTLs in lymphoblastoid cell lines, showing that these SNPs are likely to have function. SNPs from the NHGRI catalog of published GWAS containing reproducibly associated SNPs for a variety of diseases are also enriched for SNPs in conserved elements, indicating these SNPs are also relevant for disease.

477W

Genetic of LCT gene in Amerindian populations from Brazil. *D.C. Friedrich[1], S.M. Callegari-Jacques[2], F.M Salzano[1], M.L. Petzl-Erler[3], L.T. Tsuneto[3], M.H. Hutz[1].* 1) Department of Genetics, UFRGS, Porto Alegre, RS, Brazil; 2) Department of Statistics, UFRGS, Porto Alegre, RS, Brazil; 3) Department of Genetics, UFPR, Curitiba, PR, Brazil.

The LCT gene encodes lactase enzyme, which is responsible for the digestion of lactose. During early childhood, lactase level declines dramatically but some people maintain lactase secretion during their whole life, a condition known as lactase persistence (LP). LP is due to mutations in the LCT gene enhancer, located 13Kb upstream the gene. In Europeans, the -13910T allele is associated with LP. In Africans this polymorphism is almost absent while other mutations in this same region were related to LP. The LCT gene is highly polymorphic in human populations, a total of 36 haplotypes have been described, but so far Amerindians were not investigated for these polymorphisms or for the presence of LP mutations. In this study, the LCT gene and the presence of LP allele were characterized in four Native Brazilian populations. The sample consisted of 316 individuals from Guarani-Kaiowá, Guarani-Ñandeva, Kaingang, and Xavante populations. Twelve polymorphisms were genotyped by PCR-based methods or by sequencing. The -13910T allele associated with LP varied from 0.5% in the Xavante to 7.6% in the Guarani-Ñandeva, and the frequencies differ significantly between Kaingang and Xavante, Xavante and Guarani-Ñandeva, and between Guarani groups. The -13910T frequency is probably of European origin and its prevalence correlates with non-native admixture proportions previously described for these populations. The LCT gene was highly polymorphic in this ethnic group, 15 haplotypes were observed with a heterogeneous distribution among these Native Brazilian groups. All populations had significant differences in haplotype frequencies. The Guarani-Kaiowá presented an excess of LCT A, E, and U haplotypes, while the C was scarcity. An excess of the A haplotype was also observed in the Guarani-Ñandeva. The Xavante showed an excess of C, and a lack of A, and E haplotypes, whereas the Kaingang did not differ from the average haplotype frequencies. Multidimensional Scaling using F_{ST} distance showed a gradient on the distribution of these four populations, the Xavante was the most isolated and Guarani-Kaiowá was closer to Asian and Southern European populations. In each population, the LP -13910T allele occurred in a different haplotype. The absence of new mutations in the -13Kb enhancer, the absence of pastoralism reports in these groups, and LCT haplotype distribution in present day-Amerindians suggest that drift was the main factor that explains the high heterogeneity observed.

478W

Human genes with unusually recent or ancient common ancestors. *M. Hu, Y. Xue, C. Tyler-Smith.* Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

Which genetic changes have contributed to the characteristics specific to modern humans is a question of great interest to biologists. Among other approaches, this question could be addressed by identifying regions that share a very recent common ancestor among all humans. We performed a genome-wide scan for regions with a recent coalescent time (<200,000 years) using published high-coverage genome sequences of 11 unrelated individuals from European, Asian and African populations, applying the genetree* package. However, due to the lack of consistency of those genome sequences produced by different platforms, data had to be heavily filtered so the global distribution of the coalescent times across the genome was skewed to younger ages. We are applying the same approach to a diverse collection of high-coverage whole genome sequences from 54 unrelated humans released by Complete Genomics Inc. (http://www.completegenomics.com/), which have higher data consistency. We also calculate the divergence/diversity ratio among those genomes compared to other primates. Regions with high divergence/diversity ratios or recent common ancestors may have contributed significantly to the evolution of modern humans. On the other hand, regions sharing very ancient common ancestors (>2 million years ago) among humans may have entered our gene pool from other species of archaic hominin, or undergone balancing selection, where maintaining a high level of polymorphism for a long period has been advantageous. Our scan of the 11 genomes also unveiled regions in the human genome with potential ancient common ancestors, and we are also looking for ancient regions in our scan on the new 54 genomes. Our preliminary results point to some novel genes showing evidence of potential balancing selection. *Bahlo, M. & Griffiths, R. C. Inference from gene trees in a subdivided population. Theor Popul Biol 57, 79-95 (2000).

479W

Population history and paternal gene flow in the Ch'orti' Maya of Eastern Guatemala. *A.E. Justice, S.M. Johnson, M.H. Crawford.* Department of Anthropology, University of Kansas, Lawrence, KS.

Ch'orti' Maya is spoken mainly in Eastern Guatemala, western Honduras, and El Salvador. The Ch'orti' Maya have occupied eastern Guatemala and western Honduras for ~2,000 years. When the Spanish invaded the Ch'orti' in 1524, there were approximately 120,000 Mayans inhabiting the region. During the 19th and early 20th century German, French, and U.S. investors moved to the region. As a result of the introduction of African slaves in the 1500s, the attempted Ladinoization of the Indians from the 1700s through 1940s, and the negativity associated with being an Indian into the 1990s, there was cultural and biological admixture among the Indians, Africans, and Europeans. This study aims to characterize the source and level of admixture within the Ch'orti' Maya while making inferences into their historical relationships with surrounding Central Americans. DNA was extracted from 21 males residing around Jocotán, Chiquimula, Guatemala. Y SNPs were characterized using HyBeacons® PCR probes, and STRs (DYS-19, 385 a/b, 389 I/II, 390, 391, 392, 393, 438, and 439) were characterized using AFLP on an Applied Biosystems ABI Prism 3130 for haplogroup assignment. Haplotype diversity, locus diversity, and MPD were computed to characterize the intra-population variation. Slatkins Rst, R-matrix analysis, and SAMOVA were used to highlight the relationship among populations in Central America. Native American haplgroups are present in the majority of the sample, (62% haplotype Q1a3a, and 10% Q1). The majority of non-native admixture is from Europe, most likely the Iberian Peninsula (Haplogroups R1b, I, and J). The Ch'orti exhibit higher than expected haplotype diversity compared to surrounding populations, but average gene diversity and MPD. The difference in diversity measures for the Ch'orti' is likely the result of a high occurrence of low frequency haplotypes. Regardless of their geographic proximity and historical relationship to Honduran Native Americans, the Ch'orti' have a closer relationship to the paternal lineages present in El Salvador natives and Panamanian natives. Despite a long history of colonization and slavery, the Ch'orti' retain a majority of Native American Y lineages. However, the effects of colonization, including population decline and forced migration, have had an effect on the genetic structure of these paternal lineages. These events are reflected in the presence of Mediterranean Y Haplogroups and a high occurrence of low frequency haplotypes.

480W

Genetic affinities of Lakshadweep Islanders. *S. Justin Carlus[1], K. Gopal[1], A. Mishra[1], P.S. Gireesha[2], A. Rastogi[3], A.J. Francis[1], R. Tamang[1], A.G. Reddy[1], L. Singh[1,4], K. Thangaraj[1].* 1) Centre for Cellular and Molecular Biology, Hyderabad, India; 2) Yogi Vemana University,Kadapa, Andhra Pradesh, India; 3) Amity Institute of Biotechnology, Amity University, Noida, India; 4) Genome Foundation, Hyderabad, India.

The Indian subcontinent is legendary for the cultural, linguistic and genetic diversity of its inhabitants. Lakshadweep is one of the union territories of India, consisting of 36 islands located in the Arabian Sea. Till date there is no genetic study that has been carried out on the populations of this islands. Therefore, we have undertaken this study to establish the genetic affinities of the Lakshadweep islanders. About 5 ml of blood samples from 880 healthy and unrelated individuals inhabited in different Islands of Lakshadweep (Kavaratti, Agatti, Kiltan, Andrott, Kalpeni, Minicoy and Chetlat Islands) were collected with informed written consent. We have typed with the uniparentally inherited Y-chromosome and mitochondrial DNA (mtDNA) markers. Based on the mutations observed, we have assigned haplogroup to every individual. Our results showed that all the samples fell under the Indian- and WestEurasian-specific haplogroups; M and N, respectively. Further analysis showed that existence of several sublineages of Macrohaplogroups M (M2, M25, M3, M3a, M33a, M4, M4'30 and M5) and N (U2, U7, U9 R5, R7 and R9). In order to establish the paternal lineages, we screened with 12 Y-chromosomal biallelic markers viz. M9, M175, M45, M82, M69, M70, M173, M62, M124, M17, M89 and APT. Haplogroups M17-R1a and M172-J2 were observed in high frequency. Additionally, haplogroups such as M69-H, M11-L and M124-R2 were noticed in significant proportion. There are some M45-P*, M9-K*and M89-F* derived samples in less frequencies. Our mtDNA and Y-chromosomal DNA analysis revealed the existence of WestEurasian and Indian gene pool among the Lakshadweep populations.

481W

Inferences of demographic history and selection in Bornean and Sumatran Orangutan genomes. *J.L. Kelley[1], X. Ma[1], K.E. Eilertson[2], R.N. Gutenkunst[3], C.D. Bustamante[1].* 1) Department of Genetics, Stanford University, Stanford, CA; 2) Department of Statistical Science, Cornell University, Ithaca, NY; 3) Molecular and Cellular Biology, University of Arizona, Tucson, AZ.

Analysis of genetic variation in orangutans, our most distantly related Great Ape, can provide insight into forces that shape the Great Ape genome. To understand recent orangutan demographic history, we first analyze the Bornean and Sumatran Orangutan joint site frequency spectrum from 10 individuals. Five individuals from each species were sequenced with a median read depth of over 8x. Model-fitting using DaDi indicates that the two populations diverged 400,000 years ago, similar in time to the human-Neanderthal split. Our demographic inference shows that the Sumatran population has a current effective population size four times larger than the Bornean population. Additionally, the distribution of selection coefficients we infer from the frequency spectrum of non-synonymous polymorphisms is consistent with the previously estimated distribution in humans. To identify particular genes under selection, we further apply a generalized linear mixed model to estimate genome-wide variability among coding sites in the genome. Of the over 7,200 annotated orthologs queried using SNIPRE (selection inference using Poisson random effects), only 32 are best explained by a model of positive selection between orangutans and humans. Nearly 50% of loci have negative selection coefficients between human and orangutan, suggesting that constraint and conservation are predominant drivers of gene evolution in Great Ape genomes. The distribution of deleterious alleles between the two species, as measured by variation at conserved sites, is surprisingly similar given their different demographic histories. Together, these findings suggest that although the diets and environments experienced by orangutan and human are quite different, similar pressures may have acted on both orangutan and human genomes.

482W

Comparing genetic diversity and evolutionary implication of 439 obesity-related genes between populations in the Human Genome Diversity Panel (HGDP). *J. kim[1], M. Lee[1], Y. Yoo[2], J. Sung[1].* 1) Graduate School of Public Health, Seoul National University, Seoul, Korea; 2) Department of Mathematics Education, Seoul National University, Seoul, Korea.

BACKGROUND: Obesity and its complications are important risk factors of many common diseases and have become a priority problem in global health. Genome-wide association (GWA) studies have accumulated a list of genes responsible for obesity, but only a part of genes are replicated across populations. Previous studies have indicated population differences in susceptibility to obesity and to complications of obesity. To explain this difference, a “thrifty gene hypothesis” has been proposed, without being vindicated by objective evidence. Recent deluge of GWA studies has revealed hundreds of obesity-related genes, which provide targets for systematic population genetic evaluation. OBJECTIVE: We identify genes that show significant population differences in allele frequency across populations of HGDP, and for those different genes we test evidence for selection. METHOD: We examine 439 obesity related gene regions that have been replicated by independent genome-wide association studies using Human Genome Diversity Panel (HGDP) and Korean Association Resource (KARE) project samples. SNP genotypes in these regions obtained from 500~600k genome-wide arrays are used. With HGDP data, we select top 30 genes that show largest population differences in allele frequencies. For these 30 genes, extended haplotype homozygosity (EHH) and cross population extended haplotype homozygosity (XP-EHH) tests were performed to evaluate the presence or degree of positive selection in seven populations including East Asians, Europeans, Sub-Saharan Africans. RESULTS : Several obesity genes showed substantial variation in allele and haplotype frequency patterns across populations groups and we found evidence for possible positive selection in some populations. For MCHR1 gene, one core haplotype (4 SNPs) is most frequent in Americans (89%), but not in Other population (0~17%). In addition, this haplotype contains rs133072 that is replicated in previous GWAS and demonstrated relatively long range LD with slow EHH decays in Americans but not in other population groups suggesting positive selection in Americans. For ALDH2 gene, similar result is found in East Asians. CONCLUSION: We identified some obesity related genes with different SNP frequency distribution patterns and found some evidences for positive selection in some populations for those genes. This result may help to reveal the cause of obesity.

483W

Ascertainment Bias in Microsatellites: Impact on Estimates of Mutation Rates. *M. Kimmel, B. Li.* Departments of Statistics and Bioengineering, Rice University, Houston, TX, USA.

Microsatellite loci play an important role as markers for identification, disease gene mapping and evolutionary studies. Mutation rate, which is of fundamental importance, can be obtained from interspecies comparisons, which however are subject to ascertainment bias. This bias arises when a locus is selected based on its large allele size in one species (cognate species 1), in which it is first discovered. This bias is reflected in average allele length in any non-cognate species 2 being smaller than that in species 1. This phenomenon was observed in various pairs of species, including comparisons of allele sizes in human and chimpanzee. Various mechanisms were proposed to explain ascertainment bias. Here, we examine the framework of a single-step asymmetric and unrestricted stepwise mutation model with genetic drift. Analysis is based on coalescence theory. The mechanism of ascertainment bias in this model is a tighter correlation of allele sizes within a cognate species 1 than of allele sizes in two different species 1 and 2. We present computations of the expected bias, given the mutation rate, population sizes of species 1 and 2, time of separation of species 1 and 2, and the age of the allele. In particular, we show that when the past demographic history of the cognate and non-cognate taxa are different, the rate and directionality of mutations will impact the allele sizes in the two taxa differently than the simple effect of ascertainment bias. This effect may exaggerate or reverse the effect of difference in mutation rates. We reanalyze literature examples, and conclude that despite the bias, the microsatellite mutation rate in human exceeds that in chimpanzee.

484W

Lactase persistence: New variants and evidence for function. *A. Liebert[1], T. Danielsen[2,3], A. Krüger Olsen[2,3], J.T. Troelsen[2,3], D.M. Swallow[1].* 1) Research Department of Genetics, Evolution and Environment, University College London, Darwin Building, Gower Street, London WC1E 6BT, UK; 2) Department of Sciences, Systems and Models, University of Roskilde, Universitetsvej 1, DK-4000 Roskilde, Denmark; 3) Department of Cellular and Molecular Medicine, University of Copenhagen, Panum Institute, Building 6.4, Blegdamsvej 3B, DK-2200 Copenhagen N, Denmark.

Background The ability to digest lactose in milk in adulthood differs between people. Only 35% of the world population show the phenotype of lactase persistence (LP), as lactase expression is usually down regulated after weaning. LP is at highest frequency in Northern Europe where it is caused by a mutation upstream of the lactase gene (*-13.910*T* in intron 13 of the adjacent *MCM6* gene). However, *-13.910*T* is absent in many milk drinking pastoralist groups in Africa where several other putative causal alleles have been identified. These nucleotide substitutions are located within 100 bases of each other, in a region that has been shown *in vitro* to have enhancer function. Four of the variants have been studied functionally so far but there is patchy information on the distribution of these and the other variants. *Aims and methods* We have examined the distribution of *-13910*T* around the periphery of Europe and the extent of spread of the African alleles out of Africa, as well as searching for new functional variant alleles. We have sequenced DNA from individuals from 40 populations of Europe, Asia and the Middle East, and conducted transfection and gel shift assays on some of the variants. *Results -13910*T* is the predominant derived allele throughout the whole of Europe while this and the alleles first described in Africa are present together in the Middle East, although other rare alleles were also found. In all, we identified a total of 17 variants in the enhancer region, of which 8 are described here for the first time. We selected 3 variants that had not previously been studied and showed that they also have function *in vitro*. The altered binding of transcription factors to probes containing these derived alleles has been confirmed by supershift and/or competition experiments. Higher enhancer activity has also been shown in reporter gene assays. *Conclusion* Many different mutations in the enhancer region appear to alter lactase gene activity.

485W

Studying the impact of Old World diseases on Native American populations via ancient DNA. *B. Llamas[1], L. Quintana-Murci[2], A.L. Hughes[3], A. Cooper[1], W. Haak[1].* 1) Australian Centre for Ancient DNA, University of Adelaide, Adelaide, SA, Australia; 2) Genome and Genetics, Institut Pasteur, 75015 Paris, Fance; 3) Department of biological Sciences, University of South Carolina, Columbia, SC 29208, USA.

There is good evidence to suggest that prior to the arrival of the Spanish in 1492, South and Central American populations were the largest of any in the world. However, censuses performed by Spanish functionaries less than a century later show a drastic decline in both population size and distribution. Several accounts estimate that up to 95% of the indigenous population of the Americas died between the period of initial contact and the beginning of population recovery. Consequently, contact between the Eastern and Western Hemispheres in the sixteenth century represented an adaptive transition that shaped modern human biocultural diversity on a global scale.

We propose to use ancient DNA techniques to explore the selective pressures exerted by new pathogens on human populations. Our project aims are: i) to generate the first real-time picture of the genetic changes induced by epidemics in human populations using ancient DNA from a large number of specimens from pre- and post-Contact Native populations from South and Central America; and ii) to describe the genetic diversity of Native populations prior to European contact to test the hypothesis that they were immunologically `naïve' to Old World diseases, and suffered very high levels of mortality as a consequence.

Ongoing research efforts include mitochondrial and Y-chromosome genotyping using multiplex SNP analysis, the development of DNA capture methods targeting immunogenes in ancient DNA libraries, and the use of Next Generation Sequencing platforms.

Major outcomes expected from this project are a better comprehension of the biological impact of colonisation on indigenous groups, as well as a better understanding of the parameters and driving forces behind human evolutionary history.

486W

Phylogenetics and phase: Comprehensive mapping of human genetic variation associated with the age of onset of late-onset Alzheimer's disease. *M.W. Lutz[1,2], D.G. Crenshaw[1,2], A.M. Saunders[1,3], I. Grossman[4], O. Chiba-Falek[1,2], D.K. Burns[4], A.D. Roses[1,2,3,4].* 1) Deane Drug Discovery Institute, Duke University, Durham, NC; 2) Institute for Genome Policy and Sciences, Duke University, Durham, NC; 3) Neurology, Duke University Medical School, Durham, NC; 4) Zinfandel Pharmaceuticals, Inc, Durham NC.

The unique nucleotide content of each of the two homologous chromosomes that an individual carries (genetic phase) has been shown to have a profound effect on allele-specific gene expression and protein function that may contribute to clinical phenotypes or disease. Analysis of phylogenetic trees and networks is a powerful approach to investigate the relationships between phased sequence data and phenotype, particularly when mechanisms of reticulate evolution are incorporated into the analysis. We analyzed data available from two late-onset Alzheimer's disease (LOAD) association studies: 1) Lescai et al. (2010) reported association for three APOE promoter SNPs (-219, -491 and -427) and; 2) Roses et al. (2010) reported association for a poly-T length polymorphism in TOMM40 locus (rs10524523 referred as “523”). A phylogeny was constructed from DNA sequences in the TOMM40-APOE linkage disequilibrium (LD) region (Chr19 50,092,405 - 50,101,584, NCBI version 36) from LOAD patients and controls. Analysis revealed that the three SNPs in the APOE promoter were in linkage with specific “523” alleles. The major findings were: 1) The T allele of -219 always maps to a major clade on the phylogenetic tree that is associated with a higher risk for LOAD and an earlier age of onset for LOAD relative to the other major clade; and 2) the T allele of -219 always co-occurs with either a Long “523” allele (20-29 Ts) linked to APOE ,4 or a Very Long “523” allele (>29 Ts) linked to APOE ,3 while the -219 G allele co-occurs with Short “523” alleles (<20 Ts). A specific SNP in TOMM40 (rs8106922) was found as the most significant variant which separates the two major clades of the tree, while “523” remains the variant most highly associated with differences in age of onset of LOAD. Other SNPs, including the APOE promoter polymorphisms, map uniquely to specific “523” alleles. Our results demonstrate that phylogenetic analysis can reconcile results of independent studies of phased sequence data and the age of onset of LOAD and provide a comprehensive mapping of human genetic variation associated with LOAD age of onset. These data further demonstrate the importance of using phylogenetic analysis with phased sequence data for other disease gene identification studies.

487W

From Essentiality to Redundancy: Evolutionary Genetics Dissection of the Human Interferon Families. *J. Manry[1,2], E. Patin[1,2], G. Laval[1,2], S. Fornarino[1,2], L.B. Barreiro[3], M. Tichit[4], M. Fumagalli[5], M. Sironi[5], Y. Itan[6], J.L. Casanova[6], C. Bouchier[4], L. Quintana-Murci[1,2].* 1) Human Evolutionary Genetics, Department of Genomes and Genetics, Institut Pasteur, Paris, France; 2) Centre National de la Recherche Scientifique, Paris, France; 3) Department of Pediatrics, University of Montréal, Montréal, Canada; 4) Pasteur Genopole, Institut Pasteur, Paris, France; 5) Scientific Institute IRCCS E. Medea, Bosisio Parini, Italy; 6) St Giles Laboratory of Human Genetics of Infectious Diseases, New York, USA.

Detecting how natural selection has acted upon genes involved in host defense and immunity-related processes can provide insights into the mechanisms that mediate the eradication of infection. Interferons (IFNs) are helicoidal cytokines that play a key role in innate and adaptive immune responses. Most IFNs present an antiviral activity and are intercellular mediators able to modulate several major biological functions, such as cell proliferation and differentiation, or lymphocyte activation. Given the multiple genes that encode IFNs, it is now a priority to delineate those that are essential with respect to those that are more redundant in the immune response. Through the resequencing of the 27 genes encoding the human IFNs and their receptors in a panel of individuals from different ethnic backgrounds, our population genetics data revealed that some members of the type-I IFN family, with the most extreme cases being *IFNA6* and *IFNA8*, as well as the only member of the type-II IFN family, *IFNG*, fulfill essential functions in host defense. By contrast, the fact that other type-I IFNs, such as *IFNA10* and *IFNE1*, accumulate missense and nonsense mutations at high population frequencies suggests higher redundancy and pseudogenization. Finally, type-III IFNs appeared to be mainly targeted by recent positive selection, especially among non-African populations, owing to increased resistance to viral pressure. Taken together, our evolutionary data reveal that the different members of the human IFN families differ in their biological relevance, given their varying essential or adaptive behaviors, suggesting that some genes may represent better targets for clinical studies than others.

488W

Transmission distortion observed in human pedigrees. *W.K. Meyer[1], C. Ober[1, 2], R.R. Hudson[3], M. Przeworski[1,3,4].* 1) Dept. of Human Genetics, University of Chicago, Chicago, IL; 2) Dept. of Obstetrics and Gynecology, University of Chicago, Chicago, IL; 3) Dept. of Ecology and Evolution, University of Chicago, Chicago, IL; 4) Howard Hughes Medical Institute, University of Chicago, Chicago, IL.

Children of a heterozygous parent are expected to carry either allele with equal probability. Exceptions can occur, however, due to meiotic drive, competition among gametes, or viability selection, processes which we collectively term “transmission distortion” (TD). Although there are a number of well-characterized examples of these phenomena, the existence of transmission distortion in humans remains unknown. We therefore performed a genome-wide scan for regions experiencing transmission distortion within human populations. To this end, we applied the transmission disequilibrium test (TDT) to nuclear families belonging to three large sets of pedigrees of European descent that have been genotyped on genome-wide single nucleotide polymorphism (SNP) arrays: the Framingham Heart Study (FHS), a founder population of European origin (HUTT), and a subset of the Autism Genetic Resource Exchange (AGRE). Genotyping error is an important confounder in this type of analysis. In the FHS and HUTT, despite extensive quality control, we did not find sufficient evidence to exclude genotyping error as the source of the strongest signals. In the AGRE, however, many TD signals extend across multiple SNPs, a pattern highly unlikely to arise from genotyping error. These point to several promising candidate regions, notably a locus on chromosome 10q26.13 displaying strong evidence for transmission distortion and the signature of recent positive selection. Focusing on paternal transmissions, we also detected a signal in 6p21.1, a region that had been previously been suggested as experiencing TD from the analysis of a small sample of European males. In signals derived from maternal transmissions, we found a strong enrichment for genes involved in cell junctions. We found no strong maternal TDT signals near centromeres or telomeres, the chromosomal regions predicted to be most susceptible to meiotic drive in females. These results demonstrate the existence of ongoing segregation distortion or strong viability selection in contemporary human populations and suggest the involvement of cell junction-related loci in maternal distortion.

489W

Impact of restricted marital practices on genetic variation in a distinctive endogamous population. *T.J. Pemberton[1,2], F-Y. Li[1,3], E.K. Hanson[4], N.U. Mehta[1,5], G. Mendoza-Fandino[1,5], J. Ballantyne[4], J.W. Belmont[3], N.A. Rosenberg[2,6,7], C. Tyler-Smith[8], P.I. Patel[1,5,9].* 1) Institute for Genetic Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA; 2) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 4) National Center for Forensic Science, University of Central Florida, Orlando, FL; 5) Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA; 6) Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI; 7) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 8) The Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 9) Center for Craniofacial Molecular Biology, Herman Ostrow School of Dentistry, University of Southern California, Los Angeles, CA.

Social stratification in India is evident as social classes that are defined by endogamous groups known as castes. Within a caste, there exist endogamous groups known as *gols* (marriage circles), which comprise a small number of exogamous *gotra* (lineages). Thus, while consanguinity is strictly avoided and there is some randomness in mate selection within the *gol*, gene flow is limited with populations outside of the *gol*. Gujarati Patels practice this form of “endogamic exogamy.” We have analyzed genetic variation in one such group, the Chha Gaam Patels (CGP), which comprises Patels from six villages. Population structure analysis of 1,200 autosomal loci offers support for distinctive multilocus genotypes in the CGP with respect to both non-Gujaratis and other Gujaratis, and indicates that individuals from the six villages are genetically extremely similar. Analysis of Y-chromosomal and mitochondrial haplotypes provides support for both patrilocal and patrilineal practices within the *gol*, and a low-level of female gene flow into the *gol*. Time to the most recent common ancestor estimates based on Y-chromosomal haplotypes are consistent with historical accounts of the settlement of the six villages by Patels between 1155-1575 CE. Our study illustrates how the practice of *gol* endogamy has likely created isolated groups of individuals throughout India and thereby introduced fine-scale genetic structure into the population of India.

490W

Patterns of shared polymorphism between humans and chimpanzees. *S. Pfeifer[1], E. Leffler[2], A. Fledel-Alon[2], A. Auton[3], R. Bowden[1,3], O. Venn[3], S. Melton[2], Z. Iqbal[3], I. Turner[1], L. Segurel[2], R. Hernandez[4], J. Maller[1,3], J. Broxholme[3], R. Bontrop[6], G. Lunter[3], P. Humburg[3], S. Myers[1,3], P. Donnelly[1,3], M. Przeworski[2,5], G. McVean[1,3].* 1) University of Oxford, Department of Statistics, 1 South Parks Road, OX1 3TG Oxford, United Kingdom; 2) University of Chicago, Department of Human Genetics, 920 East 58th Street, Chicago, IL, 60637, United States; 3) Wellcome Trust Centre for Human Genetics, Roosevelt Drive, OX3 7BN Oxford, United Kingdom; 4) University of California, Department of Bioengineering and Therapeutic Sciences, San Francisco, CA 94143-0912, United States; 5) Howard Hughes Medical Institute, 920 East 58th Street, Chicago, IL, 60637, United States; 6) Biomedical Primate Research Centre, Lange Kleiweg 161, 2288 GJ Rijswijk, Netherlands.

Genetic polymorphisms that are shared between related species may result from either recurrent mutation or the maintenance of ancestral variation. As part of a project to map genetic variation in our closest living relatives, we have sequenced 10 Western chimpanzees (*Pan troglodytes verus*) to 8-10X and have identified over 4 million high confidence SNPs and short indels in regions of orthology to humans. We find that approximately 1% of polymorphic sites in chimpanzees are also variable among the 60 individuals from the Yoruba population sequenced as part of the 1000 Genomes Project; roughly two times the number expected by chance. Approximately two thirds of shared polymorphic sites also share the same alleles and of these over 60% represent mutations at putative CpG sites. However, even after accounting for known sequence context effects and local mutation rate variation, we still find a strong excess of certain mutation types, not all at CpG sites, indicating substantial heterogeneity in mutation rates at very fine scales and/or ancient-shared polymorphism. By characterising patterns of genetic variation and linkage disequilibrium (LD) around shared sites and, more specifically, searching for closely-sited variants in strong LD in both species we assess the evidence for shared ancient polymorphism.

491W

Evolutionary dynamics of co-segregating gene clusters associated with complex diseases. *C. Preuss, M. Stoll.* Genetic Epidemiology , Leibniz Institute for Arteriosclerosis Research, Muenster, NRW, Germany.

With the advent of the HapMap project and the 1000 Genomes project, genetic variation accounting for disease susceptibility and population specific differences within the human genome have become more tractable. Recent studies suggest that gene order and single nucleotide polymorphisms (SNPs) associated with complex traits are not randomly distributed throughout the genome, but tend to cluster in regions of long-range linkage disequilibrium (LD). Here, we analyzed the distribution of disease-associated SNPs based on pilot data from the 1000 Genomes project, a set of publicly available GWAS data and meta-analyses for three HapMap populations (CEU, AFR, ASN). We observed distinct clustering of disease-associated SNPs in regions of long-range LD, which harbor genes involved in immunity i.e. the interleukin cluster on 5q31 or RhoA on 3p21. In-depth analysis of these genomic regions using integrated haplotype scores (iHS) and LD patterns revealed strong signs of recent selective sweeps for disease variants within immune clusters. Interestingly, disease-associated SNPs are common in populations under positive selection but absent from chromosomes in populations without signs of recent selection. Our data suggest that clustering of immune genes is the result of continuing selection pressure (adaptive or positive) throughout the evolution leading to physical clustering to facilitate a rapid adaptation of the immune system to changing environments and challenges. As a result, genomic regions of long-range LD evolved, which facilitated hitchhiking of susceptibility variants for common, complex diseases with an inflammatory background such as Crohn's disease (CD), type I diabetes and rheumatoid arthritis.

492W

Rate and Molecular Spectrum of Spontaneous Mutations in the Human Genome Inferred from Rare Variants Found by Sequencing 202 Drug Target Genes in 14,000 Individuals. *V.M. Schaibley[1], M. Zawistowski[2], D. Wegmann[3], D. Kessner[3], M.G. Ehm[4], J.C. Whittaker[4], S.L. Chissoe[4], V.E. Mooser[4], M.R. Nelson[4], G. Abecasis[2], J. Novembre[3], S. Zöllner[2], J.Z. Li[1].* 1) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 2) Department of Biostatistics, University of Michigan, Ann Arbor, MI; 3) Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA; 4) Quantitative Sciences, GlaxoSmithKline.

Understanding the rate, variability, and genomic context of spontaneous mutation in human populations is fundamental to understanding our evolutionary history. One approach to studying spontaneous mutation is to examine extremely rare variants in humans, which have arisen recently and are largely unaffected by natural selection, biased gene conversion and population demographic history. Because rare variants are for the most part immune to these confounding factors, we can characterize mutations throughout the genome, particularly within coding regions, where common variants are influenced by selection. In collaboration between GlaxoSmithKline and the University of Michigan, we have sequenced the exons of 202 genes in 14,000 individuals. In all, we discovered >34,000 variants with an allele frequency <= 0.001. Using these variants, we analyzed the overall mutation rate, the rates for specific types of mutation (e.g. A:T>G:C, G:C>A:T, etc.) as well as their correlation to specific genomic features which have previously been shown to impact mutation, including GC content, recombination rate, and adjacent nucleotide context. Regions of high GC content show decreased rates of mutation for most mutation subtypes, although the strength of this association is often confounded by read depth differences due to GC content. Still, mutations toward G or C nucleotides have weak yet significant negative correlations with GC content, suggesting that mutations that increase the overall GC content may be suppressed in GC-rich regions. Recombination has been previously shown to affect mutation along the human chimpanzee lineage. In rare variants, we show that local recombination rate or distance to recombination hotspot has little impact on the overall mutation rate or those of subtypes, suggesting that recombination has not had a significant influence on the mutation pattern in these variants. We also examined the mutation rate for specific types of mutations depending on the nucleotides surrounding the variant site and found that the adjacent nucleotide context impacts the mutation rate. These results show that local genomic context has substantial and specific effects on spontaneous mutation. The ability to examine extremely rare variants provides a new opportunity to study mutation as a fundamental process in biology without previous limitations, and generates precise estimates of local mutation rates for better interpretations of medical resequencing data.

493W

Unprogrammed presentation number

494W

Evolutionary genetics evidence for strong differences in the biological relevance of host microbial sensors. *E. Vasseur[1], S. Pajon[1], J. Manry[1], B. Crouau-Roy[2], L. Quintana-Murci[1].* 1) UP Human Evolutionary Genetics, CNRS URA 3012, Institut Pasteur, Paris, France; 2) Evolution et Diversité Biologique, UMR CNRS 5174, Université Paul Sabatier, Toulouse, France.

Microbial sensors, or pattern-recognition receptors (PRRs), are responsible for the detection of pathogens by the host, and constitute key actors of the innate immune system. Detecting how natural selection has targeted immunity-related genes has proven to be a useful tool for identifying genes playing an essential role in host defence. Here, we studied the levels of naturally-occurring variation of two major families of intracellular PRRs; the NOD-like Receptors (NLRs) and the RIG-I-like receptors (RLRs), which are mainly involved in the sensing of bacteria and viruses, respectively. To this end, we resequenced the 24 genes encoding these receptors in a panel of individuals (HGDP-CEPH) representing the major continental populations and used this dataset to perform a number of inter- and intra-specific neutrality tests. Our results revealed that the NALP subfamily of the NLRs, probably the least known family of PRRs, has been globally targeted by strong purifying selection. Indeed, amino-acid altering variation at 9 out of the 14 NALPs has been strongly constrained since the human/chimpanzee divergence. This suggests that most NALPs fulfil an essential function, thus constituting excellent candidates to be further studied in the context of infectious and autoimmune diseases. By contrast, such selective constraints have been relaxed for the remaining NLRs (i.e. some NALPs and 6 of the 7 NODs) and the 3 RLRs, which in turn seem to evolve more adaptively. Specifically, we found signatures of local positive selection targeting the RLRs MDA5 and LGP2 as well as the NLR CIITA among Eurasian populations, and identified at least 3 non-synonymous mutations as putative targets. More generally, when comparing the patterns of diversity and selection depicted at the major families of PRRs - the NALPs, the NODs, the RLRs and the TLRs - a clear pattern emerged. Among the families of PRRs specialised in the sensing of nucleic acids particularly from virus, the endosomal TLRs fulfil a more essential role than RLRs. Likewise, among the PRR families specialised in the sensing of products principally from bacteria, parasite and fungi, the intracellular NALPs appear to fulfil more crucial functions with respect to most NODs and the cell-surface TLRs. Our data allow us to propose a general hierarchical model, which indicates strong differences in the relative biological importance of PRRs and pave the way for future biochemical, immunological and clinical genetics studies.

495W

A SNP in EDAR is associated with a number of phenotypes of epidermal appendages - a population study in China. *S. Wang[1], Y.G. Kamberov[2], J. Tan[3], P. Gerbault[4], L. Tan[1], Y. Yang[3], S. Li[3], B.A. Morgan[5], D.E. Lieberman[6], M.G. Thomas[4], C.J. Tabin[2], L. Jin[3], P.C. Sabeti[1].* 1) FAS Ctr Systems Biol, Harvard Univ, Cambridge, MA; 2) Harvard Medical School, Department of Genetics, Boston, MA; 3) MOE Key Laboratory of Contemporary Anthropology, Fudan University, Shanghai, China; 4) Department of Genetics, Evolution and Environment, University College London, U.K; 5) Massachusetts General Hospital, Cutaneous Biology Research Center, Charlestown, MA; 6) Department of Human Evolutionary Biology, Harvard University, Cambridge, MA.

Ectodysplasin-A Receptor (*EDAR*) is involved in the development of epidermal appendages, including hair, sweat glands and teeth. The derived allele of SNP rs3827760 in *EDAR* was previously reported to be under strong positive selection in East Asian populations. In order to uncover the adaptive mechanism of this high frequency allele in East Asia, we carried out a systematic study in different ethnic groups in China with various allele frequency. rs3827760 is associated with a number of phenotypes, including sweat gland density, shovel-shaped incisor, and Carabelli's cusp. Through haplotype analysis of 280 SNP within 1cM of rs3827760 in 52 worldwide populations, we inferred the age of the derived allele and its associated long-range haplotype. It falls into late Pleistocene, and should predate the prehistoric colonization of the Americas. Using Approximate Bayesian Computation, and by incorporating paleoclimate data, we reconstructed the scenarios of the arising and spreading of the derived allele in East Asia. A model, in which selection coefficient depending on humidity, provided the best fit to real data. This finding, together with the genetic association of rs3827760 with sweat gland density, provides evidence that the local adaptive mechanism probably involved an advantage of thermoregulation in the humid weather in East Asia.

496W

Parental Age and Assisted Reproductive Technology in Autism Spectrum Disorders , Attention Deficit Hyperactivity Disorder, and Tourette Syndrome in the a Japanese populationPopulation. *T. Shimada[1], H. Kuwabara[2], Y. Kano[2], K. Kasai[1], N. Kato[3], T. Sasaki[4].* 1) Department of Neuropsychiatry, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 2) Department of Child Psychiatry, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 3) Department of Neuropsychiatry, Showa University School of Medicine, Tokyo, Japan; 4) Division of Physical and Health Education, Graduate School of Education, University of Tokyo, Tokyo, Japam.

We investigated whether advanced parental ages and assisted reproductive technology (ART) are risk factors in autism spectrum disorders (ASD), attention deficit hyperactivity disorder (ADHD), and Tourette syndrome (TS). Clinical charts of were reviewed in Japanese outpatients with ASD (n=552), ADHD (n=87), and TS (n=123) were reviewed. Parental ages of individuals with ASD, ADHD, or TS wasere compared with parental age in the general population of Tokyo (GP) after adjusting for year of birth years. Paternal and maternal ages were significantly higher in persons with ASD and ADHD, but not those with in TS. Logistic regression analysis revealed indicated that both maternal and paternal age were associated with ASD after controlling for the other parent's age, gender, singleton or multiple birth, gestational age, birth weight, and birth order. In cases where the presence or absence of ART could be ascertained (ASD n=467; ADHD n=64; TS n=83), Tthe frequency rate of ART in cases of 467 persons with ASD subjects (4.5%) was 1.8 times of the expected frequency expected in the GP, while ART was not observed present in cases of 64 persons with ADHD and 83 TS subjects. These preliminary results remain tentative pending replication with larger, community-based samples.

497W

GENETIC AND CLINICAL ANALYSIS OF SPINOCEREBELLAR ATAXIA TYPE 8 REPEAT EXPANSION IN A SICILIAN PEDIGREE. *F. Cavalcanti[1], P. Tarantino[1], M. Sframeli[2], F. Annesi[1], E.V. De Marco[1], M. Caracciolo[1], M. Gagliardi[1,3], A. Gambardella[1,4], C. Rodolico[3], A. Quattrone[4,5], G. Annesi[1].* 1) Institute of Neurological Sciences, National Research Council, Mangone (CS), Italy; 2) Department on Neurosciences, Psychiatry and Anaesthesiology, University of Messina, Messina (ME), Italy; 3) University of Magna Graecia, Catanzaro (CZ), Italy; 4) Institute of Neurology, Department of Medical Sciences, University of Magna Graecia, Catanzaro (CZ), Italy; 5) Neuroimaging Research Unit, Institute of Neurological Sciences, National Research Council, Catanzaro (CZ), Italy.

Spinocerebellar ataxia type 8 (SCA8) is the first example of dominantly inherited ataxia reported to be caused by a dynamic mutation of the untranslated CTA/CTG trinucleotide repeat. Although it is typically described as an automal dominant condition the SCA8 expansion exhibits variable penetrance and considerable intergenerational instability with a maternal transmission bias. The repeat lengths most often associated with ataxia range from 80 to 250. In this study we describe the clinical and genetic features of a Sicilian family affected by SCA8. We obtained clinical and genealogical information from seven people of a three generation pedigree. All family members signed an appropriate informed consent form and genetic studies were performed. Blood samples were obtained from all subjects and genomic DNA was extracted by standard methods. Triplet expansion in SCA8 was detected by PCR amplification with fluorescent primers. PCR products were separated onto a capillary sequencer. The proband of the family, a 41-year women, at the age of 37,has been diagnosed clinically with ataxia. The initial symptom, was gait instability, and she progressively experienced manifestation of limb and gait ataxia, dysarthria. Molecular analysis of the family members revealed no evidence of SCA1-SCA17. However, in three members of seven examined, a heterozygous CAG repeats expansion in ATXN8 gene was detected. We investigated the presence of the SCA8 triplet expansion in a Italian family, we found 3 subjects (1 patient with ataxia, 1 patient with parkinsonism and 1 unaffected subject) with abnormally expanded CTA/CTG repeats. In this study we confirmed the reduce penetrance of disease and the marked heterogeneity of the SCA8 clinical picture.

498W

GRIK2, IL1B, NEDD8 and NEDD9 genes may act as modifiers of the Machado Joseph Disease/ SCA 3 phenotype. *V.E. Emmel[1], K.C. Donis[2], T.C. Gheno[1], J.A.M. Saute[3], L. Vedolin[6], L.B. Jardim[1 2 5], M.L. Sraiva-Pereira[1 2 4].* 1) Genetic Identification Laboratory, Hospital de Clinicas de Porto Alegre, Porto Alegre, RS, Brazil; 2) Medical Genetics Service, Hospital de Clinicas de Porto Alegre, Porto Alegre, RS, Brazil; 3) Neurology Service, Hospital de Clínicas de Porto Alegre, Brazil; 4) Department of Biochemistry, Universidade Federal do Rio Grande do Sul, Brazil; 5) Department of Internal Medicine, Universidade Federal do Rio Grande do Sul, Brazil; 6) Neuroradiology Center, Hospital Moinhos de Vento, Porto Alegre, Brazil.

Machado-Joseph disease is a neurodegenerative disease caused by expansion of a polyglutamine tract in ataxin-3 that is coded for by ATXN3 gene. CAG length is inversely correlated with disease age at onset but is responsible for about 45-60% its variation in Machado-Joseph disease. **Aims**: to investigated if single-nucleotide polymorphisms in candidate genes BAX (rs1805419), CRYAB (rs14133), GRIK2 (rs2227281), IL1B (rs16944), ITPR1 (rs17786144), NEDD8 (rs2144487), and NEDD9 (rs760678) would modify age at onset, severity of disease, and volumes of infratentorial structures at MRI, in a large group of Brazilian patients with Machado-Joseph disease. **Methods**: DNA samples of 273 Brazilian MJD patients (119 families) were studied. Specific SNPs were determined by qualitative real time PCR using the TaqMan® PCR Assay (Applied Biosystems). A subgroup of 100 patients were clinically evaluated by NESSCA score: disease severity was defined as the quotient NESSCA/disease duration. MRI was done in 30 individuals. The normalized volumes of the brainstem, midbrain, pons, medulla oblongata, and cerebellum of SCA3 patients were measured on fluid-attenuated inversion recovery (FLAIR), using semi-automated segmentation techniques and voxel count volumetry using the software ImageJ. **Results**: significant associations were found between GRIK2 polymorphism (rs2227281) and cerebellum volume (P=0.006), IL1B polymorphism (rs16944) and age at onset (P=0.042), NEDD8 polymorphism (rs2144487) and pons volume (P=0.023), and a NEDD9 polymorphism (rs760678) and disease severity (P=0.003). The effect of combined protective genotypes for AO was investigated, and combined genotypes for GRIK2 (CC) + NEDD8 (GG) (P=0.008) and IL1B (AA) + NEDD8 (GG) (P=0.055) are associated with increased AO when compared with any other genotype combination. **Conclusions**: These results suggest that polymorphisms in GRIK2, IL1B, NEDD8, and NEDD9 genes may contribute to the phenotypic expression of Machado-Joseph disease.

499W

Residual Age of Disease Onset Variance in Spinocerebllar Ataxias in a Cuba and US Cohort. *K.P. Figueroa[1], H. Coon[1], C.M. Gomez[2], H.L. Paulson[3], S.L. Perlman[4], N. Santos[9], J.D. Schmahmann[5], L. Velázquez Pérez[9], G.R. Wilmot[6], T.A. Zesiewicz[7], S.H. Subramony[8], T. Ashizawa[8], S.M. Pulst[1], Clinical Research Consortium for Spinocerebellar Ataxias (CRC-SCA).* 1) Neurology, University of Utah, Salt Lake City, UT; 2) University of Chicago, Chicago, IL; 3) University of Michigan, Ann Arbor, MI; 4) University of California Los Angeles, Los Angeles, CA; 5) Harvard University, Boston, MA; 6) Emory University, Atlanta, GA; 7) University of South Florida, Tampa, FL; 8) University of Florida, Gainesville, FL; 9) Centro para la Investigación y Rehabilitación de las Ataxias Hereditarias, Holguín, Cuba.

Disorders caused by the expansion of a coding CAG DNA trinucleotide repeat, collectively referred to as polyglutamine (polyQ) diseases, show a strong inverse correlation between CAG repeat length and age of disease onset (AO). The square of the correlation coefficient (r2) in polyQ disease ranges in most studies from 0.6 to 0.8. Despite the overall good inverse correlation between AO and repeat length in all polyQ diseases, there is tremendous variability of AO within each repeat class. This variation suggests that other factors, in addition to the mutant allele itself, exist that influence disease onset and progression. The Clinical Research Consortium for Spinocerebellar Ataxias (CRC-SCA) is an NIH-funded multi-site clinical research group. As of 6-1 2011, clinical data and CAG genotypes have been collected from 168 patients. Age of onset was considered the first sign of disease usually manifested by unsteadiness of gait. A comparison group consisted of 326 patients were recruited from the SCA2 founder population in Holguin, Cuba. We examined the age of onset and SCA2 CAG repeat length in 326 Cuban SCA2 patients from a founder population and 168 US patients from the CRC-SCA (34 SCA1, 26 SCA2, 67 SCA3, 41 SCA6). Linear and log(AO) correlations with CAG repeat length were computed. For the Cuban SCA2 population, GLM and GENMOD were used to estimate variance explained by repeat adjusted for family membership. For the Cuban cohort, the mutant SCA2 CAG repeat allele explained 69% of AO variance in the entire sample, but this decreased to 50% when adjusted for pedigree structure. Analysis of affected first degree relatives (parents & sibs) indicated that half of the residual variance was explained by shared genetic factors. In contrast, the r2 for the CRC-SCA cohort was 0.26 (SCA2), 0.34 (SCA3), and 0.22 (SCA6). Only SCA1 (r2: 0.54) showed an r2 close to values previously reported. The values for r2 in the Cuban SCA2, and US SCA1 cohorts are similar to prior reports for other polyQ diseases including Huntington disease. However, the values for SCA2, SCA3, and SCA6 are much lower than previously reported. Initial results of the first US multi-center study of spinocerebellar ataxias appear to show significant differences compared to pedigree or founder population-based single-center studies. The amount of variance attributable to the CAG repeat may be smaller than assumed by prior studies when geographically diverse unrelated individuals are included.

500W

A new case of Ataxia type 17 in a Mexican female. *D. Garcia-Cruz[1], C.M. Moran-Moguel[2], J. Sanchez-Corona[2], G. Castañeda-Cisneros[3], N.O. Davalos-Rodriguez[1], I.P. Davalos-Rodriguez[4], J.A. Alcaraz-Ochoa[1], D.M. Sanchez-Garcia[5], M.H. Orozco-Gutierrez[1], N.Y. Nuñez-Reveles[1], J. Corral[6], H. San Nicolas[6], L. de Jorge[6], V. Volpini[6].* 1) Instituto de Genetica Humana, Depto. Biología Molecular y Genómica, CUCS, Guadalajara, Jalisco, Mexico; 2) División de Medicina Molecular, Centro de Investigación Biomédica de Occidente, CMNO, IMSS, Guadalajara, Jalisco; 3) Servicio Neurocirugía, UMAE Hospital de Especialidades, CMNO, IMSS, Guadalajara, Jalisco, México; 4) División de Genética, Centro de Investigación Biomédica de Occidente, CMNO, IMSS, Guadalajara, Jalisco, México; 5) Psicologia, ITESO, Tlaquepaque, Jalisco, México; 6) Centro de Diagnóstico Genético Molecular IDIBELL, Barcelona, España.

Introduction. The Spinocerebellar Ataxia type 17 (SCA17) is an autosomal dominant polyglutamine disease caused by expansion of a trinucleotide repeat encoding glutamine (CAG or CAA) in the general transcription factor TATA box-binding protein (TBP; 600075). It is characterized by ataxia, pyramidal and extrapyramidal signs, cognitive impairments, psychosis, seizures, dementia, a clinical phenotype and mode of inheritance similar to Huntington disease. Material and Methods. A female aged 33 year-old was studied clinically due to SCA (gait ataxia, dysmetria, severe dysarthria and aggression; MRI revealed moderate cerebellar atrophy. Laboratorial studies were performed with 5mL of peripheral blood to isolate patient's DNA by GeneCatcher Kit (Invitrogen). The molecular analysis was made by identification of expansion repeats in tandem by RED assay with probes to analyze the repeats.RED products were identified by Southern Blot and sequencing. Results. Molecular analysis revealed a number of expansion repeats of 34/52 (exp).Discussion. In previous studies done in Mexico the frequency of SCA17 was 2.8 percent. This is the first time that it is detected a patient affected by SCA17 in Northwest Mexico.

501W

Screening *ANO10* mutations in a Japanese cohort of cerebellar ataxia. *K. Ishikawa[1], M. Furuki[1], H. Matsuo[1], T. Yamashita[2], H. Mizusawa[1].* 1) Dept Neurology, Tokyo Med & Dental Univ, Tokyo, Japan; 2) Department of Neurology, Kumamoto University, Kumamoto, Japan.

Autosomal-recessive cerebellar ataxia (ARCA) is a group of rare heterogeneous neurodegenerative disorders. Its cardinal clinical feature is progressive cerebellar ataxia, although various phenotypes such as pyramidal tract signs, ocular apraxia, peripheral neuropathy, or spinocerebellar long tract signs may accompany. Molecular backgrounds of ARCA are complicated and the currently known mutations are responsible for only a small portion of those patients. Recently, Vermeer and others identified mutations in anoctamin 10 (*ANO10*), a gene encoding a putative calcium-activated chloride channel, in three European ARCA families (Vermeer S. et al. Am J Hum Genet 87, 813-819, 2010). To clarify whether *ANO10* mutations are also present in Japanese patients, we screened this gene in our families with ARCA. Based on clinical features of European ANO10 patients, 4 patients from different families were identified as candidates among our cohort of 225 families with adult onset ataxias of unknown cause. Mutation screening was performed by amplifying each of 15 exons with polymerase chain reaction (PCR) followed by direct nucleotide sequencing. We found a novel, homozygous single-nucleotide deletion in exon 6, resulting in frameshift and premature translation stop, in one patient. The same mutation was not seen in 50 control subjects. Clinical features of this patient were late-onset cerebellar ataxia with gaze-evoked nystagmus and exaggerated tendon reflexes. The remaining three patients were negative with *ANO10* mutation despite that their clinical phenotypes are indistinguishable from those of the patient with mutation. We conclude that mutations in *ANO10* are also seen in Japanese, and should be screened in patients with late onset cerebellar ataxia with hyper-reflexes and nystagmus.

502W

A Genome-wide Expression Profiling to unravel effect of missense mutations in SCA28 patients. *C. Mancini[1], P. Roncaglia[3], N. Lo Buono[1], A. Brussino[1], C. Cagnoli[1], F. Maltecca[7,8], H. Krmac[4], T. Limongi[6], G. Stevanin[5], S. Forlani[5], G. Casari[7,8], A. Funaro[1], A. Durr[5], N. Migone[1,2], S. Gustincich[4], A. Brusco[1,2].* 1) Department of Genetics, Biology and Biochemistry, University of Torino, Italy; 2) SCDU Medical Genetics, S.Giovanni Battista Hospital, Torino, Italy; 3) European Bioinformatics Institute, Cambridge, United Kingdom; 4) Neurobiology Sector, SISSA/ISAS, Trieste, Italy; 5) CRICM, UPMC/Inserm UMR_S 975, CNRS UMR 7225 GHU Pitié-Salpêtrière, Paris, France; 6) Italian Institute of Technology (IIT), Genova, Italy; 7) Human Molecular Genetics Unit, Center for Genomics, Bioinformatics, and Biostatistics, Milano, Italy; 8) San Raffaele University, 20132 Milano, Italy.

SCA28 is an autosomal dominant ataxia associated with oculomotor anomalies, due to *AFG3L2* gene missense mutations. The encoded protein is a member of the mitochondrial m-AAA protease (ATPases Associated with a variety of cellular Activities), and forms an hexameric complex in the Inner Mitochondrial Membrane, which exerts proteolytic activity and protein quality surveillance functions. We performed a whole genome expression profiling (based on Affymetrix Human Genome U133A 2.0 Chip Array) using lymphoblastoid cell lines (LCLs) from four SCA28 patients (mutations p.T654I, p.M666V, p. M666T and p.G671R), and six unrelated healthy controls matched for sex and age. We found 117 probes whose expression was statistically different, 60 of which up-regulated (Fold Change - FC = 1.5-16) and 57 down-regulated (FC = 0.7-0.1).The differentially expressed genes (n=88) were clustered, in five functional categories: (1) Cell Growth and Metabolism; (2) Apoptosis activation; (3) Oxidative Stress Response; (4) Cell Adhesion and Membrane Components; (5) Calcium Binding Proteins. To verify these pathways, we performed functional experiments on seven patients' LCLs which showed: 1) a delayed growth compared to controls ($p<0.005$), and an increased number of cells (> 15%) in G0/G1 phase ($p<0.001$); 2) an increased mortality of patients' cells due to apoptosis (AnnexinV/Propidium Iodide test) ($p<0.05$); 3) normal intracellular ROS levels in basal conditions (DCFH-DA test at FACS analysis). We also evaluated the respiratory chain activity in LCLs mitochondria, measuring ATP synthesis after treatment with specific respiratory chain blocking agents. No significant difference compared to controls was measurable, likely because an impairment in ATP production is absent or below detectable levels. We did not find mitochondrial DNA large deletions by long-range PCR. In conclusion, whole genome expression profiling in SCA28 LCLs allowed to identify several altered pathways that may be related to the disease. It is conceivable that the activation of the G1-S checkpoint and apoptosis is due to a cell cycle checkpoint surveyor responding to an abnormal cellular stress coming from the mitochondria. In more sensible cell types or tissues, such as Purkinje cells in cerebellum, this may result in a detectable alteration leading to the disease.

503W

Generation and characterization of transgenic rat models of spinocerebellar ataxia type 17 (SCA17). *H. Nguyen, A. Kelp, O. Rie) , P. Bauer.* Dept Med Gen, Univ Tuebingen, Tuebingen, Germany.

Spinocerebellar ataxia 17 (SCA17) is an autosomal-dominant, late-onset neurodegenerative disorder caused by CAG repeat expansions and consecutive polyglutamine (polyQ) expansions in the TATA-box-binding protein (TBP), an ubiquitously expressed transcription factor. To further investigate this devastating disease, we generated a transgenic rat model of SCA17, which carries a full human cDNA fragment of the TBP gene with 64 CAG repeats under the control of the murine prion protein promoter (PrP-TBP64Q). We obtained ten founders and were able to establish five lines with transmission of the transgene. On the basis of the mRNA expression level and the protein level as well as the distribution of TBP in the different brain regions we chose one of these five lines (line 8.4) for further characterization. This line shows a strong expression of the mutant TBP protein in the cerebellum and a moderate expression in the olfactory bulb, brainstem and cortex. Transgenic SCA17 rats exhibited a progressive phenotype with significantly lower body weight, ataxia, motor dysfunction and premature death around 10 months of age. At this time point neuropathological changes, such as misshaped Purkinje cells and corkscrew like dendrites were observed in the cerebellum of heterozygous transgenic SCA17 rats compared to wildtype littermates. Our results indicate that this model will be a valuable tool for investigating pathogenesis and for preclinical trials.

504W

A novel mouse model of spinocerebellar ataxia type 6 develops distinct Purkinje cell degeneration. *T. Unno[1], H. Mizusawa[1], K. Watase[2].* 1) Dept. of Neurology and Neurological Science,Tokyo Medical and Dental Univ. Grad. Sch. of Medicine, Bunkyoku, Japan; 2) Dept. of Center for Brain Integration Research, Tokyo Medical and Dental Univ. Grad. Sch. of Medicine.

Spinocerebellar ataxia type 6 (SCA6) is a dominantly inherited neurodegenerative disorder caused by small CAG repeat expansions within P/Q-type voltage-gated calcium channel ($Ca_v2.1$) gene (*CACNA1A*). *CACNA1A* undergoes alternative splicing such that one splice variant translates the polyglutamine tract from a CAG repeat tract residing in the last coding exon (exon47). The mutant allele produces the channel with an abnormally long polyglutamine tract (EXP-$Ca_v2.1$) and confers a toxic gain-of-function. For the elucidation of molecular pathways involved in SCA6 pathogenesis, it would be helpful to dissect the mammalian models that develop cerebellar Purkinje cell (PC) degeneration mirroring that seen in the disease. In an effort to engender such animals by enhancing the expression of mutant channels, here we created two novel knockin mouse models of SCA6 (named as Sca6-MPI-11Q and Sca6-MPI-118Q) carrying a splice-site mutation so that only the MPI-type splicing occurs from the exon47 locus. The animals were designed to express humanized MPI channels with either 11 or 118 polyglutamine tract. Immunoblot analysis using cerebellar extracts confirmed that these mice indeed expressed humanized $Ca_v2.1$ channel protein. Homozygous SCA6-MPI-118Q developed gait ataxia as early as 6 weeks of age while homozygous SCA6-MPI-11Q mice were indistinguishable from their wild-type littermates even at 1 year of age. Neuropathological examination of the cerebella of 6-week-old homozygous SCA6-MPI-118Q mice revealed that mutant PCs developed cytoplasmic inclusions concomitant with marked neuritic changes such as reduced dendritic arborization and axonal swelling. Many of these PCs were found to have irregularly shaped nuclei. The number of remaining PCs progressively declined as the SCA6-MPI-118Q mice aged. Interestingly, the cytoplasmic inclusions mostly co-localized with a couple of lysosome marker proteins such as cathepsin B and D. These results suggested that homozygous Sca6-MPI-118Q mice developed distinct PC degeneration and faithfully reproduced many features of SCA6. Lysosome may play a role in the inclusion formation in SCA6.

505W

Genotype-Phenotype correlations in Spastic Paraplegia type 7. *K.L.I. van Gassen[1], C. van der Heijden[1,2], E.J. Kamsteeg[1], H. Scheffer[1], B.P. van de Warrenburg[2].* 1) Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 2) Department of Neurology, Donders Institute for Brain, Cognition, and Behavior (Centre for Neuroscience), Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

Spastic Paraplegia type 7 (SPG7) is an autosomal recessive neurodegenerative disorder mainly characterized by a progressive bilateral lower limb spasticity. Complex features, often of cerebellar origin, may also be observed. Many different *SPG7* mutations have been observed, but no genotype-phenotype correlations have been found so far. From a total of more than 700 patients referred for SPG7 testing, we identified 60 patients with mutations in the *SPG7* gene. We identified 13 previously unreported mutations and detected a high recurrence rate of several earlier reported mutations. Since the phenotype of SPG7 is quite variable we here investigated a possible genotype-phenotype correlation.

We were able to collect detailed clinical data of 48 patients. Patients were ranked based upon age at disease onset, pure vs. complex phenotype, ataxia vs. no ataxia and missense vs. NUL-mutations, the latter defined as at least one allele that presumably does not lead to a protein product. A generally complex phenotypic presentation occurred in 65% of all patients and was associated with a younger age of onset (p=0.065). Ataxia was observed in 53% of all patients. We found that NUL-mutations were significantly ($p<0.05$) associated with the co-occurrence of cerebellar ataxia, which was independent from age at disease onset. The c.1409G>A (p.Arg470Gln) mutation, which was found homozygously in two sibs, was associated with a specific complex phenotype that included optical nerve atrophy.

In this large Dutch SPG7 cohort, we have identified the first genotype-phenotype correlation by observing a significant association between the cerebellar phenotype of SPG7 and *SPG7* NUL-alleles. There is thus an overlapping phenotypic presentation with its biological counterpart *AFG3L2*, which when mutated causes spinocerebellar ataxia type 28, possibly suggesting that abnormal levels of the SPG7 protein impact the function of the mitochondrial AAA-protease complex in the cerebellum. In addition, a missense mutation in exon 10 resulted in concomitant optical nerve atrophy, which suggests deleterious interactions of this *SPG7* gene variant with its substrate *OPA1*, the mutated gene product in optic atrophy type 1. Functional studies are required to investigate these interactions.

506W

A novel Ataxin-7 variant associated with slurred speech, clumsiness, learning difficulties, pes cavus and ataxia. *J. Warner[1], J. Pagan[1], D. FitzPatrick[1], M. Porteous[1], A. Diamond[1], P. Kennedy[3], D. Wilcox[2].* 1) S.E Scotland Gen Service, Western Gen Hosp, Edinburgh, United Kingdom; 2) Medical Genetics, Duncan Guthrie Institute of Medical Genetics, Yorkhill Hospital, Glasgow, United Kingdom; 3) Institute of Neurological Sciences, Southern General Hospital, Glasgow, United Kingdom.

We describe a three generation family with ataxia, dysarthria, dyspraxia and learning difficulties. The male proband presented with slowly progressive slurred speech, deteriorating memory, marked cerebellar signs and pes cavus (onset age 7). Genetic testing of the dominant cerebellar ataxia genes was requested. Screening of the SCA1, SCA2, SCA3, SCA6 and FRDA genes failed to reveal any pathogenic expanded repeats. PCR amplification using primers flanking the ataxin-7 CAG repeat gave one apparently normal repeat size in combination with a smaller product than any previously described repeat. Sanger dideoxy sequencing identified the variant c.110_139del; p.Glu37_Glu46del (accession number NM_000333.3). This in phase deletion ablates the first poly proline stretch of the ataxin-7 SH3 domain. An SH3P12 gene product has been shown to colocalise with Ataxin-7 in brain. The likely pathogenic variant is present in the proband's mother and sister who both have similar symptoms and signs. We present detailed clinical descriptions including MRI of affected family members. Further in vitro studies of this variant may throw light on ataxin 7 function.

507W

Non-AUG initiated homopolymeric proteins in microsatellite expansion disorders. *T. Zu[1], N. Doty[1], B. Gibbens[2], M. Swanson[1], L. Ranum[1].* 1) Dept Mol Genet & Microb, Univ Florida, Gainesville, FL; 2) Univ Minnesota.

For a group of neurological diseases caused by microsatellite expansions, mutations within or outside of predicted coding or non-coding regions are thought to cause disease by protein gain-of-function, protein loss-of-function or RNA gain-of-function mechanisms. In the absence of AUG or other known alternative start codons, we discovered that the canonical rules of translation do not apply for CAG•CTG expansions and that CAG and CTG trinucleotide repeats express homopolymeric expansion proteins in all three frames. This Repeat-Associated Non-AUG (RAN) translation occurs in mammalian tissue culture and in lentiviral-vector transduced cells and tissues. We now demonstrate that RAN-translation can occur across a variety of disease-causing microsatellite expansion motifs including CAG, CTG and CCTG, and that the hairpin structures are required. Additionally, we show non-AUG transcripts are associated with polyribosomal fractions and that RAN-translation occurs without frameshifing or RNA editing. RAN-translation results in the accumulation of polyGln, polyAla and polySer in HEK293T and neuronal N2a and T98 cells but not in several cell-free translation systems. These results suggest that specific cellular factors increase this novel type of translation initiation. Mass spectrometry of RAN-translated polyAla protein identified homopolymeric peptides with N-terminal alanine or N-terminal-acetylated alanine. Additionally, we show that RAN translation across human spinocerebellar ataxia type 8 (SCA8) and myotonic dystrophy type 1 (DM1) CAG-expansion transcripts results in the accumulation of SCA8 polyAla and DM1 polyGln expansion proteins in multiple organ systems in affected patients and mice. More specifically, the SCA8-Ala protein has been detected in the Purkinje cell soma and dendrites in SCA8 human and mouse and the DM1 CAG-polyGln protein is found in skeletal muscle and cardiomyocytes in affected human and DM1 mouse tissue. Infiltrating apoptotic (caspase-8 positive) leukocytes have been identified in thromboses within mouse cardiac tissue suggesting polyGln toxicity in DM1 cells. Understanding the mechanisms underlying RAN-translation has the potential to dramatically change ideas about the complexity of the proteome and how perturbations at this novel level impact biology and disease.

508W

A SNAP25 promoter variant is associated with schizophrenia and a disturbed SNAP25b/SNAP25a expression ratio in patients. *A. Dumaine[1,2,3], A. Meary[1,2,3,4], P.M. Llorca[3,5], C. Lancon[3,6], F. Schurhoff[1,2,3,4], A. Szoke[1,2,3,4], M. Leboyer[1,2,3,4], S. Jamain[1,2,3].* 1) Psychiatrie Génétique, Inserm U955, CRETEIL, France; 2) Université Paris Est, Faculté de Médecine, UMR-S 955, Créteil, 94000, France; 3) Fondation FondaMental, Créteil, 94000, France; 4) AP-HP, GHU (Chenevier-Mondor), Pôle de Psychiatrie, Créteil, 94000, France; 5) CHU Clermont-Ferrand, Hopital Gabriel Montpied, Service de Psychiatrie, Clermont Ferrand, 63000, France; 6) AP-HM, Hopital Sainte Marguerite, Service de Psychiatrie, Marseille, 13000, Marseille.

There is compelling evidence in favor of a common genetic vulnerability to schizophrenia and bipolar disorder. In a recent study, we identified one variation in the promoter region of the gene encoding the synaptosomal associated protein SNAP25, which was significantly more frequent in patients with an early-onset form of bipolar disorder as compared with healthy controls. This variation was also associated with an increased level in prefrontal cortex of SNAP25b, the major isoform of SNAP25 in adulthood. SNAP25 is a presynaptic plasma membrane protein essential for the triggering of vesicular fusion and neurotransmitter release and for which altered protein levels have been already reported in patients with schizophrenia. In the current study, we genotyped this promoter variant of SNAP25 in a sample of 288 schizophrenic patients consecutively admitted in three French university-affiliated psychiatry departments and 137 unaffected controls, with no personal or family history of axis I psychiatric disorders. In addition, we analyzed the expression level of the two SNAP25 isoforms in the dorsolateral prefrontal cortex of 33 schizophrenic patients and 30 controls from the Array Collection of the Stanley Medical Research Institute. We showed that this bipolar disorder-associated variation was more frequent in patients with schizophrenia as compared with controls (p=0.0009). Although no difference in expression level was observed neither for SNAP25a nor for SNAP25b between patients with schizophrenia and controls, we found a significant difference for the ratio SNAP25b/SNAP25a between genotype categories in patients (p=0.04). The `AA' genotype carriers had a lower ratio than the `AC' and `CC' genotypes carriers. We found similar results using ANOVA with Postmortem interval, Refrigerator Interval, Brain pH and Sex as covariates. This association was not found in controls. Our results suggest that a variation in SNAP25 might be a common vulnerability factor to schizophrenia and bipolar disorder. Moreover, the SNAP25b/SNAP25a ratio is crucial during brain development and switch at puberty, a high-risk period for schizophrenia onset, with SNAP25b becoming the major isoform. According to our results, we thus assume that this switch might be impaired by the promoter variant of SNAP25, increasing the vulnerability to schizophrenia.

509W

Evaluating the NLGN3 gene alternative splicing pattern and its potential role in autism. *Z. Talebizadeh, R. Aldenderfer.* Dept Pediatrics, Children's Mercy Hosp, Kansas City, MO.

An increased prevalence of autism in males suggests a role for the X chromosome. Evaluating X chromosome inactivation (XCI) in autistic females has been recommended by us and other groups. Despite some interesting hypotheses (e.g., imprinting and epigenetic mechanisms), it is not yet clearly understood if and how XCI might be important to understanding the mechanism of autism. One way to tackle this important topic is to assess if autistic females with X-inactivation skewness (XIS) have a unique genomic profile. Exploring such a question first requires knowing how this gene regulatory process is connected with other genomic processes. X chromosome inactivation is a DNA methylation process. Previously we have shown the trend of a higher degree of XIS in autistic females compared to non-autistic females. We also reported on the potential role of aberrant alternative splicing in X-linked neuroligin genes in autism. More recent findings have shown a cross link between DNA methylation and alternative splicing. These lines of evidence prompted us to systematically evaluate alternative splicing of NLGN3 in relation to autistic females' XCI status. We evaluated the expression level of multiple alternative splicing transcripts for NLGN3, an X-linked autism gene, in autistic females with and without XIS. Subjects were ascertained from the Autism Genetics Resource Exchange (AGRE). XCI status was determined using blood-derived genomic DNA. TaqMan gene expression experiments for multiple NLGN3 splice variants were conducted using RNA from AGRE lymphoblastoid cell lines. In addition to the two known NLGN3 variants, we identified three novel splice variants for this gene. A distinct, statistically significant pattern was detected in the expression level of examined alternatively spliced NLGN3 isoforms for autistic females with XIS compared to those without XIS and controls. This is the first study to evaluate the expression level of NLGN3 at the alternative splicing level taking into consideration the X-inactivation status of autistic females. Our study indicates that NLGN3 undergoes complex splicing resulting in multiple splice variants. Furthermore, our expression data demonstrates that autistic females with XIS may represent a more homogeneous subset of this highly heterogeneous population. Applying such a stratification method may provide a way to better understand underlying genetic and epigenetic mechanism in autism.

510W

GJB2 (Connexin26) is associated with age-related hearing impairment in humans and mice. *C-C. Wu[1,2], J-H. Hwang[3,4], Y-C. Lu[1], Y-H. Lin[1], P-J. Chen[3], W-S. Yang[2,3], M-F. Chen[5], I-S. Yu[6], S-W. Lin[6], H-C. Lin[7], C-C. Kuo[7], Q. Chang[8], X. Lin[8], Y-L. Lee[9,10], T-C. Liu[1], C-J. Hsu[1].* 1) Department of Otolaryngology, National Taiwan University Hospital, Taipei, Taiwan; 2) Department of Medical Genetics, National Taiwan University Hospital, Taipei, Taiwan; 3) Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei, Taiwan; 4) Department of Otolaryngology, Buddhist Dalin Tzu-Chi General Hospital, Chiayi, Taiwan; 5) Health Management Center, National Taiwan University Hospital, Taipei, Taiwan; 6) Transgenic Mouse Models Core (TMMC), Division of Genomic Medicine, Research Center For Medical Excellence, National Taiwan University, Taipei, Taiwan; 7) Department of Physiology, National Taiwan University College of Medicine, Taipei, Taiwan; 8) Department of Otolaryngology, Emory University School of Medicine, Atlanta, GA, USA; 9) Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University, Taipei, Taiwan; 10) Research Center for Genes, Environment and Human Health, College of Public Health, National Taiwan University, Taipei, Taiwan.

Age-related hearing impairment (ARHI), the most common form of hearing impairment in humans, is a complex disease caused by an interaction between environmental and genetic factors. It has been postulated that genes leading to monogenic hearing impairment might also be susceptibility genes for ARHI. The purpose of this study is to investigate the association between ARHI and variant alleles of GJB2, of which the mutations are the most important cause of monogenic hearing impairment worldwide. A total of 1481 volunteers (all were Han Chinese, aged 40 to 80 y) were included in the clinical analyses, and their audiological phenotypes were determined according to the Zlow, Z4-tone and Zhigh scores converted from their original frequency-specific hearing thresholds. Seven sequence variants in the coding region of GJB2, including c.79G>A, c.109G>A, c.235delC, c.299_300delAT, c.341A>G, c.368C>A and c.608T>C, were genotyped and then correlated to the audiological phenotypes under the assumption of various inheritance models. We identified an association between GJB2 variant alleles and ARHI in the clinical cohort, with the strongest association between the c.109G>A allele and ARHI under the assumption of the co-dominant model ($p<0.001$, for Zlow, Z4-tone and Zhigh). The pathogenetic effects were then confirmed in a knock-in mouse model segregating the c.109G>A variant: mice homozygous for c.109G>A (Gjb2tm1Dontuh/tm1Dontuh mice) developed pre-senile hearing deterioration, revealed compromised gap-junction-mediated metabolite transfer in the inner ear sensory epithelium, and appeared more vulnerable to noise exposure. The association between GJB2 and ARHI supports the hypotheses that genes responsible for monogenic hearing impairment and genes regulating the potassium homeostasis in the inner ear might contribute to ARHI.

511W

Microarray technology and GhostMiner software in modelling genetic risk factors of neurometabolic disturbance in patients with Parkinson's disease. *M. Bodzioch[1], K. Lapicka-Bodzioch[1], J. Slawek[3,4], A. Roszmann[3,4], M. Rudzinska[2], A. Szczudlik[2], A. Slowik[1].* 1) Dep. of Neurogenetics, Jagiellonian University Medical College, Krakow, Poland; 2) Chair of Neurology, Jagiellonian University Medical College, Krakow, Poland; 3) Department of Neurology, St. Adalbert Hospital, Gdansk, Krakow, Poland; 4) Department of Neurological and Psychiatric Nursing, Medical University of Gdansk, Gdansk, Poland.

Introduction: Parkinson's disease (PD) is a mutlifactorial disease with a plentitude of genetic and environmental determinants. Most studies have focused on a limited number of genetic variants and only developed simple risk models.

Goals: To genotype multiple variants in genes involved in neurometabolism and to develop complex models of susceptibility to PD.

Materials and methods: Using the OpenArray technology, we genotyped 128 single nucleotide polymorphisms (SNP) in genes involved in neural signalling and neurometabolim, including those encoding neuroprotective humanin peptides, in 190 PD patients and 196 healthy controls (including 44 sib pairs). The genotype data were analyzed with the GhostMiner software package.

Results: The decision tree algorithm with a separability of split value method was used to create a precise statistical model, differentiating with a very high accuracy (accuracy coefficient = 0,974) the affected and non-affected. The model comprises 57 logical rules, which enable correct classification of nearly all study participants into the PD group (26 rules) and healthy controls (31 rules). The highest differentiating value was attributed to genetic variants in humanin receptors - FPR1 and FPR3, genes involved in neurotransmission - COMT, HTR3A, HTR1B, ALDH5A1 and DRD1, as well as mediators of lipid metabolism - NIPSNAP3A and ABCA1. Sex and neurodegeneration-associated genes, LRRK1 and MAPT, were also important.

Conclusions: Bioinformatic analyses of data sets including multiple gene variants result in more complex but also more precise genetic risk models of susceptibility to PD.

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512W

Pathway analysis approach for interpreting nicotine dependence GWAS. *O. Harari, J.C. Wang, L.J. Bierut, P.A.F. Madden, A.M. Goate, NAG and SAGE collaborators.* Department of Psychiatry,Washington University School of Medicine, St Louis, MO.

The identification of the genetic architectures of complex traits, such as Nicotine Dependence, requires the analysis of multiple genetic risk factors [1]. Gene Ontology (GO) analysis exploits the concept that susceptibility alleles for a trait are distributed among sets of genes that share in common biological characteristics [2]. This approach allows us to examine thousands of putative signals conferring small risk, complementing standard analysis of the top most significant findings [1]. We analyzed two Genome-wide association studies (GWAS): the Study of Addiction: Genetics and Environment (SAGE) consisting of 1,294 cases (Fagerström Test for Nicotine Dependence score⁄ 4) and 2,071 controls [3]; and the Australian twin-family study (OZ-GWAS) which includes 1,935 and 2,262 nicotine dependent and non-dependent subjects respectively [4]. We evaluated the cigarettes per day phenotype GWAS signals by employing the gene overrepresentation method [2] and selected those terms that resulted significant for both studies. We considered both the genotyped SNPs and the imputed ones, to circumvent the different genotyping platforms employed in each study (i.e. Illumina 370K chip for the majority of the samples for OZ-GWAS and Illumina 1M chip for SAGE). Also, we estimated the statistical significance of the identified terms by the gene set enrichment analysis method [1]. We found significant several GO terms (GO:0015464, GO:0007271, GO:0005892, GO:0035095, GO:0042166, and GO:0004889) that group cholinergic receptors. Importantly, these terms were also statistically significant for the Atherosclerosis Risk in Communities GWAS (7221 subjects). Additionally, we found other terms significant for the 3 studies (GO:0007606, GO:0007608, and GO:0004984) that group genes involved in the sensory perception of chemical stimulus.

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513W

Transplantation of P75 down-regulated rat bone marrow stromal cells promotes functional behavior in a rat model of spinal cord injury. *Z. Hajebrahimi[1,2], H. Edalat[2], M. Tavallaei[3], M. Movahedin[4], S. Amiri[2], S.J. Mowla[2].* 1) Department of physiology, Aerospace Research Institute, Tehran, Iran; 2) Department of Molecular Genetics, School of Biological Sciences, Tarbiat Modares University, Tehran, Iran; 3) Molecular Biology Research Center, Baqiyatallah Medical Sciences University, Tehran, Iran; 4) Department of Anatomy, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

It is hoped that stem cells will provide an inexhaustible source of neurons for therapies aimed at cell replacement or neuroprotection in disorders affecting the brain and spinal cord. Bone marrow stromal cells (BMSCs) which are capable of differentiating into neural cells, has been employed for cell and gene therapy of neurodegenerative diseases, including spinal cord injury (SCI). One of the main difficulties of cell transplantation into central nervous system (CNS) is the high proportion of cell death specifically after neural differentiation. P75 receptor, known as the death receptor, has been shown to cause apoptosis of neural cells after binding to neurotrophines. Our previous data revealed a significant reduction of apoptosis in engineered neural-like cells derived from BMSCs after inhibition of p75 expression by siRNA approach. Thus, here we have examined whether p75-siRNA expressing rat BMSCs (rBMSCs) are able to survive at the site of implant, and if they can improve functional behavior after grafting into lesion site of spinal cord injured rats. Our findings demonstrate that p75 siRNA expressing rBMSCs (labled with fluorescent carbocyanine dye DiI) could effectively integrate into host tissue and survive in injured rat spinal cord for at least 4 weeks. Additionally, functional recovery was obviously observed in these genetically modified rBMSC treated SCI animals, compared with control (intact rBMSCs) and untreated ones. Thus, rBMSCs that endogenously express p75 siRNA could be a good candidate for treatment of SCI.

514W

Genetic factors associated with the predisposition to late onset Alzheimer disase. *A. Alpman Durmaz[1], E. Kumral[2], B. Durmaz[1], H. Onay[1], F. Ozkinay[1], O. Cogulu[1], S. Pehlivan[3], M. Orman[4], C. Ozkinay[1].* 1) Deparment of Medical Genetics, Ege University, Izmir, Turkey; 2) Department of Neurology, Ege University, Izmir, Turkey; 3) Department of Biology, Gaziantep University, Gaziantep, Turkey; 4) Department of Biostatistics and Medical Informatics, Ege University, Izmir, Turkey.

Alzheimer disease is a progressive, irreversible neurodegenerative disorder characterized by loss of memory and cognitive skills with an incidence of 6-8% between the ages of 70-85. More than 90% of cases are sporadic and have later age of onset. Many studies have shown a genetic predisposition for late onset Alzheimer disease (LOAD). The most studied genetic factor that has been found to be associated with LOAD is apolipoprotein E gene besides other susceptibility genes involved in vascular pathologies, homocysteine metabolism, and neuronal growth and differantiation such as methylenetetrahydrofolate reductase (MTHFR), angiotensin-converting enzyme (ACE), APOB and brain derived neurotrophic factor (BDNF). In this study Factor V Leiden (G1691A) and H1299R, prothrombin G20210A, Factor XIII V34L, B-fibrinogen -455G>A, PAI-1 5G/4G, HPA1 b/a, MTHFR C677T, MTHFR A1298C, APOE, ACE I/D, BDNF C270T and G196A polimorphisms were evaluated in 100 LOAD patients and 100 age matched healthy controls. Factor V Leiden A allele, APOE4 allele, MTHFR CC (A1298C) and BDNF TT (C270T) genotypes were significantly higher in LOAD patients compared to the control group (p=0.05, p<0.001, p=0.04 , p=0.03, respectively). There were no significant associations between other genotypes and allel frequencies. Mini-Mental State Examination (MMSE) scores and age at onset of the patients were also evaluated for each and combined genotypes. Age at onset was significantly lowered by about approximately 4 and 5 years in patients carrying BDNF TT (C270T) and MTHFR TT (C677T) genotypes, respectively. As a conclusion Factor V, APOE, MTHFR and BDNF genes may be associated with LOAD and BDNF and MTHFR alleles may play a in the age at onset of the LOAD.

515W

Genome-wide association analysis of age-at-onset in Alzheimer's disease. *M.M. Barmada[1], F.Y. Demirci[1], R.L. Minster[1], M.M. Carrasquillo[2], V.S. Pankratz[2], S.G. Younkin[2], A.J. Saykin[3,4], R.A. Sweet[5,6], E. Feingold[1], S.T. DeKosky[7], O.L. Lopez[6], M.I. Kamboh[1].* 1) Human Genetics, GSPH, Univ Pittsburgh, Pittsburgh, PA; 2) Neuroscience, Mayo Clinic College of Medicine, Jacksonville, FL; 3) Radiology and Imaging Sciences, SOM, Indiana Univ, Indianapolis, IN; 4) Medical and Molecular Genetics, SOM, Indiana Univ, Indianapolis, IN; 5) Psychiatry, SOM, Univ Pittsburgh, Pittsburgh, PA; 6) Neurology, SOM, Univ Pittsburgh, Pittsburgh, PA; 7) Neurology, SOM, Univ Virginia, Charlottesville, VA.

The risk of Alzheimer's disease (AD) is strongly determined by genetic factors and recent genome-wide association studies (GWAS) have identified several disease susceptibility loci and genes. In addition to the disease risk, age-at-onset (AAO) of AD has also strong genetic component with an estimated heritability of 42%. Identification of genes that influence the AAO of AD may help to understand the biological mechanisms that regulate the onset of the disease. Here we report the results of a GWAS focused on identifying genes for the AAO of AD. We performed a 3-stage analysis on a total of 2,222 AD cases. As expected, the most significant associations were observed in the *APOE* region on chromosome 19 where several SNPs surpassed the conservative genome-wide significant threshold (*P*<6.22E-08). The most significant SNP outside the *APOE* region was located in a gene on chromosome 4q31 (*P*= 4.95E-07) and there were 17 additional significant SNPs in this region at *P*<1E-04. The associated gene is expressed in the cerebral cortex and thus is a strong potential candidate for affecting the AAO in AD. Confirmation in additional well-powered samples will be necessary in order to establish these findings.

516W

Characterization of Lamin B1 duplication breakpoints and expression analysis in ADLD patients. *A. Brussino[1], E. Di Gregorio[1], E. Giorgio[1], D. Lacerenza[1], F. Talarico[2], G. Vaula[3], P. Mandich[12], C. Toro[4], E.E. Pierre[8,10], P. Labauge[9], S. Capellari[5], P. Cortelli[5], F.P. Vairo[11], W. Gahl[4], O. Boespflug-Tanguy[6,7,8], A. Brusco[1,2].* 1) Department of Genetics, Biology and Biochemistry, University of Torino, Italy; 2) SCDU Medical Genetics, S.Giovanni Battista Hospital, Torino, Italy; 3) Department of Neurosciences, University of Torino, Italy; 4) NIH Undiagnosed Diseases Program and National Human Genome Research Institute, NIH, Bethesda MD, USA; 5) Department of Neurological Sciences, University of Bologna, Italy; 6) Assistance Publique des Hopitaux de Paris (APHP), Reference Center for Rare Diseases “Leukodystrophies,” Child Neurology and Metabolic Disorders Department, Robert Debré University Hospital, Paris F-75935, France; 7) University Paris 7-Denis Diderot, Paris F-75013, France; 8) Institut National de la Santé et de la Recherche Médicale, UMR 931, CNRS 6247, Génetique Reproduction et Développement Medical School, Clermont-Ferrand F-63000, France; 9) CHU Nimes-Montpellier, Neurology Department, Nimes, France; 10) CHU De Clermont-Ferrand, Cytogenetic Department, Clermont-Ferrand 63000, France; 11) Hospital de Clínicas de Porto Alegre & Universidade Federal do Rio Grande do Sul; Brasil; 12) Department of Neurology Oftalmology and Genetics, University of Genova, Italy.

Autosomal dominant leukodystrophy (ADLD) is a slowly progressive demyelinating disease, with onset in the 4th to 6th decade of life. It is characterized by autonomic dysregulation, pyramidal signs, cerebellar dysfunctions, and symmetrical primary myelin loss in the central nervous system. Duplication of the lamin B1 gene (*LMNB1*) has been implicated as the cause of disease. Eleven ADLD families of different ethnic origins have been published so far, but the duplication breakpoint has been characterized at the nucleotide level in only three. Here, we characterize the *LMNB1* duplication in eight patients with ADLD originating from Italy (n=3), France (n=3), Brasil (n=1) and North America (n=1). A custom array-CGH allowed us to define the extent of the duplications, which ranged from 128 to 474 Kb. Seven duplications were unique, proving their origin from separate mutational events. Two French families, coming from the same geographic region, shared the same duplication, and are therefore likely to have a common ancestor. The largest duplication boundaries extend centromerically up to *GRAMD3* (also involving *ALDH7A1* and *PHAX*), and telomerically up to *MARCH3*. Using long-range PCR and sequencing, the duplication breakpoints were characterized in six patients; a microhomology region was found in two, an insertion in two, and a microduplication in one, suggesting microhomology-mediated, break-induced replication (MMBIR) as a possible mechanism of origin. We studied the expression of the *LMNB1* alleles using a primer extension assay of the SNP rs1051644 in the 3'UTR (c.*239C>T); lymphoblastoid cell lines of three different patients showed a 2:1 ratio of the duplicated allele vs. the non-duplicated allele on both genomic DNA and cDNA. This proves that the duplication increases *LMNB1* expression by only 50%, whereas published real-time PCR data show several fold increases in *LMNB1* expression. In cell models, lamin B1 overexpression alters the transcription of many genes, and we suggest this may affect the real-time experimental results. In summary, we define seven novel *LMNB1* duplications in ADLD patients. Expression analysis experiments using primer extension suggest that in lamin B1 duplication carriers the gene and protein levels are increased approximately 50%. (Work supported by Telethon grant GGP10184 and S.Paolo Neuroscience grant to A. Brussino, ELA foundation 2009-007I4 grant to O Boespflug-Tanguy).

517W

Quantification of the LINE-1 copy number in postmortem brains of psychiatric disorder patients. *M. Bundo[1], T. Miyauchi[2], A. Komori-Kokubo[2], K. Kasai[3], T. Kato[2], K. Iwamoto[1].* 1) Dept. of Molecular Psychiatry, Grad. Sch. Med., Univ. Tokyo, Tokyo, Japan; 2) Lab. for Molecular Dynamics of Mental Disorders, RIKEN BSI, Wako, Japan; 3) Dept. of Neuropsychiatry, Grad. Sch. Med., Univ. Tokyo, Tokyo, Japan.

Some retrotransposons have the activity of moving through the genome, resulting in the generation of variations in the human genome. Recently, it has been shown that one of the well-characterized retrotranposons, the LINE-1 (long interspersed nuclear element 1), possess the retrotranspose activity in neural progenitor cells. Interestingly, copy number of the LINE-1 was higher in brains compared with other tissues, and that it was differed among brain sub-regions. These findings implicate that aberrant LINE-1 activity might be associated with the pathophysiology of psychiatric diseases such as schizophrenia. Here, we quantified the copy number of the LINE-1 in genomic DNA derived from postmortem livers and brains of patients with psychiatric diseases. Postmortem samples were obtained from the Stanley Medical Research Institute. Samples consist of 15 schizophrenia, 15 bipolar disorder, 15 major depression and 15 controls. Copy number of the LINE-1 was determined by realtime PCR method, according to the previous report (Coufal et al, Nature 2009). There were significant increases in brain LINE-1 content in the ORF2 probes in schizophrenia, but not differed in the 5' UTR probe. These results indicate that the increased insertion of the incomplete reverse-transcribed LINE-1 in schizophrenia. Confounding factors such as age, gender, postmortem interval, and sample pH did not affect the LINE-1 content in brains. These results suggest that aberrant retrotranposon activity in the neural progenitor cells may be associated with the pathophysiology of schizophrenia.

518W

A novel putatively autism-associated adenosine A3 receptor (A3AR) variant causes dysregulation of and altered physical association with the serotonin transporter. *N. Campbell, C.-B. Zhu, K. Lindler, R.D. Blakely, J.S. Sutcliffe.* Center for Molecular Neuroscience, Vanderbilt Univ, Nashville, TN.

Autism is a neuropsychiatric disorder expressed across a spectrum of abnormalities that includes deficits in social interactions and development of language, along with patterns of rigid-compulsive behaviors. Autism spectrum disorders (ASDs) affect approximately 1 in 110 individuals, with studies pointing to a highly complex genetic etiology. Recent discoveries have highlighted the importance of rare genetic variation that cause or confer susceptibility for ASDs, and that multiple loci within particular gene networks can have coding or structural variants. The serotonin network is one strongly implicated in autism, and rare autism-associated variants in the serotonin transporter (SERT) lead to increased activity and dysregulation. Therefore, we hypothesized that SERT regulatory proteins will harbor rare genetic variation that alter SERT function consistent with previously seen mechanisms. We tested this hypothesis on the adenosine A3 receptor locus (ADORA3). The adenosine A3 receptor (A3AR) is an attractive target as it has been show to be active in 5-HT clearance and has a role in modulating SERT activity. Initial reporting from sequencing ADORA3 exons in autism probands revealed carriers of an undocumented and functional variant (Leu-90Val) leading to increased SERT activity. He we report a second variant (Val171Ile) identified in cases (3/184) and not seen in controls (0/305). This nonsynonymous change affects a highly conserved position in the protein and is predicted to be damaging. To address this variant functionally, we co-expressed the variant (3AR with SERT in CHO cells and found SERT stimulation (i.e. 5HT uptake) to be unresponsive with a selective A3AR agonist (IB-MECA). In this report, we further demonstrate a modest overall excess of rare nonsynonymous variants in the ADORA3 gene in ASD cases vs controls; the physical association between Val171Ile-A3AR and SERT to be decreased; and a lower SERT surface expression upon IB-MECA stimulation of Val171Ile-A3AR. Here, we discuss our latest findings on the significance of this A3AR variant and its consequences for disruption of 5-HT homeostasis.

519W

The use of aripiprazole in the management of schizophrenia associated with 22q11.2 deletion syndrome. *E. Chow[1,2,3], A.S. Bassett[2,3].* 1) Clinical Genetics Service, Ctr Addiction & Mental Hlth, Toronto, ON, Canada; 2) Clinical Genetics Research Program, Ctr Addiction & Mental Hlth, Toronto, ON, Canada; 3) University of Toronto, ON, Canada.

Aripiprazole is an atypical antipsychotic medication that works as a partial dopamine agonist. It has been shown to be as effective against psychotic symptoms associated with general population schizophrenia as other atypical antipsychotic medications, with a response rate of about 75%. Unlike other atypical antipsychotic medications, it is not associated with significant weight gain or adverse metabolic effects. Aripiprazole has been approved for general use in Canada since the fall of 2009. 22q11.2 deletion syndrome (22q11DS) is the most common microdeletion syndrome in humans, and is associated with a 25% risk of developing schizophrenia in adulthood. We studied the use of aripiprazole in 5 patients with 22q11DS and schizophrenia, and compare their experiences to those in two patients with schizophrenia and other genetic or neurodevelopmental syndrome. In the 5 adult patients with 22q11DS (2 M, 3 F) and stable DSM-IV schizophrenia, their average age at time of initiation of aripiprazole was 29.4 years. The most common reason for starting aripiprazole was excessive weight gain or metabolic changes associated with the atypical antipsychotic medication(s) used previously. The median starting dose for aripiprazole was 5 mg daily, about half of the recommended starting dose. The median maximum daily dose reached was 25 mg, in the recommended therapeutic range. On average, the patients were treated with aripiprazole for 3.2 months, and an average weight loss of 2.9 kg occurred during treatment. However, 4 of the 5 patients decompensated into psychosis when switched to aripiprazole, requiring hospitalization and discontinuation of the medication. The remaining patient's psychotic symptoms were not affected by the use of aripiprazole. In contrast, in two other patients (one with Fragile X syndrome, one with a non-verbal learning disability syndrome) who also suffered from DSM-IV schizophrenia, both responded well to an average daily dose of 25 mg of aripiprazole, with complete resolution of psychotic symptoms, and remained on the medication after 18 months. Although the sample size is small, these preliminary data suggests that unlike other patients in the general population, and unlike patients with other genetic conditions, aripriprazole may not be an effective antipsychotic medication in patients with 22q11DS, even if its use is associated with less weight gain and less metabolic changes than other atypical antipsychotic medications.

520W

Identification of Bipolar Disorder Genes by Exome Sequencing. *C. Cruceanu[1], R.G. Lafrenière[2], D. Spiegelman[2], P. Grof[3], M. Alda[4], G.A. Rouleau[2,5], G. Turecki[1].* 1) McGill Group for Suicide Studies, Douglas Mental Health University Institute, McGill University, Montreal, Quebec, Canada; 2) Center of Excellence in Neuroscience of the Université de Montréal (CENUM), Montreal, Quebec, Canada; 3) Mood Disorders Centre of Ottawa, Ottawa, Ontario, Canada; 4) Department of Psychiatry, Dalhousie University, Halifax, Nova Scotia, Canada; 5) Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), and Department of Medicine, University of Montreal, Montreal, Quebec, Canada.

Bipolar disorder (BD) is a complex psychiatric condition characterized by alternating manic and depressive episodes. In spite of the strong support for the role of genetics in BD, molecular studies have by-and-large had minimal success in identifying disease-causing genes. This is likely because of high levels of heterogeneity in the sample sets used, but the problem may be minimized by focusing on a well-defined sub-phenotype of BD. Our group has led efforts to characterize BD patients that respond positively to Lithium (Li) therapy, and shown that Li-response clusters in families and can predict symptom recurrence. Research in BD genetics to date has consisted of linkage and genome-wide association studies, which presume that common variants in a small subset of genes are the cause for BD. However, the minimal success in finding specific genes suggests that BD is caused by highly penetrant rare variants in a large number of different genes. Our research focuses on a well-defined clinical subtype of BD (Li-responsive) to minimize clinical heterogeneity, and we are using massively-parallel next-generation sequencing to resequence the exomes of over 200 affected individuals from multi-generational family units with Li-responsive BD. Focusing on the exome allows us to restrict our analysis to rare and potentially highly penetrant (e.g. protein-truncating or missense) variants across a large majority of genes. To identify relevant BD susceptibility genes we prioritized variants present in the affected and missing from the unaffected individuals within a family. Subsequently, we consolidated these findings across familial units, hoping to identify genes that contain an excess of rare, potentially deleterious mutations in BD cases. To validate candidate variants we used Sanger sequencing in our families as well as an extended sample set of non-familial Li-responsive BD cases. By focusing on highly penetrant rare variants, rather than common variants, we hope to have narrowed in on the key genes and biochemical pathways that play an important role in this disorder and can lead directly to clinically relevant diagnostic and therapeutic applications.

521W

Genome-Wide Search for Pleiotropic Genetics Effects on Obesity and Brain Structure in Mexican American Individuals. *J.E. Curran[1], A.M. Winkler[2,3], R.L. Olvera[4], M.A. Carless[1], T.D. Dyer[1], J.W. Kent Jr.[1], P. Kochunov[5], M.C. Mahaney[1], A.G. Comuzzie[1], P.T. Fox[5], L. Almasy[1], R. Duggirala[1], D.C. Glahn[2,3], J. Blangero[1].* 1) Department of Genetics, Texas Biomedical Research Institute, San Antonio, TX; 2) Olin Neuropsychiatry Research Center, Institute of Living, Hartford, CT; 3) Department of Psychiatry, Yale University School of Medicine, New Haven, CT; 4) Department of Psychiatry, University of Texas Health Science Center at San Antonio, San Antonio, TX; 5) Research Imaging Institute, University of Texas Health Science Center at San Antonio, San Antonio, TX.

Obesity is a common, chronic disorder reaching pandemic proportions worldwide and now represents an immediate major public health problem. Obese individuals are at a greater risk of decreased longevity and increased morbidity from various diseases including diabetes, coronary heart disease, stroke, hypertension, arthritis and some common forms of cancer. Given the role of the neural networks in satiety and appetite regulation, it is becoming clear that the brain plays a significant role in obesity. In this study, we performed the first genetic analyses searching for evidence of pleiotropy acting on both brain anatomy (obtained from MRI) and BMI in a large cohort of Mexican American individuals from large extended pedigrees. We identified multiple brain phenotypes that exhibit significant genetic correlation with BMI. Our most significant results show a highly significant overall genetic correlation ($p = 5.3\times10^{-4}$) between entorhinal cortex thickness and BMI. The entorhinal cortex is proximal to the hippocampus and is very closely related to hippocampal function, including memory function. Given this evidence for obligate genetic pleiotropy influencing these two phenotypes, we performed genome-wide bivariate association analysis using 932,000 SNPs to localize quantitative trait loci influencing these two phenotypes. Using this novel bivariate procedure (implemented in SOLAR), we localized two putative QTLs that cannot be found via standard univariate analyses, showing the power-increasing benefits of multivariate analysis. A locus in the region of 7q23 near the ataxin 7-like 1 gene was detected ($p = 3.4\times10^{-7}$) with a focal effect on entorhinal cortex. Ataxin-related genes have been previously implicated in neuronal function, hypophagia, and obesity which suggests that we have identified an interesting empirical candidate gene. A second putative locus was mapped to 14q11 near the *MAP45* gene ($p = 7.9\times10^{-7}$). The sentinel marker evidencing this QTL shows additional pleiotropic effects on major depression-related phenotypes (e.g., Beck depression inventory, $p = 0.0007$) which is interesting given the strong relationship between obesity and depression. Our results strongly support a role for the medial temporal lobe, which includes the hippocampus and entorhinal regions, in obesity and document, for the first time, that pleiotropic genetic effects help determine the brain/obesity relationship.

522W

Network analysis reveals functional convergence between genes involved in Alzheimer's disease and molecular targets of lithium. *S.D. Detera-Wadleigh, F.J. McMahon.* NIMH/NIH, Bethesda, MD 20892-3719.

Our previous analysis of biological networks generated by molecular targets of lithium showed a striking enrichment of apoptosis functional clusters and neurotrophin signaling (Gupta et al 2011), a possible functional signature for lithium's efficacy as a mood stabilizer. Inhibition of GSK3) is one of the best-documented responses to lithium. This effect has aroused interest in lithium as a potential therapeutic agent for Alzheimer's disease (AD). Since GSK3 seems to be important in the amyloid-tau cascade thought to play a role in AD pathogenesis, inhibition of GSK3 by lithium could slow the progression of disease processes leading to AD. Motivated by this idea, we explored evidence for a functional convergence between genes involved in AD and molecular targets of lithium. We performed separate network analysis on APP and presenilin 1 (PSEN1), two genes that have been shown to play a causative role in early-onset familial AD (EOFAD). We implemented 2 network tools, MiMI on Cytoscape (www.cytoscape.org) and STRING 8.3 (http://string-db.org/). Functional annotation to identify significantly enriched pathways and functional clusters was performed using DAVID (http://david.abcc.ncifcrf.gov/tool.jsp). We found that the most enriched functional cluster in both APP and PSEN1 gene networks was regulation of apoptosis/regulation of programmed cell death, reminiscent of the highly-enriched functional cluster generated by molecular targets of lithium. We extended this analysis to genes involved in late-onset AD (LOAD), including APOE and 11 genes well-supported by GWAS (e.g., Naj et al 2011; Hollingsworth et al. 2011; Wijsman et al. 2011). Functional annotation showed that the LOAD network recruited SH3 domain-containing molecules, complement activation, plasma lipoprotein particle remodeling, regulation of cholesterol transport, and positive regulation of apoptosis as the topmost enriched clusters. These results suggest that regulation of apoptosis is a functional link between EOFAD, LOAD, and molecular targets of lithium.

523W

Imprinted small nucleolar RNA in autism spectrum disorders. *A. Eran[1,2], K. Vatalaro[1], E.N. Brown[2], I.S. Kohane[2,3,4], L.M. Kunkel[1,4].* 1) Department of Genetics, Children's Hospital Boston, Boston, MA; 2) Health-Science and Technology, Massachusetts Institute of Technology, Cambridge, MA; 3) Department of Informatics, Children's Hospital Boston, Boston, MA; 4) Department of Pediatrics, Harvard Medical School, Boston, MA.

Autism Spectrum Disorders (ASDs) are highly heritable neurodevelopmental disorders of mostly idiopathic etiology. The most common cytogenetic abnormalities associated with ASD are duplications of the imprinted 15q11-13 locus. Two small nucleolar RNA (snoRNA) clusters are transcribed from the paternal allele, HBII-52 and HBII-85, both highly expressed in the brain. The precise function of these snoRNAs is unknown, but HBII-52s have been shown to regulate splicing and adenosine-to-inosine (A-to-I) RNA editing of the serotonin receptor HTR2C, a functional and positional candidate for ASD. For an initial examination of 15q snoRNAs in ASD and their impact on HTR2C editing, we used ultradeep (>5000x) sequencing to interrogate HBII-52 and HBII-85 snoRNAs from postmortem cerebella of 25 individuals with ASD and neurotypical controls. The 454 platform was used to sequence 57 of 71 known HBII snoRNAs, as well as HTR2C editing and splicing, in cases and controls. We report differential expression and sequence variation of HBII-52 and HBII-85 snoRNAs in individuals with ASD and neurotypical controls, as well as their correlation with HTR2C editing and splicing. This study represents a first large-scale characterization of these snoRNAs in human subjects. Our results suggest that snoRNA-mediated regulation of A-to-I editing may be part of the complex epigenetic basis of ASD.

524W

Active and passive MDMA (`ecstasy') intake induces differential transcriptional changes in the mouse brain. *N. Fernàndez-Castillo[1,2,3], M.J. Orejarena[4], M. Ribasés[5,6], M. Casas[5,7], P. Robledo[4,8], R. Maldonado[4], B. Cormand[1,2,3].* 1) Genetics Department, University of Barcelona, Barcelona, Spain; 2) The Biomedical Network Research Centre on Rare Diseases (CIBERER), Barcelona, Spain; 3) Biomedicine Institute of the Universitat de Barcelona (IBUB), Spain; 4) Neuropharmacology lab, Department of Experimental Sciences and Health, University Pompeu Fabra, PRBB, Barcelona, Spain; 5) Department of Psychiatry, Hospital Universitari Vall d'Hebrón, Barcelona, Spain; 6) Psychiatric Genetics Unit, Vall d'Hebron Research Institute, Barcelona, Spain; 7) Department of Psychiatry and Legal Medicine, Universitat Autònoma de Barcelona, Spain; 8) Neuropsychopharmacology Program, IMIM (Hospital del Mar Research Institute), PRBB, Barcelona, Spain.

3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) is a recreational drug widely used by adolescents and young adults. Although its rewarding effects are well established, there is controversy on its addictive potential. We aimed to compare the consequences of active and passive MDMA administration on gene expression in the mouse brain since all previous studies were based on passive MDMA administration. We used a yoked-control operant intravenous self-administration paradigm combined with microarray technology. Transcriptomic profiles of ventral striatum, frontal cortex, dorsal raphe nucleus and hippocampus were analyzed in mice divided in contingent MDMA, yoked MDMA and yoked saline groups, and several changes were validated by qRT-PCR. The comparison of contingent MDMA and yoked MDMA versus yoked saline mice allowed identification of differential expression in several genes, most of them with immunological and inflammatory functions, but others being involved in neuroadaptation. Interestingly, the Lcn2 gene, coding for lipocalin2 that mediates astrocytosis under inflammatory conditions, was highly upregulated (between 30 and 60 times over the control expression) in all four brain regions in mice after contingent or yoked MDMA administration. In the comparison of contingent MDMA versus yoked MDMA intake, hippocampus and the dorsal raphe nucleus showed statistically significant changes. The altered expression of several genes involved in neuroadaptative changes and synapse function, which may be related to learning self-administration behaviour, could be validated in these two brain structures. In hippocampus, downregulation of the genes Bzrap1, Nlgn2 and Axin2 was identified in the contingent mice, and Camk2a, Kalrn, Egr-3 and Ddn were all upregulated in the dorsal raphe nucleus of contingent mice. In conclusion, our study shows a strong effect of MDMA administration on the expression of immunological and inflammatory genes in all the four brain regions studied. In addition, experiments on MDMA self-administration suggest that the dorsal raphe nucleus and hippocampus may be involved in active MDMA seeking behaviour, and show specific alterations on gene expression that support the addictive potential of this drug.

525W

Expression analysis in dental pulp stem cells of idiopathic autistic patients reveals alterations in important processes for neurogenesis. *K. Griesi-Oliveira[1], D.Y. Sunaga[1], L.A. Cruz[1], E. Vadasz[2], M.R. Passos-Bueno[1].* 1) Biosciences Institute - University of Sao Paulo, Sao Paulo, SP, Brazil; 2) Psychiatric Institute - School of Medicine - University of Sao Paulo, Sao Paulo, SP, Brazil.

The investigation of autism spectrum disorders (ASD) causes is hampered by the genetic heterogeneity of these neurodevelopmental diseases. However, it has becoming evident that, although genetically heterogeneous, all the different ASD-related genetic alterations should be implicated in common molecular pathways or biological processes. In this scenario, the search for differentially expressed genes (DEGs) between ASD patients and controls is a good alternative to examine the molecular etiology of such disorders. In the present study, we employed genome-wide expression analysis to compare the transcriptoma of dental pulp stem cell (DPSC) of idiopathic autistic patients and control samples. Among the 404 differentially expressed genes (DEGs) identified, 335 (83%) are expressed in nervous tissue, showing that DPSC are good source of material to explore expression differences in ASD patients. Functional annotation analysis revealed that the ASD DEGs are enriched for gene ontology categories related to nervous system development and function. The great majority of these genes are involved in the formation of axons and dendrites, particularly, genes implicated in cell adhesion and regulation of cytoskeleton dynamics, suggesting that these mechanisms can be a common core undelaying ASD etiology. Notably, “Axonal guidance signaling” was the most significantly enriched canonical pathway found in our analysis, a pathway that was already pointed as implicated in ASD etiology by functional annotation analysis of genes compromised by ASD-specific CNVs. We also verified that most of the functionally relevant DEGs can be combined in a large network of functional relations, where they also interact with others genes that are part of canonical pathways already implicated in ASD, such as PTEN and Glutamatergic signaling. We identified 83 drugs that target the genes in this network, which are, therefore, a source of molecules that can be used for future in vitro drug screening studies for ASD. The use of cellular reprograming to investigate ASD patients' neurons can be employed to verify the effects of these drugs as well as the role of the identified pathways in neuronal phenotype.

526W

Association of SNPs in the FKBP5 gene region with hippocampal and amygdala volume in a healthy control sample. *L. Gschwind, C. Vogler, D. de Quervain, A. Papassotiropoulos.* Molecular Neurosciences, University of Basel, Basel, Basel, Switzerland.

Background: FKBP5 has been reported to play a role in response to antidepressant treatment and in post-traumatic stress disease (PTSD). Furthermore, hippocampal and amygdala volume have been linked to the risk of developing depression, PTSD and a variety of other psychiatric disorders. We hypothesized that the implication of FKBP5 in psychiatric diseases might be mediated through effects of genetic variation on differences in amygdala and hippocampal volumes. Therefore, we examined whether single nucleotide polymorphisms (SNPs) in the FKBP5 gene region are associated with hippocampal and amygdala volume differences in a sample of healthy young subjects. Methods: We used automated segmentation procedures of structural magnetic resonance images to determine measures of hippocampal volume in 329 healthy young Swiss individuals. Hippocampal and amygdala volumes were corrected for age, gender and intra-cranial volume. Genetic variation in FKBP5 and additionally 20kb up- and downstream of the gene was assessed through 46 SNPs represented on the Affymetrix Genome-Wide Human SNP Array 6.0. Genetic associations were tested under the assumption of a dominant and an additive model using the Plink software package. Results: None of the investigated SNPs withstood multiple testing corrections. The number of SNPs reaching nominal significance of association with hippocampal or amygdala volumes corresponds to the number of nominally significant results that are expected by chance if an (-error rate of 5% is assumed. Conclusion: In a group of 329 healthy young individuals, which is sufficiently powered to detect medium effect sizes, we could not reveal a potential influence of FKBP5 on hippocampal and amygdala volume. It is unlikely that the impact of FKBP5 on psychiatric diseases is mediated through volumetric effects of FKBP5 genetic variation in brain regions related to psychiatric disease.

527W

A new locus for autosomal dominant Charcot-Marie-Tooth disease type 2 maps to chromosome 10p13-14. *M. Gu[1], W. Xu[1], L. Sun[1], W. Guo[1], J. Ma[2], S. Chen[2], Z. Wang[1].* 1) Dept Med Gen, E-Institutes of Shanghai Universities, Shanghai Jiao Tong Univ Sch Med, Shanghai, China; 2) Dept Neurology, Rui-Jin Hospital Affiliated to Shanghai Jiao Tong Univ Sch Med, Shanghai, China.

Charcot-Marie-Tooth disease (CMT) is one of the most common inherited neurological disorders with a prevalence estimated at 1/2500. Mutant alleles underlying CMT can segregate in an autosomal dominant, recessive, or X-linked manner. On the basis of clinical manifestation and electrophysiological properties, CMT has been divided into two main types: a demyelinating form (CMT1) affecting the glia-derived myelin and an axonal form (CMT2) affecting axon. CMT2 is characterized by distal muscle weakness and atrophy, mild sensory loss, and normal or near-normal nerve conduction velocities. Here, we report a six-generation pedigree of CMT including 8 patients, from Shandong Province, China. After informed consent was signed, 29 members in the family took part in our research. All patients presented muscle weakness and a predominating weakness of distal parts of the lower limbs, no significant sensory impairments were observed. The electrophysiological examinations show that both of motor nerve conduction velocities (MCV) and sensory nerve conduction velocities (SCV) were normal, but some compound motor action potentials (CMAP) were decreased at upper limbs. MCV were lower than normal one, but SCV was normal. CMAPs were greatly reduced at lower limbs. All evidence supported the diagnosis of CMT2. Some known loci including CMT2A, CMT2B, CMT2D, CMT2E, CMT2F and CMT2L were excluded by linkage analysis. A genome-wide scanning was carried out, and the results revealed linkage of CMT2 to a locus at chromosome 10p13-14. The fine mapping and haplotype analyses localized this novel locus to 5.41 Mb intervals between microsatellite markers D10S585 and D10S1477. The maximal two-point LOD score of 4.56 for marker D10S506 at a recombination fraction (7) of 0 strongly supported linkage to this locus. Thus, CMT2 neuropathy in this family represents a novel genetic entity. In the ALEXA-Seq data viewer (http://obiweb.bcgsc.ca/solexa/ALEXA/Sutent/genes), more than 70 genes have been mapped at above-mentioned region, twenty of them functionally similar or related to known genes which are being sequenced. We are looking forward to identifying a novel causing gene in the pedigree with CMT2.

528W

Variants in the 15q25 locus are associated with schizophrenia diagnosis. *K.J. Jackson[1], A.H. Fanous[1,2,3], J. Chen[1], K.S. Kendler[1], X. Chen[1].* 1) Dept Psychiatry, Virginia Commonwealth University, Richmond, VA; 2) Washington VA Medical Center, Washington, DC; 3) Georgetown University Medical Center, Washington, DC.

The 15q25 locus, which contains the CHRNA5/CHRNA3/ CHRNB4 genes, coding for (5, (3 and) 4 nicotinic receptors respectively, is the most significant candidate region to date associated with smoking behavior and nicotine dependence (ND). It is well known that rates of tobacco smoking are much higher in patients with schizophrenia (_80-90%) compared to the general population (_20-30%), though the underlying mechanism for this comorbidity is unclear. One hypothesis is that there are common genetic factors that predispose to both ND and schizophrenia. To explore this hypothesis, we examined the association between schizophrenia diagnosis, negative symptoms, and 5 variants in the 15q25 gene cluster (rs951266, rs16969968, rs1051730, rs8040868, rs17477223) in 7 schizophrenia datasets (n=9,448). Results from each study were used to perform a meta-analysis. Two variants were significantly associated with schizophrenia diagnosis (rs8040868, rs17477223). After inclusion of 3 bipolar sets into the meta-analysis (total n=15,780), four markers were significant after correction for multiple testing (rs951266, rs16969968, rs8040868, rs17477223). No significant association was found with the presence of negative symptoms. Gene expression analysis was also conducted using the SNPExpress database. Preliminary results indicate an association between genotypes of the rs1051730 variant and CHRNA5 expression in brain and peripheral blood mononuclear cells. A follow-up meta-analysis using publically available post-mortem brain expression data (n= 632) from the Myers Lab (http://labs.med.miami.edu/myers/LFuN/papers.html) and Stanley Medical Research Institute (http://www.stanleyresearch.org) also suggest a marginal association with the rs1051730 variant and CHRNA5 expression. Taken together, these results suggest that variants in the 15q25 gene cluster are associated with risk for schizophrenia diagnosis, and may influence CHRNA5 expression in the brain. Because the direction of the association is the same as what was found for ND, these results also support the notion that there are genetic mechanisms common to schizophrenia, ND, and potentially bipolar disorder. Future studies involve elucidation of potential gene x gene interactions with the (5, (3 and) 4 nicotinic receptor genes and (7, (4, and) 2 nicotinic receptor genes, as these genes have also been found to be associated with schizophrenia, and may contribute to maintenance of smoking behavior.

529W

Genomewide Association Study Implicates NDST3 in Schizophrenia and Bipolar Disorder. *T. Lencz[1,2,3], S. Guha[1], C. Liu[4], J. Rosenfeld[1], P. DeRosse[1], L. Cheng[5], J. Badner[5], J. Kane[1,2,3], A. Lee[2,3], P. Gregersen[2,3], I. Pe'er[6], A. Malhotra[1,2,3], A. Darvasi[7].* 1) Dept Psychiatry Res, Zucker Hillside Hosp, Glen Oaks, NY; 2) Feinstein Institute for Medical Research, Manhasset, NY; 3) Hofstra University School of Medicine, Hempstead, NY; 4) Dept of Psychiatry, University of Illinois at Chicago, Chicago, IL; 5) Dept of Psychiatry and Behavioral Neuroscience, University of Chicago, Chicago, IL; 6) Dept of Computer Science, Columbia University, New York, NY; 7) Dept of Genetics, Institute for Life Sciences, Hebrew University, Jerusalem, Israel.

Background Schizophrenia and bipolar disorder are major psychiatric disorders with high heritability and overlapping genetic variance, yet few susceptibility loci have been identified and replicated in large-scale studies. **Methods** We performed a genome-wide association study (GWAS) in an ethnically homogeneous cohort of 904 schizophrenia cases and 1640 controls drawn from the Ashkenazi Jewish (AJ) population, and sought to replicate our top result in 10 case-control cohorts (6 schizophrenia and 4 bipolar disorder cohorts containing a total n=20,446 subjects) drawn from multiple ethnicities. We also examined the relationship between alleles at our top GWAS SNP and expression of a neighboring gene in 119 postmortem cerebellar tissue samples. **Results** In our Ashkenazi GWAS discovery cohort, we identified a genome-wide significant risk locus at chromosome 4q26 (rs11098403, $P = 6.55 \times 10^{-9}$). This SNP demonstrated significant association across the 10 replication cohorts regardless of diagnosis or ethnicity (heterogeneity I2=0), resulting in a meta-analytic $P = 7.82 \times 10^{-10}$ (OR=1.12, 95%CI=1.08-1.16). Additionally, this intergenic SNP was significantly associated with postmortem cerebellar expression of the neighboring gene NDST3, which encodes an enzyme critical to heparan sulfate binding. **Conclusions** Our study suggests a possible role of NDST3 in susceptibility to schizophrenia and bipolar disorder. Heparan sulfate binding is critical to neurite outgrowth, axon formation, and synaptic processes thought to be aberrant in major psychiatric disorders.

530W

Analysis of the *FBXO7* gene in early-onset parkinsonism. *Y. Li[1], M. Funayama[1,2], H. Yoshino[1], H. Tomiyama[2,3], N. Hattori[1,2,3].* 1) Research Institute for Diseases of Old Age, Graduate School of Medicine, Juntendo University, Tokyo, Japan; 2) Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan; 3) Department of Neuroscience for Neurodegenerative Disorders, Juntendo University School of Medicine, Tokyo, Japan.

Background and Objectives: Mutations in the *FBXO7* gene located within the *PARK15* locus on chromosome 22q12-q13 have recently been identified in European patients with autosomal recessive early-onset parkinsonism-pyramidal syndrome, however, it has been detected in only three European families. To clarify the distribution and frequency of *FBXO7* mutations, we investigated *FBXO7* gene mutations in Japanese patients with early-onset parkinsonism. **Methods:** All coding exons and exon-intron boundaries of the *FBXO7* gene were sequenced in 110 Japanese patients (age at onset; 11 to 50 years, mean ±SD; 33.3±8.2 years) with early-onset parkinsonism. None of the subjects had mutations in the *parkin* gene and the *PINK1* gene. **Results:** None of pathogenic mutations in the *FBXO7* gene were detected. **Conclusions:** Our study suggests that *FBXO7* mutation is rare among Japanese patients with early-onset parkinsonism.

531W

Mesial temporal lobe epilepsy gene expression profiling: a comprehensive gene set enriched-based analysis. *I. Lopes-Cendes[1], J.F. Vasconcellos[1], C.V. Maurer-Morelli[1], C.S. Rocha[1], C.L. Yasuda[2], H. Tedeschi[2], E.D. Oliveira[2], F. Cendes[2].* 1) Department of Medical Genetics, Faculty of Medical Sciences, State University of Campinas, and CINAPCE Program, Campinas, SP, Brazil; 2) Department of Neurology, Faculty of Medical Sciences, State University of Campinas, and CINAPCE Program, Campinas, SP, Brazil.

Gene Set Enrichment Analysis (GSEA, http://www.broadinstitute.org/gsea/index.jsp) is a web tool for interpreting gene expression profiles, whose analysis is based on the enrichment of gene sets (groups of genes that share common biological function, chromosomal location and/or regulation). Mesial temporal lobe epilepsy (MTLE) is one of the most common forms of human epilepsy, and causes both neuronal loss and synaptic reorganization in the hippocampus. MTLE is largely described as a sporadic disease; however, we and others have shown that MTLE can also have familial recurrence. The present study applied GSEA to interpreted data from gene expression profiles obtained from hippocampal tissue of MTLE patients with positive and negative family history (FH) of MTLE, compared to normal controls. Transcriptional profiling by microarray analysis revealed that patients with MTLE and a positive-FH had 170 differentially expressed genes and patients with a negative-FH had 341, when compared to control samples. GSEA of the gene expression profile from patients with positive-FH demonstrated 7 enriched gene sets ($p<0.05$ and FDR q-value<0.05), which are involved in cellular proliferation, MYC and AKT1 signaling pathways, inhibition of DNA methylation and histone deacetylation, and synthesis of myelin constituents. MYC is associated with apoptotic induction in neurons, while AKT1 indirectly mediates neuron apoptosis through the regulation of FKHR and Bim. Epigenetic modifications, such as DNA methylation, may be involved in the pathogenesis of epilepsy. Finally, myelin constituents may present a differential expression profile due to neuronal plasticity following brain tissue lesion. GSEA of the gene expression profile from patients with negative-FH demonstrated 5 enriched gene sets ($p<0.05$ and FDR q-value<0.05), which are mainly involved in oxidative stress and electron transport chain. Oxidative stress was identified as an important mechanism in the etiology of seizures, and induces neuronal death. The electron transport chain may play an indirectly role in the pathogenesis of epilepsy, through the production of reactive oxygen species. In conclusion, our data shows for the first time the application of GSEA in epileptic tissue and the results support previous experimental data indicating the complex nature of the mechanisms involved in the pathogenesis of MTLE. Supported by: FAPESP.

532W

A frequentist enrichment approach links bipolar affective disorder to genes involved in long term potentiation phenotypes. *S. Meader[1], L. Winchester[2], E. Domenici[3], C. Francks[4], J. Ragoussis[2], C. Webber[1].* 1) MRC Functional Genomics Unit, Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford OX1 3QX, United Kingdom; 2) The Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, United Kingdom; 3) Biomarker & Pathway Analysis, F. Hoffmann-La Roche Ltd, CH-4070 Basel, Switzerland; 4) Max Planck Institute for Psycholinguistics, Wundtlaan 1, 6525 XD Nijmegen, The Netherlands.

Copy number variants (CNVs) have been identified repeatedly as a cause of neurological disorders. However, despite several major studies, there have been few reports that have causally implicated CNVs in the occurrence of bipolar affective disorder. We examined a novel set of CNVs identified among 888 patients diagnosed with type I or type II bipolar disorder. When compared to matched controls, we found that the CNV burden within these patients was significantly increased.

We developed a novel and statistically-robust frequency-based approach to determining functional gene enrichments in case/control CNV data which is demonstrably free from functional gene length biases. Using phenotypic data from >5,000 mouse gene knockouts and considering each of 444 reasonably populated behavioural and nervous system phenotypes, we asked whether the orthologues of the genes yielding that phenotype in mice were significantly more frequently affected among individuals with bipolar disorder compared to individuals without the disorder.

Among the bipolar patients, we identified a significant association of genes whose mouse orthologues, when disrupted, result in an *abnormal long term potentiation phenotype* (3.2-fold enrichment; $p < 4 \times 10^{-5}$; FDR < 5%). This enrichment identifies 17 bipolar candidate genes whose protein-coding sequence is disrupted by deletions within the case population, two of which have been previously associated with bipolar disorder.

To confirm the relevance of this specific phenotypic association, we formed a wholly independent set of 39 bipolar candidate genes taken from the recent literature. Among those mouse orthologues for which phenotypic information was available, the phenotype *abnormal long term potentiation* was found to be the most specific among those phenotypes most significantly enriched (6.7-fold enrichment; $p < 2 \times 10^{-4}$; FDR < 5%). Ongoing analysis exploiting detailed patients records is revealing the segregation of this novel signal with both disorder type and onset, and treatment outcome. Taken together, our findings support a significant role for copy number variation in bipolar affective disorder, and demonstrate the utility of mouse phenotypic data in gaining functional insights into, and identifying the genes underlying, neurological disease.

533W

CACNA1C is associated with memory function in healthy young adults. *A. Milnik[1,4], B. Auschra[1,2,3], D.J.-F. de Quervain[3,5], A. Papassotiropoulos[1,2,3].* 1) Division of Molecular Neuroscience, Department of Psychology, University of Basel, Basel, Switzerland; 2) Life Sciences Training Facility, Department Biozentrum, University of Basel, Basel, Switzerland; 3) University Psychiatric Clinics, University of Basel, Basel, Switzerland; 4) University Department of Neurology, University Medical Center Magdeburg, Magdeburg, Germany; 5) Division of Cognitive Neuroscience, Department of Psychology, University of Basel, Basel, Switzerland.

Background: An association of single nucleotide polymorphism (SNP) rs1006737 with depression and schizophrenia and disease-related cognitive phenotypes has been shown previously. This SNP is located within CACNA1C, which encodes for the alpha subunit of the L-type voltage-dependant calcium channel. Imaging studies have shown impaired hippocampal function during memory recall in carriers of the disease-associated allele. However, no association between this SNP and memory performance in healthy subjects has been hitherto shown, possibly due to small effect and sample sizes. Method: We analyzed behavioral and genetic data of 1471 young healthy participants carefully characterized for episodic and working memory performance and for additional psychological traits (e.g. personality, depression, anxiety). Results: Young healthy risk-allele carriers have significantly impaired episodic memory performance, albeit the effect size is modest. No association with working memory performance or additional psychological traits was observed. Conclusion: We observed a significant association of CACNA1C SNP rs1006737 with memory function in a large group of young healthy adults and hypothesize that increased CACNA1C-related vulnerability to psychiatric disorders is moderated through impaired memory capacity.

534W

Intragenic CNVs identified by exon-level microarray analysis in individuals with autism plus epilepsy. *A. Moreno-De-Luca[1], E.B. Kaminsky[1], D.H. Ledbetter[2], C.L. Martin[1].* 1) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) Geisinger Health System, Danville, PA.

Autism spectrum disorders (ASD) and epilepsy are two of the most common, heterogeneous and heritable neurodevelopmental disorders. Both conditions can present with a wide range of co-morbidities that often include cognitive and developmental impairments. ASD and epilepsy have both been associated with multiple genetic etiologies, such as single gene mutations and copy number variants (CNVs); however, the specific cause remains elusive in most cases. The prevalence of epilepsy in individuals with ASD (~30%) and vice versa (~30%) is significantly higher than that of either individual disorder in the normal population (<1%), suggesting the existence of shared genetic and molecular mechanisms that may account for their co-occurrence. The causative role of large CNVs harboring multiple genes has been well established in individuals with ASD and epilepsy. However, the relevance of small intragenic imbalances has yet to be determined. To explore the contribution of exonic CNVs to the ASD/epilepsy phenotype, we custom-designed an oligonucleotide copy number microarray with whole genome plus exon-level targeted coverage of >1,200 known and candidate ASD and epilepsy genes. We tested 144 individuals with ASD plus epilepsy from the Autism Genetic Resource Exchange who previously screened negative for any large (>250 kb), pathogenic CNVs. We identified 6 families with exonic deletions and 8 with exonic duplications (9.7% yield). To establish the inheritance pattern of the deletion cases, we performed parental microarray analysis and showed that two siblings inherited a deletion in *CNTNAP2* from their father. Three additional cases also had inherited deletions in *LIN7C* (mat), *CHRM3* (mat) and *OR1C1* (mat). These results suggest incomplete penetrance, variable expressivity or a familial benign CNV. In one case with a deletion in *ADAM22*, the inheritance could not be determined since the father was unavailable. Interestingly, we identified a *de novo* deletion of exon 24 in *EHMT1* in an individual with clinical features similar to those of Kleefstra syndrome (ASD, epilepsy, craniofacial dysmorphic features, scoliosis, and clinodactyly), suggesting a potential pathogenic role for this exonic CNV. Our results highlight the contribution of exonic CNVs to ASD plus epilepsy and support further investigations to continue unraveling the underlying shared pathophysiological pathways.

535W

Characterization of VCP R155H Homozygote Mouse Model Exhibits Pathology Typical of IBMPFD and ALS. *A. Nalbandian[1], E. Dec[1], S. Donkervoort[1], H. Yin[2], G.D. Watts[3], C. Smith[4], V. Caiozzo[5], A. Wang[2], T. Mozaffar[2], J. Weiss[2], V. Kimonis[1].* 1) Department of Pediatrics, University of California, Irvine, CA; 2) Departments of Neurology, University of California Irvine, CA; 3) School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, Norfoik, UK; 4) Department of Neurology, University of Kentucky Medical School, Lexington, KY; 5) Department of Orthopedic Surgery, University of California, Irvine, CA.

Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia (IBMPFD) is a progressive, fatal, genetic disorder with variable penetrance, predominantly affecting three main tissue types: muscle (IBM), bone (PDB) and brain (FTD). IBMPFD is caused by mutations in the ubiquitously expressed valosin containing protein (VCP) gene, a member of the AAA-ATPase super-family. It has been reported that VCP is involved in a plethora of distinct cellular processes including homotypic membrane fusion, transcription activation, nuclear envelope reconstruction, DNA repair, post-mitotic organelle reassembly, cell cycle control, apoptosis and endoplasmic reticulum associated degradation of proteins (ERAD), which is capable of destroying both integral membrane proteins and luminal proteins. The majority of individuals develop IBM with progressive proximal muscle weakness. VCP mutations have been seen in 2-3 % of familial amyotropic lateral sclerosis (ALS) and 15% of individuals with VCP hereditary inclusion body myopathy have an ALS phenotype. Cellular and animal models indicate pathogenic disturbances in IBMPFD tissues including ERAD, autophagy, apoptosis, and mitochondrial dysfunction. In the current study, we aim to characterize the R155H homozygote mouse model and to utilize this as a useful tool for investigating the pathogenesis of IBMPFD and ALS. Homozygosity of the R155H mutation is lethal and these animals die by 14 to 21 days of age, the cause(s) of which remains undetermined. Preliminary histological results have shown pathology of the heart and muscle. These mice are smaller and reveal abnormal muscle architecture of the skeletal muscle and large vacuoles in the cardiac muscle fibers. Thus, elucidating the basic molecular signaling cascades involved in IBMPFD and ALS diseases and improving protein homeostasis will ultimately lead to strategies and therapies.

536W

haplogroup U indicates proneness to autism but on the contrary, haplogroup H is likely to be the least affected mtDNA variant. *F. Piryaei[1], M. Houshmand[1,2], O. Aryani[2], S. dadgar[2], Z.S. Soheili[1].* 1) Medical genetics, National Institute of Genetic Engineering and Biot, Tehran, Iran; 2) Special medical center, Tehran, Iran.

Autism is a disorder of neural development characterized by impaired social interaction and communication, and by restricted and repetitive behaviour which appears to result from developmental factors that affect many or all functional brain systems. Autism is noticeably suggested to be affected by mitochondrial dysfunction since Brain function crucially depends on ATP production so that over 95% of total brain ATP is provided via oxidative phosphorylation. Employing direct sequencing of mtDNA fragments, the objectives of this study was to investigate probable mutations within mtDNA genes which might be causative in autism in 24 affected individuals and to compare the distribution of those individuals and 100 control subjects among mitochondrial haplogroups. 9 nucleotide substitutions were identified including G8251A, G8269A, A8271G, C8472T, C8684T, G8697A, A8701G, A8836G and G8865A in patients. G15928A and A16149C were observed in two groups as A16149C was a novel substitution. A15973G was another novel detected mutation observed in patients. Sequencing of the D loop resulted in different haplogroups. Distribution of affected and control groups among different haplogroups revealed that in all probability haplogroup U indicates proneness to autism. On the contrary, haplogroup H is likely to be the least affected mtDNA variant.

537W

Characterization of interacting partners of WNK1/HSN2 in hereditary neuropathy sensory and autonomous type II (HSAN2). *S. Ramalingam[1], J.-B. Rivière[1], V. Lavastre[1], M. Shekarabi[1], S. Holbert[1], J. Lafontaine[2], M. Srour[1], N. Merner[1], D. Rochefort[1], P. Hince[1], R. Gaudet[1], A.-M. Mes-Masson[2,3], J. Baets[4], H. Houlden[6], B. Brais[1], G. Nicholson[7], H. Van Esch[8], S. Nafissi[9], P. De Jonghe[4,5], M.M. Reilly[6], V. Timmerman[4], P.A. Dion[1,10], G.A. Rouleau[1,11].* 1) Center of Excellence in Neuroscience of the Université de Montréal (CENUM), Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), and Department of Medicine, University of Montreal, Montreal, Quebec, Canada, H2L 4MI; 2) CHUM Research Center, Montreal Cancer Institute, Montréal, Québec, Canada; 3) Department of Medicine, Université de Montréal, Montréal, Québec, Canada; 4) Department of Molecular Genetics, VIB and University of Antwerp, Antwerpen, Belgium; 5) Department of Neurology, Antwerp University Hospital, Antwerpen, Belgium; 6) MRC Centre for Neuromuscular Diseases, Department of Molecular Neurosciences, UCL Institute of Neurology, London, UK; 7) Northcott Neuroscience Laboratory, ANZAC Research Institute, Concord Hospital, and Faculty of Medicine, University of Sydney, Australia; 8) Center for Human Genetics, Katholieke Universiteit Leuven, Leuven, Belgium; 9) Department of Neurology, Shariati Hospital, Tehran University of Medical Sciences, Tehran, I. R. Iran; 10) Department of Pathology and Cellular Biology, Université de Montréal, Montréal, Québec, Canada; 11) Research Center, CHU Sainte-Justine, and Department of Pediatrics and Biochemistry, Université de Montréal, Montreal, Quebec, Canada.

Hereditary sensory & autonomic neuropathies (HSANs) are a heterogeneous group of disorders characterized by variable sensory and autonomic dysfunction due to peripheral nerve involvement. HSAN2 is a rare autosomal recessive and progressive disease with onset in the 1st decade of life. We previously established that mutations in the nervous system-specific HSN2 exon of WNK1 cause HSAN2. Mutations in WNK1/HSN2 were shown to cause HSAN2 in familial cases. The clinical and pathological data suggest that HSAN2 is characterized by abnormal development and/or degeneration mainly of sensory fibers. First, we screened for human nervous system proteins that bind to HSN2 exon using a yeast two-hybrids approach (Y2H). Positive clones were isolated based on their activation of reporter genes and the cDNAs from positive clones were rescued, sequenced and analyzed using NCBI BLAST. Following this Y2H screen, a list of potentially interesting interactors was generated and the interactions validated using co-immunoprecipitation (Co-IP) and Western blots using commercial antibodies recognizing the interactors identified. From these results, we are focusing on some specific partners of HSN2 who seems to have an important role in this disease. This project will lead to a better understand of the pathological mechanisms leading HSANII.

538W

Penalized regression to detect multiple genetic associations with motor symptom progression in Parkinson's Disease. *S.L. Rhodes[1], M. Creek[2], J. Sinsheimer[2,3,4], B. Ritz[1].* 1) Dept Epidemiology, UCLA Sch Public Hlth, Los Angeles, CA; 2) Dept Biostatistics, UCLA Sch Public Hlth, Los Angeles, CA; 3) Dept Human Genetics, Geffen School of Medicine at UCLA, Los Angeles, CA; 4) Dept Biomathematics, Geffen School of Medicine at UCLA, Los Angeles, CA.

Idiopathic Parkinson's disease (PD) is characterized by a highly variable progressive decline in motor function such that some patients become wheelchair bound within a few years while others remain without major restriction for longer periods. While current therapies treat the symptoms, no treatment prevents the neurodegeneration or the progressive decline in motor function. Few studies have investigated predictors of motor decline despite its significant impact on quality of life for PD patients. Recognized predictors include age of symptom onset, motor symptom subtype, and dementia prior to or concomitant with PD diagnosis; but, to date, no genetic predictors of progression have been identified. Given the complex etiology of PD, we hypothesized that subjects with an increased number of genetic variants in recognized PD candidate genes would be at increased risk for more rapid decline of motor function. To test this hypothesis used two complementary approaches, (1) we constructed an allelic dosage score, and (2) built models using lasso penalized regression, a continuous model selection that tests the effects of all variants simultaneously. The lasso approach allows discovery of gene-gene interactions where two-way, three-way, and even higher order interactions can be considered. This study was approved by the UCLA Human Subjects Committee and all subjects provided written informed consent. 228 incident PD patients had sufficient baseline and follow-up data to contribute to the analysis. We observed no differences between rapid and slow progressors for gender, family history of PD, duration of disease prior to baseline, interval between baseline and follow-up, or PD motor subtype. Similar to prior observations, rapid progressors were older at symptom onset than slow progressors ($p=0.018$); dementia at baseline was an exclusion criteria and thus could not be assessed. Consistent with our hypothesis, we observed a significant increase in the number of risk SNPs in rapid progressors compared with slow progressors ($p<0.001$). Furthermore, we observed a cluster of genes related to metal and iron metabolism associated with faster progression of motor symptoms. If replicated in animal models and other PD studies, these findings suggest an avenue of investigation for new therapeutics aimed to slow the progression of motor function decline in PD.

539W

Increased sociability and social interaction in mice lacking glutamate receptor interacting proteins. *R. Rose[1], R. Mejias[1], A. Adamczyk[1], R. Huganir[2], T. Wang[1].* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Department of Neuroscience, Johns Hopkins University, Baltimore.

Glutamate receptor interacting protein 1 and 2 (GRIP1/2) are homologous and neuron-enriched scaffolding proteins that interact directly with the C-termini of glutamate receptors 2/3 (GluA2/3) via its PDZ domains 4-6 (PDZ4-6). GRIP1/2 play an important role in regulating GluA2/3 trafficking and neuronal plasticity. Loss of GRIP1/2 was found to impair activity dependent GluA2 recycling in neurons. Gain-of-function GRIP1 variants clustered around PDZ4-6 have recently been reported to alter GluA2 recycling and contribute to the severity of deficits in social interaction in autistic patients. To understand the role of GRIP1/2 in social function and autism spectrum disorders, we characterized the social behavioral phenotype of adult Grip1/2 double knockout (DKO) mice. Grip1/2 DKO mice were generated by crossing Grip2 conventional KO with Grip1 conditional (neuron-specific deletion via nestin-cre expression) KO mice. Grip1/2 DKO mice and wild type (wt) control mice were matched for age, sex, and strain background. Compared to wt, Grip1/2 DKO mice were found to have a significant increase in sociability towards stranger mice (time with stranger mice in seconds: wt: 44±5; DKO, 81±8, $p=0.0022$; $n=10$); in preference for social novelty towards novel versus familiar mice (time with novel mice in seconds; wt: 35±3; DKO: 59±6, $p=0.0024$; $n=10$); and in male-male social interactions in open arena (time of social interaction in seconds; wt: sniffing, 18.9±1.1; following, 7.1±0.7; DKO: sniffing, 38.6±1.9; following, 12.6±1.9; $p<0.001$; $n=10$). These results strongly support a role of GRIP1/2 in modulating social behaviors and implicate glutamate-signaling disturbance in social behavioral disorders including autism.

540W

Deletion of MAOA and MAOB in male siblings with severe mental retardation and autistic phenotype. *M. Saito, T. Yamagata, Y. Shiba, N. Nakashima, M. Nagashima, E. Jimbo, M.Y. Momoi.* Dept Pediatrics, Jichi Med Univ, Shimotsuke, Japan.

Copy number variation (CNV) took major place on the pathogenesis of autism spectrum disorder (ASD). On the array complementary genomic hybridization (aCGH) analysis for CNV detection in ASD patients, we detected deletion of *monoamine oxidase (MAO) A* and *MAOB* in the siblings with severe mental retardation and ASD. Patient 1 was five-year-old boy. He could sit without support and spoke only several words. He was hypotonic and showed impaired sociability, stereotypic movements, excitability, self-injury and insomnia. Patient 2 was a three-year-old brother of patient 1. He could sit, but could not speak any words. Other symptoms were resembled with patient 1. Both patients were not blind. On array CGH analysis using Agilent Human genome aCGH 44K in patient 1, about 600kb deletion on Xp11.3 was detected. In this deleted region, only *MAOA* and *MAOB* were existed. In patient 1 and 2, *MAOA* and *MAOB* deletion was confirmed by PCR analysis designed to amplify several exons of each gene on genomic DNA. *NDP*, the gene for Norrie disease that showed blindness and mild mental retardation, were not deleted on aCGH analysis and the expression of *NDP* was confirmed by RT-PCR. MAO catalyzes serotonin and catecholamine. Therefore, serotonin and catecholamine levels in cerebrospinal fluid (CSF), serum and urine were analyzed. CSF serotonin level were markedly elevated to 3,000pg/ml (normal <10) in both patients, and 5-HIAA was <1.0ng/ml (24.6±1.8). Catecholamine level was mildly elevated such as CSF norepinephrine 409pg/ml(180±17), and urine dopamine 1.88mg/gCr (0.77±0.22) in patient 1. *MAOA* were reported to relate with ASD from the association study with 5' VNTR or SNPs on *MAOA*. However, mutations of *MAOA* were detected on the patients with mild mental retardation and anti-social behavior. Mutation of *MAOB* showed no symptoms. Deletion of *MAOA, MAOB* and *NDP* caused severe mental retardation and blindness. Deletion of *MAOA* and *MAOB* were reported in only one sibling with severe developmental delay and stereotypical hand movements, and our patients showed similar phenotypes with them. From our results, these symptoms such as severe mental retardation, ASD, exitability and hypotonia were considered to be caused by serotonin toxicity from the loss of catalyzing ability of MAO.

541W

Genome-wide linkage analysis suggests oligogenic regulation of brain auditory responses. *E. Salmela[1], H. Renvall[2], M. Vihla[2], J. Kere[1,3], R. Salmelin[2].* 1) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 2) Brain Research Unit, Low Temperature Laboratory, Aalto University, Finland; 3) Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden.

All sounds entering the ear produce robust auditory evoked responses (AERs) in the human brain. AERs are variable between individuals but typically stable over time for a given individual, and they have been widely used to probe cortical functions in both healthy and clinical populations. However, little is known about their underlying molecular mechanisms.

To study the possible genetic determinants of the AERs in a normal population, we have measured AERs from 113 pairs of healthy siblings using magnetoencephalography (MEG). The subjects were presented with 1 kHz tones in alternating ears, and the responses were recorded with a 306-channel Elekta Neuromag neuromagnetometer. The subjects were also genotyped on Affymetrix 250K *StyI* SNP arrays, yielding genotypes for ca. 150,000 single-nucleotide polymorphisms (SNPs) after filtering.

In both cerebral hemispheres, the amplitudes of the responses at ~100 ms (N100m) to both ipsi- and contralateral stimuli were highly heritable ($h^2 > 0.70$). A variance component-based analysis revealed linkage for the right- but not for the left-hemispheric amplitudes: a significant linkage of the ipsilateral amplitudes to a region on chromosome 2q37 (LOD = 3.32, $p < 0.04$ based on 330 permutations with Merlin), and a suggestive linkage of the contralateral amplitudes to the same region (LOD = 3.01, $p < 0.15$); additional suggestive linkage peaks were seen on chromosome 3p12 (LOD = 2.55, $p < 0.44$) for the contralateral and on chromosome 8q24.3 (LOD = 3.27, $p < 0.06$) for the ipsilateral amplitudes. Several of the highlighted regions contain functionally plausible genes, and further analyses of them are ongoing. Overall, the results demonstrate the potential of genetic analysis in linking macroscopic cortical phenotypes with molecular-level processes controlled by specific genes.

542W

Copy Number Variants and Agenesis of the Corpus Callosum: A Significant Etiologic Mechanism that Overlaps with Autism. *E. Sherr[1], S. Sajan[2], L. Fernandez[1], E. Rider[1], S. Esmaeeli[1], J. Glessner[3], H. Hakonarson[3], S. Christian[2], W. Dobyns[2].* 1) Departments of Neurology & Pediatrics, Univ California, San Francisco, San Francisco, CA; 2) Center for Integrative Brain Research, University of Washington, Seattle, WA; 3) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA.

Background: One major causative hypothesis for autism is that alterations in long-range cortical connectivity underlie the pleiotropic clinical manifestations. Agenesis of the corpus callosum (AgCC) is a common CNS malformation in which many individuals have autism or have deficits on the autism spectrum. Like autism, the genetics of AgCC is likely to be complex and prior small reports from our lab and others have suggested that de novo copy number variants (CNV's) may play an important causative role. Hypotheses: We hypothesized that AgCC patients would have a significant number of large candidate CNV's and that these CNV's may overlap with those identified in autism. Methods: We identified patients with AgCC by a comprehensive radiological and clinical review of our IRB approved AgCC cohort (n>500). Blood samples were obtained from the proband and both biological parents, when available. DNA was extracted and run on an Illumina 610 Quad Chip array. Data were analyzed using PennCNV. CNV's that were selected for analysis contained greater than 9 SNPs, were longer than 30 kb, had a PennCNV confidence level above 10 and contained one or more genes. Manual curation and merging of contiguous large CNVs were conducted. Results: 271 patient samples were run and high quality data were obtained from 96%. These data were compared against 1953 ethnically matched controls that were run and analyzed on the same platform. We compared both CNVs binned by size and grouped by associated genes. Rare deletion CNV's larger than 500 kb and gene rich (>20) were significantly associated with AgCC patients as compared to controls (OR = 14.33; p = 0.000016). A complex deletion-duplication on chromosome 8p was recurrent in the AgCC cohort and at other loci, both the deletion and reciprocal duplication were found both with AgCC. We also analyzed the enrichment of genes within CNVs previously associated with autism and found that CNV deletions in AgCC patients were significantly correlated with CNVs duplications in autism (OR = 9.74; p = 0.0062). Conclusions: Large CNV's are commonly found in AgCC patients and there is a specific enrichment of autism genes with AgCC CNV's. These data support a strong etiologic link between autism and AgCC and likely shared molecular and developmental pathways.

543W

A genetic study associating brain volume abnormalities with serotonin system gene variants in pediatric obsessive-compulsive disorder. *V.M. Sinopoli[1,2], K. Wu[1], F. MacMaster[4], P. Easter[4], J. Kennedy[3], G. Hanna[5], D. Rosenberg[4], A. Arnold[1,2].* 1) University of Toronto, Toronto, ON, Canada; 2) Hospital for Sick Children, Toronto, ON, Canada; 3) Centre for Addiction and Mental Health, Toronto, ON, Canada; 4) Wayne State University, Detroit, MI, USA; 5) University of Michigan, Ann Arbor, MI, USA.

Background: Obsessive-compulsive disorder (OCD) is a heritable and common neuropsychiatric disorder of unknown etiology. A number of candidate gene studies have been conducted; serotonergic system genes being of particular interest, since serotonergic medications are highly efficacious in treatment of this disorder. Genetic variations attributed to the serotonin transporter gene (SLC6A4), serotonin receptor 2A gene (HTR2A), and serotonin receptor 1B gene (HTR1B), have been implicated. In addition, studies have attributed the disorder to regional volumetric brain abnormalities, with the striatum (caudate and putamen) being among the most consistently associated regions. Purpose: This study attempts to associate serotonergic system gene polymorphisms with volumetric brain region abnormalities in a clinical, pediatric OCD sample. Methods: Genetic and structural magnetic resonance imaging (sMRI) brain data were collected from a clinic-based, pediatric sample of 20 medication-naïve OCD patients, ages 7 to 17, at Wayne State University in Detroit, Michigan. Association analysis was conducted for SLC6A4, HTR2A, and HTR1B, which have been previously implicated in OCD. A total of 40 single nucleotide polymorphisms (SNPs), spanning each gene, were examined and tested for association with volumes of brain regions with `a priori' evidence of association with OCD: thalamus, anterior cingulate cortex (ACC), orbitofrontal cortex (OFC), and caudate. Results: In HTR2A, the C allele of single nucleotide polymorphism (SNP), rs1923886, was found to be significantly associated with reduction in volume of the left putamen (adjusted P-value of 0.035). The same SNP was nominally associated with total and right putamen volume (not significant after adjustment for multiple comparisons). Conclusions: We identified an association between volume of the putamen, a component of the striatum, with a specific genetic variant in children with OCD. These results were consistent with previous neuroimaging findings implicating the striatum in OCD pathophysiology. Regional brain volumes provide potentially valuable endophenotypes (intermediate phenotypes) that may be associated with decreased heterogeneity compared to complex behaviours, thereby facilitating the identification of specific genotypic associations.

544W

Multi-variant analysis of "dopamine pathway" genes in skiers and snowboarders reveals no association with sensation seeking behaviours. *C.J. Thomson[1], S.R. Carlson[2], J.R. Rupert[1].* 1) Kinesiology, Univ British Columbia, Vancouver, BC, Canada; 2) Department of Psychology, University of Minnesota Duluth, Duluth, Minnesota, U.S.A.

The personality trait sensation seeking (SS) involves a desire to seek out new and thrilling experiences and has been associated with risky behaviours ranging from substance abuse to high-risk sports (*e.g.*, surfing, skiing). Approach-related traits, of which sensation seeking is one, are to some extent regulated by the neurotransmitter dopamine. Genes involved in dopamine synthesis, transport, and metabolism have been investigated in numerous studies of approach-related traits, externalizing disorders (*e.g.*, attention deficit hyperactivity disorder and conduct disorder), and substance abuse; however, few studies have measured sensation seeking specifically, and none have investigated the trait in a sporting context. We predicted that variants in genes contributing to the dopamine pathway would associate with sensation seeking behaviour in athletes. To test this, we developed a reliable questionnaire to evaluate SS behaviours in the context of skiing and snowboarding (the CSSQ-S). We administered this questionnaire, along with the commonly used Zuckerman-Kuhlman Personality Questionnaire (ZKPQ) that includes the Impulsive Sensation Seeking subscale (ImpSS) to a sample of male and female skiers from Western Canada (N = 575). Using DNA isolated from buccal cells, 32 SNPs in eight genes that encode proteins related to dopamine synthesis or function (*DRD1, DRD2, DRD3, DRD4, DAT1, COMT, MAO-B, DBH*) were genotyped. Many of the SNPs we examined had been previously explored in association with other externalizing phenotypes. Alleles at four genes (*DRD1* rs251937, *DRD2* rs6277 and rs1800497, *DRD3* rs167771, and *DAT1* rs27072 and rs463379) were associated with ImpSS score and/or CSSQ-S score (p < .05); however, these associations were not significant when the results were corrected for number of tests (*i.e.*, post FDR correction). While our data did support a correlation between skiing behaviour (as measured by the CSSQ-S) and SS in general (as measured by the ZKPQ), we did not find any evidence that alleles at the SNPs we examined were associated with SS behaviour in our cohort of skiers/snowboarders.

545W

Comparative analysis of Dopamine D4 Receptor (DRD4) rare variants in Schizophrenia and Attention Deficit and Hyperactivity Disorder (ADHD): Further evidence of allelic heterogeneity contribution to ADHD etiology. *L. Tovo-Rodrigues[1], L.A. Rohde[2], T. Roman[1], A. Salatino-Oliveira[1], J.P. Genro[1], F.B. Kohlrausch[1], C. Kieling[2], C. Zeni[2], G.V. Polanczyk[3], M. Schmitz[2], E.S. Gama[2], M.I. Lobato[2], M.H. Hutz[1].* 1) Department of Genetics, UFRGS, Porto Alegre, RS, Brazil; 2) HCPA, Porto Alegre, RS, Brazil; 3) Department of Psychiatry, USP, São Paulo, SP, Brazil.

The dysregulation of the dopaminergic system has been implicated in the pathophysiology of ADHD and Schizophrenia. Some studies have suggested that the susceptibility genes underlying both disorders might partially overlap. DRD4 is one of the most variable genes in the human genome. Its third exon contains a VNTR of 48bp and differences of single nucleotide polymorphism inside the repeats. VNTR polymorphism can vary from 2 to 11 and 7 repeat has been suggested as a susceptibility allele for psychiatry diseases. However, association studies have shown conflicting results. As an excess of rare variants was observed in ADHD probands, these inconsistences could be explained by the presence of the mutation sites inside VNTR. This study aims to compare the VNTR sequence variability of 4R and 7R alleles in predominantly Euro-Brazilian ADHD, schizophrenic and control subjects. Out of 650, 208 and 330 genotyped ADHD, schizophrenic and control individuals respectively, all 7R and some 4R homozygous individuals were resequenced, comprising 132 ADHD alleles, 68 schizophrenia alleles, and 74 control alleles. DNA was amplified and PCR product was sequenced. Haplotypes were estimated using a Bayesian method implemented in PHASE2.1 software. Fisher's Exact Test was used to compare DRD4 variants distribution among the three groups. A total of 17 haplotypes were observed in this sample. When only 4R variants are considered, no difference were observed among groups (P=0.124). Considering 7R allele, a difference was observed among groups (P=0.009). The frequency of rare variants in ADHD, schizophrenia and control groups were 0.210 (33 variable sites), 0.000 and 0.033 (6 variable sites) respectively. Multiple comparison followed by Bonferroni correction revealed an excess of rare variants in ADHD when compared to control (P=0.042) and schizophrenia groups (P=0.014) while no difference was observed between control and schizophrenia groups (P=0.927). In conclusion, this study indicates the major effect of DRD4 exon 3 VNTR rare variants and allelic heterogeneity is on ADHD genetic susceptibility. Probably this finding is exclusive to this disorder and could not be extended to other psychiatry conditions. It is likely that all previous studies of the DRD4/ADHD association modestly underestimated the relative risk by only examining repeat length rather than DNA sequence.

546W

Mutation Screening of Microtubule Genes in Sporadic and Familial Patients with Bilateral Perisylvian Polymicrogyria. *S.S. Tsuneda[1], F.R. Torres[1], M.M. Guerreiro[1], C.A. Guimarães[1], V.C. Terra-Bustamante[2], M.A. Montenegro[1], F. Cendes[1], I. Lopes-Cendes[1].* 1) Unicamp, Campinas, SP, Brazil; 2) USP, Ribeirão Preto, SP, Brazil.

Polymicrogyria (PMG) is a malformation caused by abnormal brain cortex organization, characterized by multiple small gyri with abnormal cortical lamination. The most common site for PMG is the Sylvian fissure (80%), of which 40%; are bilateral. In these patients, the cerebral cortex on the border and in the depth of the Sylvian fissure is thickened and abnormally infolded, more vertically oriented and may be extending more posterior up to the parietal lobes, characterizing the syndrome called bilateral perisylvian polymicrogyria (BPP). Approximately 28 familial cases of BPP have been described, from which 75%; are compatible with an X-linked heritance pattern. Our group has recently described a candidate locus for BPP on chromosome Xq27.2-27.3. However, most patients with BPP are sporadic and mutations in microtubule genes, acting in microtubules dynamics, such as TUBA1A, TUBB2B, TUBA8A and WRD2, have been recently implicated with several types of cortical malformations. Among them, only TUBA1A has been related to BPP, in a single family. The objective of the present study is to search for mutations in TUBA1A, TUBB2B, TUBA8A and WRD2 genes in familial and sporadic patients with BPP. Genomic DNA obtained from 26 unrelated patients with BPP was used as template for polymerase chain reactions (PCR) with primers for coding regions and intron/exon boundaries of the candidate genes. Amplicons were submitted to automatic sequencing and the chromatograms obtained were compared to nucleotide sequences in the NCBI database. We identified four base substitutions in TUBA1A gene: c.453G>C, c.510T>C, c.522G>A and c.541G>A. The first three sequence variations have been already described as synonymous substitutions. However, c.541G>A has not been yet reported and was identified in three unrelated patients. This base change results in the translation of an isoleucine for a valine at the position 181 of the protein. Preliminary analysis with SNPs&GO ans SIFT algorithms show that this aminoacid substitution is probably neutral. Since both aminoacids (isoleucine and valine) have a very similar structure, raising the possibility of a non-pathogenic mutation. However, further experiments, including the investigation of a large number of control subjects are under way, in order to better investigate the possible role of this newly described sequence variation in the etiology of BPP. Financial support: CNPq, FAPESP and EMBRAPA.

547W

Rare deletions at the *NRXN3* locus in individuals with Autism Spectrum Disorder. *A.K. Vaags[1,2], A.C. Lionel[1,2,3,4], D. Sato[1,2], J.L. Howe[1,2], C.R. Marshall[1,2], S. Lamoureux[1,2], I.E. Drmic[5], L. Senman[5], C. Chrysler[6], A. Thompson[6], C. Russell[6], D. Pinto[1,2], A. Prasad[1,2], S. Walker[1,2], W. Roberts[5,7], P. Szatmari[6], S.W. Scherer[1,2,3,4].* 1) The Centre for Applied Genomics, Hospital for Sick Children, Toronto, ON, Canada; 2) Genetics and Genome Biology, Hospital for Sick Children, Toronto, ON, Canada; 3) Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 4) The McLaughlin Centre, University of Toronto, Toronto, Ontario, Canada; 5) Autism Research Unit, The Hospital for Sick Children, Toronto, Ontario, Canada; 6) Offord Centre for Child Studies, Department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, Ontario, Canada; 7) Bloorview Kids Rehabilitation, University of Toronto, Toronto, Ontario, Canada.

The three members of the human neurexin gene family, *NRXN1*, *NRXN2* and *NRXN3*, encode neuronal adhesion proteins with important roles in synapse development and function. Recent research has implicated rare exonic copy number variants (CNVs) and point mutations at the *NRXN1* and *NRXN2* loci in Autism Spectrum Disorder (ASD) and other neurodevelopmental disorders. Illumina 1M and Affymetrix 6.0 microarray analysis of 1,600 ASD cases and their family members identified rare CNVs within the *NRXN3* gene. We now present clinical characterization of four subjects, from two unrelated families, with rare deletions at 14q24.3-31.1 overlapping exons of the *NRXN3* gene. Deletions with similar breakpoints to those seen in the probands were not observed in high-resolution microarray data from 8,427 population-based controls. The main phenotypic features in our subjects include characteristic behaviors associated with Asperger's syndrome and ASD, the severity of which, were correlated with the extent of the *NRXN3* deletion. These cases are now undergoing exome-sequencing to identify additional genetic changes that may contribute to ASD. In addition, Sanger sequencing of the coding region of the *NRXN3* gene in 350 ASD cases identified four individuals with *NRXN3* point mutations. Of note, these changes were also present in their unaffected siblings and thus may not be directly associated with the development of an ASD phenotype. This is the first report of ASD subjects with exonic deletions at the *NRXN3* locus, and adds to the evidence linking rare genetic variants affecting the neurexin gene family, with risk for neurodevelopmental disorders.

548W

Expression-based *in silico* screening of candidate therapeutic compounds for mesial temporal lobe epilepsy. *J.F. Vasconcellos[1], C.V. Maurer-Morelli[1], C.S. Rocha[1], C.L. Yasuda[2], H. Tedeschi[2], E.D. Oliveira[2], F. Cendes[2], I. Lopes-Cendes[1].* 1) Department of Medical Genetics, Faculty of Medical Sciences, State University of Campinas and CINAPCE Program, Campinas, SP, Brazil; 2) Department of Neurology, Faculty of Medical Sciences, State University of Campinas and CINAPCE Program, Campinas, SP, Brazil.

Mesial temporal lobe epilepsy (MTLE) is one of the most common forms of human epilepsy, whose pathological hallmarks are neuronal loss and synaptic reorganization in the hippocampus. The Connectivity Map (C-MAP, http://www.broadinstitute.org/cmap/index.jsp) is a web tool for gene expression signature screening *in silico* (represented by a set of genes with up- or down-regulated labels) that potentially reveals interactions among drugs, genes and diseases, providing information to identify potential drugs and targets based on gene expression analysis. MTLE is largely described as a sporadic disease; however, we and others have recently shown that MTLE can also segregate in families. The present study was conducted to investigate new potential therapeutic targets for the treatment of patients with MTLE based on their gene expression signatures through the C-MAP analysis. Transcriptional profiling by microarray analysis revealed that patients with MTLE and a positive family history [(+)FH] of MTLE had 170 differentially expressed genes and patients with a negative family history [(-)FH] had 341, when compared to healthy controls. C-MAP analysis of patients with (+)FH gene signature demonstrated a negative correlation with IL-6 and glycogen synthase kinase-3 inhibitors (connectivity score -0.692 and -0.568, respectively). IL-6 activation in epilepsy may play a role protecting the central nervous system (CNS) from seizure-induced damage, while glycogen synthase kinase-3 activates WNT pathway and may contribute to the regulation of neuronal survival and homeostasis in the CNS. C-MAP analysis of patients with (-)FH gene signature demonstrated a negative correlation with the signatures for TNF-(/NF-k) and HSP90 inhibitors (connectivity score -0.697 and -0.238, respectively). These inhibitors mediate the inactivation of main cell survival pathways. Interestingly, there was no overlap between the molecules from the C-MAP database that had a significant negative correlation in the (+)FH MTLE gene signature and those compounds which were identified in the (-)FH MTLE gene signature group. In conclusion, our data demonstrate for the first time the use of an *in silico* approach to search for potential molecular targets for the treatment of patients with MTLE based on its gene expression signature. Furthermore, the C-MAP revealed a clear difference in the gene expression profiles of MTLE patients with and without familial recurrence of the disease. Supported by: FAPESP.

549W

White Matter Lesion on Brain MRI can be Diagnostic for ATR-X syndrome: The Study of Brain MRI/CT findings in ATR-X syndrome Patients in JAPAN. *T. Wada[1], H. Ban[2], K. Enomoto[3], K. Kurosawa[4], N. Aida[5], ATR-X Syndrome Network Japan.* 1) Pediatric Neurology, Kanagawa Children's Med Center, Yokohama, Japan; 2) Pediatrics, Himeji Red Cross Hospital, Himeji, Hyogo, Japan; 3) Pediatrics, Tokyo Medical and Dental University, Tokyo, Japan; 4) Genetics, Kanagawa Children's Med Center, Yokohama, Japan; 5) Radiology, Kanagawa Children's Med Center, Yokohama, Japan.

X-linked a-thalassemia/ mental retardation (ATR-X) syndrome (MIM#301040) is among X-linked mental retardation syndromes, which is due to mutations of ATRX gene, and is characterized by boy/male patients, central hypotonic facies, severe cognitive dysfunction, HbH disease (a-thalassemia), genital abnormality, skeletal abnormality, and autistic and/or peculiar behavior. More than 200 patients in the world, including more than 70 Japanese cases, have been diagnosed as ATR-X syndrome. ATR-X syndrome seems a rare disease, however, we estimated that the prevalence of ATR-X syndrome is 1/ 30,000-40,000 newborn boys, which is much higher than that have been reported formerly. So more patients should remain to be diagnosed. Since 2010, we have established ATR-X syndrome Japan Network for patients and their families, and we have surveyed ATR-X syndrome patients in Japan. In this study, we investigate the brain MRI and/or CT findings of 25 Japanese ATR-X patients who had ATRX gene mutations. The MRI/CTs of all other than five patients showed nonspecific brain atrophy. Interestingly, the T2 and FLAIR MRI of seven patients, which we could analyze precisely, showed high intensity of the white matter around the trigones with/without multiple small spherical foci that parallel CSF intensity, and some patients were misdiagnosed as a leukodystrophy or white matter disease. These findings did not seem to correlate with their clinical severity of the patients. The sequential MRIs of another patient, who had infantile spasm at 1 year old, showed progressive brain atrophy and ventricular enlargement. We should consider ATR-X syndrome as differential diagnosis in patients with intellectual disabilities, whose T2 or FLAIR MRI shows high intensity regions in white matter, especially around the trigones. And we propose these findings as associated (<50) features or supporting features of the diagnostic criteria of ATR-X syndrome or ATRX gene mutation positive patients with atypical ATR-X syndrome features. We are now establishing the diagnostic criteria, which facilitate further clinical study of individuals with proven ATR-X molecularly, and assist in the evaluation of those who appear to have clinical features of ATR-X with no ATRX mutations. We should evaluate brain MRI of more patients to validate these findings and update the consensus for it in the near future.

550W

Association of candidate genes conferring susceptibility to schizophrenia in a sample from Indonesia with 1097 cases and 1112 controls reveals association with rs1344706 located in the ZNF804A gene. *D. Wildenauer[1], N. Dai[1], W. Qin[1], M. Wildenauer[1], A. Kusumawardhani[2], S. Schwab[3], Indonesia Schizophrenia Genetic Consortium.* 1) CCRN Sch Psychiatry, UWA, Claremont, Australia; 2) Department of Psychiatry, University of Indonesia; 3) Department of Psychiatry, University of Erlangen-Nuremberg.

Schizophrenia is a complex genetic disorder with heritability of up to 80%. World-wide prevalence (life time risk) is on average about 1%. We hypothesize that genes conferring risk for schizophrenia are present in all populations and can therefore be detected in populations with different ethnic background. However, population specific susceptibility alleles may exist. Identification of these alleles may aid in gene identification in schizophrenia as well as in characterization of the reported differences in course and outcome between developing and developed countries. We have consecutively ascertained a sample of 1097 individuals with schizophrenia and 1112 non-psychiatric controls in five Mental State Hospitals in the area of Jakarta, Indonesia. Diagnosis was made according to the DSMIV criteria by psychiatrists, trained in the use of a structured interview (DIP) in Bahasa Indonesia translation. In addition, from previous studies we have a sample of 124 families from Indonesia with 2 or 3 affected siblings with parents (318 affected, 686 individuals in total) available. This family sample revealed genome-wide significance for a schizophrenia locus on chromosome 3p26-21. Using a TDT based approach we obtained some evidence for association for SNP rs 1703183 which is located in the linked area in the region of the GRM7 (Metabotropic Glutamate Receptor 7) gene. For our current association studies based on the sample of 1097 case and 1112 controls we have followed up this initial finding. In addition, we followed up on findings from GWAS studies in Caucasian samples, i.e rs2958182 (TCF4, chr18q), rs1344706 (ZNF804A, chr2), rs795009 (SYN2, chr3p), and rs2660304 (MIR137, chr 18). In order to assess homogeneity of our case control sample, we characterized all samples in respect to ethnicity, sampling error and genotyping quality, using a DNA test panel with 360 evenly spaced SNPs (Illumina). Evidence for association was obtained for rs1344706 (ZNF804A) with a P-value of 0.006 (chi2 test). Genotype distribution was also statistically significantly different between cases and controls (P=0.027). The odds ratio was 1.179 (95% confidence interval 1.047 - 1.327). Controls revealed a T-allele frequency of 0.488, cases a frequency of 0.529, which is different from Caucasian samples, but similar to Han Chinese samples. Our results support a potential role of ZNF804A in the etiology of schizophrenia and may confirm that this is not population specific.

551W

Loss-of-function mutation of collybistin is responsible for X-linked mental retardation associated with epilepsy. *T. Yamamoto[1], K. Shimojima[1], M. Sugawara[1], M. Shichiji[1,2], S. Mukaida[3], R. Takamaya[3], K. Imai[3].* 1) Inst Integrated Medical Sci, Tokyo Women's Med Univ, Tokyo, Japan; 2) Department of Pediatrics, Tokyo Women's Medical University, Tokyo, Japan; 3) National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan.

Microarray-based comparative genomic hybridization analysis identified a 737-kb microdeletion of Xq11.2 including the cdc42 (cell division cycle 42) guanine nucleotide exchange factor (GEF)-9 gene (ARHGEF9), encoding collybistin which plays a pivotal role in formation of postsynaptic glycine and gamma-aminobutyric acid (GABA)A receptor clusters, in a male patient with severe mental retardation and epilepsy. No overlapping deletion with this was identified in the database of genomic copy number variations. A cohort study of ARHGEF9 nucleotide sequence identified a nonsense mutation in another male patient with severe mental retardation and epilepsy. This mutation affects one of the 3 transcript variants of ARHGEF9, which was confirmed to be expressed in the brain by RT-PCR. Although this nonsense mutation was shared with the patient's mother, it was not observed in 100 normal individuals. Both male patients suffered epileptic seizures after 1 year of age. Brain magnetic resonance imaging revealed mild frontal atrophy in the first patient and right frontal polymicrogyria in the second patient. Three previously reported mutations of ARHGEF9 consisted with a missense mutation in a male patient with hyperekplexia and two chromosomal disruptions in 2 female patients. The common phenotypic effects of all ARHGEF9 mutations were mental retardation and epilepsy. Therefore, ARHGEF9 is likely to be responsible for syndromic X-linked mental retardation associated with epilepsy.

552W

Variants in NRG3 at 10q23.1 correlated with a subtype of schizophrenia with severe delusions. *M. Zeledón[1,2,3], M. Taub[4], N. Eckart[1,2], R. Wang[3], M. Szymanski[1,2], P. Chen[5], A. Pulver[3,6], D. Avramopoulos[1,3], A. Sawa[3], D. Valle[1].* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins Medical Institute, Baltimore, MD; 2) Predoctoral Training Program in Human Genetics, Johns Hopkins Medical Institute, Baltimore, MD; 3) Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD; 4) Biostatistics, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD; 5) Department of Medical Genetics, National Taiwan University Hospital, Taipei City, Taiwan; 6) Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD.

Schizophrenia (SZ) is a disabling psychiatric disease affecting 1% of the world's population. Previously, we reported a linkage peak (NPL of 4.7) at 10q22 in the Ashkenazi Jewish (AJ) population and followed this up with a fine mapping association study that found significant association between a phenotypic quantitative trait “delusion” and 3 SNPs in intron 1 of NRG3 (Chen et al., AJHG 84:21, 2009). Two other groups have replicated our findings in outbred samples. To identify causative variants in the 162kb LD block covering the 5' end of NRG3 and containing the 3 associated SNPs we performed Illumina sequencing of this LD block in 47 AJ SZ patients at the extremes of the delusion trait distribution. We found 5 variants in a 10kb segment of intron 1 with significant frequency differences (including 2 of the previously reported SNPs) and an inherited 1.8kb microdeletion 21.5kb upstream of the transcription start site. An individual homozygous for the deletion allowed us to unambiguously define the deletion haplotype which includes the risk alleles for all 5 associated SNPs. The deletion frequency among the AJ SZ patients with the highest delusion scores (MAF=10.1% n=69, 7 deletion carriers) was higher than in patients with the lowest delusion scores (MAF=0%, n=67, Fisher's exact p=0.013). We tested the correlation between delusion scores and deletion frequency for 658 AJ SZ patients across the range of delusion scores, and found the deletion significantly correlated with higher delusion factor scores (p genotype effects=0.0069). Due to its location 5' of the transcription start site, this deletion may be perturbing regulation of NRG3 expression. Alternatively, it may tag a haplotype containing the causative variant(s). In this regard, preliminary data on RNA from 180 temporal lobe samples show that one of the 5 delusion associated SNPs (rs10748842) is also strongly associated with the use of alternative NRG3 transcription start sites in adult brains. Our data suggest that the deletion or, more likely, one of the associated SNPs perturb expression of NRG3 isoforms and lead to increased risk for a high-delusion SZ phenotype. Understanding how variation in this region affects NRG3 regulation will allow us to decipher the mechanistic pathways leading to risk for SZ or modifications of the SZ phenotype.

553W

GWAS scoring routines and serial, permutated enrichment analyses reveal a substantial polygenic component to the risk of alcohol dependence, with biological ontologies implicated in both European-American and African-American subjects. *M. Zlojutro[1], D.M. Dick[2], A. Agrawal[3], K.K. Bucholz[3], M. Schuckit[4], S. Kuperman[5], J. Kramer[6], J.A. Tischfield[7], J.I. Nurnberger, Jr.[8], V. Hesselbrock[9], B. Porjesz[10], L. Bierut[3], H.J. Edenberg[8], L. Almasy[1].* 1) Department of Genetics, Texas Biomedical Research Institute, San Antonio, TX; 2) Virginia Commonwealth University, Richmond, VA; 3) Washington University School of Medicine, St. Louis, MO; 4) University of California, San Diego VA Medical Center, San Diego, CA; 5) University of Iowa College of Medicine, Iowa City, IA; 6) Department of Psychiatry, University of Iowa College of Medicine, Iowa City, IA; 7) Human Genetics Institute of New Jersey, Rutgers University, Piscataway, NJ; 8) Indiana University School of Medicine, Indianapolis, IN; 9) University of Connecticut Health Center, Farmington, CT; 10) Department of Psychiatry, SUNY Downstate Medical Center, Brooklyn, NY.

Alcohol dependence (AD) is a complex disorder in which both genetic and environmental factors affect susceptibility. Linkage, candidate gene and genome-wide association studies (GWAS) have identified several genes associated with AD, although explaining only a small fraction of the overall risk. To better understand the polygenic structure of the hereditary component underlying AD risk, we developed a series of scoring routines for aggregating genotypic information. Routines were delineated by P-value thresholds, both in cumulative and non-cumulative manners, and weighted by AD odds ratios derived from case-control GWAS data for European-American (EA; n=1,274) and African-American samples (AA; n=457) from the Collaborative Study on the Genetics of Alcoholism (COGA). Scores, representing hundreds or thousands of SNP variants, were calculated in population-matched target samples from the Study of Addiction: Genetics and Environment (SAGE) GWAS data. In addition, SNPs used in the various scoring routines were annotated to gene locations and tested for gene enrichment of biological pathways and ontologies using a permutated approach. SAGE scores explained a significant, yet small percentage of the variation in AD status, with peak values occurring at P-value thresholds of <0.05 and <0.30 for EA and AA target samples, accounting for 0.73% (P= 1.64 ×10-3) and 2.14% (P=2.08×10-4) of the variation, respectively. For non-cumulative routines, significant scores were observed at 0.50<P<0.55 and 0.55<P<0.60 for EAs and AAs, indicating that a substantial polygenic component underlies AD, involving thousands of common alleles, many of small effect. The COGA-derived scoring routines were also applied in the alternate population target samples, which showed no significant evidence of association, suggesting that population-specific alleles play an important role in AD risk. However, gene enrichment analysis of the various routines found several biological ontologies and pathways enriched in both the EA and AA samples. These include glucocorticoid receptors (significantly enriched for routines with P-value thresholds <0.20) and Maf transcription factors (P thresholds <0.35), as well as chloride transport (0.30< P thresholds <0.55) and glycine metabolism (0.30< P thresholds <0.85) that include gene variants of much smaller effect. These results underscore the complex, polygenic nature of AD and provide key insights into its underlying etiological mechanisms.

554W

RAAS pathway genes in susceptibility to Intracranial aneurysm in Indian population. *M. Banerjee[1], L.V Koshy[1], S. Sathyan[1], H.V. Easwer[2], S. Premkumar[3], J.P. Alapatt[3], R.N. Bhattacharya[2].* 1) Dept Human Molec Gen, Rajiv Gandhi Ctr Biotech, Trivandrum Kerala, India; 2) Dept. Neurosurgery, SCTIMST, Trivandrum Kerala, India; 3) Dept. Neurosurgery, Calicut Medical college, Calicut, Kerala, India.

Intracranial aneurysm (IA) is a fairly common condition that is often asymptomatic until the time of rupture, resulting in Aneurysmal subarachnoid hemorrhage (aSAH), ensuing significant morbidity and mortality. It is the cause for an estimated five to 15 percent of all strokes. The reported prevalence rate of intracranial aneurysms in India varies from 0.75 to 10.3 percent as determined in angiographic and autopsy studies. Current evidence supports the concept, that the pathogenesis of intracranial aneurysms has a multifactorial origin, where the essential defect of the arterial wall may, at least in part be genetically determined. Hypertension is one of the most common population attributable risk factor for aSAH in Indian population. Renin-Angiotensin-Aldosterone System (RAAS) plays a very prominent role in regulation of blood pressure by influencing vascular volume, homeostasis and vascular tone. The genes that encode components of the RAAS are thought to play a role in determining genetic susceptibility to essential hypertension, and may also be viewed as a candidate pathway for IA. The mechanism of decreased expression of local RAAS could be attributed to mutations in genes that encode components of the RAAS systems, thereby conferring a genetic susceptibility to IA. These genetic mutations could act as effect modifiers that could interact with environmental risk factors like hypertension to influence the presence or absence of IA, or even the rupture of IA. In the present study we focused on six primary blood pressure-regulating genes of the RAAS: renin (REN), angiotensionogen (AGT), angiotensin I-conveting enzyme (ACE), angiotensin II receptor, subtype 1 (AGTR1) , cytochrome b-245 alpha polypeptide (CYBA) and endothelin-1(EDN1) as potential candidate genes. The most significant association was seen with EDN1 -2212G/A polymorphism, which was also found in genotypic and haplotypic combination. ACE 2350G/A polymorphism also revealed significant association. Gene x gene interactions also supported the interaction among these risk conferring alleles of EDN1 and ACE while the gene x environment interactions were independent of EDN1. The genetic risk indicated by a 5' regulatory region of EDN1 -2212G/A, and epigenetic risk indicated by a miRNA binding region of the AGTR1 1166A/C, interacted independently with a common denominator ACE 2350G/A for developing IA. Our results indicate novel genetic and epigenetic interactions of RAAS pathway genes.

555W

Common genetic susceptibility to autism spectrum disorder (ASD) and dyslexia (DYS). *M.L. Cuccaro[1], D. Ma[1], E.R. Martin[1], J.L. Haines[2], J.R. Gilbert[1], M.A. Pericak-Vance[1].* 1) Dept Med, Univ Miami Sch Med, Miami, FL; 2) Center for Human Genetics Research, Vand Univ, Nashville, TN.

Autism spectrum disorder (ASD) is a neurobehavioral phenotype with a strong genetic component. A common finding in ASD is an increased prevalence of other neurobehavioral disorders (e.g., epilepsy, intellectual disability, and learning disabilities) in affected individuals and first-degree relatives. For example, in families with both ASD and epilepsy, we found an increased rate of dyslexia (DYS) in first-degree family members vs. families with ASD alone. This supports the emerging model that select genetic variants give rise to diverse neurobehavioral phenotypes. We hypothesize that DYS genes will be more strongly associated with both ASD and DYS in the subset of families with DYS relatives compared to the ASD-only families. To test this hypothesis, we examined whether variants in DYS genes tend to have p-values ranked lower among the ASD+DYS families compared to the ASD-only families. We first identified nine high-profile DYS candidate genes (KIAA0319, CMIP, DYX1C1, CNTNAP5, DOCK4, DCDC2, PCSK6, SLC2A3, GRNI2B) based on the existing literature. Then, from our overall ASD data set (ascertained based on DSM-IV/ADI-R criteria) we identified a subset of DYS families (N=33). Using family history data, a family was classified as DYS if at least one non-ASD individual within the nuclear family was positive for DYS (36 parents; 3 siblings). Using data from high-density SNP arrays, family-based association analysis was carried out using Combined Association in the Presence of Linkage test (CAPL) treating individuals with ASD and DYS as affected and all others as unaffected. In addition, CNV detection was carried out on samples and high-confidence CNVs were detected by two distinct algorithms. There were 673 SNPs falling in the nine candidate gene regions. Fifty-five SNPs presented a p-value of less than 0.05 [0.001-0.049]. A total of 542 case-unique CNVs were identified among the 37 DYS individuals (two dropped due to QC) who were genotyped using Illumina 1M chip. However, none of the nine candidate genes appear to be disrupted by these CNVs. An investigation of CNVs from a unique subset of eight patients who had both ASD and DYS did not identify any shared CNVS. While none of the association results survived multiple testing corrections, we did observe a greater frequency of significant p-values in DYS candidate genes than expected by chance in our DYS subset (8.2% vs. 5%). This suggests that DYS genes may be involved in risk for both ASD and DYS.

556W

Direct genetic effects of putative functional SNPs in CRH pathway genes associated with anxious temperament in rhesus macaques. *G.L. Fawcett[1,2], M. Raveendran[2], J.A. Oler[5], A.S. Fox[5], S.E. Shelton[4], D.M. Muzny[2], R.A. Gibbs[1,2], N.H. Kalin[4,5], J. Rogers[1,2,3].* 1) Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX 77030; 2) Human Genome Sequencing Center, Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX, 77030; 3) Southwest National Primate Research Center, San Antonio, TX 78245; 4) Department of Psychiatry, The HealthEmotions Research Institution, University of Wisconsin-Madison, Madison, VI 53719; 5) Department of Psychology, Waisman Laboratory for Brain Imaging and Behavior, University of Wisconsin-Madison, Madison, WI 53705.

Children who exhibit extreme anxiety in the face of novel challenges are predisposed to developing clinical anxiety problems as adults. The corticotrophin releasing hormone (CRH) pathway has been implicated in mediating individual variation in later risk of anxiety and depression. Various lines of evidence demonstrate that increased activity in the CRH pathway results in elevated anxious behavior. Using a well validated rhesus monkey model to assess anxiety, brain metabolic rates in the amygdala and anterior hippocampal regions were shown to be associated with anxious temperament (AT), a composite behavioral phenotype including blood cortisol measurements, freezing behavior, and vocalization behavior among juveniles in response to a brief mildly stressful challenge. Both AT and hippocampal metabolic rates during assessment of anxious behavior show strong heritability (h^2=0.50 and h^2=0.52, respectively). Additionally, we have previously associated an intronic SNP (*SNP9111*) in *CRHR1* with AT in this population (n_220). In our current work, 447 rhesus macaques from a single pedigree were resequenced (Sanger) for four genes (*CRH*, *CRHR1*, *CRHR2*, and *CRHBP*). Using measured genotype analysis in SOLAR, we replicated the association of *SNP9111* with AT. Of particular interest, we identified a putative functional nonsynonymous SNP (*SNP5043*) in strong linkage disequilibrium with *SNP9111* that is significantly associated with AT (p=0.002). *SNP5043* is located in exon 6 of *CRHR1*, the sole exon in *CRHR1* displaying significant conservation only within higher order primates. Several other SNPs and indels in other genes in the CRH pathway also showed significant association with AT; one in *CRH* (*SNP3155*) was detected as probably damaging using Polyphen-2. We conclude that a complex genetic architecture composed of direct effects in multiple genes contributes to the strong heritability for AT in this rhesus macaque model. Efforts to characterize the direct genetic and possible epistatic contributions to AT and its neural substrates are underway to gain further insight into the childhood risk to develop anxiety and depression.

557W

Association Study of Five Genes and Early-Onset Generalized Anxiety Disorder. *Y. Feng[1], K. Manassis[2], D. Avery[2], CL. Barr[1, 2].* 1) Genetics & Development Division, Toronto Western Hospital, Toronto, ON, Canada; 2) Hospital for Sick Children, Toronto, ON, Canada.

Introduction: Anxiety disorders are common with the prevalence of the group of disorders estimated as high as 25% in the population. Twin studies indicate moderate heritability for anxiety disorders with a very high genetic correlation (.99) with major depressive disorder. Here we investigate the relationship of five genes (AVP, AVPR1B, CRH, GABRD, and NTRK3) to early-onset anxiety in a sample of families with children/adolescents with generalized anxiety disorder (GAD). We focused on markers we previously identified as associated with childhood-onset depression because of the genetic overlap indicated from family and twin studies. Methods: We genotyped 17 markers in those five genes in 161 families with a child/adolescent proband (165 affected children) with a diagnosis of GAD and tested for association using the Transmission Disequilibrium Test (TDT). Results: We found evidence for biased transmission of the alleles of the SNP rs1870393 in the CRH gene (p=0.022) and a trend for alleles of the SNP rs2376803 in the GABRD gene (p=0.059). Conclusions: While preliminary, these results support the role of hypothalamic-pituitary-adrenal (HPA)-axis and GABAergic system in early-onset generalized anxiety disorders. Further studies are in progress to determine the nature of this association.

558W

Increased exonic de novo mutation rate in probands affected with schizophrenia. *S.L. Girard[1], J. Gauthier[1], A. Noreau[1], L. Xiong[1], S. Zhou[1], L. Jouan[1], A. Dionne-Laporte[1], D. Spiegelman[1], E. Henrion[1], O. Diallo[1], J.Y.J. Bao[2], A.H.Y. Tong[2], C.H. Lin[2], B. Millet[3], N. Jaafari[3], R. Joober[4], P. Dion[1], S. Lok[2], M.-O. Krebs[3], G.A. Rouleau[1].* 1) CENUM, Universite de Montreal, Montreal,; 2) Genome Research Centre, The Li Ka Shing Faculty of Medicine, University of Hong Kong; 3) Université Paris Descartes, Faculté de Médecine Paris Descartes, Paris, France; 4) Douglas Mental Health University Institute, Department of Psychiatry, McGill University, Montréal.

Schizophrenia (SCZ) is a severe psychiatric disorder that profoundly affects cognitive, behavioral and emotional processes. The wide spectrum of symptoms and clinical variability in SCZ suggest a complex genetic etiology, which is consistent with the numerous loci thus far identified by family linkage, copy number variation (CNV) and association studies. While SCZ heritability may be as high as ~80%, the genes responsible for much of this heritability remain to be identified. Using high-throughput sequencing technologies, we sequenced the exome of 14 patients with schizophrenia and their parents. We identified 15 de novo mutations (DNMs) in 8 probands, which is significantly more than expected considering the previously reported DNM rate. In addition, 4 of the 15 identified DNMs are nonsense mutations, which is more than expected by chance. Our study supports the notion that DNMs may account for a fraction of the heritability reported for SCZ while providing a list of genes possibly involved in disease pathogenesis.

559W

Evaluation of Candidate Genes for Autism using Vineland Adaptive Behavior scales-II as covariates in APL-OSA. *J. Jaworski[1], I. Konidari[1], p. Whitehead[1], E. Martin[1], M. Cuccaro[1], J. Gilbert[1], J. Hussman[2], M. Pericek-Vance[1], J. Haines[3], R. Chung[1].* 1) MIHG, University of Miami - Miller School of Medicine, Miami, FL; 2) Hussman Foundation, Ellicott City, Maryland; 3) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN.

Autism is a complex disorder of neurodevelopment and is characterized by social impairments, language problems, and interfering repetitive behavior. Although a broad range of methods have implicated a number of candidate genes to be associated with Autism, no single gene has shown consistent replication across datasets and methods. Genetic heterogeneity can be one of the reasons that causes this inconsistency. If clinically derived traits are correlated with genes responsible for a subset of families with autism, they can be used to dissect the entire sample and identify the homogeneous subset. The Vineland Adaptive Behavior scales (VABS-II)is a widely used measure of adaptive functioning and has been used as a quantitative trait in prevous studies of ASD. The VABS-II consists of four domains (Communication, Daily Living, Socialization, and Motor Skills) and the Adaptive Behavior Composite score, an overall index. APL-OSA is an association method that can identify a genetically homozygous subset of families based on a trait-related covariate. Every individual from each family is assigned a domain score and the single marker association is evaluated from lowest to higher scoring families. The subset of families with the largest association statistic is selected and a permutation procedure is used to test the relationship between the family-specific covariate values and the association statistics. The dataset consisted of 428 Autistic Caucasian multiplex families with a total of 1585 individuals of which 708 were affected. SNPS of various candidate genes were typed for the dataset using the Illumina1Mduo chip. Using the Adaptive Behavior Composite score as a covariate yielded a significant SNP, RS757775 (p-value 0.03) within the candidate gene OXTR on chromosome 6. In addition, another SNP in the OXTR gene was significant for the communication skills domain (RS6443206, p-value 0.02). Both observations were observed as the covariate was sorted from low to high indicating a deficiency in the domain. Although the markers did not pass multiple testing corrections, this gene had previously been seen to be associated with autism as well as the VABS and IQ. We have independently replicated previously reported association with the OXTR gene and autism. The use of the VABS-II domain scores with APL-OSA on other candidate genes for autism could lead to the discovery of more genes associated with the disease.

560W

Evidence of imprinting at the autosomal recessive intellectual disability gene, TRAPPC9, and a possible role for CNVs at this locus in autism. *L. Kaufman[1], M. Ishida[2], K. Nakabayashi[3], A. Mir[4], G. Moore[2], J. Mill[5], S. Lewis[6], B. Fernandez[7], M. Ayub[8], J. Vincent[1].* 1) Neurogenetics, Univ Toronto, Toronto, Canada; 2) Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH; 3) Department of Maternal-Fetal Biology, National Research Institute for Child Health and Development, Tokyo 157-8535, Japan; 4) Department of Bioscience, COMSATS Institute of Information Technology, Islamabad, Pakistan; 5) King's College London, MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, De Crespigny Park, London, UK; 6) Department of Medical Genetics, UBC, Vancouver, BC, Canada; 7) Disciplines of Genetics and Medicine, Memorial University of Newfoundland, St John's, Newfoundland, Canada; 8) School of Medicine and Health, Durham University, Durham, UK.

TRAPPC9 is a gene that has been implicated in autosomal recessive intellectual disability (ID). Its protein product, NIBP, is an activator of the NF-2b transcription factor and may be involved in cellular trafficking. Many ID genes have been implicated in autism and we hypothesized that *TRAPPC9* mutations may contribute to the autism phenotype as well. Unique CNVs overlapping *TRAPPC9* have been identified in 7 individuals with varying neurodevelopmental phenotypes. Four of these CNVs are maternally inherited, one is paternally inherited, and two are of unknown inheritance. Two are duplications, five are deletions, and all of them overlap exons of the gene. We have validated two of these CNVs, a deletion and duplication, using qPCR. *TRAPPC9* displays a recessive inheritance pattern in individuals with ID, however, it is partially paternally imprinted in mouse brain. Two other genes at the same locus, *Kcnk9* and *Eif2c2,* are also imprinted in mouse brain. Our group has confirmed preferential maternal expression (~80%) of *Trappc9* in mouse brain using a pyrosequencing assay and has demonstrated that expression is relatively consistent across brain regions and development periods. We also found that imprinting is specific to NPCs and neurons. Imprinting status in humans is still unknown, however, we hypothesized that maternally inherited CNVs may contribute to an observable phenotype by disrupting the dosage of the gene. To assess if maternally inherited truncating mutations have an effect on phenotype we reassessed unaffected carriers from an ID family with a *TRAPPC9* mutation. We found that unaffected carriers with a maternally inherited mutation were phenotypically normal, whereas those with a paternally inherited mutation were borderline ID. It is also possible that CNVs overlapping *TRAPPC9* result in the disruption of imprinting at this locus. No imprinting control region (ICR) is known for this locus however, *Peg13*, a paternally expressed, non-coding RNA that overlaps intron 17 of *Trappc9* in mice, has been proposed as a potential candidate. While there is no known homolog in humans, mRNAs that are proximally similar to *Peg13* have been identified. CNVs overlapping *TRAPPC9* have been identified in individuals with autism, however it is unclear if and how they contribute to the phenotype. Further studies, including the analysis of *TRAPPC9* imprinting in human brain and identification of an ICR will allow for better insight into the role of this genetic variation.

561W

Response to Antidepressants is associated with Polymorphisms in the Leptin Gene and reduced Leptin availability. *S. Kloiber[1], S. Ripke[2], B. Puetz[1], J. Hennings[1], P. Weber[1], M. Ising[1], M. Uhr[1], EB. Binder[1], B. Muller-Myhsok[1], F. Holsboer[1], S. Lucae[1].* 1) Dept Psychiatry, Max Planck Inst, Munich, Germany; 2) Center for Human Genetic Research, MGH Simches Research Center Boston, MA, USA.

Leptin, one of the key players in weight regulation, has been shown to exert antidepressant-like effects. Therefore we analyzed genetic variants in the leptin gene, leptin mRNA-expression, and leptin plasma concentrations in a sample of depressed inpatients from the Munich Antidepressant Response Signature (MARS) project. SNPs in the leptin gene were associated with response to antidepressant treatment. Results remained significant after correction for multiple testing and could be partially replicated in a second sample. Diminished leptin mRNA expression and decreased leptin levels could be linked to unfavorable treatment outcome. Our data thus point towards a role of leptin in antidepressant treatment response.

562W

Targeted sequencing of *ALDH2* and ADH cluster loci shows differences in distribution of rare SNPs in European American alcohol dependent subjects and controls. *J.B. Listman[1], H. Zhao[2,3], X. Chen[3,8], H.R. Kranzler[4,5], J. Gelernter[1,3,6,7].* 1) Department of Psychiatry, Yale U Sch Med, New Haven, CT; 2) Department of Epidemiology and Public Health, Yale U Sch Med, New Haven, CT; 3) Department of Genetics, Yale U Sch Med, New Haven, CT; 4) Department of Psychiatry, U of Pennsylvania Sch Med, Philadelphia, PA; 5) VISN 4 MIRECC, Philadelphia VAMC, Philadelphia, PA; 6) VACT Healthcare Center, West Haven, CT; 7) Department of Neurobiology, Yale U Sch Med, New Haven, CT; 8) Program in Computational Biology and Bioinformatics, Yale U Sch Med, New Haven, CT.

Genes encoding the main components of the ethanol metabolism pathway, alcohol dehydrogenase cluster loci (*ADH1a,1b,1c,4,5,6,7*) and aldehyde dehydrogenase 2 (*ALDH2*) harbor numerous common variants that affect drinking behaviors. Alcohol dependence (AD) has a heritability of 0.5-0.6 in populations of European ancestry but known risk alleles cannot account for this. Consistent with what is now known about rare and non-coding variant effects on complex phenotypes, we hypothesized that we could identify regions within the ADH cluster on chr4 and *ALDH2* on chr12 that are particularly sensitive to variation that affects risk for AD. We also hypothesized that some of the regions that are likely regulatory would be conserved in controls but show greater variation in the form of rare variants in cases. Samples from 16 European American (EA) AD cases and 8 EA AD controls were sequenced for the seven ADH cluster genes and *ALDH2* including introns, exons, 20kb upstream to the 5' end and 10kb downstream from the 3' end for a total of approximately 385kb. Regions were amplified using RainDance-designed primers and a droplet-based microfluidic PCR process. Twenty-four tagged samples were sequenced in two lanes of an Illumina GAII. Mapping was performed using the Burrows-Wheeler Alignment Tool with 3 mis-matches and no gaps allowed and SAMtools was used for genotype calling and SNP calling. We used Polyphen to test for potentially damaging non-synonymous variants and identified potential regulatory regions through UCSC Genome Browser ChIP-Seq and DNAse study data. We found differences between cases and controls in patterns of locations of novel rare SNPs. Consistent with the hypothesis, non-coding, putative regulatory regions of *ADH5* and *ADH7* contained multiple SNPs in 2/3 of cases but in no controls. SNPs in controls did not show clustering in a particular genomic region. Our preliminary findings require detailed follow-up.

563W

Single nucleotide polymorphism (SNP) fine mapping of the *AVRP1a* and *OXTR* region in families with autism: associations in the *OXTR/CAV3* locus. *A. Lori[1], I. Lee[2], J.F. Cubells[1,3], T. Lehtimäki[4], K. Puura[5], D. Skuse[2], L.J. Young[3], E.B. Binder[1,6].* 1) Departments of Human Genetics, Emory University School of Medicine; Atlanta GA, USA; 2) Behavioural and Brain Sciences Unit, University College London, Institute of Child Health, London, UK; 3) Departments of Psychiatry and Behavioral Sciences, Emory University School of Medicine; Atlanta GA, USA; 4) Dept of Clinical Chemistry, Tampere University and University Hospital, Tampere, Finland; 5) Dept of Child Psychiatry, Tampere University and University Hospital, Tampere, Finland; 6) Max-Planck Institute of Psychiatry, Munich Germany.

Autism spectrum disorders (ASDs) are neurodevelopmental disorders characterized by deficits in social behavior and communication, and by idiosyncratic interests and repetitive behaviors. Although highly heritable, few association studies have successfully identified genes that contribute to risk for ASDs. Vasopressin (AVP) and Oxytocin (OXT) and their receptors appear to play important roles in a social behaviors such as social attachment, and several studies have reported associations of the genes encoding these peptides and receptors with ASD, or with variation in human social cognitive function. We tested whether variation at *AVPR1A* and *OXTR1*, respectively encoding the AVP-1a receptor and OXT receptor, is associated with ASD in 170 families ascertained for ASD, from Finland and the UK. SNPs were selected using HAPMAP and SNP Browser. Criteria for selection included a minor allele frequency of >0.01 and pairwise r2 of 0.85 among validated SNPs. A total of 31 SNPs for *AVRP1A* and 62 SNPs for *OXTR1* were successfully genotyped using a Sequenom platform. A TDT analysis using PLINK was performed in the 170 families using single SNP and 2 and 3 SNP sliding window haplotype analyses. In the single SNP analyses, no association was observed for *AVRP1A* for any of the SNPs. In the *OXTR1* region, however, rs237865 associated with ASD with experiment-wide significance after correcting for multiple testing (p=0.0004). Two- and 3-SNP haplotypes including this SNP also showed significant associations (0.02 > p > 0.0002). The SNP is located in the 5' region of *Caveolin3* (*CAV3*) and 3' of *OXTR1* and ongoing eQTL analyses might resolve which genes' expression is affected by the associated polymorphisms. CAV3 is expressed in skeletal and smooth muscle, glial cells and early postnatal peripheral nerve. CAV3 is also involved in glucose homeostasis and obesity. Mutations within this gene cause neuromuscular and cardiac disorders, but also idiopathic elevation of serum creatine kinase, and sudden infant death syndrome. Further research is warranted to confirm associations between this locus and ASD, and to investigate the hypothesis that *CAV3* associates with metabolic dysfunction sometimes observed in patients with ASD, or whether the observed SNP association, if replicated, represents an association to functional variation at *OXTR1*.

564W

The SNP and haplotype analysis of CD200 gene with schizophrenia in a Japanese population. *F. Nishimura[1], A. Yoshikawa[1], M. Tochigi[1], Y. Kawamura[1,2], T. Umekage[3], T. Sasaki[4], K. Kasai[1], C. Kakiuchi[1].* 1) Department of Neuropsychiatry, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 2) Yokohama Clinic, Kanagawa, Japan; 3) Division for Environment, Health and Safety, University of Tokyo, Tokyo, Japan; 4) Office for Mental Health Support and Graduate School of Education, University of Tokyo, Tokyo, Japan.

Introduction: Schizophrenia is one of the major mental disorders with approximately 1% prevalence rate. It is the most debilitating psychiatric disorder, inflicting much burden upon the patient, the family, and the society. The family, twin and adoption studies have established that the genetics play a significant role in the etiology of schizophrenia. On the basis of these studies, the heritability of schizophrenia is approximately 80%. However, genetic variants with decisive effect of schizophrenia have not been completely identified yet. We previously used DNA microarray analysis to examine the mRNA expression patterns in the lymphoblastoid cells derived from two pairs of monozygotic twins discordant for schizophrenia. We found the downregulation of the CD200 in all of the five independent replicates for each twin pairs. CD200 is expressed on B-cells and neurons and it downregulates microglia activation through CD200R. The notion that the immune system in schizophrenics may be involved in its susceptibility and the neuropathology of schizophrenia has recently been reported to be closely associated with the above-mentioned microglia activation. On the other hand, the upregulaion of CD200R1/CD200 pathway in the animal model of rheumatoid arthritis has been implicated. Interestingly, there is an inverse correlation between the occurrence of schizophrenia and rheumatoid arthritis. Hence, we considered CD200 as the possible common susceptibility gene for schizophrenia and rheumatoid arthritis. Method: 384 patients with schizophrenia diagnosed by DSM-IV criteria and 384 sex and age matched healthy control subjects were recruited. The objective of this study was clearly explained and written informed consent was obtained from all subjects. The study was approved by the Ethical Committee of the Faculty of Medicine, University of Tokyo. We selected the 23 tag SNPs from the JSNP database of Japanese population. We genotyped these SNPs by TaqMan Probe. We assessed the SNP and haplotype association. Results: The distribution of all 23 SNPs followed Hardy-Weinberg equilibrium in both the schizophrenia and control subjects. No significant association was observed in single SNPs and haplotype for CD200. Conclusion: We need further studies to analyze the CD200R which regulates microglia activation with CD200 and to determine the reason why CD200 expression was downregulated in the discordant monozygotic twins.

565W

Survey of effects of the DRD4 7R allele on Substance Use Disorder across schizophrenia, bipolar and other psychiatric phenotypes. *V.F.G Oliveira, A.K Tiwari, C.C Zai, M. Tampakeras, O. Likodi, B. Mackenzie, J.L Kennedy.* Neuroscience, CAMH, Toronto, Ontario, Canada.

Background: The D4 dopamine receptor gene (DRD4) contains a 48bp-tandem repeat polymorphism (VNTR) in exon 3 that impacts the length of the third cytoplasmic loop of the receptor protein. The DRD4 7R allele (7R) has undergone strong evolutionary selection in humans (Ding et al 2002). There is evidence supporting an association between the 7R allele and substance use disorders (SUDs). Psychiatric disorders are frequently complicated by substance abuse and this comorbidity is generally associated with more severe course of disease. A longstanding question has been: Is comorbid substance abuse arising from the same biological mechanisms involved in the primary psychiatric disorder, or does it have separate neural substrate? Genetic investigations may help resolve this issue. We propose that the DRD4 7R allele may play an important role in the comorbidity of SUDs and psychiatric disorders, and affect the age of onset. Methods: Overall, we had 344 schizophrenia (SCZ) and 613 bipolar (BD) European-Caucasian individuals, of which 105 were classified with alcohol abuse or dependence (lifetime) and 437 as smokers (lifetime) (DSM-IV criteria). Smoking status was assessed using the Fagerstrom test (schizophrenia) or similar sections from the SCAN interview (bipolar). The sample had >80%; power to detect an odds ratio as low as 1.7 (alcohol) and 1.49 (smoking). The DRD4 48bp repeat alleles were determined by PCR (Lichter et al., 1993) and sequence-based genotyping in an ABI 3130 platform. Results: Linear regression model indicated a trend toward a positive association between presence of the DRD4 7R allele and late age of onset for females, but not males, in SCZ while controlling for alcohol abuse/dependence (uncorrected p=0.021) and smoking (uncorrected p=0.027) variables. For bipolar individuals, 7R allele was not associated with age of onset while controlling for sex and alcohol abuse/dependence (p=0.632). For smoking, linear regression model indicated a positive association between presence of the 7R allele and age of onset (uncorrected p=0.009) while controlling for sex. Conclusions: The 7R allele may play a role in comorbid SUD in both schizophrenia and bipolar disorders. The results are somewhat limited by the modest sample size, and replication is required. DRD4 may influence risk for smoking in both SCZ and BP, but for alcohol abuse/dependence only in SCZ. Thus the risk effect of DRD4 is likely complex and in some cases disease specific.

566W

ApoE-SNCA-LRRK2 genes interaction in Parkinson's disease and controls. *A. Parsian[1], J.H. Zhao[2].* 1) Div Neuroscience & Behavior, NIAAA/NIH, Bethesda, MD; 2) MRC Epidemiology Unit, Cambridge, UK.

Our earlier reports of multiple variations in the ApoE, SNCA and LRRK2 genes have indicated that they are at most have modest increase of susceptibility to Parkinson's disease. In the current investigation, we focused on their interactions. The sample included 227 familial and 355 sporadic cases of Parkinson's disease and 236 unrelated controls. In the analysis we selected haplotypes showing increased risk according to haplotype specific analysis in SNCA as covariate to those in LRRK2 or repeat variation in ApoE in an interaction analysis both using R/haplo.stats, and vice versa for a pair of genes. As in our earlier report, haplotype 112 of 770-int4-Rep1 in SNCA has frequency of 0.04817 (haplo.em) and shows moderate significance (haplo.score) score statistic 2.53, asymptotic and simulated p values = 0.01; haplo.glm t-statistic=2.37 and p = 0.0182). In contrast, haplotypes 211212 and 212211 for rs10506151-rs10784486-rs1365763-rs1388598-rs1491938-rs1491941 in LRRK2 have score statistics -1.79 and -1.42 and t-statistic -1.79 (p = 0.0744) and -1.76 (p = 0.0794). The strongest evidence of statistical significance is the presence of SNCA haplotype 112 and haplotypes 222111 and 222221 in LRRK2 (both with t-statistic>5, p<0.0001), or presence of haplotypes 211212 and 212211 in LRRK2 with haplotype 122 in SNCA (t-statistic=1.77, p=0.078). However, such analysis between ApoE, LRRK2 and SNCA did not produce significant results. Given the recent successes of genome-wide association studies (GWASs) which largely indicate that SNPs established accounted for a small amount of phenotypic variations, it is hopeful that study of gene-gene interactions can help to identify further genotypic contributions to the “missing heritabilities”. Our results are in line with earlier reports that specific haplotypes in SNCA and LRRK2 might contribute to increased risks of Parkinson's disease.

567W

A polymorphism on CHRNA3 gene is associated with tobacco smoking in patients with ADHD in a Brazilian sample. *E.R. Polina[1], K.L. Silva[2], C.A.I. Salgado[2], E.H. Grevet[2], P. Belmonte-de-Abreu[2], C.H.D. Bau[1,2].* 1) Department of Generics, UFRGS, Porto Alegre, RS, Brazil; 2) Adult ADHD Outpatient Clinic, HCPA, Porto Alegre, RS, Brazil.

There is an important relationship between attention-deficit/hyperactivity disorder (ADHD) and tobacco smoking. Adults diagnosed with ADHD smoke at significantly higher rates than individuals in general population and have a threefold increased risk to become nicotine dependents. Some studies have shown that ADHD patients may smoke because of an improvement in attention and impulsivity control. Although the relationship between cigarette smoking and ADHD is not clear, it has been suggested that they may share a common neurotransmitter system. One theory is the “nicotinic receptor hypothesis” based on the finding that nicotinic receptors modulate the dopaminergic activity, which is involved in the underlying pathophysiology of ADHD. The CHRNA5-CHRNA3-CHRNB4 gene cluster located on chromosome 15q.25 has been associated with nicotine and alcohol dependence and lung cancer. This study aims to investigate a possible association between the polymorphism on nicotinic receptor gene and tobacco smoking in ADHD patients. We analyzed the polymorphism rs3743078 of the (3 nicotinic acetylcholine receptor subunit (CHRNA3), a transition C>G on intron 4, already associated with tobacco smoking. The sample comprises 435 Brazilians of European descent (188 smokers and 247 non-smokers) evaluated in the adult ADHD outpatient clinic of Hospital de Clínicas de Porto Alegre. The diagnostic of ADHD followed the DSM-IV criteria and tobacco smoking was evaluated with the Fagerstrom Test of Nicotine Dependence. TaqMan assay for allelic discrimination (Applied Biosystem) was used to determine SNP genotypes. Our results showed a significant association between the G allele and tobacco smoking in ADHD patients, after adjustment for gender (OR=2.455; P=0.002; 95% CI=1.378-4.371). These findings confirm, in patients with ADHD, the previous evidence for association between rs3743078 and nicotine dependence. Several other SNPs will be studied in this gene cluster in individuals with and without ADHD in order to verify how ADHD impact the relationships between nicotinic receptors and smoking. Future studies should also evaluate possible mediating effects of neuropsychological deficits in this association.

568W

Homozygosity Mapping in 7 Pakistani Intellectual Disability Families Identifies 3 New Autosomal Recessive Loci. *MA. Rafiq[1], A. Mir[2], A. Noor[1], K. Mittal[1], E. Wiame[3], L. Kaufman[1], A. Mikhailov[1], F. Naeem[4], E. Van Schaftingen[3], T. Nasir[5], M. Ansar[6], M. Ayub[7], JB. Vincent[1,8].* 1) Molecular Neuropsychiatry, Centre for Addiction and Mental Health, Toronto, Ontario, Canada; 2) Deaprtmrnt of Biosciences, International Islamic University, Islamabad, Pakistan; 3) de Duve Institute, Université catholique de Louvain, Brussels, Belgium; 4) Lahore Institute of Research & Development, Lahore, Pakistan; 5) Mayo Hospital, Lahore, Pakistan; 6) Department of Biochemistry, Quaid-I-Azam University, Islamabad, Pakistan; 7) Tees, Esk and Wear Valleys NHS Foundation Trust United Kingdom; 8) Dept. of Psychiatry, University of Toronto, Toronto, Ontario, Canada;.

Intellectual disability (ID), or mental retardation (MR), is a neurodevelopmental disorder that has a devastating impact on the affected individuals and their families, as well as on health and social system. Here we report a study on non-syndromic autosomal recessive intellectual disability (NS-ARID) in consanguineous Pakistani families. Seven families (MR28, MR62, MR64, MR70, MR72, MR74 and PK11) were genotyped using Affymetrix 500K single-nucleotide polymorphism (SNP) microarrays. This approach allowed us to identify seven homozygous-by-descent (HBD) loci: 18q22.3-q23, 7p14.1-q11.22, 8q23.1-q24.21, 6q13-q16.2, 16q12.2-q21, 8p11.21-q13.2 and 11p11.2-q13.4.Three out of the seven loci mapped (8q23.1-q24.21, 6q13-q16.2 and 11p11.2-q13.4) overlap with previously reported studies and reduce the critical HBD region. Three other loci (18q22.3-q23, 16q12.2-q21 and 8p11.21-q13.2) have not been reported previously. We have sequenced DNA from four families (MR70, MR72, MR74 and PK11) by whole exome sequencing and exome data is being analyzed to discover disease causing mutations. Genes within the HBD region for family MR64 on 8q23.1-q24.21 have been sequenced and no causative mutation found so far. Sequencing of all genes within the HBD region discovered in family MR28 did not expose any exonic or splice junction changes. The remaining locus of MR62 (7p14.1-q11.22) family already mapped in patients with phosphoserine phosphatase (PSPH) deficiency, which is responsible for mental retardation and additional clinical phenotypes. Compound heterozygous mutations in the PSPH gene have been reported in patients with PSPH deficiency. We sequenced PSPH and found a homozygous missense mutation, Ala35Thr, in all affected members. This is first report of a homozygous change in PSPH. Studies to measure the effect of mutation on enzymatic activity reveal that the enzyme with the Ala35Thr, mutation has about a 5-fold reduced catalytic activity. The side chain of Ala35 is in a small hydrophobic pocket near the catalytic site. Threonine may make the side chain too bulky and polar to fit in the small pocket.

569W

Association study shows positive correlation between inflammatory *IL1B* gene and mesial temporal lobe epilepsy with hippocampal sclerosis. *R. Santos[1], M. Silva[1], R. Secolin[1], C. Yasuda[2], T. Velasco[3], A. Sakamoto[3], F. Cendes[2], I. Lopes-Cendes[1], C. Maurer-Morelli[1].* 1) Department of Medical Genetics, Faculty of Medical Sciences, University of Campinas, Campinas, SP, Brazil; 2) Department of Neurology, Faculty of Medical Sciences, University of Campinas - UNICAMP, Campinas, SP, Brazil; 3) Department of Neurology, Psychiatry and Clinical Psychology, University of São Paulo, Ribeirão Preto, SP, Brazil.

The epilepsy syndromes presenting complex inheritance are still elusive when it comes to the identification of susceptibility genes even in the era of large genome wide association studies. Therefore, the use of candidate gene approach to study a more limited, but well characterized clinical sample still a valuable strategy. Several studies describe the inflammatory processes as a key point in the generation and progression of acute and chronic neurodegenerative diseases. The *IL1B* gene, which encodes a proinflammatory cytokine, has been associated to increased susceptibility to mesial temporal lobe epilepsy (MTLE) with mesial temporal sclerosis (MTS). The aim of this study was to investigate the association between *IL1B* gene with MTLE with magnetic resonance imaging (MRI) signs of MTS in a large group of patients. DNA samples were obtained from 204 unrelated patients with MTLE and MTS signs, as well as 204 unrelated controls, with no history of epilepsy. We selected seven SNPs within *IL1B* from HapMap database. SNPs were genotyped using the SNPlex^{TM} genotyping system (Applied Biosystems). Minor allele frequency (MAF>0.05), linkage disequilibrium (r^2>0.8) and Hardy-Weinberg equilibrium (HWE pvalue>0.05) were estimated using the HAPLOVIEW software. To evaluate the possibility of sample stratification we genotyped 86 additional SNPs distributed throughout the genome and located outside of the candidate genes loci. Statistical analysis was performed by logistic regression model and by Bonferroni correction for multiple comparisons. We found association between the SNP rs3730364 in the IL1B gene and MTLE [p=1.4x10^{-14}, OR=0.11; 95%CI: 0.06 - 0.21]. The genomic control indicated the absence of population stratification (Fst = 0.00821) between patient and control groups. Our association study shows a relationship between one SNP (rs3730364) in the IL1B gene and MTLE with MRI signs of MTS. However, rs3730364 do not appear to be a functional variant. Genomic control was critical to validate the groups as not stratified, giving robustness and reliability to our study. Although much progress has been made in the characterization of genes for the monogenic and rare forms of epilepsy the common epilepsy syndromes, usually showing complex inheritance remain a major challenge for gene identification, our study hopes to shed some light into this area.

570W

Exploring the contribution of ATP1A2 and SCN1A to genetic susceptibility in non hemiplegic migraine. *M.J. Sobrido[1,2], M. Camiña-Tato[2], A. Sampayo[3], J. Pardo[4], R. Cruz[2], A. Carracedo[1,2], P. Cacheiro[2].* 1) Fundación Pública Galega de Medicina Xenómica-SERGAS, Santiago de Compostela, Spain; 2) Genomic Medicine Group, University of Santiago de Compostela, CIBERER-ISCIII, Spain; 3) PAC Casa do Mar-SERGAS, A Coruña, Spain; 4) Neurology Department, Hospital Clínico Universitario, Santiago de Compostela, Spain.

Mutations in three genes have been identified in Familial Hemiplegic Migraine (FHM): CACNA1A (type I), ATP1A2 (type II), and SCN1A (type III). While most previous studies have found no evidence of association of CACNA1A polymorphisms with common forms of migraine, fewer data exist on ATP1A2 and SCN1A. Objectives: To analyze the role of genetic variants in the Na/K ATPase alpha-2 subunit (ATP1A2) and sodium channel voltage-gate type I alpha subunit (SCN1A) in common forms of migraine in a population from North-West Spain. Methods: We performed a case-control study of 641 subjects from Galicia, a region without significant population substructure. All patients were diagnosed by International Headache Society 2004 criteria for MO (188) and MA (145). Case and control groups were matched for age and gender. Recurrent headache was ruled out in control individuals and their first degree relatives. Genotyping was carried out with the SNPlex platform (Applied Biosystems, Foster City, CA) for 11 SNPs in ATP1A2 and 13 SNPs in SCN1A, with complete gene coverage based on HapMap haplotype blocks. Statistical analysis was performed using the R package SNPassoc. Single SNP association was assessed through Likelihood Ratio Test considering different genetic models. SNPs passing quality control threshold for genotyping rate (95% SNPs, 75% samples), MAF (0.10) and HWE in controls (alpha=0.05). While global case-control comparison did not show association, a statistically significant genotype association was observed in women for SNP rs13004083 under a recessive model (AA-AG vs. GG) for both migraine types (p=0.0025; OR 2.94, 95% CI 1.40- 6.18) and MA (p= 0.0031; OR 3.51, 95% IC 1.51- 8.16). Upon stratification by migraine type, the same genotype was associated with MA (p=0.0225; OR 2.17 95% CI 1.13-4.18). None of the individual associations remained significant after Bonferroni correction for multiple testing. Conclusions: A SNP located in the 5'-near SCN1A region, between SCN1A and SCN9A showed association with common migraine in a population of North-West Spain, particularly in women with MA. These data, together with previously reported nominal association of SCN9A and MA suggest that genetic variation in these voltage dependent sodium channel genes or in their proximity may play a role in common forms of migraine. Financial support: Ministerio de Ciencia y Tecnología SAF2005-07978, Fundación Pedro Barrié de la Maza, Merck-MSD.

571W

CNS pathology in the mouse model of sialidosis recapitulates features of early stage Alzheimer's disease. *I. Annunziata[1], A. Patterson[1], D. Helton[1], S. Keilani[2], S. Gandy[2], A. d'Azzo[1].* 1) Department of Genetics, St. Jude Children's Research Hospital, Memphis, TN; 2) Mount Sinai Alzheimer's Disease Research Center, Mount Sinai School of Medicine, Mount Sinai, NY.

Lysosomal sialidase NEU1 catalyses the hydrolysis of sialo-glycoconjugates by removing their terminal sialic acid residues. In humans, primary or secondary deficiency of this enzyme leads to two clinically similar neurodegenerative lysosomal storage disorders: sialidosis and galactosialidosis. Mice deficient in Neu1 recapitulate the early-onset severe form of sialidosis. We have recently discovered that loss of Neu1 activity exacerbates the process of lysosomal exocytosis in various cell types by influencing the sialic acid content of Lamp-1. This increases the ability of a pool of lysosomes to dock at the PM and engage in lysosomal exocytosis. In this study we have investigated whether excessive lysosomal exocytosis underlies some of the neurological aspects seen in the brain of Neu1-/- mice. Histopathological examination of the brain of these mice revealed a progressive and time dependent formation of dystrophic neurites positive for APP/A) , particularly in the CA3 region of the hippocampus and the adjacent fimbria. The affected regions coincide with sites of high Neu1 expression in wild-type brain. This abnormality was paralleled by abnormal expression of oversialylated Lamp-1 and activated proteases, both features linked to excessive lysosomal exocytosis. These findings represent an example of a spontaneously occurring AD-like phenotype in a mouse model of a neurodegenerative disease and could contribute to the understanding of some of the pathological mechanisms of adult neurodegenerative conditions of unknown etiology. (This study was supported in part by NIH grant GM060950 and ALSAC of St. Jude Children's Research Hospital).

572W

SCN1A mutation associated with intractable myoclonic epilepsy and migraine headache. *P. Frosk[1,3], A. Mhanni[1,3], M. Rafay[2].* 1) Genetics and Metabolism, Health Sciences Centre, Winnipeg, MB, Canada; 2) Section of Neurology, Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, MB, Canada; 3) Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, MB, Canada.

SCN1A encodes the voltage-gated sodium channel NaV1.1. Its function is to propagate action potentials within the nervous system. Mutations in this gene have been associated with a number of seizure phenotypes including generalized epilepsy with febrile seizures plus (GEFS+), severe myoclonic epilepsy in infancy (SMEI), borderline SMEI (SMEB), intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTCS), and a number of cryptogenic focal and generalized epilepsies. The severe phenotypes are usually caused by *de novo* mutations in the gene and are less likely to be hereditary. When mutations are inherited, the spectrum of seizure phenotypes can be quite broad in the family even though patients have the same mutation. More recently cases of familial hemiplegic migraine have been found to be associated with mutations in this same gene. Here, we describe a child with speech and gross motor coordination delays, and intractable epilepsy, with primarily focal but also myoclonic seizures, and a mutation in SCN1A (c.3521C>G, p.T1174S). Interestingly the patient's mother, who suffers from frequent migraines with aura but has never had seizure, was found to also carry this mutation. A recent report describes this mutation in patients with familial hemiplegic migraine and without seizures but did describe a broader phenotype in one individual (coordination difficulties, multifocal action myoclonus, an abnormal electroencephalogram but no reported clinical seizures and later development of headache). Our patient's phenotype is unique because of the presence of intractable epilepsy. This suggests that the phenotypic consequences of some SCN1A mutations may be broader than previously thought and that lack of a family history of seizures does not preclude an inherited mutation.

573W

Molecular pathogenesis for paroxysmal involuntary movements as a channelopathy: genetic analysis of human cases and an animal model. *C. Okada[1], H. Matsuo[1], S.E. Sander[2], M. Hamann[2], A. Richter[2], T. Hamada[3], A. Nakayama[1], Y. Utsumi[1], Y. Kawamura[1], H. Onoue[4], K. Kaida[4], Y. Kobayashi[3], K. Kamakura[4], N. Shinomiya[1].* 1) Dept. of Integrative Physiol. and Bio-Nano Med., National Defense Med. Col., Saitama, Japan; 2) Institute of Pharmacol. and Toxicol., Dept. of Veterinary Sci., Freie Univ., Berlin, Germany; 3) Dept. of Anat. and Neurobiol., National Defense Med. Col., Saitama, Japan; 4) Dept. of Internal Med., National Defense Med. Col., Saitama, Japan.

Paroxysmal dystonic choreoathetosis (PDC) is thought to be one of hereditary channelopathies, which is characterized by episodes of involuntary movements precipitated by caffeine, alcohol or emotional stress. We found a large Japanese family with PDC in which at least 17 members in 6 generations had been affected. Linkage analysis revealed that to chromosome 2q32-36 was the responsible locus of PDC in this family. Mutations in exon 1 of myofibrillogenesis regulator 1 (MR-1) gene which is located in PDC locus, were reported in several American PDC families. Sequence analysis showed that A7V mutation in MR-1 gene was found in all PDC-affected Japanese patients and this was also consistent with previously reported American PDC families (A7V and A9V). Since the effects of MR-1 mutation on its molecular function and intracellular localization were not clear, we first cloned distinct isoforms of MR-1 including MR-1L and MR-1S, and introduced A7V and A9V mutations into these isoforms. In spite of these disease-causative mutations, no change in the intracellular localization of MR-1 protein was found in MDCK cells, yet one isoform (MR-1L) showed clear localization to cytoplasmic membrane. An animal model of PDC (PDC hamster: dt^{sz}) has been reported. Clinical symptoms of dt^{sz} are similar to those of human PDC, but its causative gene remains unidentified. We determined the genome structure of hamster MR-1 gene and performed a mutational analysis using this model. Curiously, mutations similar to human PDC (A7V and A9V) were not detected in the MR-1 gene of dt^{sz} hamster. Therefore, we conducted a comprehensive analysis of alterations in gene expression of dt^{sz} and normal hamsters with a high coverage expression profiling (HiCEP) method. The expression profile of 109 genes in dt^{sz} hamster showed more than three-fold changes compared to normal hamster. These results imply that the causative gene of dt^{sz} may code a channel/transporter protein that is associated with MR-1 protein. Although further analysis of molecular functions and localization of PDC causative gene product will be needed, our approach will facilitate to understand the pathogenesis and will ultimately lead to the development of more effective therapies for basal ganglia disorders including other paroxysmal choreoathetosis, dystonic syndrome and Parkinson's disease.

574W

22q11.2 deletion in a Brazilian schizophrenia patient revealed by MLPA and FISH techniques. *M.I. Melaragno[1], F.T. Bellucco[1], V.K. Ota[1], S.I. Belangero[1,3], M.C. Cernach[1], A. Gadelha[2,3], J. Mari[2], R.A. Bressan[2,3], M.C. Smith[1], D.M. Christofolini[1,4].* 1) Genetics Department, Universidade Federal de Sao Paulo, Sao Paulo, Brazil; 2) Department of Psychiatry, Universidade Federal de São Paulo, São Paulo, Brazil; 3) Interdisciplinary Laboratory of Clinical Neuroscience, Universidade Federal de São Paulo, São Paulo, Brazil; 4) Human Reproduction and Genetics Division, Department of Gynecology and Obstetrics, Faculdade de Medicina do ABC, Santo Andre, Brazil.

The most important known genetic risk factor for developing schizophrenia is the chromosomal 22q11.2 deletion, since about 25% of children with 22q11.2 deletion will develop schizophrenia in adolescence or adulthood. Moreover, in adult patients with schizophrenia it is estimated that the frequency of deletion is from 1:50 to 1:100. In this study a total of 158 adult unrelated schizophrenia patients, assessed according to DSM-IV were recruited from the Schizophrenia Program of Universidade Federal de São Paulo (UNIFESP), Brazil. The presence of 22q11.2 region copy number variation was investigated using multiplex ligation-dependent probe amplification (MLPA) technique using P250 DiGeorge kit and confirmed by fluorescence in situ hybridization (FISH). A typical 1.5 Mb 22q11.2 deletion, ranging from LCR22A to LCR22B, was detected in one patient, corresponding to 0.6% of prevalence in our sample, similar to literature data (0 % a 5,3 %). This patient is a 30 years and 10 months old man who presents learning disability and socialization difficulty. His first psychotic event occurred at 26 years old. The patient presented with a long face, short forehead, downslanting and short palpebral fissures, prominent nasal bridge with a bulbous nasal tip, hypoplastic alae nasi, long and discrete philtrum, small ear lobes with a thick edge of the helices, narrow and high palate and hyperchromic spot in the left hemithorax with irregular contours with the largest diameter of 10 cm suggesting nevi. Serum calcium levels were normal. Echocardiogram showed no structural heart abnormality. The patient presented neither nasal speech nor velopharyngeal insufficiency. Patient's parents were also investigated and presented normal karyotypes and FISH results. The patient here described met clinical criteria for 22q11deletion syndrome in adults, since he presents three major phenotypic findings of the syndrome such as, dysmorphic face, learning difficulties and psychiatric disorder. In our knowledge this is the first study investigating the presence of 22q11.2 deletion in a Brazilian sample of schizophrenia patients. Our work confirmed the prevalence of the 22q11.2 microdeletion in patients with schizophrenia and also that MLPA is a valuable and accurate diagnostic tool to investigate 22q11.2 rearrangements also providing information about deletion extension not accessed by standard FISH techniques. Financial support: FAPESP, CAPES and CNPq, Brazil.

575W

Heritability analysis of behavioral traits in children of alcoholics. *V. Strumba[1], K. Shedden[3], R.A. Zucker[1], M. Burmeister[2].* 1) Addiction Research Center, University of Michigan, Ann Arbor, MI; 2) Molecular and Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, MI; 3) Department of Statistics, University of Michigan, Ann Arbor, MI.

The main goal of our study is to identify genetic variants associated with behavioral traits associated with risk for Alcohol Use Disorder (AUD). The subjects are participants in the Michigan Longitudinal Study, which has been tracking children of alcoholic parents and SES-matched control families for over 25 years. Starting with the enrollment age of 3-5 years, children were assessed annually for a variety of traits including externalizing and internalizing behaviors, temperament, and comorbid symptomatology. As a first step in identifying gene-to-behavior associations, we need to identify the most powerful set of behavioral measurements for a genetic association test. Power for genetic studies requires at least part of the variance of the trait to be inherited. Therefore, we carried out heritability analysis of a variety of behavioral traits assessed in children of alcoholic parents at different developmental stages. To carry out heritability analysis we used data collected on full siblings from over 400 families. Due to the longitudinal nature of this dataset we used trait measures at all available time points. We tested item level data for each instrument used in the study as well as composite scores as trait measures where appropriate. In this analysis we identified a number of traits with low to moderate heritability, whereas other traits show no significant heritability. Among moderate, but highly significant, heritable traits we find four personality domains as measured by NEO-FFI: agreeableness (23%), consciousness (22%), extraversion (18%) and neuroticism (23%) with p-values <0.005. There is also significant heritability of behavioral problems measured by CBCL youth self report, such as anxiousness (23%), withdrawn (28%) and internalizing behavior problems (24%) with p-values <0.005. The set of traits with the best evidence for heritability will be used in the next step to test for association with genetic variants found in or near candidate genes known or suspected to be associated with alcohol and other drug use, as selected for a commonly used drug addiction panel of SNPs (Hodgkinson CA et.al. “Addictions biology: haplotype-based analysis for 130 candidate genes on a single array” Alcohol Alcohol. 2008 Sep-Oct; 43(5):505-15.). Supported by grants from the National Institute on Alcohol Abuse and Alcoholism (R37 AA 07065 (RAZ) and T32 07477 RAZ/KB), and from the National Institute on Drug Abuse (R01 DA 17732 (RAZ, MMH and JKZ).

576W

Construction of in vitro model system to understand molecular mechanism of CGG repeat expansion in Fragile X syndrome. *Y. Nakayama[1], T. Seko[2], N. Sunamura[2], Y. Ogino[1], M. Kato[3], E. Nanba[1], M. Oshimura[2,4], H. Kugoh[2,4].* 1) Functional Genomics, The Center for Bioscience and Biotechnology, Yonago, TOTTORI, Japan; 2) Dept. of Biomed. Sci., Inst. of Regenerative Med., Tottori Univ; 3) Div. of Human Genomic Sci., Dept. of Mol. and Cellular Biology, Sch. of Life Sci.Faculty of medicine, Tottori univ; 4) Chromo. Eng. Res. Ctr., Tottori Univ.

Fragile X syndrome (FXS) is the most common inherited form of mental retardation. FMR1 gene on chromosome Xq27 region is responsible gene for FXS and (CGG)n triplet repeat has been found in its 5'UTR region. In FXS, CGG triplet repeat expands more than 200 triplets exclusively when Pre-Mutation (PM) allele between 45 to 200 CGG triplets is maternally transmitted. Following repeat expansion, CpG methylation across the promoter region of FMR1 gene occurs, leading to inactivation of FMR1 expression, and results in FXS phenotypes. However, the mechanisms of CGG repeat expansion and subsequent gene inactivation of FMR1 have not been fully elucidated. To date, several Knock-In (KI) mouse models with PM range CGG triplet repeat have been produced. Some showed CGG repeat expansion to Full-mutation (FM) range over 200 repeats in single generation, however, subsequent methylation at the FMR1 promoter region and gene silencing observed in human patients were not reproduced. These KI mice models suggested the importance of chromosomal context (cis element) for repeat expansion.

To develop a genomic model system to understand molecular mechanism of CGG triplet repeat expansion, CGG repeat region with suitable chromosome context and fully genomic reprograming activity to mimic germ cell environment should be important. In this study, to fulfill these conditions in vitro, we construct the human artificial chromosome (HAC) containing PM allele-derived CGG triplets with or without FMR1 promoter region (CGG-HAC). Then, as Embryonic Germ (EG) cells are well known to have dominant reprograming activity, CGG-HAC will be introduced into EG cells via microcell mediated chromosome transfer to see whether CGG expansion occurs and to see the effect of genomic reprogramming over CGG triplet repeat stability.

Further, to identify any specific protein(s) or non-coding RNA(s) may be associating CGG triplet repeat, we introduced lacO tandem repeats onto CGG-HAC. That enables us to harvest CGG-HAC with specific factors bound using lacO-lacI-mediated immunoprecipitation for further analysis. Thus, development of a novel method using HAC, termed chromosome immunoprecipitation (ChrIP), will be valuable to isolate chromatin-protein and/or chromatin-RNA complex toward molecular dissection of CGG repeat expansion mechanism in Fragile X syndrome.

577W

Autism Spectrum Disorders associated with 17q12 Deletions Implicate the Acetyl-CoA Carboxylase Alpha Gene (ACACA). *D. Moreno-De-Luca[1], B.D. Pearce[2], O.Y. Ousley[3], C.L. Martin[1], D.H. Ledbetter[4], Simons Simplex Collection Genetics Consortium.* 1) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA; 2) Department of Epidemiology, Rollins School of Public Health, Emory University, Alanta, GA, USA; 3) Emory Autism Center, Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA, USA; 4) Genomic Medicine Institute, Geisinger Health System, Danville, PA , USA.

The 17q12 deletion is a recurrent copy number variant (CNV), which we previously identified as a very significant risk factor for autism spectrum disorders (ASD) and schizophrenia. There are 15 genes within this CNV; however, the specific gene or genes responsible for the behavioral phenotype remain unknown. We analyzed CNV data from 1,124 simplex ASD families from the Simons Simplex Collection (SSC) and identified three cases with genic deletions in 17q12. Two cases have the typical 17q12 deletion; in one case the deletion arose de novo, whereas in the other it was inherited from a father with macrocephaly and an elevated score on the Social Responsiveness Scale (SRS). The third case has a smaller deletion including only one of the 15 genes within the CNV region, ACACA, encoding acetyl CoA carboxylase alpha, the rate-limiting step in fatty acid synthesis. Downstream in this same pathway lies the 7-dehydrocholesterol reductase gene (DHCR7), mutations in which cause Smith-Lemli-Opitz syndrome (SLO) syndrome. Interestingly, most patients with SLO syndrome have ASD. The case with this smaller 17q12 deletion has ASD and macrocephaly, two of the most prominent features of individuals with recurrent 17q12 deletions. Moreover, he inherited this smaller deletion from his mother, who has macrocephaly, depression, and an elevated SRS score; however, two unaffected siblings without the deletion also have macrocephaly, therefore it is uncertain whether the macrocephaly in this family is related to the deletion. These findings support the pathogenic role of 17q12 deletions in ASD and implicate ACACA as a strong candidate gene responsible for the neuropsychiatric phenotype.

578W

Defining the use of electronic medical records in genetic studies of multiple sclerosis. *M.F. Davis[1], J.L. McCauley[2], J.R. Oksenberg[3], S.L. Hauser[3], M.A. Pericak-Vance[2], J.L. Haines[1].* 1) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN; 2) John P. Hussman Institute for Human Genomics, University of Miami, Miller School of Medicine, Miami, FL; 3) Department of Neurology, School of Medicine, University of California, San Francisco, CA.

Multiple sclerosis (MS) is a neurodegenerative disease with complex genetic underpinnings and clinical heterogeneity. The HLA locus was identified as a critical genetic determinant of the disease in the 1970s, but no other genes or loci were discovered until the recent GWAS era. There are 23 confirmed loci in the published literature for MS at the present time. At Vanderbilt University, we have the unique opportunity to access BioVU, an opt-out DNA biobank. Over 120,000 leftover blood samples from outpatients have been collected and used to extract DNA, and each sample is linked to the individual's clinical data through a “synthetic derivative” of their de-identified electronic medical record (EMR). Currently, there are about 1000 samples with a diagnosis of MS in BioVU, and thousands of samples that can be used as controls. We have received access to the DNA samples and the synthetic derivative of all patients with MS and 2400 control subjects. We are undertaking a study to characterize the clinical information available from the EMR that can be used for genetic studies of MS. Previous studies have lacked access to long-term clinical information and few studies have analyzed the genetics of the various clinical aspects of MS. We are investigating the range and depth of information that can be extracted from the EMR, especially age of onset, progression (EDSS), first neurological symptom, and response to treatments, with the goal of performing genetic analyses using these sub-phenotypes to better understand the genetic heterogeneity of MS. As manual review of large numbers of medical records would be time-prohibitive and prone to human error, we are working to develop methods to extract this information using algorithms. Based on our preliminary manual review of 55 of these medical records, we estimate we will be able to extract an age of onset for 765 individuals, EDSS for 240 individuals, first neurological symptom for 655 individuals, and treatment information for 890 individuals. All of the samples we have extracted from BioVU have been genotyped on the Immunochip, which contains 196,524 SNPs representing genes implicated in autoimmune disorders, including MS. Once characterization of data available from the EMR is complete, we will perform association analyses with the sub-phenotypes identified.

579W

Detection of a deletion in 20p in a schizophrenia patients. *C. Zai, M. Tampankeras, V. de Luca, A. Tiwari, V. de Oliveira, N. Freeman, B. MacKenzie, J. Kennedy.* Dept Neurogenetics, CAMH, Toronto, ON, Canada.

Schizophrenia is a severe chronic neuropsychiatric disorder that affects approximately 1% of the population. Its etiology remains unclear despite decades of research. We aimed to conduct a genome-wide association study on our sample of schizophrenia samples using the Affymetrix SNP6.0 platform. Analysis of raw data was carried out on Genotyping Console 2.1. Preliminary findings revealed a male patient of African ancestry with a possible deletion in the short arm of chromosome 20. The patient had no reported history of cannabis or alcohol abuse. Of note is a history of seizures. We are currently further characterizing this deletion and looking more in depth into the personal history and family history of medical conditions and other phenotypes, including history of suicide, of this patient.

580W

MLPA in proband and carrier analysis of Spinal muscular atrophy. *C. Arthi, B.R. Lakshmi.* MDCRC, Avinashilingam Deemed University for Women, Coimbatore, Tamil Nadu, India.

Spinal Muscular Atrophy (SMA) is one of the most common autosomal recessive disorder, affecting 1 in 10000 live births and with a carrier frequency of 1 in 50. The deficiency of the ubiquitous protein Survival Motor Neuron (SMN), coded by the SMN genes, SMN1 and SMN2 causes the disorder. In majority of cases, this deficiency is due to absence of the SMN1 gene. For accurate molecular diagnosis, the results of commonly used non-quantitative methods like conventional PCR-restriction fragment length polymorphism (PCR-RFLP) should be a confirmed by a quantitative analysis. Carrier analysis is also rendered difficult due to the presence of SMN2 copies. The present study analyses the usage of a recent, quantitative dosage analysis method viz, Multiplex Ligation Probe Amplification (MLPA), to estimate both homozygous and heterozygous absence of SMN1, SMN2 copy number and carrier analysis in a consanguineous family which led to genetic and reproductive counseling. A total of 47 patients clinically suspected for SMA were taken up for molecular diagnosis by both PCR-RFLP and MLPA. All the patients who had homozygous deletion of exon 7 (and 8) of SMNI gene by PCR-RFLP method showed the similar deletion status by MLPA. Three cases of heterozygous deletion of SMN1 exon 7 and 8 were identified by MLPA. Further sequence analysis of the SMN1 gene will be done to pick the mutation in the other SMN1 allele. Two patients detected for homozygous absence of SMN1 exon 7 by PCR-RFLP also had heterozygous deletion SMN1 exon 8 by MLPA. In a family where a male proband showed deletion of exons 7 and 8 of SMN1 gene by PCR-RFLP, the proband's sister was married to the maternal cousin. The couple visited the facility after the marriage requesting carrier diagnosis and counseling. MLPA was done for the proband, his parents, the sister and her husband (maternal cousin). On analysis both the parents of the proband were confirmed for their carrier status and the sister was also found to be a carrier of SMN1 exon 7 & 8 deletion. But the maternal cousin did not carry the mutation and genetic counseling was given to the family. Carrier analysis for SMA is not offered anywhere in our country and we observe that MLPA is a very useful and robust technique for carrier diagnosis in SMA. In a country where consanguineous marriages are common, such techniques offer valuable information towards alleviating and preventing such genetic disorders.

581W

Copy number loss in Ashkenazi Parkinson's disease patients, non-carriers of founder *LRRK2* or *GBA* mutations. *A. Bar-Shira[1], M. Kedmi[1], I. Pe'er[4], N. Giladi[2,3], A. Orr-Urtreger[1,3].* 1) Genetic Inst, Tel Aviv Sourasky Med Ctr, Tel Aviv, Israel; 2) Movement Disorders Unit, Parkinson Center, Department of Neurology, Tel Aviv Sourasky Med Ctr, Tel Aviv, Israel; 3) Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel; 4) Department of Computer Science, Columbia University, NY, USA.

Parkinson's Disease (PD) is a complex disorder, associated with multiple genetic, environmental and age-related risk factors. The high level of copy number variation (CNV) identified in the general population, up to 12% of the human genome, suggests that CNVs could underlie some of the normal human variations including differences in cognitive, behavioral, and psychological features. An increasing number of human diseases result from recurrent DNA rearrangements that involve unstable genomic regions and abnormal dosage of gene(s). In PD, an abnormal copy number of the *alpha-synuclein* gene was associated with disease risk and severity (Singleton, Science 302:841, 2003). We aimed to determine whether specific CNVs are associated with disease risk and severity in our PD patient population. A pilot study was performed using Affymetrix SNP6.0 Array (~900K CNVs). Four study groups were analyzed: Ashkenazi PD patients with either *LRRK2* G2019S (75 samples) or founder *GBA* mutations (93 samples), PD-patients without these mutations (PD-NC,130 samples) and age- and sex-matched controls (128 samples). CNV changes were determined using the Genotype Console 2.1 (Affymetrix), Partek 6.3 and SVS V7.2.2 (Golden Helix). Initially, the identification of CNVs was done compared to an online HapMap database. In the 426 samples, 6595 CNVs were detected in the autosomal chromosomes: 3914 gain and 2681 loss events. Novel CNVs, which were not previously described, were also detected. For more reliable data, the above CNVs were filtered based on the average distance between the markers (number of CNV markers/length of CNV) and only CNV events with average distance of less than 10 Kb were selected. Here, 3582 gain events and 2157 loss events were detected. 1671 CNVs were in the controls and 4068 in PD-patients: 13.055 CNVs/individual in the control group and 13.65 in PD-patients. However, when dividing the patients according to the different carrier sub-groups, significant more loss events were detected among the PD-NC group (patients without founder *LRRK2* or *GBA* mutations) compared to the other groups. A candidate CNV on 8p23.2 was selected for validation-replication. Validation was fully achieved, but the replication step did not confirm its association with PD. Our results demonstrate that the Ashkenazi population is valuable for studying the complex genetic basis of PD, and suggest that additional CNVs studies are necessary to examine their potential involvement in PD.

582W

Novel genomic imbalances detected by aCGH in patients with intellectual disability: searching for the key cognition genes. *M. Barbosa[1,2], F. Lopes[1], C. Bessa[1], G. Soares[2], J. Silva[2], T. Temudo[3], M. Rocha[2], C. Gomes[1], G. Barros[1], F. Duque[4], G. Oliveira[4], C. Marques[4], M. Reis-Lima[2], A.M. Fortuna[2], J. Pinto-Basto[5], J. Weiss[6], E. Sistermans[6], B. Ylstra[7], P. Maciel[1].* 1) Life and Health Sciences Research Institute (ICVS), Porto, Portugal; 2) Center for Medical Genetics Dr. Jacinto Magalhães, National Health Institute Dr Ricardo Jorge, Porto, Portugal; 3) Hospital Geral de St. António, Porto, Portugal; 4) Hospital Pediátrico de Coimbra, Coimbra, Portugal; 5) Center for Predictive and Preventive Genetics, IBMC, Porto, Portugal; 6) Department of Clinical Genetics, VU University Medical Centre, Amsterdam, The Netherlands; 7) Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands.

The discovery of new genomic imbalances in patients with ID requires the differentiation of pathogenic copy number variations (CNVs) from benign variants. Whenever a new alteration is found, is important to prioritize the "best" candidate genes that may account for the ID phenotype, allowing the discovery of new ID causative genes. We have analyzed 130 patients with idiopathic ID (76 syndromic and 54 non-syndromic) by aCGH (Agilent 180K). The prioritization of candidate ID genes for each patient was based on bioinformatics analysis using the TS-CoExp Disease Gene Prediction web tool, leveraged with described/predicted protein function data from OMIM/PubMed. TS-CoExp predictions, based on conserved co-expression data, were performed for "Brain", "Brain and Central Nervous System", and also for all available co-expression networks, using a training set of 727 OMIM entries for which the keywords "mental retardation" or "intellectual disability" were present in the title and/or synopsis. We have discovered non-polymorphic genomic imbalances in 53 patients, of which 6 presented previously described microdeletion/microduplication syndromes, 20 had novel CNVs very likely to be pathogenic (VLP) and 27 presented smaller and less-likely pathogenic CNVs, the relevance of which needs to be further assessed. The novel VLP alterations included 1p36.21p36.23 del, 1q43-q44 del, 2p13.1-13.3 del, 2q11.2-q12.2 del, 2q11.2 dup, 3q22.1-q23 del, 5q35.3 dup, 6q25.3 deletion, 7q11.23 del, 7q33 del, 9q34.2-q34.3 dup, 9q33.2-q33.3 dup, 10q26.3 del, 12q24.21-24.2 del, 12q24.21 dup, 14q32.31-q32.33 dup, 16q24.3 dup, 20q13.12-q13.13 del and Xq24 dup. Among the genes prioritized by the TS-CoExp web tool are AKT3 (V-AKT murine thymoma viral oncogene homolog 3), SEMA4F (semaphorin 4F), CREG2 (cellular repressor of E1A-stimulated genes), RAB6B (RAB6B, member of the RAS oncogene family), SNX9 (shorting nexin 9), CNOT4 (CCR4-NOT transcription complex, subunit 4), RXRA (retinoid X receptor, alfa), EIF5 (eukaryotic translation initiation factor 5), EBF3 (early B-cell factor 3), MED13L (mediator complex subunit 13-like), ARFGEF2 (ADP-ribosylation factor guanine nucleotide exchange factor 2) and CUL4B (cullin 4B, for which only deletions and point mutations have been associated with ID so far). Our work contributes for the identification of new key genes implicated in cognition and, when mutated, in ID pathophysiology.

583W

Rare copy number variation at SEMA5A eQTLs is associated with autism. *Y. Cheng, L. Weiss.* Psychiatry, University of California, San Francisco, San Francisco, CA.

In a previous genome wide association study (GWAS), significant association with autism was detected near the SEMA5A gene, which has also shown evidence for reduced expression in autism (Weiss et al, 2009). Previously, 245 SEMA5A expression quantitative trait loci (eQTLs) were identified and shown to exhibit common polymorphism association with autism (Weiss et al., 2010, ASHG #294),. Here, we investigated rare copy number variants (CNVs) in these regions. From four datasets (Bucan et al, 2009; Marshall et al, 2008; Pinto et al, 2010; Szatmari et al, 2007), we obtained a list of autism-specific CNVs with less than 80% overlap with a reported variant in the Database of Genomic Variants (DGV , Iafrate et al, 2004). For each dataset, we calculated the total number of CNVs that overlapped with our eQTL regions and with random regions of the same length. This region sampling was repeated for 1,000 permutations to calculate a P-value. In the DGV, representing variation in the healthy population (69,694 CNVs >1kb reported in peer-reviewed literature), we observed 7% overlap (P= 0.23). Marshall et al. reported 277 CNVs from 427 probands (using 500K array and >1 algorithm) with 28% overlap (P<0.001). Bucan et al. reported 551 CNVs from 912 probands (Illumina 550K array, exonic CNVs), 22% of which overlapped our eQTLs (P=0.33). Szatmari et al. identified 209 CNVs from 173 probands (using 10K array and stringent thresholds), with 26% overlap (P=0.19). Pinto et al. reports 1,102 CNVs from 996 probands (1M array and CNVs >30kb called with >1 algorithm), which gives 18% overlap (P=0.01). The 372 records from Autism Chromosome Rearrangement Database (ACRD) has 27% overlap (P<0.001). The current analysis has some limitations, including differences in dataset size, array type, CNV calling algorithm and reporting criteria. For example, average size varies (0.1-4.6Mb), and exon-overlap might not be relevant for a CNV acting on regulatory sequence. Despite heterogeneity across studies, the proportion of overlap is 2.5- to 4-fold that seen in the DGV. In summary, our approach of defining an expression regulatory pathway for a SNP-associated candidate gene has revealed additional common, and now rare, variants associated with autism and may provide a framework for identifying which rare CNVs are likely to contribute to autism risk.

584W

Attention problems and CNVs: A study of MZ twin pairs. *E.A. Ehli[1,2], A. Abdellaoui[3], Y. Hu[1], J.J. Hottenga[3], M. Kattenberg[3], C.E. van Beijsterveldt[3], M. Bartels[3], R.R. Althoff[4], X. Xiao[5], P. Scheet[5], E.J. de Geus[3], J.J. Hudziak[4], D.I. Boomsma[3], G.E. Davies[1,2].* 1) Avera Institute for Human Behavioral Genetics, Avera Mckennan Hospital, Sioux Falls, SD, USA; 2) Department of Psychiatry, University of South Dakota, Sanford School of Medicine, Division of Basic Biomedical Sciences, Sioux Falls, SD, USA; 3) Department of Biological Psychology, VU University, Amsterdam, The Netherlands; 4) University of Vermont, College of Medicine, Burlington, VT, USA; 5) Department of Epidemiology, University of Texas M.D. Anderson Cancer Center, Houston, TX, USA.

The purpose of this study was to identify *de novo* copy number variations (CNVs) in monozygotic twin pairs and to examine whether *de novo* and/or inherited CNVs might be related to attention problems (AP) in childhood. Based on longitudinal phenotyping, subjects were selected from the Netherlands Twin Register. We selected fifty monozygotic (MZ) twin pairs (22 concordant affected, 17 concordant unaffected, and 11 discordant pairs). For 36 pairs, their parents could also be included to help detect *de novo* CNVs. Probe intensity data was generated using Affymetrix SNP 6.0 arrays. CNV calls were made using concordance between two algorithms, Birdsuite 1.5.5 and PennCNV. In total 45 complete twin pairs, four separate twins, 26 complete pairs of parents, and 7 separate parents passed quality control thresholds. We are able to detect two different types of *de novo* CNVs: (1) CNVs shared by the MZ twins, but not inherited from the parents (i.e. pre-twinning de novo CNVs), were detected by comparing copy number (CN) calls between parents and twins. (2) The presence of CNVs not shared between MZ twins that may be inherited in one twin, but not in the other (i.e. post-twinning de novo CNVs), was investigated by comparing the CN calls between MZ twins. Genome-wide CNV burden (= all detectable inherited and de novo CNVs) linked to attention problems was tested with permutation tests in Plink for seven groups of CNVs (CN=0 or 1, CN= 1, CN=0, CN=3 or 4, CN=3, CN=4, CN=0, 1, 3 or 4). Eight pre-twinning *de novo* CNV events were identified. We also identified 21 CNV segments that differed in CN between the MZ twins: 9 in the concordant low group, 6 in the discordant group and 6 in the concordant high group. Quantitative PCR is being utilized to confirm the CNVs identified from the microarray data. After corrections for multiple testing, the association tests for genome-wide CNV burden yielded a significant association with AP for the average size of the group of CNVs with any deviation from the expected CN (CN=0, 1, 3 or 4), where the affected individuals had significantly larger CNV events than the unaffected group (p = .0004). Each group of CNVs showed a larger average CNV size in the affected group, except for the CNV group with deletions of 2 copies (CN = 0). This group however was the only group to show a nominally significant result in the amount of CNV events, were the affected group had more events than the unaffected group (p = .036).

585W

Genome-Wide Analysis of Copy Number Variations in Schizophrenia. *N. Feng, G. Todarello, C. Li, B.S. Kolachana, R. Vakkalanka, D.R. Weinberger, R.E. Straub.* CBDB, GCAP, NIMH, National Institutes of Health, Bethesda, MD.

Copy number variants (CNVs) were associated with schizophrenia in several previous genome-wide analyses. However, many specific CNVs reported were unique, with minimal chromosomal overlap between studies and only a few regions have garnered support from multiple studies, and these include 1q21.1, 2p16.3 (NRXN1) and 22q11.2 (VCFS). Moreover, recent studies suggested that rare de novo CNVs 500kb and greater are more likely to be important in the etiology of schizophrenia. Here we report preliminary results from a genome-wide analysis of CNVs in a total of 1851 Caucasian individuals: 570 schizophrenia probands, 213 unaffected siblings, 535 parents, and 533 controls. There were a total of 220 full trios and 213 discordant sibling pairs available for evaluating inheritance. Genomic DNA from lymphoblast cell lines were genotyped on Illumina BeadArray chips (550K, 610K, 660K and 2.5M) and CNVs called by the PennCNV 2010 program. There were no consistently significant differences between schizophrenia probands and controls in the frequency of deletions or duplications at either the >100kb or >500kb size ranges. We examined in greater detail some previously reported regions of interest. At NRXN1, 6 of 570 schizophrenia probands had qPCR confirmed deletions, ranging in size from 25-282kb, whereas none of 533 controls had such deletions called. At 1q21.1, there were 3 schizophrenia probands (2 confirmed as de novo) with >500kb deletions and none of the controls had large deletions. At 22q11.2, 3 VCFS location deletions (2 confirmed as denovo) ranging from 1.5-2.5Mb were called in schizophrenia probands and none in controls. These results support the association of deletions in the 1q21.1, NRXN1 and VCFS regions with schizophrenia. In contrast, regions 15q11.2, 15q13.3, 16p11.2 and 16p13.1 did not show any notable frequency differences, but this may be due to our relatively small sample size. Finally, preliminary analyses indicated a few rare deletions more prevalent in schizophrenia probands in genes of potential biological relevance such as PARK2 and ASTN2 and other genes from neurodevelopmental pathways. qPCR validation and association analysis with cognitive and other phenotypes is ongoing.

586W

Genome-wide Copy Number Variation analysis for Schizophrenia in a large Ashkenazi cohort. *S. Guha[1], J. Rosenfeld[1], A.K. Malhotra[1,2,3], A. Darvasi[4], T. Lencz[1,2,3].* 1) Psychiatry Research, Zucker Hillside Hospital, NSLIJ, New York, NY; 2) Center for Psychiatric Neuroscience, The Feinstein Institute for Medical Research, Manhasset, NY; 3) Department of Psychiatry and Behavioral Science, Albert Einstein College of Medicine of Yeshiva University, NY; 4) Department of Genetics The Institute of Life Sciences, The Hebrew University of Jerusalem, Givat Ram, Jerusalem, Israel.

Copy number variations (CNVs) are recognized to contribute to normal human genomic variability and are also associated with the risk of human disease. Recent genome-wide studies have provided evidence that CNVs may be associated with schizophrenia and other psychiatric disorders. Ethnically homogeneous founder population may increase power for detection of rare and common CNVs associated with human disease. To identify CNVs that increase the risk of schizophrenia, we performed a whole-genome CNV analysis on a cohort of 1233 schizophrenia cases and 2279 controls drawn from the Ashkenazi Jewish (AJ) population from Israel. Ethnically homogeneous samples were selected from a larger AJ cohort and genotyped for 1.4 million probes using Illumina HumanOmni1-Quad arrays. After stringent quality filtering for both samples and markers, we performed PCA with AJ-specific AIMs and MDS correction in PLINK to correct for any population stratification effects. After QC, we analyzed the rare CNVs associated with schizophrenia in 904 schizophrenia cases and 1640 matched controls using three different algorithms: QuantiSNP, PennCNV, and CNVpartition. All the calls were separately assessed using the specific QC threshold recommended by each algorithm. The final CNV calls were made based on common consensus calls from all the algorithms using the following filtration criterion: minimum 20 probes, minimum 500kb in size, at least one gene in the segment and less than 50% overlapping with the common copy number polymorphism at the Database of Genomic Variants (DGV). Our analysis revealed a novel association of a ~ 700 kb deleted segment at chromosome 16, a part of which was previously associated with mental retardation, developmental delay, and associated phenotypes. This CNV successfully replicated in other datasets and the combined analysis identified a significant association (6/9516 cases and 0/10322 controls) p= 0.011, odds ratio = ¥ (1.16-¥) with a common minimum region of ~ 640 kb containing 20 genes. We have also replicated known schizophrenia associated CNVs e.g., 22q11, 3q29. Overall burden analysis showed a significant (p = 0.03) overrepresentation of CNVs in cases compare to controls. Deletions demonstrated a 1.6 fold excess in cases (p = 0.01) compare to controls, but duplications did not differ between cases and controls. Results further contribute to our understanding of the role of CNVs in etiology of schizophrenia and other neurodevelopmental disorders.

587W

A FOXP2 intragenic deletion in a patient with autism. *F. Gurrieri, N. Cannelli, G. Neri.* Genetica Medica, Univ Cattolica, Rome, Italy.

Autistic disorders (ASD) are a group of childhood neurodevelopmental disorders characterized by difficulties in socialization and communication and stereotypic behaviors. Array-CGH has recently allowed the identification of CNVs in 10% of ASD cases, often non randomly. Nevertheless, this high-throughput approach is limited by the resolution and assembly of the array-chip used in which some genes or genomic regions, still involved in ASD, may not be represented. In addition, alterations can be detected in regions not known to be related to ASD and therefore difficult to interpret as meaningful results. With the aim to develop a targeted quantitative genetic test for ASD we built an “in-house” MLPA assay with probes covering 9 candidate genes for ASD and tested it on 120 ASD patients. METHODS: All patients tested negative for Fragile-X syndrome, Rett syndrome and SHANK3 deletions/mutations. Candidate genes included in the MLPA assay were: FOXP2 (7q31), UBE2H (7q32), CALU (7q32.1), GRM8 (7q31), GRM5 (11q14), DOC2B (17p13), HOMER1 (5q14), NLGN3 (Xq13), FHIT (3p14). One or more probes were designed for each gene, according to MRC-Holland online manual and MLPA reactions were performed using EK1 reagent kit (MRC Holland, Amsterdam). Results were analysed by a developed spreadsheet in Microsoft Excel. All test/reference ratio values below 0.7(0.35 for NLGN3 in males) or exceeding 1.3 (0.65 for NLGN3 in males) were considered as CNA. The assay was first validated on 30 controls. RESULTS: We found a CNV in 5 patients (4.1%), all but one in NLGN3. In a single patient we detected a de novo deletion in exon 16 of FOXP2 gene. These probes are not included in the array-chips currently in use in our laboratory (44K and high-definition 105K from Agilent). CONCLUSIONS: Our results indicate that a targeted MLPA assay could allow detection of intragenic CNVs that might have been missed with array-CGH, even at high resolution. This approach needs to be validated in higher cohorts of patients and other combinations of candidate genes might be assembled for a targeted diagnosis in ASD patients. The NLGN3 alterations confirm already known associations of neuroligins to autism; along the same line, the FOXP2 alteration is in agreement with the role of this gene in speech and language disorders but also suggests a role in autism pathogenesis.

588W

Large-scale pathway analysis of copy number variants highlights defective GABAR-A signaling in autism spectrum disorders. *D. Hadley, J. Glessner, F. Mentch, D. Abrams, C. Kim, E. Frackelton, C. Hou, R. Chiavacci, J. Connolly, G. Lyon, H. Hakonarson, Autism Genome Project.* Children's Hospital of Philadelphia, Philadelphia, PA.

The ability to quantify individual's genomic risk for disease can facilitate the development of new interventions and improve medical practice. Many rare Copy Number Variants (CNVs) that harbor small genomic deletions and insertions have been described in the autism spectrum disorders (ASD). To identify these likely functional elements, we combined various large cohorts of autistic patients with a large number of neurologically normal controls to analyze over 3K affected cases and 7K controls. In a two-stage genome-wide association design, we uncovered 266 genome-wide statistically significant (combined P <= 2.76E-08) distinct CNV regions (CNVR). The 38 genes with exons disrupted by these robust CNVRs are most enriched in gene networks impacting neurological disease, behavior and developmental disorder. GABAR-A receptor signaling was found to be the most significant canonical pathways disrupted in ASD because case-enriched defects in GABRA5 ,GABRB3, and GABRG3 genes. Moreover, network analysis of the first-degree gene interactome of the GABAR-A receptor family suggests that ASD cases are significantly enriched for pathway defects (P <= 2.1E-21, OR = 9.9) when compared with neurologically normal controls. Taken together, the CNVRs we have identified impact multiple novel genes and signaling pathways, including genes involved in GABAR-A signaling, that may be important for new therapeutic development.

589W

Copy number aberrations affecting adhesion genes involved in the development of the cerebellar vermis are associated with autism spectrum disorders. *S. Hochreiter[1], D.-A. Clevert[1,2].* 1) Institute of Bioinformatics, Johannes Kepler University Linz, Linz, Austria; 2) Charité University Medicine, Berlin, Germany.

Motivation: We investigated neurodevelopmental dysfunctions in autism spectrum disorders (ASD) by a integrative analysis including the two largest genome-wide studies on associations between copy number aberrations (CNA) and ASD, the BioGPS tissue atlas, the Allen brain atlas, and *in situ* hybridization histochemistry data from the developing mouse brain. In contrast to the original association studies, we considered "ASD candidate genes" each of which is the only CNA-impaired gene in an ASD case, therefore, presumably causing ASD. For extracting ASD candidate genes, we developed an analysis pipeline for rare and small CNAs. Rare CNAs are supposed to be more disease-specific, because CNAs that cause ASD with high probability are assumed to be *de novo* and quickly vanish in the population due to their low reproductive fitness. Small CNAs affect only few genes and, therefore, are very specific concerning the genes they are impairing. **Results:** ASD candidate genes that are indentified independently in both CNA studies include the neurexins *CNTNAP2* and *NRXN1*, the catenin *CTNNA3*, the cadherin *CDH13*, and the contactins *CNTN5* and *CNTN6*. Gene set enrichment analysis of ASD candidate genes showed that significant biological processes are all related to cell and synaptic adhesion the postsynaptic density, membrane and synapse. At data from the BioGPS, the Cancer Genome Anatomy Project, and the Allen brain atlas, ASD candidate genes have significantly different variations in their expression values in cerebellum compared to other genes, where at the Allen brain atlas cerebellar vermis lobes I-II, III, VI, and VIII where most significant. *In situ* hybridization histochemistry data indicate that ASD candidate genes are primarily expressed in the developing mouse cerebellum. **Discussion:** Our results which hint at the cerebellar vermis as the location of ASD's pathogenesis are consistent with pathological studies of ASD cases, where, in over 90% of the examined brains, well-defined cerebellar abnormalities were found. Also studies on children with vermal lesions showed phenotypes like speech disorders and behavioral disturbances similar to autism. The high percentage, 60-80%, of ASD cases showing motoric deficits again hints at the cerebellum. We explain 4:1 male to female ratio in ASD by the regulatory influence of estrogen on the development of the cerebellum. The human estrogen 17) -estradiol enhances in the cerebellum synaptic connectivity.

590W

High coverage of copy number variants (CNVs) in Finnish patients with autism spectrum disorders using Nimblegen 2.1M array. *K. Kantojärvi[1], R. Vanhala[2], T. Lepistö[2], R. Alen[3], I. Järvelä[1], L. Muthuswamy[4].* 1) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 2) Hospital for Children and Adolescents, University of Helsinki, Finland; 3) Department of Pediatrics, Jyväskylä Central Hospital, Jyväskylä, Finland; 4) The Ontario Institute for Cancer Research, Toronto, Canada.

Autism spectrum disorders (ASD) are neuropsychiatric disorders characterized by restricted repetitive behavior and abnormalities in communication and social interaction. The model of inheritance of ASD seems to be very complex and probably involves multiple interacting genes. Several studies have shown that de novo copy number variants (CNV) contribute to autism risk in 5-15% of cases in families with one affected individual. We performed array comparative genomic hybridization (aCGH) utilizing Nimblegen 2.1M oligonucleotide array on 21 Finnish families with autism spectrum disorders (ASD). The findings were confirmed using quantitative PCR. We detected five copy-number variations that most likely are causative of autism, because they either arose de novo and/or overlapped with known microdeletions. One of the deletions was de novo NRXN1 previously shown in neuropsychiatric conditions.

591W

Familial occurrence of Asperger Syndrome associated with a 1Mb Duplication on Xq including the MCT8-Gene. *P.M. Kroisel[1], K. Wagner[1], M. Mach[1], E. Vallant[1], M. Brunner-Hantsch[2], M.R. Speicher[1], K.M. Roetzer[1].* 1) Human Genetics, Medical University of Graz, Graz, Styria, Austria; 2) Psychiatric consultant, Graz, Austria.

By array CGH using a 60k Agilent oligonucleotide array we were able to identify a maternally inherited microduplication of about 1Mb at Xq12.3-q13.3 that affects at least 5 genes in a patient with Asperger-syndrome. One of the genes involved in this microduplication is the MCT8-(monocarboxylate transporter-8)-gene, already known to cause Allan-Herndon-Dudly-syndrome(AHDS), which follows an X-linked pattern of inheritance and leads to a severe form of mental retardation combined with distinct dysmorphic features in males whereas females show a mild phenotype with no neurologic anomalities. Since different and occasionally reciprocal phenotypic features can be observed in patients with microdeletions versus microduplications of particular genomic regions or loci like the Williams-Beuren- or DiGeorge-syndrome critical region or the PMP22-gene, a different phenotype to AHDS is not unexpected in the patient described here with the identified Xq microduplication. The affected male patient as well as his maternal uncle shows a much milder phenotype compared to AHDS patients with just a few characteristic facial anomalies like smaller ears however a pronounced form of Asperger-syndrome and a general reduction of mental capacity with remarkable strength in fine coordination and spatial orientation is present. Potential additional effects related to an increased gene dosage of the KIAA2022-gene and the ZDHHC15-gene with regard to mental impairment can not be ruled out at the current state of this investigation. Because just a few similar microduplications are currently listed in databases like Decipher a comparison with these patients is difficult. From our findings we would propose that at least in this familial setting a genetic explanation for the development of Asperger-syndrome was found and gain of function mutations of the MCT8-gene might be a more common reason for this syndrome than the identified specific microduplication or mutations of the linked neuroligin-3(NLGN3)-gene.

592W

Genome-wide investigation of rare CNVs in a newly characterized Canadian autism cohort. *A.C. Lionel[1,2], B. Thiruvahindrapuram[1], D. Merico[1], J.L. Howe[1], Z. Wang[1], J. Wei[1], L. Zwaigenbaum[3], B.A. Fernandez[4], W. Roberts[5], P. Szatmari[6], C.R. Marshall[1], S.W. Scherer[1,2].* 1) The Centre for Applied Genomics and Program in Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) McLaughlin Centre and Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 3) Department of Pediatrics, University of Alberta, Edmonton, Alberta, Canada; 4) Disciplines of Genetics and Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada; 5) Autism Research Unit, The Hospital for Sick Children and Bloorview Kids Rehabilitation, University of Toronto, Toronto, Ontario, Canada; 6) The Offord Centre for Child Studies, Department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, Ontario, Canada.

Autism spectrum disorder (ASD), a common neurodevelopmental disorder with an estimated prevalence rate of nearly 1% of children, is characterized by impairment in reciprocal social interaction, communication deficits and a repetitive pattern of behavior. In recent years, several studies have highlighted the role of rare copy number variants (CNVs) in the genetic etiology of ASD. The study of these rare deletions and duplications in the genomes of ASD patients has proven to be a powerful tool for the identification of novel candidate genetic loci for further investigation by targeted gene sequencing and functional studies. We conducted, and present here for the first time, a genome-wide CNV scan of more than 700 unrelated, newly characterized Canadian ASD patients using two high resolution SNP genotyping platforms: the Affymetrix SNP 6.0 and the Illumina Omni2.5M-Quad. A multi-algorithm approach incorporating Birdsuite, Affymetrix Genotyping Console and iPattern (for Affymetrix data) and iPattern, PennCNV and CNVPartition (for Illumina data) was utilized for CNV detection. High confidence CNVs detected by this approach in the ASD cases were compared with CNVs found in array data from more than 2,000 ancestry-matched population based controls. We detected and confirmed validated rare exonic CNVs in the ASD cases that were absent in controls and overlapped previously implicated ASD loci (e.g. *NRXN1*, *SHANK3*, *PTCHD1*, 16p11.2), or identified new candidate susceptibility loci for ASD (e.g. *NRXN3*). We are currently collecting samples from the parents and extended family of the ASD cases in order to study the inheritance patterns of the CNVs of interest and their potential segregation with elements of phenotype. We will also test for gene interaction sub-networks enriched for rare CNVs in ASD cases compared to controls. Our results provide support for the role of rare CNVs in risk for ASD and identify new candidate genetic loci for further investigation.

593W

A genome-wide CNV association study on Autism Spectrum Disorder (ASD) in the Japanese population. *X. Liu[1], T. Shimada[2], K. Tokunaga[1], T. Sasaki[3].* 1) Department of Human Genetics, University of Tokyo, Tokyo, Japan; 2) Department of Neuropsychiatry, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 3) Office for Mental Health Support and Graduate School of Education, University of Tokyo, Tokyo, Japan.

Introduction: Family and twin studies have strong evidence that autism spectrum disorder has a strong genetic background; however,all know variation only accounts for a small fraction for known patitens. We conducted a genome-wide copy number variation (CNV) association study on ASD in the Japanese population.

Methodology: Participants consisted of 500 ASD trio Japanese CNVs were detected using Genome-Wide Human SNP array 6.0 and determined by PennCNV. CNVs with length <100kb were excluded. CNVs were considered to becolocalized if they overlapped by at least 50%; of their length. They were classified as rare CNVs (found in <1%; of the total sample) or common CNVs (found in ⁄ 5%;).

Results: Identification of the pathogenic CNV variaton is underway.Preliminary results support the association between 15q11.2 with ASD. To identify the pathogenic CNV variation we are focusing on denovo CNVs.

Conclusions: We discuss the Copy Number Variation study in the Japanese population, By comparing our result with the caucasian study we may get new insight into the complex genetic structure of ASD.

594W

Copy number variations reveal novel GABAergic and neural genes potentially involved in autism. *D.Q. Ma[1], A.J. Griswold[1], H.N. Cukier[1], J.M. Jaworski[1], I. Konidari[1], P.L. Whitehead[1], S.M. Williams[2], R. Menon[3], E.R. Martin[1], J.L. Haines[2], M.A. Pericak-Vance[1].* 1) John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL; 2) Vanderbilt University, Nashville, TN; 3) Department of Epidemiology and Department of Obstetrics and Gynecology, Rollins School of Public Health, Emory University, Atlanta, GA.

The underlying genetic causes of autism spectrum disorders (ASDs) are complicated given that ASDs are highly heritable yet few associated genetic loci have been identified. Rare copy number variations (CNVs) have been implicated as contributing to autism etiology with each locus explaining less than 1% of the disease population. Due to the extremely heterogeneous nature of ASDs, independent studies are important to evaluate the contribution of ASD related CNVs and discover novel regions containing susceptibility genes and implicated gene networks. In this study, a genome-wide SNP array was utilized for CNV detection. High confidence calls were defined as CNVs identified by two distinct CNV-calling algorithms in a case-control dataset of European ancestry consisting of 813 unrelated ASD cases and 592 pediatric controls. Cases have an overall significantly heavier burden in number and size of deletions, thereby disrupting more annotated genes (size: p=9.90E-06; number: p=2.00E-05). Individual CNVs of interest were validated by real time PCR. Seventeen deletions larger than 1Mb were detected exclusively in cases, implicating CNV regions at 10p15.2, 13q12.12, 13q33.1, 14q23.2-3, and 17p12, which are not reported in DGV or AUTdb. Examination of case-specific CNVs provided additional evidence for pathways previously implicated in ASDs and revealed several novel candidate genes within GABAergic signaling and neural development pathways. The experimentally validated genes include DBI, an allosteric binder of GABRA receptors, the GABRA receptor-associated protein GABARAPL1, and SLC6A11 (GAT-3) a postsynaptic GABA transporter that could affect GABAergic signaling. We also identified CNVs in COBL, deletions of which show severe defects in neuronal cytoskeleton morphogenesis in vertebrates, and DNER, a neuron specific Notch ligand required for cerebellar development. Taken together, these CNVs may be a few of the missing pieces of ASD heritability and lead to discovering novel etiological mechanisms.

595W

Chromosome 15q11.2 is associated with schizophrenia risk in a set of patients. *D. Rudd, M. Axelsen, S. Mugge, E. Epping, N. Andreasen, T. Wassink.* Univ Iowa, Iowa City, IA.

Schizophrenia is characterized by positive and negative symptoms that include hallucinations, delusions and disorganized thought as well as flat affect and decreased speech. Genomic copy number variation (CNV) has been found to underlie and increase risk for a large number of neuropsychiatric disorders including schizophrenia. This project focuses on identification and characterization of pathogenic CNVs in a sample set of 457 schizophrenia patients and 290 psychiatrically healthy controls. To date, 181 probands and 141 controls have been genotyped using the Affymetrix Genome-Wide Human SNP 6.0 Arrays and analyzed for CNVs with Genotyping Console and CNAG. CNVs were compared between cases and controls and cross-referenced with the Database of Genomic Variants. The proximal arm of chromosome 15 contains canonical segmental duplications that predispose the region to CNV formation, but only recently have disease-associated CNVs been reported between the two most proximal breakpoints (BPs), BP1 and BP2. Here we report three case subjects that have a ~1Mb deletion of the BP1-BP2 region at chromosome 15q11.2. The BP1-BP2 deletion encompasses the four genes *CYFIP1, NIPA1, NIPA2* and *GCP5*. The deletion is present in three probands, two were maternally inherited and none were found in controls. All CNVs of interest were validated by qPCR or NimbleGen 385K whole-genome array CGH. The four genes within the deletion have previous association with Prader Willi syndrome, Angleman syndrome, autism and schizophrenia and are often inherited from a mildly or unaffected parent. *CYFIP1*is of primary interest due to its direct interaction with the Fragile X Mental Retardation Protein (FMRP) and its reported role in regulation of neuronal translation during brain development. *NIPA1, NIPA2* and *GCP5* are also expressed in the brain. It is a distinct possibility that in addition to the 15q11.2 deletion in probands, a “second CNV hit” contributes to disease pathology. Thus, the two probands with maternal inheritance of the deletion were further analyzed for sporadic secondary CNVs. For example, a *de novo* duplication of four exons of *ATXN2* as well as a *de novo* deletion of the promoter and the first three exons in *SYN3*, a gene with previous association with schizophrenia, was called in one proband. In the second family the proband had an additional *de novo* duplication of two exons in two synapse-associated genes *CACNA1B* and *DLGAP2* as well as a *de novo* intronic duplication of *SYN3*.

596W

Analysis of copy number variation in Alzheimer's disease: the NIA-LOAD/NCRAD Family Study. *S. Swaminathan[1], L. Shen[1], S. Kim[1], M. Inlow[1, 2], J.D. West[1], T. Foroud[1], K.M. Faber[1], R. Mayeux[3], A.J. Saykin[1], The NIA-LOAD/NCRAD Family Study Group.* 1) Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN; 2) Department of Mathematics, Rose-Hulman Institute of Technology, Terre Haute, IN; 3) The Gertrude H. Sergievsky Center, The Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University College of Physicians and Surgeons, New York, NY.

We previously investigated the role of copy number variants (CNVs) in Alzheimer's disease (AD) and mild cognitive impairment (MCI) using participants in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort [1]. Case/control association analyses revealed CNVs overlapping the AD candidate gene *CHRFAM7A* as well as *CSMD1*, *HNRNPCL1*, *SLC35F2*, *NRXN1* and *ERBB4* genes in cases (AD and/or MCI), but not controls. Using a similar approach as ADNI, we analyzed the role of CNVs in AD using non-Hispanic Caucasian participants in the NIA-LOAD/NCRAD Family Study. Briefly, CNV calls were generated in PennCNV software for 794 AD cases and 196 neurologically evaluated controls with DNA derived from blood or brain tissue. The controls had no family history of AD and were unrelated to cases. Extensive quality control was performed and 711 cases and 171 controls were retained for a case/control association analyses. We tested for CNVs in the entire genome and also limited our analysis to candidate genes from the AlzGene database. Genes overlapped by CNVs in cases, but not controls were determined. A trend for lower CNV call rate for deletions as well as duplications was observed in cases as compared to controls. The whole genome approach identified CNVs overlapping *IMMP2L* in 13 cases and no controls. As a comparison in the ADNI cohort, CNVs were found overlapping *IMMP2L* in four AD, one MCI and one control. The *ERBB4* and *NRXN1* genes identified in the ADNI CNV study also had CNV calls overlapping them in five and four cases respectively, but no controls. These genes have been previously associated with neuropsychiatric disorders. The candidate gene approach revealed a number of AD candidate genes overlapped by CNVs only in cases. The *CHRFAM7A* AD candidate gene had CNV calls overlapping it in 12 cases and one control. This gene was identified in the ADNI CNV study using a similar candidate gene approach. The present analyses confirm previous findings (*ERBB4*, *NRXN1*, *CHRFAM7A*) and also identified a new gene (*IMMP2L*) that may play a role in susceptibility to AD. Further investigation of these genes in independent cohorts are warranted. [1] Swaminathan *et al.* Genomic Copy Number Analysis in Alzheimer's Disease and Mild Cognitive Impairment: An ADNI Study. *IJAD* (in press).

597W

Rare variants in the 15q13.3 encoded CHRNA7 gene in autism. *S. Thomson, J. Sutcliffe.* Mol Physiology & Biophysics, Centers for Mol Neuroscience and Human Genetics Research, Vanderbilt Univ, Nashville, TN.

Autism spectrum disorders (ASDs) affect about 1/110 of children and are associated with conditions such as impairments in reciprocal social interaction and communication and presence of restricted and repetitive behaviors. While ASDs seem to be highly heritable, genome-wide association studies thus far have been limited in identifying common risk variants. This suggests a highly complex architecture for the underlying genetic etiology of ASDs. Recent studies showed that ASD individuals carry a higher global burden of rare, genetic copy number variants (CNV) compared to controls. This might also suggests that an increased number of rare variances in probands within a certain gene could be associated with autism, especially since ASD is a clinically heterogeneous genetic disorder. Recurrent deletions in the 15q13-q14 region were first reported in mental retardation, seizures, autism, schizophrenia and bipolor disorder. While the recurrent deletions contain several genes, attention has been focused on CHRNA7 since mutations in ion channel genes have been associated with epilepsy and schizophrenia. CHRNA7 is a member of a superfamily of pentameric ligand-gated ion channels that are distributed throughout the central and peripheral nervous system. Naturally occurring genetic variances in nAChRs can modulate cognition and behavior as well as contribute to the risk for various neurological psychiatric disorders. In an effort to identify rare individuals variances we sequenced all ten exons of CHRNA7 in a large cohort of ASD patients (400) as well as controls (384). We found a number of non-synonymous, synonymous, and intronic variances in probands that were not present in controls indicating that the prevalence of variances are greater in probands compared to controls. To determine if probands contained overall higher number of variances that potentially could alter and/ or interfere with the function or expression of the ion channel, we performed a two-sided Fisher's exact test for non-synonymous and splice side effecting variances. We identified seven non-synonymous and two splicing effecting variances in probands (2.25%) compared to one non-synonymous variance in controls (0.26%), which when combined reached significance (p-value = 0.021). Our study provides further evidence that rare individual variances play an important role in autism and may lead to a better understanding into the genetic complexity of ASD.

598W

Whole genome copy number variation study in an Arab-Israeli sample supports the role of rare large de novo deletions. *F. Torri[1,2], A. Akelai[3], A. Calabria[4,5], D. Amann-Zalcenstein[6], E. Ben-Asher[6], K. Kanyas[3], J.S. Beckmann[7], B. Lerer[3], F. Macciardi[1,2].* 1) Department of Psychiatry and Human Behavior, University of California, Irvine, Irvine, California 92617; 2) Biomedical Informatics Research Network (BIRN), Information Sciences Institute, University of Southern California, Los Angeles, CA 90292; 3) Hadassah-Hebrew University Medical Center, Jerusalem, Israel; 4) Dept. of Medicine, Surgery and Dentistry, San Paolo Hospital, University of Milan; 5) University of Milan - Fondazione Filarete, Genomics and Bioinformatics Unit; 6) Weizmann Institute of Science, Rehovot, Israel; 7) Centre Hospitalier Universitaire Vaudois and University of Lausanne, Switzerland.

We have recently published a fine mapping on an Arab-Israeli sample of schizophrenia with the definitive evidence on the role of the neurodevelopmental gene AHI1 in schizophrenia, shedding light also on new possible candidate genes MAP7 and PDE7B that are expressed in brain and may play a role in neurodevelopment. In line with the rising evidence on the implication of rare copy number variants (CNVs) in schizophrenia, we have carried out a whole-genome CNV analysis in the same familial Arab-Israeli sample, focusing on rare de novo CNVs. With a novel approach for family-based CNVs analysis, we were able to label and filter the events starting from the Penncnv calls. We detected heterozygous deletions and heterozygous duplications previously unreported, of sizes ranging from 7.2 kb to 7 Mb. Genes affected by rare de novo CNVs in our sample show mostly expression in brain. Preliminary pathway and functional analysis revealed a different enrichment profile for genes disrupted by large deletion events (>400 kb), than all the genes disrupted by rare de novo CNVs, independently from the dimension. Genes affected by de novo CNVs detected in our sample are mainly involved in cell metabolism pathways. This evidence may be interesting per se since both genes and metabolites related to energy metabolism (and thus energy-expensive brain functions) are altered in schizophrenia and, at the same time, appear to have changed rapidly during recent human evolution, probably as a result of positive selection. Interestingly, genes disrupted by large de novo deletions in our sample showed a significant enrichment in brain development and function-related pathways and processes like axonal guidance and long term potentiation (LTP). Even if we do not propose a model of interaction between those genes and the most known candidate genes to schizophrenia, there are multiple links between them that can be found by a simple information retrieving. Genes interested by rare large de novo deletion participate also in pathways that show a high level of overlapping to those in which AHI1, PDE7B and MAP7 are involved, with the great majority of the interactors being candidate genes to schizophrenia or involved in other brain pathways and processes. All together, these evidences support the etiologic role of AHI1 as a susceptibility gene for schizophrenia and put it into a potential network of genes that play a role in neurodevelopment and brain processes.

599W

Systematic shRNA-mediated silencing of 100 genes implicated in autism spectrum disorders and schizophrenia in iPSC-derived human neurons. *C. Ernst[1,2], K. Theriault[1], S. Sheridan[1], T. Nieland[2], D. Root[2], S. Haggarty[1,2], J. Gusella[1,2].* 1) Department of Neurology, Harvard Medical School, Boston, MA; 2) RNAi platform, Broad Institue of MIT and Harvard, Cambridge, MA.

Autism spectrum disorders (ASD) and schizophrenia are highly heritable disorders of human cognition and behavior. Advances in sequencing technology have allowed for better refinement of genetic etiology of complex disorders, and numerous genes and microdeletion/microduplication regions have been associated with both disorders, with some overlap. However, it remains challenging to establish a causal role for genetic variants associated with disease risk. One aim of this study is to investigate important implicated genes in these related disorders by identifying aberrant phenotypes in a genetic knock-down system. This is particularly relevant for elucidating the relative contributions of genes encompassed within microdeletion/microduplication syndromes such as 16p11.2 or 22q11.2, where the presumed causative gene(s) has not been found. To complement mutation analysis studies, we developed and characterized a human neural progenitor cell line derived from induced pluripotent stem cells (iPSC-NPs) taken from fibroblasts from a psychiatrically screened healthy male. iPSC-NPs were karyotypically normal, differentiated into all central nervous system cell types assessed, and were capable of synapse formation upon long-term differentiation. We selected over 100 genes associated with ASD and/or schizophrenia and targeted each gene with > four short-hairpin (sh) RNA's/gene, delivered by lentivirus along with a series of control shRNAs not known to target any human gene. In total, over 400 stable cell lines were generated and all cell lines were validated by qPCR in comparison to control shRNAs. A battery of phenotypic assays at both the progenitor and mature neuron stage were developed that assess a range of phenotypes including cell growth, cell extension, and intracellular calcium release. Changes in these cellular phenotypes occur upon knockdown of several of the GWAS-nominated candidate genes suggest that these genes may play a functional role in microdeletion/microduplication syndromes. These results can help the research community prioritize and direct further investigation of the function of genes associated with ASD and schizophrenia.

600W

Dynamic Versus Static Biomarkers of Schizophrenia and Bipolar Disorder: Considering Differential Developmental Courses in Genetic Studies. *M. Maziade[1, 2], T. Paccalet[1], N. Rouleau[1, 3], A. Sasseville[1], E. Gilbert[1], C. Cellard[1, 3], M. Battaglia[4], C. Marino[5], M.A. Roy[1], C. Mérette[1], M. Hébert[1].* 1) Dept Psychiatry, Ctr Rech, Univ Laval R-Giffard, Quebec, QC, Canada; 2) Faculty of Medicine, Laval University, Quebec, QC, Canada; 3) École de psychologie, Laval University, Quebec, Canada; 4) Academic Centre for the Study of Behavioural Plasticity, Vita-Salute San Raffaele University, 20 Via Stamira d'Ancona, 20127, Milan, Italy; 5) Eugenio Medea Institute, Department of Child Psychiatry, Via Don luigi Monza, 2023842 Bosisio Parini (Lecco), Italy.

Objective: Schizophrenia (SZ) and bipolar disorder (BP) are neurodevelopmental diseases and theirphenotype eludes genetic and neurobiological research. SZ and BP patients carry cognitive and physiological intermediate phenotypes (IP) that are precursors of disease in children at risk appearing many years before disease incidence (Maziade et al. *Schizophr Bull* 2009 and 2010; Hébert et al. *Biol Psych* 2010). Few studies have investigated the longitudinal mechanisms underlying intermediate phenotypes in children at risk. Yet, timing and differences in developmental courses need consideration in genetic studies. Our objective was to investigate the developmental courses of cognitive and physiological IP from childhood to young adulthood in terms of stability or fluctuations. **Methods:** We used a step by step sampling approach to narrow-down the early disease mechanisms. Upstream, we started with a 20-year follow-up of 48 densely affected multigenerational kindreds (1500 clinically characterized adult members). We then identified 400 members affected by a DSM-IV SZ or BP. Downstream, we finally focused on the 65 offspring aged 7 to 22, who were administered a neuropsychological battery and electroretinography (ERG) testing. We then constructed cross-sectional trajectories that were compared to those of controls, in order to investigate the developmental courses of IP, cognitive as well as ERG, in these high-risk offspring. **Results:** Three shapes of developmental trajectories were detected. First, some IP (such as lower global IQ and diminished amplitude in rod b-wave, measured by ERG) displayed stability in deficit from childhood until young adulthood. The impairment in visual memory was unstable and exhibited a non-linear two-stage trajectory: a lagging period during childhood followed by a recuperation period from adolescence until adulthood, as supported by a significant Group x Age Periods interaction (p = .035). The working memory impairment was characterized by a late-onset lag, emerging during late adolescence. **Conclusion:** 1. Different cognitive or physiological IP would mark different developmental courses according to three types of trajectories. 2. Timing of intermediate phenotype measurement and targeting dynamic, instead of static, endophenotypes may be crucial when considering how a particular endophenotype relate to genotype or gene expression.

601W

Peripheral blood expression profiles act as biomarkers for postpartum depression in a high risk population. *D. Mehta[1], L. Kraus[1], M. Rex-Haffner[1], J. Newport[2], Z. Stowe[2,3], E. Binder[1,3].* 1) Max Planck Institute for Psychiatry, Munich, Germany; 2) Emory University School of Medicine, Department of Psychiatry and Behavioral Sciences, Atlanta, GA; 3) Emory University School of Medicine, Department of Obstetrics and Gynecology, Atlanta, GA.

Introduction Postpartum depression affects 13% of women and has a strong negative impact on maternal well-being and infant development. Early detection, possibly by using biomarkers, coupled with timely treatment is therefore very important for both mother and child. Aims The aim of this study was to identify biomarkers for postpartum depression by global assessment of peripheral blood expression changes during the peripartum period. Methods Multiple measurements from 67 women (220 samples) were collected longitudinally in the 1st and 3rd trimester and within 7 weeks postpartum. All women had a history of mood or anxiety disorder and included 3 groups: postpartum onset depression (n=16), no depressive symptoms (n= 33) and depressed at all timepoints (n=18). Depression was assessed using the SCID mood module and the Beck Depression Inventory (BDI). Whole blood RNA was extracted from Tempus tubes and hybridized on Illumina HumanHT12-v4 microarrays. Background correction filtering and VSN normalization resulted in 15456 probes. Statistical analysis was performed in R using mixed models. Results Transcriptional profiles of >3000 transcripts were significantly changed across pregnancy and early postpartum after Bonferroni corrections($p<3x10-6$) with significant over-representation of steroid hormone transcription factor binding sites within these transcripts. Comparison of 3rd trimester expression profiles between the post-partum onset and no depressive symptoms groups revealed 116 significant transcripts with significant over-representation of estrogen receptor transcription factor binding sites (ESR1) within these 116 transcripts. Expression levels of these transcripts allowed classifying women with and without postpartum depression with 88% prediction, 82% sensitivity and 93% specificity. Conclusions Third trimester expression profiles of a subset of transcripts allowed high classification accuracy of women who developed postpartum depression, thus serving as potential biomarkers. RT-PCR validation of results and increasing the sample size to add power to the study is currently underway. Over-representation of ESR1 fits to the previous hypothesis that differential sensitivity to changing steroid levels is a vulnerability trait for postpartum depression (Bloch,M.et al.Am J Psychiatry 157,2000). Our results suggest that postpartum depression risk may be biologically detectable in the 3rd trimester, allowing timely prevention and treatment strategies.

602W

Behaviour and learning in children with NF1: Data from a population-based study. *A. Lehtonen[1], S. Garg[3], J. Green[2], D. Trump[1], G. Evans[3], S. Huson[3].* 1) Medical Genetics, University of Manchester, Manchester, United Kingdom; 2) School of Medicine, University of Manchester, Manchester, United Kingdom; 3) Genetic Medicine, Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom.

Research in the last two decades has begun to define the behavioural and cognitive phenotype of neurofibromatosis type 1 in childhood, documenting problems with behaviour and social functioning, academic achievement, attention, executive functioning and coordination (e.g. Hyman et al, 2005; Krab et al., 2008; Ferner et al., 1996). However, there is still a paucity of large-scale, comprehensive studies with representative sampling strategies. Our study aimed to delineate the behavioural and cognitive phenotype of NF1 through a population-based sample of children with NF1. Method Participants: The study took advantage of the large, population-based cohort of NF1-patients followed in Genetic Medicine, St. Mary's Hospital, Manchester, UK. Altogether 220 families with 4-16 year old children were invited to take part. All the families were sent questionnaires about different aspects of behaviour. A hundred children, aged 6-16 years, were also invited to do a cognitive assessment battery. The families invited to the cognitive assessment were randomly selected, stratifying for age and socio-economic status. 108 families returned the questionnaires, and 49 children took part in the cognitive assessment phase. Control participants for the cognitive assessment study included 20 siblings of the children with NF1 and 29 community controls, matched for age and socio-economic status. Measures: The questionnaire measures focussed on attention, social communication and general behaviour. The cognitive assessment included measures of intelligence, attention, memory and learning, academic achievement, visuospatial perception, and facial emotion recognition. Participants were seen either at home or in the hospital. Results Preliminary data analyses demonstrate very high levels of social communication, attention and behavioural problems: 26% of participants were in the `severe' range on the Social Response Questionnaire, indicating almost certain diagnosis of autism, and further 29% were in the moderate range, indicating mild/ moderate autism. 54% of participants scored in the `abnormal' or `borderline' range on the Strengths and Difficulties Questionnaire, and 53% of participants scored beyond the clinical cut-off for ADHD on the Conners' Parent Rating Scale. Results from the cognitive assessment study are pending (data collection has only just finished), but results will be available for the Conference, and we are happy to update this abstract accordingly.

603W

Gene-environment interaction studies in multiple sclerosis: The development of a reliable retrospective survey tool. *A. Hadjixenofontos[1], W.K. Scott[1], L.F. Barcellos[2], J.L. Haines[3], M.A. Pericak-Vance[1], J.L. McCauley[1].* 1) John P. Hussman Institute for Human Genomics, Miller School of Medicine, University of Miami, Miami, FL, USA; 2) Division of Epidemiology, School of Public Health, University of California, Berkeley, CA, USA; 3) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN, USA.

Multiple sclerosis (MS) is second only to trauma as the most common cause of neurological disability in young adults. There is increasing appreciation for the existence of a fundamental and complex architecture of gene-environment (GxE) interactions contributing to complex genetic disorders, including MS. While multiple environmental risk factor studies in MS have been undertaken, most have been performed with small numbers and even fewer have incorporated genetic information. Gathering prospective environmental exposure data to study complex genetic diseases is extremely challenging and often prohibitive. We have therefore developed a scripted environmental assessment survey for use in ongoing studies of genetic and environmental risk factors in MS. We utilized the PhenX validated toolkit measures as a primary source in our survey construction, with adaptations to target MS-specific environmental hypotheses. Our survey addresses the roles of latitude, sun exposure, infectious diseases, smoking, alcohol, (environmental, food, and drug) allergies, and gender hormones in the pathogenesis of MS. Our survey is designed to be administered electronically via the use of a tablet computer, supporting both in-person and phone interviews. Before the interview begins, participants are asked to complete a Life Events Calendar which we employ as a recall aid. The phone interviews mimic the in-person design in the use of both the calendar and list cards which are mailed to the participants ahead of the interview. Over-reporting is addressed by the use of questions on infectious diseases that have been shown not to influence MS. The face validity of the resulting survey was assessed by expert epidemiologists. We attempt to minimize interviewer bias by standardized training and a detailed script, which includes scripted clarifications. Our survey has undergone reliability testing via test-retest of 10 subjects. As a result of the reliability testing, we revised and shortened sections on vaccinations and dietary supplements that participants said were overwhelming. Questions that were deemed unreliable due to recall bias (e.g. vaccinations, hours spent in pre-school, time points for contraceptive use) were also removed. The mean time to complete this survey is 31.6 minutes. We are also developing the survey in Spanish, with the reliability to be tested via reverse-translation and additional test-retest assessments.

604W

Genome wide linkage and association analysis of job stress. *K. Min[1], J. Min[2], D. Lee[3], Y. Song[4], K. Lee[5], J. Sung[3,6], S. Cho[3,6].* 1) Ajou University school of Medicine, Suwon, Korea; 2) Institute of Health and Environment, Seoul National University, Seoul, Korea; 3) Department of Epidemiology, School of Public Health and Institute of Health and Environment, Seoul National University, Seoul, Korea; 4) Department of Family Medicine, Samsung Medical Center and Center for Clinical Research, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Seoul, Korea; 5) Department of Family Medicine, Busan Paik Hospital, Inje University College of Medicine, Busan, Korea; 6) Corresponding authors.

Objective: Despite evidence for the contribution of genetic makeup to an individual's susceptibility to stress, researchers have largely focused on whether stress acts as a modifier of a genetic predisposition for the occurrence of diseases or symptoms. Mental and physical stress during the occupational activities consists in a substantial part of overall stress. We performed linkage and family-based association tests to identify genetic backgrounds related to job stress using Korean family data. Method: Affymetrix Genome-Wide Human SNP array 6.0 were genotyped in 1269 subjects participating in the Healthy Twin study. After applying extensive quality checks (Exclusion criteria: monomorphic SNPs, Mendelian error > 3 families, MAF < 0.01, HWE < 0.001), a total of 568,121 markers were included for this analysis. Job stress was measured by the short version of Effort-Reward Imbalance Model. We performed nonparametric linkage (NPL) analysis with MERIN and family based association analysis with Golden Helix (Golden Helix, Inc., Bozeman, MT, USA) package. IMPUTE program was utilized to impute the genotypes of all SNPs located in the regions based on Asian HapMap data. Results: The chromosomal region at 1q42.2 (LOD=3.71; NPL Z=4.19) showed strong linkage with job stress. Six suggestive linkage regions are observed at 1q24.3 (LOD=2.15; NPL Z =3.25), 4q35.1 (LOD= 2.79; NPL Z =3.53), 10p11.21 (LOD=2.50; NPL Z =3.59), 12p13.31 (LOD= 2.39; NPL Z =3.17), 14q23.2 (LOD=2.31; NPL Z =3.38), and 21q21.1 (LOD= 2.13; NPL Z =3.20). Moreover, under the linkage peak (LOD>1.9), rs687723 at chromosome 1q42.13 located in the upstream of GALNT2 (UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2 (GalNAc-T2)) were significantly associated with job stress. As increasing risk alleles of the polymorphisms, ER ratio was significantly increased after age and sex () =0.019, p<0.0001). Conclusion: We identified candidate region 1q42.1-2, harboring the AGT (angiotensinogen (serpin peptidase inhibitor)), DISC1(disrupted in schizophrenia 1), and TRAX (translin-associated factor X) genes, which may explain the control over reactions to stress and neuropsychiatric illnesses. In addition, strong linkage regions for job stress included cardiovascular-related genes, such as GALNT2 and KCNK1(potassium channel).

605W

Challenges in investigating novel SNPs identified from Next Generation Sequencing of the 11p13 locus in a Rolandic Epilepsy family sample. *L. Addis[1], T. Chiang[2], A. Derkach[3], J.J. Russo[4], L.J. Strug[2], D.K. Pal[1].* 1) Institute of Psychiatry, King's College London, London, United Kingdom; 2) Hospital for Sick Children, Toronto, ON, Canada; 3) Department of Statistics, University of Toronto, Toronto, ON, Canada; 4) Center for Genome Technology and Biomolecular Engineering, Columbia University, New York, NY, United States.

Rolandic Epilepsy (RE) is a common childhood epilepsy of complex genetic inheritance, characterized by the abnormal EEG endophenotype of centro-temporal spikes (CTS). CTS is linked to 11p13 (HLOD 4.30), a locus pleiotropic for Speech Sound Disorder (HLOD 7.54) with fine mapping showing association in two independent cohorts to SNPs in *ELP4. EPL4* is a subunit of the Elongator complex involved in transcriptional regulation of many neurodevelopmental genes. As resequencing of *ELP4* coding regions revealed no significant polymorphisms we have used next generation sequencing to interrogate the 650Kb linkage region in 27 RE probands. DNA was amplified by long-range PCR (Roche) before preparation of paired-end multiplexed (9x) Illumina sequencing libraries. Libraries were sequenced using 2x36 cycles on an Illumina GAIIx. Raw sequence data were aligned, sorted, merged and assembled using Mosaik. SamTools was used to call SNPs and InDels with a high quality filter of 45, minimum read depth of 5x and MAF >20%. A validation analysis pipeline incorporating Novoalign and the Broad Institute's Genome Analysis Toolkit is underway. SNPs were called as novel if absent in dbSNP, HapMap and 1000Genomes. Our methodology resulted in read depth averaging 415x, with an average of 1000 SNPs called per sample. We have discovered 252 potential novel SNPs in this region, of which 230 occur in only one individual each. Of the `common' novel SNPs, 18 cases presented with the same SNP in *ELP4* intron 3, and two SNPs within *ELP4* intron 9 were present in 10 cases each. Intron 9 contains *PAX6* enhancer elements, and is an area of alternative *ELP4* splicing. Four high frequency novel SNPs were also found in neighboring *DCDC1*. Reconciling discrepancies between Sanger sequencing for validation and NGS results has been challenging, but 4 of the 7 high frequency SNPs are validated to date. SNP frequency has been compared to control data from Complete Genomics where available, ruling out some variants. Other database releases were not comparable to our data in coverage. After validation, the remaining novel SNPs will be typed in matched controls and family members for comparable sequence data. Almost half of the novel rare variants identified in this study are present in *ELP4*, with half again located in intron 9. We are assessing the probability of this occurrence, and are conducting rare variant analysis using binning statistics and functional analysis to investigate these SNPs further.

606W

Heterogeneous Phenotypes of Idiopathic Occipital Epilepsy, Febrile Convulsion and Hemiplegic Migraine Occurring in a Family with a SCN1A Defect. *G. Annesi[1], L. Mumoli[2], A. Labate[1,2], P. Tarantino[1], G. Palamara[2], E. Ferlazzo[2,3], A. Fratto[1,2], U. Aguglia[2,3], A. Quattrone[2,4], A. Gambardella[1,2].* 1) Institute of Neurological Science, National Research Council, Mangone (CS), Italy; 2) Institute of Neurology, University of Magna Graecia, Catanzaro (CZ), Italy; 3) Regional Epilepsy Centre, Hospital of Reggio Calabria (RC), Italy; 4) Neuroimaging Research Unit, National Research Council, Catanzaro (CZ), Italy.

The voltage-gated Na_channel alfa1 subunit (SCN1A) is the most clinically relevant among all the known epilepsy genes with the largest number of epilepsy related mutations so far characterized. More recently, SCN1A mutations were identified in some families with familial hemiplegic migraine (FHM), further supporting a pathophysiological link between migraine and epilepsy. Here we report a three-generation family, in which affected individuals had heterogeneous phenotypes that included febrile convulsions (FC), focal epilepsy and hemiplegic migraine (HM). We studied a family composed by 13 members spread over three generations. All five affected individuals (5 women, mean age 32.7 years, range: 16-50) underwent a comprehensive clinical evaluation. Peripheral blood samples were obtained for DNA extraction from all members of the family. Mutation analysis was performed by direct sequencing of SCN1A. The neurologic and psychiatric status of all 5 affected subjects was normal, as also brain MRI. The proband (a 16-year-old girl) had developed benign occipital epilepsy (BOE) at the age of 7 years. Her 43-year-old mother had simple febrile seizures (FS) that resolved before the age of five, but she later developed BOE, at the age of 6. The 37-year-old sister also had a similar phenotype of simple FS at the age of 18 months, and BOE that started at the age of 8 years. In all three patients antiepileptic drugs were tapered off, between ages 10 and 12 years, with no seizure relapse. Two additional relatives (50-year-old mother and her 16-year-old daughter) had clear-cut hemiplegic migraine without epilepsy or FS. All affected members and one obliged carrier had the heterozygous T1174S missense mutation of SCN1A. Our findings illustrate that BOE may be part of the clinical spectrum associated with SCN1A mutations. Moreover, the association with FHM further strengthens the molecular links between migraine and epilepsy, two common paroxysmal disorders. Probably, assessing the biophysical and functional consequences of such a mutation will help to better understand the complex genotype-phenotype relationship that underlies SCN1A mutations.

607W

Exploring the genetic complexity of juvenile myoclonic epilepsy. *F. Conte[1], T. Peluzzo[1], F. Oliveira[1], L. Betting[2], D. Holanda[3], L. Gitaí[4], F. Gameleira[4], D. Gitaí[3], F. Cendes[2], I. Lopes-Cendes[1], CInAPCe.* 1) Dept of Medical Genetics; Faculty of Medical Sciences, University of Campinas, Brazil; 2) Dept of Neurology; Faculty of Medical Sciences, University of Campinas, Brazil; 3) Sector of Genetic and Molecular Biology, ICBS; Federal University of Alagoas, Brazil; 4) Neurology Sector, HUPPA; Federal University of Alagoas, Brazil.

Purpose: Mutations in EFHC1 were identified in patients with two forms of idiopathic generalized epilepsy (IGE), juvenile myoclonic epilepsy (JME) and juvenile absence epilepsy. EFHC1 protein has 3 domains of unknown function called DM10 and one calcium binding domain, EF Hand. We aimed to search for mutations in EFHC1 and try to establish correlations between genotype and phenotype. Method: We sequenced all 11 exons of EFHC1 searching for the presence of any variant by the alignment with sequences available in public databases. When a mutation was found, its pathogenic potential was checked by different available algorithms. Of the 184 individuals enrolled in the study, 102 have JME, 32 have other forms of IGEs and 50 are control subjects. Results: We found 5 single nucleotide variants with potential pathogenic effect only in patients with JME. In exon 3, we identified 2 single nucleotide polymorphisms (SNPs) already described previously in patients with JME. The first is a transition from cytosine to thymine at position 475 (475C>T, R159W), present in 12 patients, 10 of whom with a positive family history. The second is a transition from guanine to adenine at position 545 (545G>A, R182H), found in 5 patients, 4 of whom with a positive family history. Interestingly, 1 patient presented both SNPs. In exon 4 we found a SNP in 3 patients (1 with a positive family history); this is a transition from thymine to cytosine at position 685 (685T>C F229L), and it has already being reported previously in a patient with JME. In exon 5 we identified 2 novel mutations. The first, was found in 2 patients with sporadic JME, this is a transition from guanine to adenine at position 887 (887G>A, R296H). The second is a transition from adenine to guanine at position 896 (896A>G, K299R), present in 1 patient with a positive family history. None of these mutations were identified in our control group. Conclusion: We found five nucleotide changes in EFHC1. These changes are located in the coding region of the first and second DM10 domains of EFHC1 protein and in the region between them. Overall the frequency of mutations in EFHC1 was 17% in our IGE group of patients, but if we consider only patients with JME, the frequency raises to 22%. In addition, we found mutations in patients with positive and negative family history; however, we only found mutations in patients with JME. We did not find any phenotypic difference between JME patients with and without EFHC1 mutations.

608W

Protocadherin 19 mutations in sporadic and familial cases of epilepsy and mental retardation in females (EFMR). *L.M. Dibbens[1], R.S. Moller[2], M.A. Bayly[1], S. von Spiczak[3], T. Desai[4], H. Hjalgrim[2,5], G. Wallace[6], S. McKee[7], R.C. Dale[8], I.E. Scheffer[9].* 1) Pharmacy and Medical Sciences, University of South Australia, Adelaide, South Australia, Australia; 2) Danish Epilepsy Centre, Dianalund, Denmark; 3) University Medical Center, Schleswig-Holstein, Kiel, Germany; 4) Epilepsy Research Centre, Department of Medicine, University of Melbourne, Austin Health, Victoria, Australia; 5) University of Southern Denmark, Denmark; 6) Neuroscience Department Royal Children's Hospital and Mater Children's Hospital Brisbane, Australia; 7) 7 Northern Ireland Regional Genetics Service, Belfast City Hospital, Belfast, Northern Ireland, UK; 8) Paediatrics & Child Health, University of Sydney and the Children's Hospital at Westmead, Sydney, Australia; 9) Department of Medicine, Austin Health; Department of Paediatrics, The University of Melbourne; Royal Children's Hospital, Melbourne Australia.

The contribution of mutations in the X-linked protocadherin 19 (PCDH19) gene in epilepsy and intellectual disability was first discovered in six large families in which multiple females over several generations had the disorder Epilepsy limited to Females with Mental Retardation (EFMR) (Dibbens et al 2008). These pedigrees showed an unusual mode of reverse X-linked inheritance where transmitting hemizygous males did not have epilepsy or intellectual disability. Current evidence suggests this is due to the mechanism of cellular interference resulting from a mixture of PCDH19 protein positive and negative cells. PCDH19 encodes a transmembrane protein belonging to the cadherin superfamily and is proposed to function in calcium dependent cell-cell adhesion and /or cell-cell signalling.

The initial gene discovery has been extended to show that de novo PCDH19 mutations cause sporadic cases of EFMR. In this study we set out to further explore the spectrum of clinical phenotypes caused by mutations in PCDH19, to determine if genotype-phenotype correlations can be drawn and to analyse the inheritance of mutations. We report 14 new cases of EFMR due to PCDH19 mutations: 7 familial, 4 de novo and 3 yet to be determined.

To date a total of 66 PCDH19 sequence mutations have been reported in unrelated subjects with seizures with and without intellectual disability: 65 in girls and 1 in a boy mosaic for a PCDH19 deletion. 59 mutations are unique while 3 are recurrent. 60/66 mutations are located in the highly evolutionary conserved extracellular portion of the protein, indicating that amino acid variation in this region is not well tolerated, while 6 mutations are within the cytoplasmic region. 4 PCDH19 gene deletions have been reported, making a total of 70 mutations. 32/70 mutations have been inherited by the proband from a parent, 28/70 are de novo and 10/70 did not have parent material available. We conclude that PCDH19 analysis should be considered in cases of early onset epilepsy in females where mutations can be familial or de novo. Phenotypic analysis of our new PCDH19 mutation positive cases includes later seizure onset than previously reported (6 years) and a catatonic/Parkinsonian-like state.

609W

GENETIC STUDY OF SCN1A-RELATED EPILEPSIES IN SOUTHERN ITALY. *M. Gagliardi[1,2], P. Tarantino[1], E.V. De Marco[1], A. Labate[1,4], G. Tortorella[3], F. Annesi[1], M. Caracciolo[1], F. Cavalcanti[1], A. Quattrone[4,5], A. Gambardella[1,4], G. Annesi[1].* 1) Institute of Neurological Sciences, National Research Council, Mangone (CS), Italy; 2) University of Magna Graecia, Catanzaro (CZ), Italy; 3) Department of Neurosciences, Psychiatry and Anaesthesiology, University of Messina (ME), Italy; 4) Institute of Neurology, University of Magna Graecia, Catanzaro (CZ), Italy; 5) Neuroimaging Research Unit, National Research Council, Catanzaro (CZ), Italy.

Approximately 10% of generalized epilepsy with febrile seizures (GEFS+) families carry mutations in the gene coding for the alpha1 subunit of the neuronal channel (SCN1A). Furthermore, SCN1A mutations occur in more than 70% of patients with severe myoclonic epilepsy of infancy (SMEI) and are usually de novo. Most of the mutations are nonsense or frameshift: missense mutations are also common. Recent studies have already reported that about 12% of mutation-negative SMEI patients have microchromosomal abnormalities involving SCN1A. The aim of this study was to extend the sample number in our population to investigate the frequency of SCN1A mutations in GEFS+ and SMEI patients. Twenty-three GEFS+ probands belonging to unrelated families and 24 SMEI patients were recruited from Southern Italy. Diagnoses were based on the International League against Epilepsy criteria. Mutation analysis was performed by direct sequencing. The genomic anomalies were screened using MLPA and confirmed by real-time PCR. Among the 23 GEFS+ probands (17.4%) had SCN1A variations that we had previously published (Met1841Thr, Tyr779Cys, Ile944Thr); and one novel mutation was found (R604H). Among the 24 SMEI patients, 12 (50%) carried SCN1A mutations. Seven of them were already reported by ourselves. Five novel mutations were found (K1246fsX1268, Phe807Leu, R1636X), p.1502del, Y1492Y) in 5 unrelated SMEI cases. SCN1A is the most clinically relevant epilepsy gene. We found 12 SMEI patients carrying different mutations, thus confirming the high genetic heterogeneity related to these diseases. Moreover in our population we found frequency of GEFS+ mutations that is higher than the one reported by other authors .Our results confirm that SCN1A mutations represent an important cause of GEFS+ and SMEI also in Southern Italy.

610W

Molecular characterization of a new model of generalized epilepsy with absence seizures. *A.H.B. Matos[1], C. Rocha[1], J.F. Vasconcellos[1], V.D.B. Pascoal[1], M.T. Chamma[2], S. Botte[2], C.V. Maurer-Morelli[1], I. Lopes-Cendes[1], A.C. Valle[2].* 1) Department of Medical Genetics, Faculty of Medical Sciences, University of Campinas - UNICAMP, Campinas, SP, BRAZIL; 2) Department of Pathology, School of Medicine, University of São Paulo - USP, São Paulo, SP, BRAZIL.

BACKGROUND: A spontaneous mutant rat was identified presenting generalized epilepsy with absence seizures (GEAS). GEAS rat shows distinct behavioral and electroencephalographic characteristics as compared to previously reported genetic epilepsy rat models. The aim of the present study was to characterize the genetic profile this strain of rat, through gene expression analysis in the hippocampus of these animals. METHODS: We obtained total RNA from the hippocampus (n=3) of GEAS rats and controls Wistar (n=3) with TRIzol (Invitrogen-Life Technologies). Gene expression analysis was performed using the GeneChip® Rat Genome 230 2.0 Array (Affymetrix™), and analyzed using MAS5.0, program R with package Affy and RankProd of Bioconductor for expression measure. The analyses of overrepresented gene ontology categories were performed with DAVID software, and gene interactions and correlation networks were identified with Ingenuity Pathways Analysis software. Statistical analysis was performed by t-test with $p \leq 0.01$ for detecting a significant difference between groups. RESULTS: Forty-five differently expressed genes were identified in GEAS animals compared to controls, among these we found genes related to central nervous system development, activation of MAPK, transcription factors, neuronal migration and apoptosis, such as *Nrsn1, Hspb1, Fos, Twist1* and *Krt18*, respectively. The top enriched gene ontology categories included signal peptide, extracellular region and antigen processing and presentation of peptide antigen. Among the most activated signaling pathways were endocrine system disorders, gastrointestinal disease, cell cycle and neuron system development and function, which include genes such as *Prlr*, *Hspb1*, *Igfpb2*, *CD74* and *Fos*. CONCLUSION: Our results showed a different molecular signature in GEAS rats compared to control animals. The differently expressed genes were related to several essentials cellular functions, in particular, those related to cerebral cortex development, including neuronal migration and apoptosis.

611W

A Bayesian model for splicing QTL mapping with RNA-Seq. *E. Pantaleo[1], M. Stephens[1,2].* 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Department of Statistics, University of Chicago, Chicago, IL.

An important challenge of the post-genomic era is to make sense of how genome sequences control gene regulation. We developed a Bayesian model that uses RNA-Seq data and SNP data to understand how genetic variation can modify gene expression and splicing pattern.

For a single set of RNA-Seq data (e.g., one individual) the Bayesian model can infer the transcript model, i.e., the set of transcripts that are expressed in the RNA-Seq data (allowing for alternative splicing) and transcript abundances jointly. When applied to a set of RNA-Seq from individuals for which extensive genotype data are available, the Bayesian model can search for splicing QTLs and expression QTLs (where no alternative transcripts are observed) using a transcript model and alternative transcript abundances inferred from the data.

We applied the model to RNA-Seq data of lymphoblastoid cells derived from 69 Nigerian individuals generated as part of the International HapMap project for which we had 1.2 billion reads of either 35 or 46 base pairs. The model has mapped a set of splicing QTLs. An algorithm implementing the method for single-end (short) reads will be made publicly available.

612W

A comprehensive approach to the study of Hearing Loss: identification of new candidate genes and pathways. *G. Girotto[1], M.A Lewis[3], N. Pirastu[1], D. Licastro[2], D. Vozzi[1], K.P Steel[3], P. Gasparini[1].* 1) Med Genet, DMS, UNITS/Burlo, Trieste, IT., Italy; 2) CBM Scrl- Basovizza, Trieste, Italy; 3) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK.

Hereditary hearing loss (HHL) is a common disorder accounting for at least 60% of prelingual deafness. Although mutations on GJB2 and GJB6 genes are known to be involved in most of the cases of nonsyndromic HHL (NSHHL) in Caucasian populations, other genes could still be major players in other populations. Moreover, little is known about the molecular bases of normal hearing function and age-related hearing loss (ARHL). Thus, research still needs to identify new genes possibly involved in monogenic forms, study hearing as a complex trait and to understand genetic factors underlying ARHL. To reach this goal we have planned an integrated strategy characterized by: A) a genome-wide association study (GWAS) on hearing quantitative traits. From the meta-analysis of data from 6 isolated populations (more than 3500 samples coming from 2 European countries - G-EAR Consortium) some significant and suggestive loci (p<10-6,10-7) for quantitative traits have been found. Some of them map to known hereditary hearing loss loci whose genes still need to be identified. Data have also been used to construct a highly significant "in silico" pathway for hearing function (Girotto G. et al. JMG 2011). B) a GWAS on qualitative traits focused on ARHL. Preliminary analysis of ARHL in more than 1000 cases (G-EAR Consortium) led to the identification of other suggestive loci/genes including genes known to be involved in auditory development and function but also genes whose function is still unknown. C) a replica phase of the data, which is in progress on additional sample cohorts from Italy, UK and Central Asia. D) Next Generation Sequencing. Whole exome resequencing of selected cases from large pedigrees affected by NSHHL and whole genome sequencing of some ARHL cases belonging to two isolated Italian populations is in progress. In addition, using all the data (from A to D) we were able to define a list of strong candidate genes that have been chosen for in vivo validation. In many cases, little is known about the expression and the possible function of these genes. We are therefore investigating the expression of these candidate genes in the ear by immunocytochemistry using wildtype mice at 5 days old. In conclusion, preliminary results provide new candidate genes for normal hearing function, ARHL and HHL suggesting new targets for hearing impairment treatment and prevention.

613W

Multifactor Dimensionality Reduction 3.0: Open-Source Software for Systems Genetics. *P. Andrews, J.H. Moore.* Dartmouth Medical School, Lebanon, NH, USA.

Multifactor dimensionality reduction (MDR) was designed as a nonparametric and genetic model-free approach to identifying, characterizing and interpreting gene-gene interactions in genetic and epidemiologic studies of common human diseases. The kernel of the MDR algorithm uses constructive induction to combine two or more polymorphisms into a single predictor that captures interaction effects. This general approach has been validated in numerous simulation studies and has been applied to a wide-range of different human diseases. We describe here version 3.0 of the open-source MDR software package that has been made freely available to the genetic epidemiology and bioinformatics communities since February of 2005. Over this time period MDR has been downloaded more than 30,000 times. This new version of MDR has been significantly updated to allow users carry out a systems genetics analysis by inferring and characterizing large networks of gene-gene interactions. Here, the vertices of the network represent the single-nucleotide polymorphisms in the data while the edges or connections among vertices represent the synergistic gene-gene interactions that exceed some predefined threshold. We report the degree distribution, subgraph motifs, centrality, modularity and other measures of network complexity. We also allow users to filter their list of SNPs according to the structure of the network as a way to refine and focus their MDR analysis. These new features in the MDR software move beyond models of several SNPs to the inference of large interacting networks of SNPs enabling a systems genetics approach to complex diseases association studies.

614W

Accelerated skeletal muscle ageing is a molecular signature in OPMD. *S.Y. Anvar, P.A.C. `t Hoen, S.M. van der Maarel, V. Raz.* Center for Human and Clinical , Leiden University Medical Center, Leiden, Netherlands.

Reduced physical ability and progressive loss of skeletal muscle mass and strength are major contributors to health problems at late age. The molecular pathogenesis of muscle weakness onset and progression is poorly studied. In a longitudinal ageing study of genome-wide expression profiles in quadriceps of control individuals (17-89 years), we revealed that the major transcriptional changes occur around the age of 42. A genome-wide transcriptome comparison between muscle-ageing and muscles of patients with oculopharyngeal muscular dystrophy (OPMD) revealed significant similarities. OPMD is an autosomal dominant and late-onset muscle weakness. It is caused by a poly-alanine expansion in PABPN1 protein, leading to higher aggregation potency. Here, we found substantial similarities between OPMD and ageing on gene and pathway levels. Commonly, major transcriptional changes begin at midlife. This suggests that the onset of OPMD is triggered by ageing-associated factors. In addition, as the progression of transcriptional changes in ageing was found to be accelerated in OPMD, we suggest that progressive muscle weakness is faster in OPMD patients. From the ageing and OPMD deregulated processes, the highest association was found for genes in muscle contraction, mitochondria and ubiquitin-proteasome system. Evidently, genes encoding for aggregation-prone proteins were highly enriched in OPMD and muscle ageing. We suggest that changes in expression levels can portray the onset and progression of muscle weakness.

615W

A Comparison of Cataloged Variation between International Haplotype Map Consortium and 1000 Genomes Project. *C. Buchanan, E. Torstenson, W. Bush, M. Ritchie.* Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Since the publication of the relatively complete human genome in 2003, geneticists have been increasingly interested in risk variant associations to resolve the etiology of traits and complex diseases. The International Haplotype Map Consortium (HapMap) undertook the task of cataloging all of the common variation across the genome (variants with a frequency of at least 5%). It was the HapMap along with high-throughput genotyping technological advances that led to the era of genome-wide association studies (GWAS). GWAS have been a successful endeavor to identify common variants associated with many traits and diseases. Subsequently, another effort began in 2008 with the 1000 Genomes Project, which aims to sequence 2,500 individuals and identify rare variation across the genome. They intend to identify 99% of variants with a minor allele frequency of at least 1%. One might conclude, given the goals of both consortia that 1000 Genomes Project would include all of the variants originally discovered (or validated) in the HapMap project. Using merged Phase I/II/III HapMap data and low coverage pilot data from 1000 Genomes Project, we compared the overlap between the two resources. In a comparison of the two raw data sets, only approximately 84% of HapMap SNPs were found in 1000 Genomes data. After filtering out HapMap variants for allele frequencies greater that 95% or less than 5%, 99% of HapMap SNPs were found in 1000 Genomes Project data. Therefore, if one is interested in both common and rare variation, it is essential to note that not all of the variants cataloged in the HapMap are also cataloged in the 1000 Genomes Data. This could potentially impact decisions about which resource to use for SNPs queries, rare variant validation, or imputation. Both the HapMap and 1000genomes are useful resources for human genetics; it is important to understand the assumptions made and filtering strategies implemented to deposit into these resources.

616W

Comparison of different methods for detecting gene-gene interactions in case-control data. *T. Cattaert[1], J.A. Rial Garcia[2], E. Gusareva[1], K. Van Steen[1].* 1) Montefiore Inst and GIGA-R, Univ Liège, Liège, Belgium; 2) Universidade da Coruña, Spain.

It is generally believed that epistasis makes an important contribution to the genetic architecture of complex disease, and numerous statistical and bioinformatics methods have been developed to detect it.

We compare several state-of-the-art epistasis detection methods in terms of empirical power, type-I error control, and CPU time. The methods compared include Model-Based Multifactor Dimensionality Reduction (MB-MDR) [1, 2], BOolean Operation-based Screening and Testing (BOOST) [3], EPIBLASTER [4], Random Jungle (RJ) [5], Logistic Regression and PLINK.

Our comparative study is based on an extensive simulation study using different two-locus models, exhibiting both main effects and epistasis [3]. In these simulations, 100 SNPs are generated, no LD between them. All genotypes are assumed to be in Hardy-Weinberg equilibrium. Furthermore, 2 disease-associated SNPs are selected, with MAFs set to 0.1, 0.2 and 0.4. The MAFs of the non-disease associated SNPs are uniformly distributed on [0.05, 0.5]. In order to achieve high accuracy in empirical power estimation, all simulation settings involve 1000 replicates. All methods are applied to WTCCC Rheumatoid Arthritis data.

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617W

The OBiBa project: An international collaboration for open source software for biobanks. *V. Ferretti[1], P. Laflamme[1], P. White[2], P. Burton[4], I. Fortier[3].* 1) Ontario Institute for Cancer Research; 2) University of Western Australia; 3) Research Institute of the McGill University Health Center; 4) University of Leicester.

OBiBa is an international collaborative initiative whose mission is to develop high quality open source software for large-scale population-based biobanks and clinical studies. It is comprised of independent and self-funded teams in Canada and Australia, each of which is producing stand-alone applications that support particular biobank activities. In this presentation, we will describe briefly the OBiBa software modules:

- ETA: a participant management and recruitment application used to track communication with participants, store and manage consent as well as contact information.
- DataSHIELD: An innovative statistical analysis tools enabling pooled data analysis without sharing any individual-level data among studies.
- GenoByte: a high-performance database engine for storing and querying genomic variations and data produced by high-throughput sequencing and genotyping technologies.
- Mica: a web-based application for creating web portals used by epidemiology study consortia to disseminate various information, such as studies design, data dictionaries, and data content.
- Onyx: A comprehensive software suite used for managing participant baseline interviews and collecting data in clinics. Onyx combines web-based data collection modules for questionnaire administration, consent electronic signature and physical measurements.
- Opal: A web-based data integration platform used to manage, store, query and transform phenotypic data from multiple heterogeneous sources. Opal architecture and tools are designed to ensure and enforce participant privacy and confidentiality.

OBiBa is a core project of the Population Project in Genomics Consortium (P3G), a consortium including some of the world's largest population-based biobanks. OBiBa modules are developed in collaboration with many partners including the Dunes Environment which aims to offer methods and IT solutions to support data harmonization and synthesis.

Current main OBiBa software users include the Canadian Partnership for Tomorrow (300,000 participants), the Canadian Longitudinal Study for Aging (50,000 participants), the Leicester Cardiovascular Biomedical Research Unit (UK), the Ontario Institute for Cancer Research and the McGill University and Genome Quebec Innovation Center. OBiBa is also partner of the recently granted EU FP7 BioSHaReEU project. OBiBa software are freely available at www.obiba.org.

618W

GAMETES: A Fast, Direct Algorithm for Generating Pure, Strict, Epistatic Models with Random Architectures. *J. Fisher, R. Urbanowicz, J. Kiralis, N. Sinnott-Armstrong, T. Heberling, J.H. Moore.* Dartmouth Medical School, Lebanon, NH, USA.

Statistical epistasis can be defined as deviation from additivity in a linear mathematical model summarizing the relationship between multi-locus genotypes and phenotypic variation in a population. The detection and modeling of statistical epistasis is a bioinformatics challenge. Thorough evaluation of newly developed epistasis analysis algorithms calls for simulation studies in which a priori disease models are used to provide test data where the true underlying genetic architecture is known. However, few user-friendly software packages are available for developing epistasis models and simulating data. Here, we introduce Genetic Architecture Model Emulator for Testing and Evaluating Software (GAMETES), an algorithm and software package for the generation of complex single nucleotide polymorphism (SNP) disease models. GAMETES has the flexibility to efficiently generate n-locus strict and pure epistatic models of “random” architecture. N-locus models are purely and strictly epistatic if all n loci, but no fewer, are predictive of disease status. All models are generated to fit specified model constraints including heritability, SNP minor allele frequencies, and population prevalence. For every valid combination of these model constraints, a population of model architectures can be generated rapidly. We use the term architecture to reference the unique composition of a model (i.e. the penetrance values and arrangement of those values within a penetrance function). We tested GAMETES by simulating pure, strict, epistatic models with respective datasets, having 2-6 loci, and variable heritabilities, minor allele frequencies, and sample sizes. We evaluate GAMETES by searching for these simulated models using multifactor dimensionality reduction (MDR), an established machine learning methods for detecting epistasis. Our results indicate that GAMETES is a fast, reliable, and flexible method for generating complex genetic models of “random” architecture. Beyond data simulation, this strategy also offers a framework for the theoretical investigation of genetic models. The GAMETES software is open source, and implemented in a user-friendly manner.

619W

A SYSTEMATIC COMPARISON OF GWAS PATHWAY ANALYSIS METHODS. *H. Gui, M. Li, P. Sham, S. Cherny.* The University of Hong Kong, Hong Kong, Hong Kong.

Though rooted in genomic expression studies, pathway analysis for genome-wide association studies (GWAS) has gained increasing popularity, since it provides a way of discovering hidden disease causal mechanisms by combining statistical methods with biological knowledge. Algorithms or programs currently available can be categorized by different types of input data, null hypothesis or number of analysis stages. Due to complexity caused by SNP, gene and pathway relationships, re-sampling strategies like permutation are always utilized to derive empirical distributions for test statistics, and then evaluate the significance of candidate pathways. However, thorough performance evaluation of these algorithms on real GWAS datasets and real biological pathway databases needs to be done before these methods should become common practice in GWAS. Seven algorithms were selected to conduct pathway analysis on SNP genotypes together with simulated and real phenotypes from the WTCCC Crohn's disease study. All 7 methods control type I error rate (at 0.05) well, and are mostly slightly conservative. However, the methods varied greatly in terms of power and running time. In real data analysis, raw data-based algorithms turn out to be best, provided sufficient computation capacity is available. Given the variability in performance, in general, particularly when underlying disease causal mechanism is ambiguous, it is worthwhile to apply two or more pathway analysis algorithms on the same GWAS dataset.

620W

Integration and Visualization of Genetic and Genomic Data Using a 3-D Video Game Engine. *D. Hill, J.H. Moore.* Dartmouth Medical School, Lebanon, NH, USA.

This is an exciting time in biomedical research due to the availability of technology that allows us to measure tremendous amounts of information about genes, proteins and other biomolecules that play an important role in the molecular pathology of disease. However, it is also a challenging time due to the bioinformatics needs associated with storing, managing, analyzing and interpreting `omics' data. While we have made great progress in developing the databases and data analysis tools for measuring statistical relationships in high-dimensional datasets, the bioinformatics methods for knowledge discovery in the large volumes of statistical results generated from `omics' analyses are in their infancy. To address this interpretation challenge, we have developed an innovative three-dimensional (3-D) visualization approach to the exploration of statistical analysis results from genome-wide association studies (GWAS) that capitalizes on the power of human visual perception and our evolved ability to recognize complex patterns. The overall goal of this study is to replace the traditional approach of sifting through thousands of rows of p-values in an Excel spreadsheet with an innovative and unconventional visual approach that presents the results in an interactive 3-D graphical format that makes important local and global patterns much easier to identify by the user. To implement this we have harnessed the power of cutting-edge computer graphics technology in the form of fast Graphical Processing Unit (GPU) video card hardware and sophisticated 3-D video game engine software (Unity3D) that is designed for real time rendering of 3-D visual environments and animation. Here, we introduce a 3-D heatmap software package that is able to visualize more than five dimensions of genetic and genomic research results thus permitting the integration of SNPs, gene expression and clinical data, for example. Our 3-D heatmap software is open-source and freely available.

621W

Systems Genetics Analysis of Bladder Cancer Susceptibility using Statistical Epistasis Networks. *T. Hu, N. Sinnott-Armstrong, J. Kiralis, A. Andrew, M. Karagas, J.H. Moore.* Dartmouth Medical School, Lebanon, NH, USA.

Epistasis is ubiquitous in the genetic architecture of complex traits such as disease susceptibility. Experimental studies in model organisms have revealed extensive evidence of biological interactions among genes, and statistical and computational studies in human populations have revealed evidence for nonadditive effects of genetic variation on complex traits. The goal of this study was to use network science to determine the extent to which nonadditive interactions exist beyond small subsets of genetic variants. We inferred statistical epistasis networks to characterize the space of pairwise interactions among approximately 1500 single nucleotide polymorphisms (SNPs) spanning nearly 500 cancer susceptibility genes in a large population-based study of bladder cancer. Networks were built by incrementally adding edges between SNPs if the strength of their pairwise interactions was greater than a given threshold. By changing the value of the threshold, we obtained a series of such networks. We then statistically compared them to series of networks derived from permuted data to determine whether the topological properties were consistent with those expected under the null hypothesis of no association between genotype and phenotype. We observed that once a specific value of the threshold was reached, the real-data network clearly differentiated itself both from the other real-data networks and from the permuted-data networks. In particular, at that point, it comprised a significantly higher number of hub SNPs and a large sizeable connected component became detectable. We determined that the vertex degree distribution of this largest network was distinctively different from all others and its topology was approximately scale-free. In contrast to many existing techniques proposing a classification model of a subset of susceptibility SNPs, this network approach characterizes a global picture of genetic interactions in a populated-based genetic data.

622W

Optimized Rsq thresholds for quality control of MACH/minimac imputed genotypes. *T.A. Johnson[1], H. Fujita[2], K. Hara[2], T. Tsunoda[1].* 1) Laboratory for Medical Informatics, Center for Genomic Medicine, RIKEN Yokohama Institute, Yohohama, Kanagawa, JAPAN; 2) Department of Diabetes and Metabolic Diseases, Graduate School of Medicine, University of Tokyo, Bunkyo-ku, Tokyo, JAPAN.

Quality control of imputed genotypes for genome-wide association analysis is even more essential than for real genotype data. To evaluate the efficiency of suggested Rsq QC cutoffs for the MACH/minimac imputation program, we compared Rsq with genotype concordance for a subset of samples that had additional real genotype data. We selected 423658 autosomal SNPs for 7541 Japanese samples with Illumina 550k or 610k data, estimated haplotype phase using MACH, and imputed genotypes using minimac with 1000 Genomes ASN reference haplotypes (_$8.7x10^6$ loci after filters). Illumina 610k data for 4470 samples overlapped 52335 imputed non-550k loci, for which overall percent concordance between real/imputed genotypes was quite good (94.2±12.5%; mean±SD), although a sizable fraction of imputed loci were of lower quality (<90% concord.=14.7%, <80% concord.=9%). Concordance decreased with respect to the number of major alleles in a genotype class (major homozygotes:93.9±20.2%, heterozygotes: 90.9±22.9%, minor homozygotes: 81.0±33.6%). Since low MAF loci could exhibit high total concordance despite less accurate imputation of heterozygote and minor homozygote genotypes, we calculated concordance for GWAS purposes as the worst performing genotype class for a particular locus. For each locus, we extracted minimum concordance values across the three genotype classes ($min_{gt.concord}$) and plotted them against minimac's Rsq values for five MAF bins (MAF bin ranges: 0.0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4, 0.4-05), which indicated that Rsq filtering efficiency varied with MAF. We then categorized loci as well-imputed ($min_{gt.concord}$ ⁄ 80%, n=40217) or poorly-imputed ($min_{gt.concord}$ <50%, n=8310). We observed using the lower suggested Rsq cutoff of 0.3 that almost 100.0% of well-imputed genotypes were included ($Prop_{w.i.inc}$) and 71.2% of poorly-imputed genotypes excluded ($Prop_{p.i.exc}$), while the higher Rsq cutoff of 0.5 had $Prop_{w.i.inc}$ and $Prop_{p.i.exc}$ of 99.8% and 87.1%, respectively. While $Prop_{w.i.inc}$ was satisfactory, the high proportion of poorly-imputed loci not excluded could lead to increased false-positive associations during analyses. We therefore attempted to determine an optimized Rsq cutoff for each MAF bin by finding Rsq that maximized the product $Prop_{w.i.inc}$* $Prop_{p.i.exc}$. The optimized Rsq thresholds of 0.75, 0.70, 0.66, 0.60, and 0.55 for the five MAF bins from 0 to 1.0 allowed retention of 98.1% of well-imputed loci while removing 96.4% of those that were poorly-imputed.

623W

Gene Ontology Analysis of Genome-Wide Epistatic Interactions Supports a Role for Actin Cytoskeletal Genes in Sporadic ALS. *N. Kim[1], J. Fisher[1], P. Andrews[1], C. Read[2], K. Askland[2], B. Harris[3], J.H. Moore[1].* 1) Dartmouth Medical School, Lebanon, NH, USA; 2) Brown University, Providence, RI, USA; 3) Georgetown University, Washington D.C., USA.

Sporadic amyotrophic lateral sclerosis (ALS) has a complex and unknown multifactorial etiology that is likely influenced by multiple gene-gene and gene-environment interactions. We conducted a genome-wide interaction analysis in two publically available data sets each with approximately 500,000 single-nucleotide polymorphisms from the Illumina HumanHap 550k chip. The detection set consisted of 276 cases and 271 controls from the USA (Schymick et al. 2007) while the replication set consisted of 221 cases and 211 controls from Ireland (Cronin et al. 2008). Genome-wide interaction analysis was previously carried out using the multifactor dimensionality reduction (MDR) method implemented on graphical processing units for high-performance computing (Greene et al. 2010). All pairwise interactions were analyzed and assessed for statistical significance using a 1000-fold permutation test that corrects for multiple testing. In the present study, we carried out a Gene Ontology (GO) analysis of the interaction results in the following manner. First, we assigned each SNP an accuracy value corresponding to the model with the strongest interaction involving that SNP among all pairs. Second, we assigned each SNP a p-value from the permutation test. Third, we assigned each gene a p-value based on whether there was an overabundance of SNPs with p-values less than or equal to 0.05. For this analysis we used the Exploratory Visual Analysis (EVA) software that performs a spatial permutation test that corrects for gene size. Fourth, we assigned each GO category a p-value using EVA based on the overabundance of genes with p-values less than or equal to 0.05. This was repeated in both data sets and any GO category that was significant at the 0.05 level in both studies was considered statistically significant with replication. This analysis identified the actin cytoskeleton cellular component as the only GO category that met the replication criteria. These genetic association results are consistent with previous biochemical and cellular studies documenting cytoskeletal changes in ALS motor neurons that require structural integrity for proper function. This study highlights the power of combining epistasis analysis using MDR with GO analysis of biological annotation.

624W

The Kaiser Permanente/UCSF Genetic Epidemiology Research Study on Adult Health and Aging: Genotyping Informatics and Quality Control for 100,000 Subjects Genotyped on the Affymetrix Axiom System. *M.N. Kvale[1], S. Hesselson[1], T. Hoffmann[1], J. Gollub[3], T. Webster[3], Y. Zhan[3], Y. Lu[3], G. Mei[3], L. Walter[2], D. Ludwig[2], B. Dispensa[1], C. Schaefer[2], P.Y. Kwok[1], N. Risch[1].* 1) Human Gen, Univ California, San Francisco, San Francisco, CA; 2) Kaiser Permanente Northern California Division of Research, Oakland, CA; 3) Affymetrix Inc., Santa Clara, CA.

A goal of Kaiser Permanente/UCSF Research Study on Adult Health and Aging is to assay genotypes on 675,000+ SNPs for each of 100,000 subjects in a highly accurate and consistent fashion. The unprecedented number of subjects and the 14-month duration created unique opportunities and challenges for ensuring the highest possible quality of genotype calling across the entire dataset. One such challenge was the design and use of four different arrays, based on European, Asian, African and Latino ancestry, respectively, with the latter two using different chemistries than the first two. The improved chemistry also allowed for the tiling of up to 50% of SNPs with single (versus double) probe set representation for the latter two arrays (and hence more SNPs overall), but presented additional issues regarding genotype calling. For all arrays, we created algorithms for assaying genotype reproducibility, measuring and classifying genotype call quality, and for change-point detection of non-stationarities in the stream of data as the experiment progressed. We created methods for increasing sample call rate, optimizing genotype reproducibility across samples and reducing biases wrought by changing experimental conditions over the course of the experiment. In particular, we generated call rate statistics across all probes on each of the four arrays and demonstrated that the dQC composite metric can be used to predict sample call rates and diagnose performance problems. The use of a Fisher Linear Discriminant metric proved useful in comparing the performance of SNPs. By design, we included a single duplicate sample on each plate to measure sample reproducibility and to detect changes due to assay chemistry and performance. Axiom genotype calling is based on the BRLMM Bayesian clustering algorithm. For some arrays, we found sensitivity to characteristics of cluster location priors, and as a result devised methods to create good Bayesian priors to enhance genotyping performance and reproducibility. We also created a support vector machine classification algorithm for classifying SNP call quality and for detecting poorly performing SNPs that could be rescued through the use of alternative BRLMM algorithm parameters. Across all probes placed on the arrays and over 100,000 saliva-derived DNA samples, we achieved a SNP probe success rate (SNP call rate > 97%) ranging from 94.4% to 96.8% for the four different arrays.

625W

A Genome wide association identifies six loci associated with Chiari-like malformation in the Griffon Bruxellois breed. *P. Lemay[1], Q-H. Trinh[1], P. Knowler[2], M-P. Dubé[3], S. Blott[4], G.A. Rouleau[5], C. Rusbridge[2], Z. Kibar[1].* 1) Obstétrique et gynécologique, CHU Ste-Justine, Montreal, Quebec, Canada; 2) Stone Lion Veterinary Hospital, Wimbledon Village, United-Kingdom; 3) Institut de cardiologie de Montréal, Université de Montréal, Canada; 4) Centre for Preventive Medicine, Department of genetics, Animal Health Trust, United-Kingdom; 5) Centre de recherche du CHUM, Université de Montréal, Canada.

Chiari malformation type 1 (CMI) is a very common congenital abnormality of the craniovertebral junction affecting 1 in 1280 humans. It is characterized by a descent of the cerebellar tonsils through the foramen magnum and is frequently associated with syringomyelia. The etiology of CMI is thought to be multifactorial involving genetic factors. Human CMI is very similar to the canine condition referred to Chiari-like Malformation (CM) characterized by disproportionally small volume caudal cranial fossa and large brain. CM occurs in a variety of Toy breed dogs in particular the Griffon Bruxellois (GB). The strong genetic homogeneity of the canine population made this animal model a powerful tool for genetic mapping of complex human diseases. The high incidence of CM in the GB as well as the increased genetic homogeneity in the dog population will help us achieve our main objective of identifying genes predisposing to CM in the GB.

A GWAS was conducted using 56 GB dogs and the Canine SNP20 BeadChip array developed by Illumina. CM affectedness was determined by magnetic resonance imaging. Basic case-control association analysis of the genotyping data with PLINK v1.07 generated highly significant association scores over the Bonferroni threshold at six different genomic loci on chromosomes 2, 8, 12, 16, 38. P values for CM association at these regions ranged between 1.439×10^{-6} and 1.63×10^{-10}. After adjustment for population stratification with EIGENSOFT v.3.0, one SNP rs22804546 on chromosome 2 remained strongly associated to CM with an adjusted empirical P value of 1.9×10^{-5}.

Fine mapping of the candidate locus is currently underway. Once this locus is reduced to the minimum possible, a positional candidate gene approach will be used to identify the mutation associated with CM in the GB breed. To this day, no genes have been associated to the human and canine versions of CM. This study will give insight in the pathophysiological mechanisms of this disease, which could lead to the development of better diagnostics and prognostic tests.

626W

MaCH-Admix: Genotype Imputation for Admixed Populations. *Y. Li[1], Y. Liu[2], M. Li[3], W. Wang[2].* 1) Dept Genetics, Univ North Carolina, Chapel Hill, NC; 2) Dept Computer Science, Univ North Carolina, Chapel Hill, NC; 3) Dept Biostatistics and Epidemiology, Univ Pennsylvania School of Medicine, Philadelphia, PA 19104.

Genotype imputation has become a standard practice and a powerful tool for genome-wide association studies (GWAS) and meta-analysis, particularly when studying individuals of European or East Asian ancestry due to their higher level of LD and the existence of closely matched reference populations. However, little methodological work exists for genotype imputation in admixed populations, which comprise >20% of the U.S. population. Here, we extend our previously described MaCH hidden Markov model to incorporate two classes of approaches for imputation in admixed populations: (1) ancestry-weighted approach, where haplotypes from different reference populations are weighted according to inferred ancestry of the to-be-imputed individuals; and (2) Identity by State (IBS) based methods where a different set of reference haplotypes is selected for each to-be-imputed individual based on genetic similarity. Using HapMapIII African Americans (ASW) and Hispanics (MEX), we compared with other approaches including a baseline where all reference haplotypes are selected, random selection of reference haplotypes (from only the reference or from the reference plus constructed haplotypes of to-be-imputed individuals), and IMPUTE which uses one special case of IBS-based approaches. Based on our results, we recommend simple ancestry-weighted approach when 20-50% of the entire reference pool is selected at each Markov iteration and IBS-based approaches when a small proportion (<20%) of the reference pool is utilized at each iteration. Our IBS-based approaches resulted in 1.13% and 1.96% increased dosage r2 than the IBS-based approach implemented in IMPUTE, for ASW and MEX respectively. Our new methods, together with the functionality to infer ancestry, are implemented in MaCH-Admix, freely available at http://www.sph.umich.edu/csg/yli/MaCH-Admix/.

627W

A Graphical Representation of Quality Control Metrics for Next Generation Sequencing. *S. McGee, J. Smith, J. Furlong, I. Stanaway, I. Robertson, M.J. Rieder, D.A. Nickerson.* Genome Sciences, University of Washington, Seattle, WA.

With the wide acceptance of second-generation, massively parallel DNA sequencing methods and increases in sample throughput, it is necessary to automate the accurate and efficient determination of data quality at numerous points along the data processing pipeline to locate possible errors. These QC data need to be presented in a concise, informative, and data rich format to facilitate rapid decision-making about whether to proceed, remove, or hold a sample. To facilitate this process we have developed a "QC Dashboard" that presents an overview of quality metrics and is currently in use for exome sequencing at the Northwest Genome Center (NWGC). The QC Dashboard is a graphical and textual display of statistical information parsed from the flatfiles of sequence assembler routines (multiple assembler formats are accepted). Plots include overall number of reads, reads mapped to target and number of unique reads; allele distributions at each cycle in the analysis; plots for sample complexity and read depth coverage and uniformity. Per cycle error rates and read qualities are also plotted and summarized. In addition, a predictor is displayed detailing the number of lanes required until the depth-of-coverage goal (i.e., 90% of the sequence target with greater than 8x coverage). Each dashboard is adaptable to individual- or merged-lane data and is generated automatically to be displayed for human review.

628W

Combining gene flow with exome sequencing in large family studies of complex disease. *J.A. Morris, J.C. Barrett.* Wellcome Trust Sanger Institute, Cambridge, Cambridgeshire, United Kingdom.

In contrast to Mendelian diseases, linkage studies of large families with multiple affected individuals of complex diseases such as autism and diabetes have identified very few linked loci replicated across multiple families. Genome-wide association studies have subsequently identified dozens of common alleles of modest affect associated with such diseases, but these results do not explain either the extreme disease load, or the within-family linkage peaks of some large pedigrees. Next generation sequencing has enabled the molecular diagnosis of previously intractable Mendelian diseases and will soon be applied on a population scale to the study of complex disease. One intermediate study design between these two extremes is to sequence the exomes or genomes of individuals from large complex disease pedigrees in order to identify family-specific high-penetrance mutations. For example, the UK10K project is sequencing individuals from a number of large Finnish pedigrees with >4 cases of autism spectrum disorder. To enable this and similar studies, we have developed a tool which integrates gene flow within a family (single family linkage) and next generation sequencing data. Users can interactively filter and prioritize variants based on haplotype sharing across different sets of selected individuals, allele frequency in reference datasets, and functional annotation. The application requires a compressed and indexed VCF file containing sequence data (exome or whole genome) from one or more family members and genotypes across all family members used by the MERLIN software package to reconstruct haplotype flow within the family. Users can then interactively filter variants based on sharing among different individuals, or parametrically specify penetrance and phenocopy rates. The variants that remain after the haplotype based filtering are displayed in an interactive, annotated table view. Using this view the user can further sort and filter the variants using any metadata in the original VCF file or direct online connection to public databases, such as ENSEMBL. Once the user is happy with their list of variants it can be output in text format using a fully customizable export tool. Our application effectively harnesses the power provided by sequencing related individuals in multiply affected families for identifying disease-causing variants.

629W

Dyadic and Heterophilic Properties of SNPs with Marginal Effects Across Statistical Epistasis Networks. *D. Ng, T. Hu, A. Andrew, M. Karagas, J.H. Moore.* Dartmouth Medical School, Lebanon, NH, USA.

Susceptibility to common human diseases is determined partly by the complex interplay between multiple genetic and environmental factors. A central goal of computational genetics is to develop and apply analytical methods that are able to embrace, rather than ignore, the complexity of the genotype-phenotype relationship. The goal of this study was to use network science to determine the extent to which nonadditive interactions exist beyond small subsets of genetic variants. We previously inferred statistical epistasis networks to characterize the space of pairwise interactions among approximately 1500 single nucleotide polymorphisms (SNPs) spanning nearly 500 cancer susceptibility genes in a large population-based study of bladder cancer. Here, vertices are SNPs and edges or connections represent epistatic relationships. We discovered that statistical epistasis networks associated with bladder cancer are much larger than expected under the null hypothesis of no association. The goal of the present study was characterize the distribution of SNPs with marginal effects across these epistasis networks. First, we measured the dyadic properties of SNP-SNP pairs with states (1-1), (1-0) and (0-0) for SNPs with significant marginal effects (1) and those without (0). A network is considered dyadic if SNPs with marginal effects tend to interact with themselves and heterophilic if not. We found significant evidence for networks with heterophilic properties suggesting that SNPs with significant marginal effects are more to interact with SNPs that do not exhibit marginal effects than with each other. We then repeated the analysis labeling SNPs as included (1) or excluded (0) from different Gene Ontology (GO) categories and repeated the vertex distribution analysis. We found a diversity of dyadic and heterophilic properties across different GO categories. This study brings us a step closer to a systems genetics approach to complex disease that embraces the complexity of the genotype-phenotype mapping relationship by explicitly modeling the connections and properties among many SNPs.

630W

Phenotype-Genotype Integrator (PheGenI): Synthesizing Genome-Wide Association Study Data with Existing Genomic Resources. *E.M. Ramos[1], D.J. Hoffman[2], H.A. Junkins[1], D.R. Maglott[2], J. Paschall[2], L. Phan[2], S.T. Sherry[2], M. Feolo[2], L.A. Hindorff[1].* 1) Office of Population Genomics, National Human Genome Research Institute, NIH, Bethesda, MD; 2) National Center for Biotechnology Information, National Library of Medicine, NIH, Bethesda, MD.

Objective: Rapidly accumulating data from genome-wide association studies (GWAS) and other large-scale studies will be most useful when synthesized with existing databases. To address this, we developed the Phenotype-Genotype Integrator (PheGenI), a user-friendly web interface that integrates various National Center for Biotechnology Information (NCBI) genomic databases with association data from the NHGRI GWAS Catalog and supports downloads of search results. **Background:** The GWAS design has identified over 4,400 genetic variants associated with over 200 human traits and diseases. Rarely are the functional consequences of these variants understood, and replication, functional and follow up studies are crucial next steps. Integration of GWAS data with existing complementary databases can facilitate prioritization of variants to follow up, study design considerations, and generation of biological hypotheses. **Methods:** A number of existing genomic resources are housed at the NCBI, including dbSNP, Gene, and the Genotype-Tissue Expression (GTEx) program. GWAS association data are available through two other NIH resources, the Database of Genotypes and Phenotypes (dbGaP) and the NHGRI GWAS catalog. The availability of these centralized resources made possible the development of PheGenI (http://www.ncbi.nlm.nih.gov/gap/PheGenI), an online portal for scientists and clinicians who use or produce GWAS data, to browse, search, integrate and download results. **Results:** 7,614 association records have been integrated with approximately 38,000 records from dbSNP, 46,000 records from Gene, and 61,000 expression QTL records from GTEx. After weighting for associations that belong to multiple trait categories, 78% of these association records are distributed among a few categories: Chemicals and Drugs, Digestive System Diseases, Eye Diseases, Immune System Diseases, Mental Disorders, Neoplasms, Nervous System Diseases, Skin and Connective Tissue Diseases, and Other. PheGenI features include the ability to search by phenotype, gene, SNP, or chromosomal range. In addition, users can filter search results by association p-value or SNP functional class; display results locally on DNA sequence tracks or globally across the genome; and download data tables. **Conclusions:** PheGenI will facilitate follow up of results from GWAS, enabling scientists to investigate functionality of identified SNPs and interrogate relationships between SNPs and human disease.

631W

A software package for examining bisulfite conversion rates in methylation sequencing data. *S. Sun[1,2], X. Yu[2].* 1) Case Comprehensive Cancer Center, Case Western Reserve Univ, Cleveland, OH; 2) Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH.

DNA methylation is adding a methyl-group to the 5's cytosine. It is a very important epigenetic phenomena that can affect gene expression significantly in both normal and disease cells. Therefore, it is very important to study methylation patterns in the whole genome. With the aid of the next generation sequencing technology and the bisulfite conversion technique, it is now possible to identify methylation signals at single CG site level in a genome. However, sometimes bisulfite conversions may not be complete, and this will dramatically affect the methylation signal at cytosine sites. Therefore, it is crucial to examine the bisulfite conversion rate before any down-stream analysis. In order to meet this need, we have developed a software package that takes the ACGT-count output from BRAT (Bisulfite-treated Reads Analysis Tool) alignment (Harris et al. 2009) to access bisulfite conversion rates. Our software package has the following feature: generating summary statistics and figures for the structures of genome sequences and bisulfite conversion rates of either partial or all nonCGc sites (i.e., cytosine sites that are not followed by guanines) from (1) any specific region in the whole genome with a chromosome name, start and end positions; (2) a list of target regions with different chromosome names, start and end positions; and (3) a specific chromosome. Our software package is suitable for sequencing data from human adult samples and can help the user quickly diagnose whether a methylation sequencing data set has successful bisulfite treatments.

632W

A simulation-based comparison of statistical tests for incorporating biological information into analyses of genome-wide data. *J.L. Taylor[1], F.S. Goes[2], J.T. Leek[3], M. Pirooznia[2], F. Seifuddin[2], P.P. Zandi[4].* 1) Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD; 3) Department of Biostatistics, Johns Hopkins University School of Public Health, Baltimore, MD; 4) Department of Mental Health, Johns Hopkins University School of Public Health, Baltimore, MD.

In genome-wide association and expression studies, it is often a goal to identify biologically-defined groups of genes which collectively are more associated with a phenotype of interest than other genes. One approach, referred to here as the “over representation approach”, asks whether there are more in-group genes that meet an arbitrary threshold for significance than expected by chance. Another approach uses information from all genes in the genome-wide experiment, rather than just those that meet some threshold, and asks whether in-group genes are enriched among the most significant. A popular example of the latter approach is gene-set enrichment analysis (GSEA). GSEA typically uses the Kolmogorov-Smirinov (KS) statistic or a weighted version of it (wKS) in order to test for enrichment. However, it has been proposed that a simple t-test between in-group and out-of-group genes may alternatively be used to test for enrichment. We propose another test which borrows from the “over-representation” approach but does not rely on arbitrary thresholds. In this test, referred to here as the “modified over-representation” (MOR) test, the minimum p-value for a binomial test of over-representation at every possible threshold is taken as the test statistic and permutation is used to determine its significance. We use simulations to compare the power of the KS statistic, the wKS statistic, the t-test and the MOR test for detecting enrichment of a group of genes under different scenarios. Simulations under the null hypothesis show that all tests provide appropriate control of Type I errors. When there is true enrichment, the t-test has equal or better power than the wKS test under all conditions examined. When there are no true associations among out-of-group genes, these two tests outperform the KS test. However, when there are also true associations among out-of-group genes , the KS test outperforms the t-test and wKS test. Under all conditions examined, the novel MOR test performs as well or close to the best performing test. This simulation suggests a simple t test is more powerful for detecting enrichment than the most popular method for GSEA currently in use. However, it does not perform as well when there is genetic heterogeneity and genes outside the group of interest are also associated with the phenotype. The novel MOR test does as well as the best performing test under all conditions examined, whether there is genetic heterogeneity or not.

633W

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634W

Quantifying Genome-Wide Pleiotropy. *A. Tyler, J. Payne, A. Erives, J.H. Moore.* Dartmouth Medical School, Lebanon, NH, USA.

Pleiotropy is a widely documented phenomenon in which a single gene has the potential to affect more than one phenotype, and is identified as a single mutation that alters these phenotypes relative to a wildtype baseline. Despite there being many known examples of pleiotropy, it remains poorly understood as a genetic principle. In recent years advances in high-throughput technologies have allowed researchers to begin to quantify instances of pleiotropy at the genome-wide scale in organisms such as Baker's yeast (Saccharomyces cerevisiae) and nematodes (Caenorhabditis elegans). The vast majority of these large scale efforts to quantify pleiotropy have used one of two methods: gene deletion or quantitative trait locus (QTL) analysis. These two methods rely on different types of mutation to identify pleiotropic genes, and may therefore identify different genes as pleiotropic. Here we use computational models of gene regulatory networks to simulate both gene deletion studies and QTL analyses. We find that in simulated gene deletion studies there is a positive correlation between gene degree and pleiotropic impact; deletion of highly connected genes (hubs) results in the largest change in phenotype. In QTL analyses, on the other hand, we find that hubs are minimally pleiotropic. We show that this difference is due to the nature of the mutations used in each type of analysis. Using data from a gene deletion study and a QTL analysis in S. cerevisiae, we show that these correlations between gene degree and pleiotropic impact arise in biological systems as well. We further show that these two types of analysis identify functionally distinct sets of genes as pleiotropic. We discuss the implications of these results in light of interpreting future analyses of genome-wide pleiotropy.

635W

Genetic Heterogeneity Detection Using a Learning Classifier System. *R. Urbanowicz, A. Granizo-Mackenzie, J.H. Moore.* Dartmouth Medical School, Lebanon, NH, USA.

Machine learning algorithms within complex problem domains have been described as ``black boxes'' when it comes to data mining and interpretation. Learning classifier system (LCS) algorithms are characteristic of this description, evolving an entire population of rules or “classifiers” which collectively comprise the evolved solution. While flexible, adaptive, and adept at classification, LCSs are notoriously problematic when knowledge extraction is the goal. In the present study we combine a number of processing heuristics with a re-tasked visualization strategy to form an interpretation pipeline seeking to detect heterogeneity within single nucleotide polymorphism (SNP) association studies. Processing includes the following steps; (1) rule compaction, to remove useless rules, and condense the population to a much smaller set of non-redundant, maximally general classifiers, (2) binary re-encoding of all classifiers, to simply discriminate whether a given SNP within a classifier was specified in a rule or not, and (3) hierarchical clustering across all remaining classifiers as well as across all SNPs. Previous work had introduced a 3-D heat map for bioinformatics visualization. We apply this software to examine the processed population of LCS classifiers. The dimensional flexibility of the 3-D heat map software allows users to simultaneously visualize all classifiers, the SNPs they specify, and their numerosity and fitness. We also incorporate a weighting scheme to draw attention to SNPs which are most frequently specified within the classifier population. When an LCS is correctly learning underlying associations within the training data, it is expected that the SNPs predictive of disease classification will be more frequently specified in rules while those SNPs that are just noise, will tend to be assigned a “don't care” symbol. We demonstrate the usefulness of this methodology on datasets which simulate a complex and heterogeneous association. All datasets have two independent 2-locus epistatic interactions, representing genetic heterogeneity. We found that the implementation of this approach facilitates the identification of predictive SNPs as well the heterogeneous relationship between independent pairs of SNPs and disease status. This strategy offers an intuitive pathway to guide the identification of potential heterogeneous subgroups within a case/control disease study.

636W

TRANS-EQTL MAPPING USING 55,000 SAMPLES ALLOWS FOR DISSECTING DISEASE MECHANISMS. *H. Westra[1], J. Karjalainen[1], G.J. te Meerman[1], R.C. Jansen[2], R.S.N. Fehrmann[1], C. Wijmenga[1], L. Franke[1,3].* 1) Department of Genetics, University Medical Center Groningen, University of Groningen, 9700RB, Groningen, The Netherlands; 2) Groningen Bioinformatics Center, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, 9700AB, Groningen, The Netherlands; 3) Blizard Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London E1 2AT, UK.

In recent years, multiple genome-wide association studies (GWAS) have been performed in order to elucidate the genetic basis of complex diseases, such as Type 1 diabetes (T1D). While successful in identifying associated loci for a multitude of diseases, the challenge is now to understand the downstream effects of these associated variants. One strategy to gain such insight is by investigating whether these variants affect gene expression (i.e. expression quantitative trait locus (eQTL) mapping). A few recent eQTL studies for these traits have identified *trans*-eQTL genes that are both biologically plausible and which often contain additional SNPs that also confer susceptibility to the trait. While these results are encouraging, these studies are currently still faced with two main problems: sample sizes are often too small to detect many small-effect size *trans*-eQTLs and usually eQTL data is available for only one single tissue. As such it is impossible to make inferences on the effects of the variant in multiple tissues. To resolve both of these problems, we generated a dataset of 55,000 human Affymetrix gene expression array samples from the Gene Expression Omnibus. These samples reflect many different tissues and cell-types, such as the HapMap phase II samples, although genotype data is lacking for most of these samples. By employing principal component analysis we identified ~400 principal components (PC) that generally reflect differences between cell-types, physiological and metabolic state. We argued that the accuracy for observing the effect of genetic variation on gene expression should increase by removing the PCs from the data. As expected, we observed a considerable increase in the number of detectable eQTLs after removal of PCs from 246 HapMap samples, for which genotype data was also available. Owing to their large effect size, some of these eQTLs allowed us to use residual gene expression as perfect proxies for SNP genotypes. This allowed us to predict SNP genotypes for each of the 55,000 samples, and subsequently permitted us to perform *cis*- and *trans*-eQTL mapping. We present results using all samples, and show how these results compare to cell-type and tissue-specific eQTL analyses. Particular attention will be devoted to an important type 1 diabetes SNP, and we will show how the detected downstream effects compare to our conventional eQTL analysis in 1,469 peripheral blood samples, for which genome-wide genotype data is available.

637W

Genetic association of the Dickkopf 1 gene with Paget's disease of bone. *M. Beauregard[1,2], E. Gagnon[2], J. Morissette[2], J.P. Brown[1,2,3], L. Michou[1,2,3].* 1) Laval University, Quebec, Quebec, Canada; 2) CHUQ (CHUL) Research Centre, Quebec (Quebec) Canada; 3) Rheumatology Department, CHUQ (CHUL), Quebec (Quebec) Canada.

Introduction: Paget's disease of bone (PDB) has an autosomal-dominant mode of inheritance with incomplete penetrance. Mutations of the Sequestosome-1 gene have been identified, but account for only 37% of familial and 10% of sporadic forms of PDB. The Dickkopf 1 gene, expressed by osteoblasts and osteocytes, encodes a protein that inhibits Wnt signaling and downregulates bone remodeling. Expression of DKK1 is increased in pagetic osteoblasts and stromal cells and DKK1 serum levels are elevated in pagetic patients. The objectives of this study were to identify rare variants of DKK1 and to test for genetic association with PDB. Materials and methods: DKK1 exons, promoter and exon-intron junctions from 30 French-Canadian patients with familial PDB and four unrelated healthy individuals were amplified and sequenced. All variants identified in at least one individual and absent from NCBI's SNP database were considered as potential rare variants. An association study was also conducted by genotyping three common variants of DKK1 in unrelated French-Canadian PDB patients (N=183) and healthy individuals (N=295). The association study compared minor allele frequencies between cases and controls. Haplotypes composed of the two mostly-associated Tag SNPs were inferred by the use of SIMWALK 2.89 and PHASE. Results: Three rare variants of DKK1 were identified. The first rare variant was located in exon 2 and alters an amino acid highly conserved in evolution (Arg120Leu) and predicted in silico to be damaging, the second found in the 3'UTR, and the third found in intron 1 (IVS1 184 T/C) on a splice site. The G allele of the TagSNP rs1569198 was significantly lower in patients compared with controls (42% versus 49%, p=0.03, RR=0.77, 95% confidence interval: 0.61, 0.98), and a trend toward an association was found with the A allele of the rs2241529 (p=0.08). The haplotype GG was significantly less frequent in patients when compared to controls (41% versus 47%, p=0.047, RR=0.79, 0.62, 1.00). Conclusion: This study identified three rare genetic variants in DKK1 in patients with familial PDB. These variants are all located in regions important for the DKK1 function, especially exon 2 which encodes a cysteine-rich domain interacting with LRP5/6 co-receptors. In addition, a genetic association was found between an haplotype and a common variant of DKK1 in unrelated PDB patients. Further studies are required to investigate the functional consequences of this association.

638W

The Interaction between Killer-cell Immunoglobulin-like Receptor Genes and HLA Alleles in Susceptibility to Psoriatic Arthritis. *V. Chandran[1], F.J. Pellett[2], R. Ayearst[2], R.A. Pollock[2], D.D. Gladman[1].* 1) Medicine/Rheumatology, Unversity of Toronto, Toronto, Ontario, Canada; 2) Toronto Western Research Institute, Toronto, Ontario, Canada.

Introduction: HLA Class I genes are strongly associated with PsA. HLA Class I alleles interact with KIRs on NK cells and this interaction may play a role in PsA susceptibility. We therefore conducted a case-control study to determine the association between KIR2D & KIR3D genes and their interactions with HLA alleles in PsA. Methods: 678 PsA cases satisfying CASPAR classification criteria and 688 healthy ethnically matched controls were selected. KIR typing was performed by PCR-SSP and HLA typing by PCR-SSO with appropriate quality controls. The difference in the frequency of individual KIR genes in cases and controls was tested for significance using $\chi 2$ test and Fisher's exact test. Trends for increasing susceptibility to PsA from combined genotypes (HLA-KIR and HLA) were evaluated by the Cochran-Armitage trend test. Multivariate analyses were conducted using logistic regression. Results: In univariate analyses, KIR2DL2 and KIR2DS2 genes were significantly associated with an increased risk of PsA. Multivariate analysis revealed that only KIR2DS2 was independently associated with PsA (OR 1.258, 95% CI 1.017, 1.556, p= 0.03). The presence of HLA-C group 2 was associated with an increased risk of PsA (trend test p=0.006). The risk of PsA is increased when KIR2DS2 is present in the presence of HLA-C ligands (C group 1) for the corresponding inhibitory KIRs, and is highest when KIR2DS2 is present in the absence of HLA-C ligands for homologous inhibitor KIRs, compared to the state when KIR2DS2 is absent (trend test p=0.03). The presence of HLA-C alleles that have high cell surface expression was also associated with increased risk of PsA (trend test p<0.0001). Although association between HLA-B, Bw4 and HLA-Bw4 80isoleucine was associated with increased PsA risk (trend test p<0.0001 for both analyses), no statistical interaction between HLA-Bw4 80ile and KIR3DS1 could be demonstrated. Conclusion: This study confirms the association between the KIR2DS gene, especially KIR2DS2, with PsA. The increased risk conferred by HLA-C alleles that have high cell surface expression and HLA-B Bw4 alleles was demonstrated. These results indicate a potential role for NK cells in PsA pathogenesis since KIRs interact with these alleles to enhance the inflammatory response.

639W

Linkage Analysis in an Australian Population Identifies a Region Affecting Femoral Neck BMD. *S.C. Nguyen, J.R. Center, J.A. Eisman, T.V. Nguyen.* Osteoporosis and Bone Biology, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia.

Genome-wide association studies, with their large cohorts and dense sequencing coverage, have been extremely successful at identifying the gene regions which influence many different traits, including bone mineral density (BMD). Whilst they continue to be useful, the numbers needed to detect and replicate signals is at times prohibitively large. Given the high heritability of BMD traits, linkage studies using related individuals represent a cost-effective alternative for detecting regions associated with variation in BMD. A moderately-sized linkage study using related individuals can have the same power to detect gene regions as a much larger association study using unrelated individuals. Here we perform a linkage analysis for BMD at the femoral neck, lumbar spine and total body using 500 people across 90 extended pedigrees from the Dubbo Osteoporosis Genetics Study (DOGS). This study was designed as an off-shoot of the Dubbo Osteoporosis Epidemiology Study (DOES), and is a multi-generational family study with ongoing recruitment and follow-up. Using a multipoint linkage scan, we find evidence for linkage at chromosome 3q25 with respect to variation in femoral neck BMD (but not lumbar spine or total body BMD). Furthermore we find evidence for suggestive linkage at regions previously identified in other linkage and association studies looking at BMD. Overall there is reason to once again pursue linkage as a study design for identifying gene regions associated with BMD traits.

640W

Pathway-based analysis of bladder cancer genome-wide association study highlights metabolic detoxification, mitotic, and clathrin-mediated pathways. *I. Menashe[1*], J. Figueroa[1*], M. Garcia-Closas[1], Q. Yang[1], D. Maeder[1], W. Wheeler[2], T. Picornell[3], M. Malatas[3], F. Real[3], N. Chatterjee[1], S.J. Chanock[1], D.T. Silverman[1], N. Rothman[1].* 1) DCEG, NCI, Rockville, MD; 2) IMS Inc., Rockville, MD; 3) CNIO, Madrid, Spain.

Genetic association studies have conclusively identified ten variants associated with bladder cancer risk; however, estimates suggest additional susceptibility loci for this disease. To identify new candidate susceptibility loci, we applied a pathway analysis to a bladder cancer genome-wide association study containing data from 3,532 cases and 5,120 controls of European background (n=5 studies). 1,399 pathways were drawn from five publically available resources (Biocarta, Kegg, NCI-PID, HumanCyc, and Reactome), and we constructed 23 additional candidate pathways related to bladder cancer. In total, 1422 pathways, 5647 genes and ~90,000 SNPs were included in our study. Logistic regression models adjusting for age, sex, study, DNA source, and smoking status was used to assess the marginal trend effect of SNPs on bladder cancer risk. Two complementary pathway-based methods (gene-set enrichment analysis [GSEA], and adapted rank-truncated product [ARTP]) were used to assess the enrichment of association signals within each pathway. Eighteen pathways were detected by either GSEA or ARTP at $P < 0.01$. To minimize false positives, we used the I^2 statistic to identify SNPs displaying heterogeneous effects across the five studies. After removing these SNPs, seven pathways (`Aromatic amine metabolism', `NAD biosynthesis', `NAD salvage', `Clathrin derived vesicle budding', `Lysosome vesicle biogenesis', 'Retrograde neurotrophin signaling', and `Mitotic metaphase/anaphase transition') remained. These pathways seem to belong to three fundamental cellular processes (metabolic detoxification, mitosis, and clathrin-mediated vesicles). Identification of the aromatic amine metabolism pathway supports the ability of this approach to identify pathways with established relevance to bladder carcinogenesis and suggest additional candidate susceptibility loci.

641W

Genetic Analysis of Prostate Cancer Using Automatic Computer Programming. *J.H. Moore[1], D. Hill[1], J. Fisher[1], N. Lavender[2], L. Kidd[2].* 1) Dartmouth Medical School, Lebanon, NH, USA; 2) University of Louisville, Louisville, KY, USA.

The paradigm of identifying genetic risk factors for common human diseases by analyzing one DNA sequence variation at a time is quickly being replaced by research strategies that embrace the multivariate complexity of the genotype to phenotype mapping relationship that is likely due, in part, to nonlinear interactions among many genetic and environmental factors. Embracing the complexity of common diseases such as cancer requires powerful computational methods that are able to model nonlinear interactions in high-dimensional genetic data. Previously, we have addressed this challenge with the development of a computational evolution system (CES) that employs an automatic computer programming approach called genetic programming (GP). Our CES approach is different from most other GP-based methods in that it can learn how to solve a genetic analysis problem in addition to finding a good model. Our results have demonstrated that CES is capable of efficiently navigating these large and rugged fitness landscapes toward the discovery of biologically meaningful genetic models of disease predisposition. Further, we have shown that the efficacy of CES is improved dramatically when the system is provided with statistical expert knowledge, derived from a family of machine learning techniques known as Relief, or biological expert knowledge, derived from sources such as protein-protein interaction databases. The goal of the present study was to apply CES to the genetic analysis of prostate cancer aggressiveness in a large sample of European Americans. We introduce here the use of 3-D visualization methods to identify interesting patterns in CES results. Information extracted from the visualization through human-computer interaction (HCI) are then provide as expert knowledge to new CES runs in a cascading framework. We present a CES-derived multivariate classifier and provide a statistical and biological interpretation in the context of prostate cancer prediction. The incorporation of HCI into CES provides a first step towards an interactive discovery system where the experts can be embedded in the computational discovery process. Our working hypothesis is that this type of HCI will provide more useful results for complex problem solving than the traditional black box machine learning approach.

642W

Gastric cancer is associated with Native American ancestry in the Latin American (Peruvian) population. *L. Pereira[1], R. Zamudio[1], G. Soares-Souza[1], R. Gilman[2, 3], E. Tarazona-Santos[1].* 1) Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil; 2) Johns Hopkins School of Public Health; 3) Asociación Benéfica PRISMA.

Gastric cancer (GC) is one of the most important causes of cancer deaths worldwide. In the Peruvian population, the gastric cancer ranks second in incidence among men and third among women, and is the most lethal type of cancer. We performed an association study to test the hypothesis that ethnicity is associated with gastric cancer in the Peruvian population from the city of Lima, controlling the effect of potential confounding variables. We collected biological samples (biopsies and blood for DNA extraction) as well as clinical, socioeconomic and dietary data from 200 GC cases and 297 controls from three hospitals from Lima. To estimate admixture, we genotyped 106 ancestry informative SNPs in these samples and used published data from the following ancestral populations: HapMap Yoruba (176 individuals), HapMap Europeans (174 individuals), and Native American (296 indigenous individuals groups from Peru collected by our group). We estimated admixture using the Bayesian method implemented in the software Structure 2.3.3. Our samples from Lima, even if mostly categorized phenotipically as “mestizos”, showed a high level of Native American ancestry (78.4% for the cases and 74.6% for the controls) and very low level of African ancestry (<5%). Using a logistic regression model we found association between gastric cancer and Native Ancestry (ORs 4.02, p<0.01). Considering other variables, we observed association of gastric cancer with fruit/vegetables intake (ORs 0.58-0.63, p<0.03) and burning symptom (ORs 1.69, p=0.03). The association with Native American ancestry persists when these covariates were controlled for. Our study provides the first evidence of association of GC and ethnicity in Latin America. We are currently genotyping common variants in candidate genes (selected from immune pathways and from results of genome-wide association studies) to test the association between these genetic variants and GC, controlling the effect of admixture and other environmental covariates. Support: NCI-Fogarty, CNPq-Brazil, CAPES-Brazil, FAPEMIG-Brazil.

643W

GSTM1 and GSTT1 copy numbers and mRNA expression in lung cancer. *M. Rotunno[1], T.K. Lam[1], J. Lubin[1], A. Vogt[1], P.A. Bertazzi[2], N.E. Caporaso[1], M.T. Landi[1].* 1) Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland; 2) Unit of Epidemiology, Fondazione IRCCS Ospedale Maggiore Policlinico and Department of Occupational and Environmental Health, Università degli Studi di Milano, Milan, Italy.

Deletions in Glutathione S-transferase M1 and T1 (GSTM1/T1) are common in the general population and can affect xenobiotic metabolism. Numerous studies evaluated the effect of GSTM1/T1 deletions on lung cancer risk using a null/present model, but only two small studies estimated the contribution of the heterozygous deletion with mixed results. We measured GSTM1/T1 copy numbers in 2120 controls and 2100 cases from the Environment And Genetics in Lung cancer Etiology population (EAGLE) study. We evaluated their effect on mRNA expression from tissue and blood samples and their association with lung cancer risk overall and for different histology types. We tested the null/present, dominant and additive models using logistic regression. Cigarette smoking, sex, cruciferous-based diet, and hormone-related information for women were studied as possible modifiers of the tested associations. Gene expression from blood and lung tissue cells was strongly down-regulated in subjects carrying GSTM1/T1 deletions by both trend and dominant models ($p<0.001$). No significant associations between lung cancer and GSTM1/T1 were detectable using the null/present model. In contrast, analyses distinguishing subjects with 1 and 0 deletions of GSTM1/T1 revealed several significant associations. There was a decreased risk of lung cancer in never smokers (OR=0.44, 95%CI=0.23-0.82, p=0.01) and women (OR=0.50, 95%CI=0.28-0.90, p=0.02) carrying 1 or 2 GSTM1 deletions. Analogously, male smokers had an increased risk (OR=1.13, 95%CI=1.0-1.28, p=0.05) and women a decreased risk (OR= 0.78, 95%CI=0.63-0.97, p=0.02) of lung cancer for increasing number of GSTT1 deletions. The corresponding gene-smoking and gene-gender interactions were significant ($p<0.05$). In summary, by distinguishing individuals carrying 0 and 1 deletion of GSTM1/T1 for the first time in a large population of lung cancer, we have found a clear relationship between deletions and gene expression in blood and lung tissue cells and showed opposite effects of GSTM1/T1 deletions on lung cancer susceptibility for male smokers and for never smokers and women. Our results suggest that a decreased activity of GSTM1/T1 enzymes is 1) a risk factor for lung cancer in smokers, likely due to mechanism of impaired ability to detoxify carcinogens, 2) a protective factor in nonsmokers, possibly because of enhanced activity of protective dietary constituents, and 3) a protective factor in women, possibly due to hormonal-related mechanisms.

644W

Prevalence of beta Thalassaemia. *K. Sanwaria[1], M. Vashist[1], P. Abrol[2].* 1) Department of Genetics, M.D.University, Rohtak, Haryana, India; 2) Depart of Paediatrics,Pandit Bhagwat Dayal University of Health Sciences, Rohtak ,Haryana,India.

INTRODUCTION: India is an ethnically diverse country with an approximate population of 1.2 billion.) -thalassemia is the commonest single-gene disorder in the Indian population The frequency of) -thalassemia trait () TT) has been reported from <1% to 17% and an average of 3.3. It is characterized by reduced synthesis of Hb subunit that results in microcytic hypochromic anemia, an abnormal peripheral blood smear with nucleated red blood cells and reduced amounts of Hb A. Without treatment, affected children have severe failure to thrive and shortened life expectancy. OBJECTIVE: To investigate the) thalassemia profile. METHODS: All subjects were interviewed using a standarized proforma .Various hematological analysis and hemoglobin electrophoresis was done after the informed consent of parents/ guardian . RESULTS: Two hundred thirty patients of) thalassemia major were analysed from year 2009 to Jan 2011. Thalassaemia incidence rates varied significantly among age groups and gender. The occurance of) -thalassaemia was inversely propotional with age.Retrospective study revealed that 51%) thalassemic major patients were diagnosed before 8 months of age and 38% of cases between 8 months & 5 years.Twelve percent patients came to light after 5 years of age Sex ratio revealed prevelance of) thalassemic males(males-71.6%& females- 28.4%). Patients belonged to nine communities i.e Punjabi, Sunar, Jaat, Brahmin, Ahir, Khaati, Baniya, Chauhan and Sikh. Punjabis were found to have the highest frequency (31%) of) thalassemia followed by Jaat (11.6%), Brahman (8.3%) and Sunar (8%). As a corollary, the projected incidence of) -thalassaemia major in newborn babies greatly varied by the subcastes of the parents.Two third (75%)patients were from Urban region and 25% belonged to rural area. Highest frequency of) thalassemia was found in B+ blood group, whereas no case was reported in A- and AB- blood group. The frequency of ABO blood group in) thalassemic patients varied in the descending order of B, O, A, AB, Rh+, Rh- and O, B, A, AB, Rh+, Rh- in thalassemics and control group respectively. CONCLUSION: Specific genes were proliferated along with the extension of time. Ethnic subgroups within populations known to commonly carry the) -thalassaemia gene will provide useful information in genetic research,epidemiological and public health perspectives.

645W

Efficient Detection of Tumor Somatic Mutations using Next-Generation Sequencing Data. *Z. Song[1], J. Long[2], J. He[3], W. Zheng[2], C. Li[1,3].* 1) Center for Human Genetics Research, Vanderbilt Medical Center, Nashville, TN; 2) Vanderbilt Epidemiology Center, Nashville, TN; 3) Department of Biostatistics, Vanderbilt University School of Medicine, Nashville, TN.

Direct comparisons between normal and tumor samples allow us to identify somatic mutations that either trigger tumorigenesis or arise as a result of it. However, a tumor sample may be a mixture of tumor and non-tumor cells, and a somatic mutation may not be present in all tumor cells. As a result, a somatic mutation may exist only in a fraction of cells of a tumor sample, and thus may not be detectable through genotype calls. We develop a novel algorithm SMUG (Somatic MUtation Gleaner) for detecting two types of somatic mutations, base substitution and loss of heterozygosity (LOH), by examining the sequence reads of tumor samples. We applied our method to whole exome sequencing data of 8 breast cancer patients, and detected many somatic mutations that were missed by the traditional approach of comparing genotype calls between normal and tumor samples (denoted CALL). To detection base substitutions, we walk through the tumor data to identify potential sites, force genotype calls in blood samples, and keep the sites that are homozygous in blood. To distinguish sites that have the same mutation rate but different depth, we employ an empirical Bayes (EB) approach to adjust mutation rate. High depth sites will receive less adjustment than low depth sites. To detect LOH regions, we focus on sites that are heterozygous in the normal sample. If there is no LOH at a site, the tumor sample allele counts should follow a binomial distribution with equal probability for the two alleles in the normal sample. For each site, we define an LOH score to reflect the level of departure from the expected distribution. LOH regions and their boundaries can be determined by applying a threshold to LOH scores. For our 8 breast cancer patients, we compared the results of SMUG and CALL to the COSMIC (catalogue of somatic mutations in cancer) database and published LOH regions in breast cancer. With the same QC criteria, SMUG identified 8,207 base substitutions, while CALL found 784, in which only 6 were not reported by SMUG. At gene level, nonsynonymous substitutions found by SMUG hit 342 COSMIC genes (43 breast cancer genes), while those found by CALL hit 61 COSMIC genes (9 breast cancer genes). For LOH detection, SMUG identified an average of 265 LOH regions per subject, while CALL reported 40. Among 19 published LOH regions related to breast cancer, SMUG found 126 LOH intervals in 13 of them, while CALL found 22 intervals in 7 of them.

646W

High-resolution array-CGH analysis of germline DNA in a melanoma-prone family linked to chromosome 9p21. *R. Yang[1], J. Struewing[2], S. Chanock[1], M. Tucker[1], A. Goldstein[1], NCI Core Genotyping Facility.* 1) Dept DCEG, NCI, Bethesda, MD; 2) NHGRI, NIH, Bethesda, MD.

Germline copy number variations (CNVs) have recently been recognized as a significant source of genetic variation and have been associated with disease susceptibility. Chromosome 9p21 has been implicated in the pathogenesis of cutaneous malignant melanoma (CMM) because it contains CDKN2A, the major known high-risk CMM susceptibility gene. However, a subset of CMM families linked to 9p21 has no CDKN2A mutations. The goal of this study was to determine whether CNVs, particularly 9p21 CNVs, were related to CMM risk in a 9p21-linked melanoma-prone family without a CDKN2A mutation. The family has 13 CMM members and 6/7 genotyped CMM cases shared a common 9p21 haplotype. An extensive mutation analysis did not detect any mutations in coding or non-coding regions of CDKN2A. We therefore conducted a genome-wide search for CNVs using the Nimblegen 385K whole-genome array-CGH. We analyzed genomic DNA from 4 CMM cases and 1 spouse. We used the Nexus Copy Number™ built-in Rank Segmentation algorithm to identify significant CNVs (significant threshold=0.000001; minimal number of probes per segment=5; log2 ratio>0.2 for gains and -0.3 for losses). No CNVs were consistently shared by multiple CMM cases. However, there were some small CNVs in the 9p21 linked region that occurred in some but not all CMM cases. Given the importance of 9p21 in melanoma susceptibility and the observed linkage to this region in this family, we further evaluated CNVs in this region using two independent approaches: 1) a custom-made array-CGH design to focus on the linked region (average probe spacing, 13 bp) [analyzing 3 cases]; and 2) quantitative PCR (qPCR) targeting 4 genes (SH3GL2, CDKN2A, KIAA1797, and SLC24A2) in this region [analyzing all 26 individuals with available DNA]. No CNVs that were shared by multiple cases were identified suggesting that CNVs in the 9p21 region do not cause melanoma predisposition in this family. We are currently using next-generation sequencing technology to examine all exons in the 9p21 linkage region as well as exomic and complete genomic sequencing to identify disease-related variant(s) in the entire exome/genome in this family.

647W

Genetics factors involved in MLIA susceptibility: an association study in a Portuguese sample. *M. Alves-Ferreira[1,2], T. Pinho[3], A. Sousa[1,4], J. Sequeiros[1,2,4], C. Lemos[1,2,4], I. Alonso[1,2,4].* 1) UnIGENe, IBMC, Porto, Portugal; 2) CGPP, IBMC, University of Porto, Portugal; 3) CICS, ISCS-N/CESPU, Portugal; 4) Department of Population Studies, ICBAS, University of Porto, Portugal.

Tooth agenesis affects 20% of the world population and agenesis of maxillary lateral incisors (MLIA) is one of the most frequent subtypes, characterized by the absence of formation of deciduous or permanent lateral incisors. In the Portuguese population, we found a prevalence of 1.3% for MLIA. In a previous study, we also found evidence of a strong genetic component for this trait. Several genes such as MSX1, PAX9 and AXIN2 have been identified as being expressed during odontogenesis. Mutations in these genes can lead to abnormalities in odontogenesis, which is a complex mechanism regulated by sequential and reciprocal epithelial-mesenchymal interactions, resulting in tooth agenesis. Therefore, our aim was to study the association of polymorphisms in these genes and in other genes related with odontogenesis and MLIA susceptibility. Association studies are an essential approach in the research of candidate genes involved in MLIA susceptibility in our subjects, allowing the identification of common variants associated with this trait. A case-control study, in a total of 300 individuals, is underway; a 1:2 ratio was achieved in order to increase the study statistical power. We selected 44 tagging single nucleotide polymorphisms (SNPs), which were genotyped by SNaPshot, using a multiplex approach. After genotyping, statistical analysis was performed by logistic regression. Regarding allelic frequencies, a significant high risk was found for the A allele of rs7149262 in PAX9. The results of the logistic regression analysis show that the AA genotype of rs8004560 in PAX9 confers a significant increased risk for individuals with MLIA (OR= 7.06, 95% CI: 1.54-32.34). These results, although preliminary, allow us to hypothesize that PAX9 may have a role in MLIA susceptibility. In the end of this study, we expect to find or exclude the role of the selected candidate genes in MLIA susceptibility. Furthermore, epistasis analysis also will be performed in order to identify possible gene-gene interactions, which are important in the etiology of complex traits. This study has identified some polymorphisms potentially involved in MLIA susceptibility and may contribute to better understand the genetic basis of dental agenesis.

648W

The Genetic Underpinnings of Exercise Adherence. *M.S. Bray[1], J.R. Fernandez[2].* 1) Epidemiology/Genetics, University of Alabama at Birmingham, Birmingham, AL; 2) Nutrition, University of Alabama at Birmingha, Birmingham, AL.

The increasing prevalence of obesity is one of the most important public health issues today, with more than two thirds of the U.S. population being overweight or obese. Though physical activity is recommended to improve health and facilitate weight loss and weight maintenance, the ability of individuals to respond and/or adhere to an exercise program varies widely. Preliminary studies in our group point to a genetic basis for exercise adherence that is neural in origin. Thus, we examined a dense array of markers in genes residing in neural pathways related to satiety signaling and pleasure/reward systems in a large cohort of young adults (18-35 y) from the Training Interventions and Genetics of Exercise Response (TIGER) study. Subjects in the study underwent a 30-week exercise intervention, which involved 30 minutes of aerobic exercise at 65-85% of maximum heart rate reserve three days per week. Exercise adherence was defined by more than 42,000 computerized exercise sessions in which exercise duration, frequency, and intensity were documented. Of 12 tag SNPs located across the fat mass and obesity associated (FTO) gene, five loci (rs13334933, rs9924072, rs1125392, rs7205987, and rs10492872) significantly raised risk of exercise dropout ($p<0.02$). Combinations of any three risk raising alleles in these loci more than doubled exercise dropout risk (OR=2.22; 95%CI: 1.45-3.40). A linear decrease in exercise intensity/duration was observed with increasing number of risk alleles, suggesting a putative functional role for the combined variants in limiting the capacity for high endurance exercise. The fat mass and obesity associated (FTO) gene is the most highly replicated GWAS finding for obesity to date, and several studies have demonstrated that the risk-raising effects of the FTO locus may be ameliorated by physical activity. The results presented here are the first to document a role for the FTO gene in influencing both exercise adherence and duration.

649W

Association analysis of rare variants and alcohol level of response in the San Diego Sibling cohort. *H. Choquet[1], G. Brush[1], J. Kasberger[1], M. Robertson[1], M. Schuckit[2], R. White[1], E. Jorgenson[1].* 1) Ernest Gallo Clinic and Research Center, University of California, San Francisco, Emeryville, California; 2) Department of Psychiatry, University of California, San Diego, and the Veterans Affairs San Diego Healthcare System, San Diego California.

BACKGROUND: Evidence suggests that rare genetic variants may play an important role in complex disease etiology. Genome-wide association studies, however, have limited power to examine the effect of rare variants; for this reason, understanding of the effects of rare variants on complex diseases remains limited. Recently, a number of statistical "collapsing" methods have been proposed to examine rare variants from sequence data by testing the aggregate effect of the variants in a gene on disease susceptibility. The aims of the present study are to apply collapsing methods to investigate the effect of variants identified through sequencing of alcohol use disorder candidate genes in subjects with a familial background of alcohol dependence to identify genes that influence level of response to an alcohol challenge. METHODS: The coding regions and exon-intron boundaries of 240 candidate genes were sequenced in 295 Caucasian subjects with a familial background of alcohol dependence from the San Diego Sibling cohort who had been tested for level of response to an alcohol challenge. To classify the variants detected either as pathogenic mutations or as neutral, we used functional in silico analyses. To assess the association between rare genetic variants and level of response to an alcohol challenge, we used the "step-up" statistical approach to combine multiple rare variants in each gene and analyze them as a single group. RESULTS: Genetic screening of 240 genes in 295 subjects has led to the identification of 4,207 genetic variants (8.8% of them are novel or rare (MAF<5%)). Out of these variants detected, 29.7% were non-coding variants, 30.8% silent variants and 39.5% missense variants. Approximately 65.5% of these missense variants have been considered as deleterious mutations using in silico functional predictions. Finally, using the "step-up" statistical method, a number of novel associations with a level of response to alcohol were identified. CONCLUSIONS/PROSPECTS: This study demonstrates that the evaluation of rare variants using sequence data is possible. Furthermore, the "step-up" approach that groups rare variants allowed the identification of response to alcohol traits-associated genes. To confirm our findings, identified rare missense variants will be investigated for cosegregation with response to alcohol in families. Furthermore, replication studies using large sample sizes will need to be carried out.

650W

No genetic association with *CCR5, CD226* or *RFX* polymorphisms in Norwegian Addison's disease patients. *M.C. Eike[1], B. Skinningsrud[1], A. Stormyr[1], B.A. Lie[1], E.S. Husebye[2, 3], D.E. Undlien[1].* 1) Department of Medical Genetics, Oslo University Hospital, University of Oslo, Oslo, Norway; 2) Section of Endocrinology, Institute of Medicine, University of Bergen, Bergen, Norway; 3) Department of Medicine, Haukeland University Hospital, Bergen, Norway.

Autoimmune Addison's disease (AAD) is a rare disease characterised by autoimmune destruction of the adrenal cortex. As AAD patients frequently suffer from concomitant autoimmune diseases of other organs, AAD is an interesting model disease for susceptibility factors common to autoimmunity. Accordingly, many of the genes involved in other autoimmune diseases have also been associated with AAD. In this study, we investigated two candidate polymorphisms strongly associated with other autoimmune diseases, a 32 bp deletion (Δ32) in the *chemokine C-C motif receptor 5 gene* (*CCR5*) and the rs763361 C>T Gly307Ser SNP in the *CD226* gene. In addition, SNPs in the *class II MHC transactivator* (*CIITA*) gene has previously been associated with disease in our AAD patients and a number of other autoimmune diseases. We therefore investigated the possibility for presence of susceptibility factors in the genes of the three coregulatory factors of CIITA, the *regulatory factor X* genes (*RFXANK*, *RFX5* and *RFXAP*), with a total of eight tagging SNPs. 416 patients with Addison's disease (321 for rs763361) and 1029 healthy blood donors were genotyped, all of Norwegian origin. *A priori*, we had ⁄ 80% power to detect an OR above 1.41-1.85 or below 0.70-0.45, depending on minor allele frequency. Two of the tagging SNPs, in *RFX5* and *RFXAP* respectively, were not in Hardy-Weinberg equilibrium in the control population ($p<0.05$), and were therefore excluded. No significant associations were found between Addison's disease and either polymorphism, nor with haplotypes of any of the *RFX* SNPs ($p>0.05$). However, a tendency for a protective effect was observed for patients homozygous for *CCR5*Δ32 (recessive OR 0.39, 95% CI 0.11 to 1.31, $p=0.11$), similar to what has been reported earlier for patients with type 1 diabetes. In conclusion, no significant disease association was found in this study, possibly due to limited power. However, a tendency was observed for the 32 bp deletion polymorphism in the *CCR5* gene, which could merit follow-up studies in other AAD cohorts.

651W

Genetic Analysis of *CAPN10* gene: a candidate region for the susceptibility to Polycystic Ovary Syndrome. *F.R. Faucz[1,2], I.R. Hauer[1,3], R.I. Werneck[1], M. Olandoski[1], R.B. Alexandre[1,2].* 1) Laboratory of Human Molecular Genetics, Group for Advanced Molecular Investigation (NIMA), PPGCS/CCBS, Pontifícia Universidade Católica do Parana, 80215, Curitiba, PR, Brazil; 2) Section of Endocrinology and Genetics, Program on Developmental Endocrinology Genetics, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, 20892, Bethesda, Maryland, USA; 3) Laboratory of In Vitro Fertilization of Fertway Clinic, 80250, Curitiba, PR, Brazil.

The Polycystic Ovary Syndrome (PCOS) is a heterogeneous endocrinological disease, usually characterized by enlarged and containing numerous small cysts ovary (polycystic appearance) and excessive hormone production. PCOS affects between 4 and 12% of women in reproductive age, and while the exact cause is undetermined, a genetic component is suggested by several association studies. Recent evaluation of the *CAPN10* gene in PCOS patients has suggested that different alleles may play a role in PCOS susceptibility. We searched for possible association between four single nucleotide polymorphisms in the CAPN10 gene (SNP-19 rs3842570, -43 rs3792267, -44 rs2975760 and -56 rs2975762) and Brazilian women with PCOS. Fifty women with PCOS (34,9 +/- 5,27y) and 150 healthy women (35,6 +/- 5,79y) were genotyped, and uni-and multi-variate analysis were conducted. Applying Fischer Exact Test and logistic regression model, we observed strong association between markers SNP-43 (p=0,026; OR=2.25, IC 1.1 - 4.6) and SNP-56 (p=0,005; OR=3.05, IC 1.4 - 6.63). Allele A of SNP-56 showed highest disease association (p=0.031). Stronger association with the PCOS disease status was observed after construction of haplotypes, which was in line with previous studies. The construction of haplotypes was performed by the Thesias software package. As reported before, haplotype TGG3 showed protective effect against the development of PCOS (OR= 0.25; CI=0.143-0.445; p=0.000431). While haplotypes TGA2 (OR=3.73778; CI=1.759-7.941; p=0.00065) and TAG3 (OR=3.01389; CI=1.425-6.376; p= 0.003902) showed high probability to increase the predisposition for the disease. Further studies on greater sets of samples are needed to confirm this observation.

652W

Genetic and serologic evidence implicate *IL6* as a susceptibility gene for leprosy type 2 reaction. *V.M. Fava[1], A.L.M. Sousa[2], L.H.F Sampaio[2], C.M.T Martelli[2], M.B. Costa[2], M.T. Mira[1], M.M.A. Stefani[2].* 1) Pontifical Catholic University of Paraná, Curitiba, Brazil; 2) Federal University of Goiás, Goiânia, Brazil.

Type 1 (T1R) and Type 2 (T2R) leprosy reactions (LR) are aggressive inflammatory episodes of sudden onset that occur in 30% of leprosy affected individuals, often several months after successful completion of treatment. Today, these unpredictable episodes are the major cause of permanent disabilities associated with leprosy. In this scenario, the description of innate predictive factors for LR will have a major impact over preventive strategies against the effects of such a stigmatizing event, which only recently has come under leprosy research focus. In a previous comparative screening of a large panel of cytokines in the serum of individuals affected by T1R and T2R, our group identified *IL6* as a strong candidate for genetic analysis. Here, we present the results of a prospective follow-up study involving 409 leprosy-affected individuals monitored for at least one year for the occurrence of LR. By the end of the follow-up, 154 individuals had developed T1R, 39 had developed T2R and the remaining 216 were considered as controls. Fluorescence-based TaqMan technology was applied to produce genotypes of all individuals for three tag SNPs capturing the entire information of the *IL6* locus. Allele frequencies were compared between cases and controls matched by leprosy clinical form. No association was observed between IL6 variants and occurrence of T1R. However, we found independent, positive evidence for association between T2R and all three tag SNPs: rs2069832 (p=0.002), rs2069840 (p=0.03) and rs2069845 (p=0.04); Interestingly, SNP rs2069832 is in the same bin as rs1800795, a known functional *IL6* regulatory variant. Genotyping of rs1800795 confirmed association with T2R (p=0.005). Finally, we produced functional data showing statistically significant correlation between risk genotypes of rs2069832 (p=0.04), rs2069840 (p=0.02) and rs1800795 (p=0.04) and serum levels of IL6 in a sample of 49 selected individuals. Taken together, these results strongly implicate *IL6* polymorphisms in the control of susceptibility to leprosy T2R.

Fava, V.M; Sousa, A.L.M, share first authorship; Stefani, M.M.A; Mira, M.T, share senior authorship

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653W

A combination of variations near NMU and NMUR2 coding regions identifies young women with significant lower bone mineral density at femoral neck. *S. Giroux[1], V. Clément[1], A. Bureau[2,3], D.E.C. Cole[4,5], F. Rousseau[1,2,6].* 1) Centre de Recherche de l'Hôpital St-François d'Assise du Centre hospitalier universitaire de Québec, Québec, Canada; 2) Faculté de Médecine, Université Laval, Québec, Canada; 3) Centre de recherche Université Laval Robert-Giffard, Québec, Canada; 4) Department of Clinical Pathology, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada; 5) Department of Laboratory Medicine & Pathobiology, University of Toronto,Toronto, Ontario, Canada; 6) Centre for the Development, Evaluation and Rational Implementation of New Diagnostic Tools in Medicine (CEDERINDT) Québec, Canada.

Bone mass is highly variable among healthy individuals and genetic variations are thought to be an important factor explaining this diversity. In addition, pathway specific gene-gene interactions are likely to affect the determination of complex traits such as bone mineral density (BMD). Recently, bone remodeling was shown to be under neuronal control mediated by neuropeptides such as leptin and neuromedin. We analyzed selected candidate genes involved in these pathways (LEP, LEPR, ADRB2, NMU, NMUR2, NPY, NPY2R and CART) with HapMap data tools to select a set of Tag SNPs. 79 Tag SNPs were analyzed in a group of women from Québec city (709 women) using the Sequenom technology. SNPs were analyzed for association with BMD measured at lumbar spine (LS) and femoral neck (FN). 14 significant associations ($p < 0.05$) were tested in a second group of women from Toronto (673 women) using the same technology. Genetic variants remaining associated with BMD in the combined samples of women were tested against all others for interactions. We found a significant association between LS BMD and one SNP in the NMU gene in the Quebec sample ($p = 0.017$) and the same SNP was associated with FN BMD in the Toronto sample ($p = 0.025$). After samples were combined, the same SNP showed a significant association with FN BMD ($p = 0.011$) and a trend with LS BMD ($p= 0.077$). We also found a significant interaction between that SNP and one SNP in the NMUR2 gene ($p = 0.035$). After stratification, we identified a subgroup of women with significantly lower FN BMD (gene effect size = 0.53SD) representing 3.1% of the population. The same group also exhibited a significantly lower LS BMD (0.32SD) when compared to all other women combined. Interestingly, the SNP in NMUR2 was correlated ($r = 0.33$) with a deletion located 200kb downstream from NMUR2 gene. Two individuals identified as deleted with a TaqMan assay were sequenced to determine the deletion boundaries. The sequence revealed a complex organization with a loss of 4kb and an addition of a 250bp originating from a region some 4kb downstream from the deletion. A PCR assay was designed to genotype the entire sample. The deleted allele was also associated with a lower BMD in interaction with NMU. Although the potential for a CNV to be functional is greater than that of a SNP, functional assays will be needed to prove that the CNV is causal. Replication in different samples of women will also be required to validate this association.

654W

Testing GWAS SNPs for COPD and lung function in a Polish cohort with severe COPD. *M. Hardin[1], J. Zielinski[2], E. Wan[3], C.P. Hersh[3], P.J. Castaldi[3], E. Schwinder[3], P. Sliwinski[2], I. Hawrylkiewicz[2], M. Cho[3], E.K. Silverman[3].* 1) Pulmonary Critical Care, Channing Laboratory, Brigham and Women's Hospital, Boston, MA; 2) National Tuberculosis and Lung Diseases Research Institute, Warsaw, Poland; 3) The Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Rationale: Previous genome-wide association studies (GWAS) have demonstrated genetic loci associated with COPD. More recently, in the CHARGE and SpiroMeta consortiums, additional loci associated with lung function (FEV1 and FEV1/FVC) have been identified. In order to confirm previously identified COPD loci and to assess pulmonary function loci as COPD genetic determinants, we performed candidate gene analyses of loci previously associated with COPD and lung function in a novel cohort with severe to very severe COPD. **Methods:** All subjects were Caucasian smokers from Poland. 315 COPD cases and 338 controls were enrolled. All subjects completed a respiratory questionnaire, performed standardized spirometry, and provided blood samples for testing. From previous GWAS studies, we included four single nucleotide polymorphisms (SNPs) that had been associated with COPD: FAM13A(rs7671167), HHIP(rs13118928), IREB2(rs13180), and CHRNA3/5(rs8034191), as well as four SNPs that have been associated with lung function in general population samples: AGER(rs2070600), ADCY2(rs11134242), THSD4(rs4316710), and INTS12(rs17096090). We tested for association with COPD using logistic regression, controlling for age, gender and pack-years. **Results:** Subjects with COPD were older (62 vs 58 years, p<0.01) with greater pack-years (45 vs 34 pack-years, p<0.01). There were more males than females but gender was equally distributed among cases and controls. Cases had significantly worse lung function (FEV1 31% predicted vs 103% predicted, p<0.01; FEV1/FVC 36% vs 76%, p<0.01). Among SNPs previously associated with COPD, CHRNA3/5 (OR 1.8 [1.4,2.4], p = 1x10-8), IREB2 (OR 0.7[0.5, 0.9], p = 0.003), and HHIP (OR 0.7 [0.5,0.9], p= 0.001) demonstrated significant association with COPD. FAM13A (OR 0.8 [0.7,1.1], p=0.06) approached statistical significance. The CHRNA3/5 locus was no longer significant when adjusting for the presence of the IREB2 locus. Among the SNPs associated with lung function, ADCY2 demonstrated a significant association with severe COPD (OR 1.3 [1.1,1.7], p=0.007); however, SNPs near AGER (p= 0.25), THSD4 (p=0.18) and INTS12 (p=0.3) did not demonstrate association. **Conclusion:** In a population of Polish subjects with severe to very severe COPD, we demonstrate replication of association between three SNPs and COPD (CHRNA3/5, IREB2 and HHIP) as well as association with COPD of two loci that have been associated with lung function (HHIP and ADCY2).

655W

Imputation-based association analysis within the ITGAM-ITGAX region identifies multiple common variants associated with SLE susceptibility in multiple ethnic populations. *X. Kim-Howard[1], C. Sun[1], K. Kaufman[1], J. Kelly[1], J. James[1], P. Gaffney[1], K. Moser[1], G. Gilkeson[2], C. Langfeld[3], R. Kimberly[4], T. Vyse[5], M. Alarcón-Riquelme[1], A. Adler[1], G. Wiley[1], J. Harley[6], S.K. Nath[1], SLEGEN, PROFILE.* 1) Oklahoma Med Res Foundation, Oklahoma City, OK; 2) Medical University of South Carolina, Charleston, South Carolina; 3) Wake Forest University Health Sciences, Winston-Salem, North Carolina, USA; 4) University of Alabama at Birmingham, Birmingham, Alabama; 5) King's College London, Guy's Hospital, London, UK; 6) Cincinnati Children's Hospital Medical Center and the US Department of Veterans Affairs Medical Center, Cincinnati, Ohio.

Introduction: We identified a novel non-synonymous ITGAM variant, rs1143679, associated with systemic lupus erythematosus (SLE). Subsequent GWAS confirmed association with ITGAM and expanded susceptibility to the ITGAM-ITGAX region. We hypothesize that there are multiple independent, possibly ethnic-specific, SLE-predisposing variants within the ITGAM-ITGAX region. Objective: We performed a comprehensive analysis to assess whether single or multiple causal variants from ITGAM-ITGAX are involved in SLE susceptibility for independent data sets (N=13,064), including European-American (EA) (3980 cases/3491 controls), African-American (AA) (1527/1811), Hispanic (HS) (961/336), and Native-Americans (NA) (531/471). Methods: We genotyped 79 SNPs from ITGAM-ITGAX and imputed SNPs using sequence data as a reference panel for EA, AA, and HS. For EA and AA we used dense fine-mapping data from the ImmunoChip SNP array and 1000Genomes as reference panels for a confirmation set. This allowed single- and multiple-SNP association analysis of nearly all common SNPs within this region. LD patterns and conditional logistic regression analysis were used to assess independence between SNPs. Results: After imputation and quality control we analyzed 164-304 SNPs. We found multiple significantly associated SNPs, many of which were in strong LD. Using differences in LD structure between populations and conditional analysis we identified 2 SNPs independently associated in at least 3 populations. As expected, rs1143679 was significantly, independently associated (EA p=1.9E-29, AA p=1.4E-8, HS p=0.001, NA p=2.6E-8). ITGAM intronic SNP rs35472514 was independently associated in AA (p=7.3E-5) and HS (p= 0.003). Though rs35472514 is significant in EA (p=6.9E-30) we could not assess independence due to strong LD. These results were confirmed. Interestingly, 2 SNPs (1910 bp) were independently associated in HS (rs62051471 p=0.001) and NA (rs12928810 p=9.7E-11). While we could not confirm that these SNPs are independently associated, they may represent a population-specific association in HS and NA. There is also a population-specific independent association for HS in ITGAX (rs13332545 p=0.0007). Conclusion: Using recently available unpublished sequence data we confirmed that ITGAM SNP rs1143679 is highly associated with SLE. We identified an independent, SLE-predisposing ITGAM SNP rs35472514, ITGAX SNP rs13332545 in HS, and potential population specific SNPs rs62051471 and rs12928810.

656W

Replication in Korean women of an association between a common variant in the breast cancer susceptibility gene, ZNF365, and a mammographic density measure that predicts breast cancer: The Healthy Twin Study. *M. Lee[1], J. Lee[1], D.H Lee[1], Y.M Song[2], K. Lee[3], J. Stone[4], J. Hopper[4], J. Sung[1].* 1) Complex Disease and Genetic Epidemiology Branch, Department of Epidemiology and Institute of Environment and Health, School of Public Helath Seoul National University, Seoul Korea; 2) Department of Family Medicine, Samsung Medical Center, and Center for Clinical Research, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Seoul Korea; 3) Department of Family Medicine, Busan Paik Hospital, Inje University College of Medicine, Busan Korea; 4) Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, University of Melbourne, Australia.

Mammographic density (MD) for age and BMI is a major risk factor for breast cancer (BC), the most frequent cancer in women worldwide. BC is one of the most rapidly increasing cancers in Asian women; age at onset of BC in East Asian women tends to be younger and mammographically `dense' breasts more common than in Western women. Although an association between MD and BC risk has yet to be formally established in Asian women, the authors have shown that MD has similar genetic variance in Korean and Caucasian women (heritability of 0.6~0.7) despite the differences in their BC and MD epidemiology. We attempted to further dissect the genetic etiology of MD by identifying candidate genetic variants and comparing them with the recent findings from genome-wide association (GWA) studies on MD and BC. The Healthy Twin study recruited twin-families from 2005 to 2011 in Korea. Clinical information, epidemiological data and MD values were collected. A total of 583 women with MD values from 314 families were included in the genetic analysis. Family-based GWA method was adopted to investigate causative variants for MD variation (FBAT and QTDT). The common variant rs10995205 in the gene ZNF365, a known susceptibility variant for BC in Caucasian women, was replicated as being associated with MD adjusted for age in Korean women ($p = 0.008$). The C allele (frequency = 80 %) was associated with an increase in MD by 32% and 42% for having one or two more C alleles, respectively. Significantly associated SNPs were also found in 13q14.11 and 13q31.1 as well as in 1p13.3 ($p< 10-6$). Additive effects of specific allele in several SNPs on MD values were investigated. Additionally possible candidate variants with p-value less than 10-5 were identified in chromosomes 2, 3, 6, and 8. This study replicated in Korean women the association between ZNF365 which has been associated with MD and BC in studies with Caucasian women. We also found variants in several regions responsible for variation in MD. These findings suggest that at least some of the genetic causes of the association between MD measures and BC risk are common to Asian and Caucasian women.

657W

Searching for modifiers of age-at-onset in familial amyloid polyneuropathy (FAP) ATTRV30M : a candidate gene approach. *D. Santos[1], T. Coelho[2], J.L. Neto[1], J. Pinto-Basto[1,3], J. Sequeiros[1,3], I. Alonso[1,3], C. Lemos[1,3], A. Sousa[1,3].* 1) UnIGENe, IBMC, Porto, Porto, Portugal; 2) Unidade Clínica de Paramiloidose, Centro Hospitalar do Porto (CHP), Porto, Portugal; 3) ICBAS, Instituto Ciências Biomédicas Abel Salazar, Univ. Porto, Portugal.

Familial amyloid polyneuropathy (FAP) is a monogenic disease caused by mutations in the transthyretin (TTR) gene (chr18q12.1). This disease is an autosomal dominant type of systemic amyloidosis and is characterized by amyloid deposition of mutated fibrillar TTR. The main clinical expression is a progressive peripheral sensori-motor and autonomic neuropathy. Of all FAP-related mutations described in the TTR gene, the most frequent is V30M which is associated with clusters in Portugal, Japan, Sweden, Majorca and Brazil, known for their remarkable differences in age-at-onset (AO). However, and most importantly, among Portuguese families although the same mutation is present, FAP shows a wide variation in AO (17-80 yrs) and even with asymptomatic carriers aged 93. This variability is also evident between generations. Also, significant differences in AO regarding gender are known in Portuguese serie, suggesting that sex hormones may have a modifier role in the disease onset, affecting the expression levels of TTR. In mice, 5-alpha-dihydrotestosterone has a stronger inducer effect in TTR expression than 17-beta-estradiol, which may explain a significantly earlier onset in men, in most populations. Genes found to be upregulated in FAP may also act as modifiers. Our aim was to study 11 candidate-genes as genetic modifiers of AO in FAP ATTR V30M. We analysed a sample of 100 FAP families with at least 2 generations affected. Through the degree of linkage disequilibrium (LD) existing between SNPs, 73 tagging SNPs were selected (with a minor allele frequency of 0.1%). The SNPs frequencies in the European population were obtained resorting to the Hapmap (http://www.hapmap.org/), using Haploview v.4.1 to plot LD patterns and to select the tagging SNPs. The SNPs in study were divided in four sets, and genotypes are being analysed in three 19-plex and one 16-plex multiplex SNaPshot® (Applied Biosystems) assays. SNaPshot® is an efficient and sensitive multiplexing SNP typing method. Results are being analyzed with the GeneMapper™ v4.0 software. The samples' genotyping for 73 SNPs is currently in an optimization stage, and a preliminary analysis is underway. Allelic or haplotypic frequencies will be compared in classic- (early-) and late-onset patients groups. We expect to find or exclude the potential role of a selected number of candidate genes as modifiers of FAP ATTR V30M, in order to better understand the mechanisms involved in AO variability.

658W

***In silico* candidate gene study for childhood tooth decay.** *J.R. Shaffer[1], Z. Zeng[1], X. Wang[2], M. Lee[2], K. T.Cuenco[2], M.M. Barmada[1], D.E. Polk[3], R.J. Weyant[3], R. Crout[4], D.W. McNeil[5], D.E. Weeks[1], E. Feingold[1], M.L. Marazita[1,2].* 1) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 2) Center for Craniofacial & Dental Genetics, Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA; 3) Department of Dental Public Health and Information Management, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA; 4) Department of Periodontics, School of Dentistry, West Virginia University, Morgantown, WV; 5) Dental Practice and Rural Health, West Virginia University, Morgantown, WV.

Dental caries (*i.e.*, tooth decay) is one of the most common chronic diseases in children and leads to several associated health and social problems including chronic pain, tooth loss, difficulty hearing, eating, and sleeping, failure to thrive, poor school performance, poor social relationships, and decreased success later in life. As a multi-factorial disease, it is the result of a complex interplay of genetic and environmental factors. Despite the high heritability (30-60%), few specific risk genes have been identified. We generated a list of 140 candidate genes on the basis of a variety of lines of evidence supporting possible roles in cariogenesis including known enamel, saliva, and taste preference genes, MMP genes, FGF genes, and WNT signaling pathway genes (all regulators of odonogenesis), and other genes implicated in linkage or association studies and/or animal models. As a complementary analysis to our genome-wide association scan (GWAS), we performed an *in silico* candidate gene study by testing 1440 SNPs in these *a priori* candidate genes for association with childhood dental caries. Two phenotypes were considered: the number of carious pit and fissure tooth surfaces (PF, *i.e.*, molar surfaces where the enamel folds inward) and smooth tooth surfaces (SM, *i.e.*, all non-PF surfaces). 596 samples of European ancestry were included for analysis of SM caries (ages 3 to 12 years); 572 samples were included for PF caries (age 4 to 14 years). All analyses were adjusted for the effects of age and age^2. Bonferroni adjustment was used to correct for multiple comparisons at the gene-level (*i.e.*, 140 genes, (= 0.00036). After correcting for multiple comparisons, the known cariogenic gene CD14 was associated with both SM caries and PF caries (rs702399, p = 0.0001 for both). The osteogenesis gene BMP6 (rs11760020, p = 0.0003) and the SHH pathway gene GLI1 (rs=3741414, p = 0.00002) were associated with SM caries only. Taken together, SNPs in *a priori* candidate genes exhibited a modest excess of association with SM caries than expected by chance alone (*i.e.*, 8.3% yielded p-values < 0.05). This finding suggests that our list may include other potential caries genes. Additional work is needed to determine the effects of these genes, if any, on dental caries. Support: DE018903, DE014899, DE021425.

659W

Goldilocks variants: prevalence and significance in association studies. *D. Waterworth[1], L. Warren[2], M. Ehm[2], M. Nelson[2], V. Mooser[1].* 1) Genetics, GlaxoSmithKline, King of Prussia, PA; 2) Genetics, GlaxoSmithKline, Research Triangle Park, NC.

As the GWAS era wanes and sequencing approaches come to the fore, questions around the utility of rare or low frequency variation arise. A few tantalizing functional “goldilocks” variants, here defined as ~0.5-2% MAF and putatively functional, have been discovered in early sequencing experiments, such as in the example of PCSK9, which proved to be informative for understanding this gene, its role in disease and the potential for pharmacotherapy. How often will goldilocks variants be present? 202 drug target genes were sequenced in 14,002 mostly Caucasian individuals with a variety of diseases and medically relevant traits. Variants were considered putatively functional if they met the criteria for any of the 3 in silico methods (SIFT, PolyPhen and PhyloP). Out of a total number of 38535 variants detected in Caucasians, 53 were goldilocks (0.14%). On a per gene basis, these 53 goldilocks variants were found in 41 genes, representing 20% of the genes sequenced. A higher percentage of non-synonymous variants were putatively functional within the goldilocks frequency range (53/105-50%) as compared to variants with greater than 2% frequency (45/154-29%). Two deeply phenotyped studies, GEMS (dyslipidemia case-control, n=1583) and Lausanne (population-based, n=2086) were analyzed for association over a range of cardiovascular and inflammatory phenotypes. Variants with MAF>0.2% were analyzed individually whilst putative functional variants including non-synonymous, nonsense, splice and readthrough variants with MAF < 0.2% were aggregated (104157 tests - 42 traits and 108329 tests - 45 traits, respectively). We evaluated how many of our top association results were with goldilocks variants. In both the GEMS and Lausanne analysis, 5 out of 11 significant associations (p<3.6e-5) were with goldilocks-type variants, whereas 0 would be expected by chance. These included ADIPOQ with adiponectin (MAF=0.015; P = 2.2E-17), EDNRA and IL-18 (MAF= 0.004; P=8.8E-8), and TACR2 and waist (MAF=0.004; P=1.5E-5). These variants exhibited particularly large effect sizes, from 0.7-2SD difference between carriers and non-carriers. In summary, goldilocks are particularly informative variants, however only ~20% of genes were found to contain putative goldilocks variants and they represent only 0.14% of the total variants generated by sequencing. Additionally, only about one third of them are imputable (r2 > 0.7), thus resequencing is often required to detect these variants.

660W

Common genetic variants underlie endometriosis and obesity-related traits. *N. Rahmioglu[1], H.R. Harris[2], S. Macgregor[3], A. Morris[1], G.W. Montgomery[3], S.A. Missmer[2], C.M. Lindgren[1], K.T. Zondervan[1], The International EndoGene Consortium, The GIANT Consortium.* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 2) Brigham and Women's Hospital and Harvard Schools of Medicine and Public Health, Boston, MA, USA; 3) Queensland Institute for Medical Research, Herston, Queensland, Australia.

Lower body mass index (BMI) and waist-hip ratio (WHR) have previously been associated with an increased risk of endometriosis in epidemiological studies. As both endometriosis and obesity-related traits are heritable, we aimed to investigate the potential commonality (pleiotropy) in genetic variants underlying their aetiology and identify common pathogenic pathways, using data from three large-scale genome-wide association studies (GWAS). The IEC GWAS included data for ~500,000 genotyped SNPs on 3,194 endometriosis cases (42.7% rAFS III/IV) and 7,060 controls from the UK and Australia; the GIANT Consortium BMI GWAS included data on ~2,400,000 genotyped and imputed SNPs for 32,387 individuals; the GIANT WHR-adjusted for BMI (WHR-adjBMI) dataset on ~2,850,000 genotyped and imputed SNPs for 190,803 individuals. All individuals in the three datasets were of European ancestry. Overlap between endometriosis signals (all cases and rAFS III/IV only) vs. BMI and WHR-adjBMI signals, effectively ~500,000 SNPs, were investigated systematically through genetic enrichment analyses using binomial exact tests and polygenic prediction modelling. The top SNP previously found to be significantly associated with increased risk of endometriosis, rs12700667 on chromosome 7 (all endo: p=2.6x10-7; rAFS III/IV: p=1.5x10-9), had a p-value of 4.4x10-5 in the WHRadjBMI GWAS and marked one of 13 reported genome-wide significant loci associated with lower WHRadjBMI. Further comparison of results showed significant enrichment for 5 SNPs (binomial p≤0.009) and similarly opposite directions of effect between the traits, including rs10919299 in close proximity to the selectin gene family, rs7521902 upstream of WNT4 (wingless-type MMTV integration site family), rs1250248 in FN1 (fibronectin1), and rs6556301 in FGFR4 (fibroblast growth factor receptor). Enrichment between genetic variants involved in endometriosis and BMI was less pronounced (3 SNPs with binomial p≤0.01). Here we identify support for pleiotropy in genetic variants underlying endometriosis and obesity-related traits, and pinpoint several novel genetic loci with plausible biological candidacy. Ongoing work, involves replication and regression modeling of these pleiotropic loci in the Nurses Health Study on both endometriosis and obesity-related traits to investigate their correlation and the potential influence of covariates further.

661W

Genes associated with cephalometric variation in the Tricho-Dento-Osseous syndrome. *M.E. Cooper[1], M. Govil[1], M.A. Torain[2], T.C. Hart[3], M.L. Marazita[1], J.T. Wright[2].* 1) Center for Craniofacial and Dental Genetics, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA; 2) Dept. of Pediatric Dentistry, University of North Carolina, Chapel Hill, NC; 3) Department of Periodontics, University of Illinois at Chicago, Chicago, IL.

Individuals with the extremely rare tricho-dento-osseous syndrome (TDO) exhibit marked variation in their hair, tooth enamel thickness and progressive bone density. TDO syndrome results from a 4G deletion in the DLX3 gene on 17q21, a transcription factor that is important in the development of many different tissues. The objective of this study is to evaluate genotypes contributing to observed variations in the TDO bone density phenotype in TDO affected individuals. **Methods**: Twenty-two Cephalometric measures were evaluated from radiographs. These measures were then normalized using age-by-gender published standards if available, otherwise the individual's age and gender. Thirty-seven TDO individuals with 2-22 Cephalometric measures were successfully genotyped using 10K SNP Chips (Affymetrix USA). Simple linear regression, as implemented in PLINK, was used to test for association. The analysis was repeated with a subset of 18 unrelated or very distantly related TDO individuals. **Results**: The full sample had 73 SNPS from 22 chromosomes for 18 phenotypes with p-values <E-04. Six SNPs achieved p=E-06. Three SNPS with p=3.55E-06 were associated with the ANS_Me (Anterior vertical mandibular height) (chr1_rs953653, chr3_rs1398735, chr14_ rs728497 in RGS6 gene). Six SNPS were associated with multiple cranial measures at the p<E-05 level; four SNP pairs involve the Go landmark (gonion- posterior mandible) extending to S (sella) or to Ar (articulare-point at the mandible ramus) ï¿½ essentially posterior mandibular heights. SNP chr2-rs699662 is associated with S-GO(Posterior mandibular vertical position) as well as Co-Gn (total mandibular length). SNP chr3-rs1391962 is associated with 2 facial depth measures: (S-N or anterior cranial base)and N-Ba. The cranial measure Glabella had 3 SNPs associated at the p=E-06 level and another 5 SNPS at the p=E-05 level. The 18-person subset had 28 SNPs associated with the N_S_Ar angle (p=4E-05, 9 individuals); but this is very possibly an inflated result as the regression had to be adjusted using the subjects age and sex as no published data for children less than 18 years existed. **Conclusions**: Despite the small sample size, several associations approached Bonferroni significance levels of p ≤ 5E-06, providing evidence for a relationship between TDO cranial densities and particular SNPs and potentially several genes of interest that may be important in bone formation. NIH grant # R01-DE015196, K99DE018085, R00DE018085.

662W

Clinical and Genetic Epidemiology of Bardet-Biedl syndrome in Tunisia. *O. MHAMDI[1], I. Ouertani[1,2], H. CHAABOUNI BOUHAMED[1,2].* 1) Department of Human Genetics, Faculty of Medicine Tunis, Tunis, Tunisia; 2) Department of congenital and Hereditary diseases, Charles Nicolle hospital Tunis. Tunisia.

Abstract Bardet-Biedl syndrome is a ciliopathy causing multivisceral abnormalities. This disease is defined by a combination of cardinal features: obesity, post axial polydactyly, hypogonadism, intellectual disabilities, pigmentary retinopathy and renal deficiency. The prevalence of BBS in Europe ranges from 1/125000 to 1/160000. In some isolated populations over the world, it was estimated 1 in 17000 in Bedouins of Kuwait and 1 in 18000 in Newfoundland. We report in the present study the first epidemiological study of BBS in North Africa. Aim: The aim of this study was to evaluate the epidemiological criteria and estimating the current prevalence of BBS in Tunisia. Methods: A three pages of clinical analysis index card requesting details of the patient's birth and delivery, age at diagnosis. Information's was sought including consanguinity, geographic origin, height, weight, presence or absence of limb abnormalities, presence of rod/cone dystrophy, renal abnormalities, development delay, hypogonadism in males and other minor criteria including learning disabilities, disorders of speech language, ataxia and other medicals problems. Results: From 1984 to 2009, 46 Tunisian families including 67 affected members were diagnosed as BBS. Consanguinity was highly in our group 93.47 %. The overall minimum prevalence of BBS in Tunisia was found to be approximately 1 in 156 000. This frequency increases in the north, 1 in 87000, which is about 2 times higher than the figure from the overall country. In spite of limited study, our report showed that BBS was an uncommon disease in Tunisia and this result may be reflecting the actual frequency of BBS in North Africa where the disease seems uncommon.

663W

A Comprehensive Evaluation of SNP Weighting Schemes for the Analysis of Uncommon Genetic Variants. *A. Byrnes[1], Y. Li[1, 2], M. Li[3].* 1) Department of Biostatistics, University of North Carolina, Chapel Hill, NC; 2) Department of Genetics, University of North Carolina, Chapel Hill, NC; 3) Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, PA.

Genome Wide Association Studies (GWAS) have, and continue to, generate a wealth of genomic data on millions of individuals. As this technology becomes less costly, larger studies become possible thus increasing the power to detect even variants of low frequency or small effect size. Simultaneously, massively parallel sequencing is generating an unprecedented amount of data, including direct measurements of uncommon genetic variants. These data provide an opportunity to more systematically examine the effects uncommon variants exert on complex human diseases and traits. Several methods currently exist to aggregate information across SNPs to implicate regions in which uncommon variants contribute to phenotypic variation. However, there is little consensus on which method is most effective. Two of the most important determinants are the weighting scheme adopted when aggregating information across SNPs and whether a method is robust when both risk and protective variants are present. Here we present a comprehensive evaluation of multiple weighting schemes through a series of simulations intended to mimic large case-control studies genotyped with a current-era GWAS chip (e.g. the Affymetrix Axiom and Illumina HumanOmni chips). We evaluate data-independent and data-dependent methods, as well as a novel method that constructs weights by combining information from regression coefficients, minor allele frequency, and functional annotation. We examine the performance of each of these methods in simulations where there are both single and multiple contributing uncommon variants. In addition, we investigate situations in which variants contribute in the same direction and those in which variants contribute in different directions. The results provide practical guidelines regarding the choice of weighting scheme under a wide range of scenarios so that current era association data can be utilized to implicate regions where uncommon variants may play an important role.

664W

Association Testing Combining Family-Based and Case-Control Designs. *J.E. Cerise, W.C.L. Stewart.* Div. Stat. Genetics, Biostat, Sch. Pub. Health, & Psychiatry Dept., Columbia U., New York, NY.

Many genetic studies use case-control data as well as overlapping family data. Each contains information about association that the other lacks but, because these designs share an overlapping set of patients, the family-based and case-control association tests are correlated. Researchers often ignore the correlation, reporting each p-value separately. But this approach is inefficient and the overall evidence for association is often unclear. We present an efficient test that summarizes all the evidence for association by scaling the weighted average of the family-based and case-control test statistics; the weights vary in proportion to the corresponding sample size. In the simplest setting, our test is the standardized weighted average of the transmission disequilibrium test and the two-sample test of proportions (i.e. allele frequencies). The scaling factor, estimated from the parametric bootstrap, depends on the covariance between the family-based and case-control test statistics. Due to its increased efficiency, our combined test has notably more power to detect association than either test alone. Another attractive feature is that the same bootstrap procedure can provide estimates of the critical value for testing. This is useful if either the family-based or case-control test is non-normal (e.g. chi-squared). Also, if controls are unavailable, an appropriate reference data set may be used. To assess the power of our combined test, we generated data at a single SNP using different genetic models, minor allele frequencies (MAFs), and levels of linkage disequilibrium (LD) between the SNP and a simulated disease locus. The phenotypes and family structures were taken from a real type 1 diabetes study. There were 325 controls, 323 disjoint cases, and 108 overlapping cases shared between the family-based and case-control designs. When the MAF, LD, gene frequency, and penetrance were 0.4, 0.3, 0.1, and 0.1, respectively, the family-based test had 33% power; the case-control test had 25% power; and our combined test had 58% power to detect an association. Other scenarios showed similar gains in power. In summary, we've developed a general framework for detecting association that integrates case-control and family data to increase power. Our test is implemented in the software package EAGLET, and is freely available for download from the web. This research was supported in part by the National Institutes of Health grants MH48858, MH65213, and NS27941.

665W

Evaluating type 1 error in large pedigree analyses. *L.N. D'Aoust[1], A.C. Cummings[1], E. Torstenson[1], M.F. Davis[1], W.K. Scott[2], M.A. Pericak-Vance[2], M.D. Ritchie[1], W.S. Bush[1], J.L. Haines[1].* 1) CHGR, Vanderbilt University, Nashville, TN; 2) Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami, Miami, FL.

Studying population isolates with large, complex pedigrees has many advantages for discovering genetic susceptibility loci; however, analyses can be quite complicated. Association tests need to be corrected for relatedness, and linkage analysis requires subdividing and simplifying the pedigree structure. The Modified Quasi-Likelihood Score (MQLS) test developed by Thornton and McPeek corrects for all pedigree relationships while testing for association with a binary trait, but simulations to evaluate type 1 error rates using pedigree structures as large and as complex as pedigrees in the Amish population have not been published. Additionally, the effect of pedigree splitting on linkage type 1 error rates also has not been evaluated extensively. We have extended GenomeSIMLA to simulate SNP data in complex pedigree structures based on template pedigrees to generate the same structure and distribution of sampled individuals. Using 1000 simulated pedigrees (124 null SNPs each) with the same structure as an Amish pedigree, we have evaluated type 1 error rates for MQLS and for 2-point and multipoint analyses using PedCut for pedigree splitting and Merlin for linkage analyses. We see no inflated type 1 error rate (5.06% for p-values ≤ 0.05 and 0.13% for a p-values ≤0.001) when using MQLS. Using PedCut-derived sub-pedigrees for linkage analysis, we see low type 1 error rates of 0.01% and 0.02% under dominant and recessive two-point models using an HLOD threshold of 3.0. Under dominant and recessive multipoint linkage models, we see type 1 error rates of 2.50% for both models using an HLOD threshold of 3.0. The average peak HLOD scores under the dominant and recessive multipoint models were 0.66 and 0.57, respectively. We also ran MQLS on the sub-pedigrees, and we see no change in type 1 error compared to running MQLS on the entire pedigree. Therefore, pedigree splitting does not seem to inflate type 1 error in large complex pedigrees such as those seen in the Amish population.

666W

A fast algorithm to optimize SNP prioritization for gene-gene and gene-environment interactions. *W.Q Deng, G. Paré*. Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, Ontario, Canada.

Detection of gene-environment interactions using exhaustive search necessarily raises the multiple hypothesis problem. While frequently used to control for experiment-wise type I error, Bonferroni correction is overly conservative and results in reduced statistical power. We have previously shown that prioritizing SNPs on the basis of heterogeneity in quantitative trait variance per genotype leads to increased power to detect genetic interactions. Our proposed method, Variance Prioritization (VP), selects SNPs having significant heterogeneity in variance per genotype using a pre-determined P-value threshold. We now suggest prioritizing SNPs individually such that the optimal heterogeneity of variance P-value is determined for each SNP. The large number of SNPs in genome-wide studies calls for a fast algorithm to output the optimal prioritization threshold for each SNP. In this report, we present such an algorithm, the Gene Environment Wide Interaction Search Threshold (GEWIST), and show that the use of GEWIST will increase power under a variety of interaction scenarios. Furthermore, by integrating over possible interaction effect sizes, we provide a framework to optimize prioritization in situations where interactions are *a priori* unknown.

667W

Interpreting Meta-Analyses of Genome-Wide Association Studies. *B. Han[1], E. Eskin[1,2].* 1) Department of Computer Science, University of California, Los Angeles, Los Angeles, CA; 2) Department of Human Genetics, University of California, Los Angeles, Los Angeles, CA.

Meta-analysis is an increasingly popular tool for combining multiple genome-wide association studies in a single analysis to identify associations with small effect sizes. The effect sizes between studies in a meta-analysis may differ and this difference, or heterogeneity, can be caused by many factors. If heterogeneity is observed in the results of a meta-analysis, interpreting the cause of heterogeneity is important because correct interpretation can lead us to a better understanding of the disease and an effective design of a replication study. However, interpreting heterogeneous results is difficult. The traditional approach examining the association p-values of the studies does not effectively predict if the effect exists in each study and utilizes only within-study information. In this paper, we propose a framework facilitating the interpretation of the results of a meta-analysis. Our framework is based on a new statistic representing the posterior probability that the effect exists in each study, which is estimated utilizing cross-study information. Simulations and application to the real data show that our framework effectively segregates the studies predicted to have an effect, the studies predicted to not have an effect, and the ambiguous studies that are underpowered. In addition to helping interpretation, the new framework also allows us to develop a new association testing procedure taking into account the existence of effect, which is shown to improve p-values at all six associated loci showing high heterogeneity in the Crohns disease data.

668W

Novel susceptibility loci for Crohn's disease, rheumatoid arthritis and type 1 diabetes identified using a data mining approach designed to detect weak associations. *G.E. Hoffman[1], B.A. Logsdon[1,2], J.G. Mezey[1,3].* 1) Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY; 2) Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA; 3) Department of Genetic Medicine, Weill Cornell Medical College, NY, NY.

There has been mounting interest in identifying weak associations in genome-wide association studies (GWAS) using multiple-locus methods that incorporate tens to hundreds of thousands of genetic markers in a single statistical model using the widely applied framework of regularized regression. Yet high computational costs and concerns about performance have prevented widespread adoption by the GWAS community.

We applied a class of highly scalable regularized multiple-locus methods to mine datasets from the original Wellcome Trust Case Control Consortium (WTCCC) to identify new disease susceptibility loci. Our likelihood methods incorporated four penalties suggested to date for regularized regression analysis of GWAS data and we additionally implemented a variational Bayes algorithm that incorporates a statistically justified mixture penalty. Our algorithmic and implementation improvements allow us to simultaneously analyze hundreds of thousands of genetic markers and overcome the previous computational limitations that have prevented wider adoption of regularized multiple-locus methods. We also provide a novel method for assessing significance and the data-adaptive tuning of the strength of the penalties to facilitate substantial gains in the statistical power. We have made these implementations available in a user-friendly software package.

Our re-analysis of the WTCCC identified associations for Crohn's disease, rheumatoid arthritis and type 1 diabetes. These associations re-capitulated all of the non-marginal associations discovered in the original WTCCC analysis and also identified additional susceptibility loci that were too weak to be significant in the original analysis. Of these latter associations, over 25 were identified in an independent GWAS of the same or etiologically similar phenotype. Another 20 novel associations were too weak to be detected by standard single marker methods, yet they implicate loci known to play a role in disease etiology or are known to function in a relevant biological pathway. We also identified additional novel loci with no previously reported link to these autoimmune diseases. These results establish the power of multiple-locus methods to extract additional biologically relevant information from existing GWAS datasets as well as emerging next-generation sequencing datasets.

669W

A combined functional annotation score for non-synonymous variants. *M.C. Lopes[1,2], C. Joyce[1], F. Cunningham[3], S.L. John[4], J. Asimit[1], E. Zeggini[1].* 1) Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 3) European Bioinformatics Institute, Hinxton, Cambridge, United Kingdom; 4) Pfizer, Groton, Connecticut, United States.

Next generation sequencing has opened the possibility of large-scale sequence-based disease association studies. A major challenge in interpreting whole-exome data is predicting which of the discovered variants are deleterious or neutral. To address this question, in the absence of further experimental research, several functional-annotation tools focusing on the analysis of non-synonymous coding variants (ns variants) have been implemented. We have developed a score called **C**ombined **A**nnotation sco**R**ing to**OL** (CAROL), which combines information from two bioinformatics tools: PolyPhen-2 and SIFT, in order to improve the prediction of the effect of ns variants. We used a weighted-*Z* method, which combines the ratio of the probability of the variant amino acid ($P_{(v,i)}$) to the probability of the wild-type amino acid ($P_{(w,i)}$) occurring at each position (*i*) from protein alignments. The algorithm used for the weighting method equals the inverse of the log-likelihood probability of substituting wild-type amino acid (*w*) for the variant amino acid (*v*). We defined two dataset pairs to train and test CAROL using information from the dbSNP, `HGMD-PUBLIC' and 1000 Genomes Project databases. The training pair comprises a total of 1000 positive control (disease-causing) and 5,100 negative control (non-disease-causing) variants. The test pair consists of 1,944 positive and 10,206 negative controls. CAROL was found to have good predictive power for the effect of non synonymous variants, and had a higher predictive accuracy than each individual annotation tool (Polyphen-2 and SIFT). In addition to this, CAROL had the distinct advantage of higher coverage. The combination of annotation tools can help improve automated prediction of whole-genome/exome non-synonymous variant functional consequences.

670W

Comparison of genome-wide association genotyping products for imputation of low-frequency and rare variants. *A. Mahajan[1], B. Howie[2], C. Fuchsberger[3], R.D. Pearson[1], K.J. Gaulton[1], N. Robertson[1], N.W. Rayner[1,4], Y. Chen[1], I. Prokopenko[1,4], M.I. McCarthy[1,4], A.P. Morris[1] on behalf of the GoT2D Consortium.* 1) WTCHG, University of Oxford, Oxford, United Kingdom; 2) Department of Human Genetics, University of Chicago, Chicago, USA; 3) Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, MI, USA; 4) OCDEM, University of Oxford, Oxford, UK.

Imputation methods are widely used in the analysis of genome-wide association studies (GWAS). These methods predict genotypes at untyped variants present on high-density reference panels, like those made available through the 1000 Genomes project. For any reference panel, the success of genotype imputation at an untyped variant depends crucially on: (i) the "scaffold" of variants typed in the GWAS samples; and (ii) the minor allele frequency (MAF) of the variant. We undertook imputation in 314 type 2 diabetes (T2D) cases genotyped using two GWAS scaffolds: (i) the Affymetrix GeneChip 500K Mapping Array Set (A500K); and (ii) the Illumina Omni2.5 BeadChip (O2.5M). Imputation was performed using IMPUTEv2 and the 1000 Genomes European (EUR) reference panel (August 2010 release) across 27 established T2D susceptibility loci (6.2Mb of the genome). For each scaffold, we evaluated the proportion of variants, $\hat{\beta}$, not genotyped on either chip, that were "well" imputed (info $\geq$ 0.4). There was little difference in imputation quality between scaffolds for common variants (MAF $\geq$ 5%; A500K $\hat{\beta}$=0.912, O2.5M $\hat{\beta}$=0.941). However, there were substantial improvements in quality using the O2.5M scaffold for low-frequency variants (1%$\leq$MAF<5%; A500K $\hat{\beta}$=0.686, O2.5M $\hat{\beta}$=0.879; 28% improvement) and rare variants (MAF<1%; A500K $\hat{\beta}$=0.150, O2.5M $\hat{\beta}$=0.245, 63% improvement). We assessed concordance between "predicted" genotypes (posterior probability $\geq$ 90%) at well imputed variants with "truth" genotypes in 108 overlapping samples sequenced by the UK component of the GoT2D Consortium. However, our analysis showed no clear difference in concordance rates between scaffolds. Our empirical observations were confirmed through simulations using the EUR reference panel for chromosome 22. Our simulations demonstrated that imputation from the O2.5M scaffold substantially increased the posterior probability of the correct genotype call for common variants (A500K 91.6%, O2.5M 96.2%). This increase was most noticeable when the correct genotype was heterozygous (A500K 86.3%, O2.5M 93.3%) or homozygous for the minor allele (A500K 67.9%, O2.5M 75.0%). The results of our study highlight that a dense GWAS scaffold, such as O2.5M, provides improved quality of imputation for low-frequency variants, and increased accuracy in genotype prediction at well imputed common variants. However, more costly re-sequencing experiments will be required to recover high-quality genotype calls at rare variants.

671W

ParaHaplo 3.0: A program package for imputation and a haplotype-based whole-genome association study using hybrid parallel computing. *K. Misawa[1], N. Kamatani[2].* 1) Research Program for Computational Sciences, Riken, Yokohama, Kanagawa, Japan; 2) Center for Genomic Medicine, RIken, Yokohama, Kanagawa, Japan.

Background: Use of missing genotype imputations and haplotype reconstructions are valuable in genome-wide association studies (GWASs). By modeling the patterns of linkage disequilibrium in a reference panel, genotypes not directly measured in the study samples can be imputed and used for GWASs. Since millions of single nucleotide polymorphisms need to be imputed in a GWAS, faster methods for genotype imputation and haplotype reconstruction are required.

Results: We developed a program package for parallel computation of genotype imputation and haplotype reconstruction. Our program package, ParaHaplo 3.0, is intended for use in workstation clusters using the Intel Message Passing Interface. We compared the performance of ParaHaplo 3.0 on the Japanese in Tokyo, Japan and Han Chinese in Beijing, and Chinese in the HapMap dataset. A parallel version of ParaHaplo 3.0 can conduct genotype imputation 20 times faster than a non-parallel version of ParaHaplo.

Conclusions: ParaHaplo 3.0 is an invaluable tool for conducting haplotype-based GWASs. The need for faster genotype imputation and haplotype reconstruction using parallel computing will become increasingly important as the data sizes of such projects continue to increase. ParaHaplo executable binaries and program sources are available at http://en.sourceforge.jp/projects/parallelgwas/releases/.

672W

Privacy preserving meta-analysis of sequencing-based association studies. *I. Pe'er, R. Banerjee, S. Zafer.* Dept Comp. Sci, Columbia Univ, New York, NY.

Human genetics recently transitioned from GWAS to studies based on NGS data. For GWAS, small effects dictated large sample sizes, typically made possible through meta-analysis by exchanging summary statistics across consortia. NGS studies groupwise-test for association of multiple potentially-causal alleles along each gene. They are subject to similar power constraints and therefore likely to resort to meta-analysis as well. The problem arises when considering privacy of the genetic information during the data-exchange process. Many scoring schemes for NGS association rely on the frequency of each variant, and therefore standard meta-analysis would require exchanging the identity of the sequenced variant. As such variants are often rare, potentially revealing the identity of their carriers, such disclosure jeopardizes privacy. We tackle the challenge of tallying frequency counts of rare, sequenced alleles between consortium members, for meta-analysis of sequencing data without disclosing the allele identity and counts, thereby protecting sample identity. This apparent paradoxical exchange of information is achieved through cryptographic means. The key idea is that parties encrypt identity of genes and variants. When they transfer information about frequency counts in cases and controls, the exchanged data does not convey the identity of a mutation, therefore does not expose carrier identity. The parties use identical encryption key, thus identical variants will be encrypted identically. One could therefore sum up the counts for identical variants, without knowing the identity of the alleles whose counts are being tallied. We have developed a protocol for such meta-analysis of genomewide sequencing data, scoring association for rare variants pooled per gene. The exchange relies on a 3rd party, trusted to follow the protocol although not trusted to learn about the raw data. The 3rd party only sums up counts of encrypted variants within encrypted-name genes and computes the per-gene score without learning about the sequenced variants. For some groupwise testing methods, the counts may also be encrypted, with the 3rd party tallying counts through partially homeomorphic encryption, without knowing the counts themselves. We show applicability of this method to publicly available exome-sequencing data from multiple studies, simulating phenpotypic information for meta-analysis. The protocol is publicly available as open source.

673W

Hardy-Weinberg Equlibrium in Triad Designs. *M. Rao[1], S. Venkatesan[2], S. Kasala[3].* 1) Environmental Hlth, Univ Cincinnati, Cincinnati, OH; 2) Department of Biomedical Engineering, Univ Cincinnati, OH; 3) Department of Mathematics and Statistics, Univ North Carolina, Wilmington, NC.

The focus of a triad design is identifying a bi-allelic SNP associated with a disease of interest. The case is one with the disease. The parents of the case are inducted into the study thus constituting a triad. All three members of the triad are genotyped at the SNP. The data consist of a random sample of such triads. The goal of this research is to test Hardy-Weinberg Equilibrium of the population at the SNP. A large sample chi-squared test can be used to test the validity of Hardy-Weinberg Equilibrium. There is no direct exact test a la Fisher available. We develop an exact test in a novel way using algebraic statistics methodology and invoking Markov Chain Monte Carlo algorithm. The key idea is to enumerate all possible triad data sets (fiber) with exactly the same allele frequencies provided by the given triad data set. Algebraic statistics methodology provides a way to get a handle on the fiber and a minimal markov basis provides a deep insight into the fiber.

674W

KELVIZ: A Graphing and Annotating Tool for Statistical Evidence in Human Genetics. *S. Seok, B. Nouanesengsy, V. Vieland.* BCMM, Nationwide Children's Hospital, Columbus, OH.

KELVIZ is a custom built graphing application designed to plot output from KELVIN, a software package implementing the PPL statistical framework for linkage and/or association analysis. The program can also be used to graph other statistics, such as p-values, LODs, etc. Current options in KELVIZ include plots over the genome, groups of chromosomes, or specified regions, while allowing for overlap of multiple sets of results. Additional features include a flexible set of options for custom annotations, either input from files or manually edited, zooming, adding comments, and overlaying marker maps. A variety of options for displaying and saving graphs are provided. Graphs can also be saved in a custom file format, for reuse and further editing at a later data. This feature, similar to the “FIG” file used in MATLAB, efficiently facilitates collaboration efforts for graph creation. KELVIZ also provides a non-GUI command-line interface to expedite and automate the generation of graphs. KELVIZ is a graphical user interface (GUI) program written in Python and it uses the Matplotlib graphing library. It is a cross-platform application that has been shown to work on Windows, Mac, and Linux.

675W

A tool to test for functional enrichment of GWAS hits. *C. Tang, M. Ferreira.* Genetic Epidemiology, Queensland Inst Med Research, Brisbane, Australia.

To facilitate the interpretation of GWAS results, we developed a tool that integrates and annotates the genomic, transcriptional and epigenetic properties of genetic variants (e.g. protein-coding, conservation, CpG islands, eQTL and transcriptional factor binding site) from various biological databases. Our software allows users to upload summary statistics from a GWAS and not only pinpoint the functionally important variants amongst GWAS hits but also to directly test various biological hypotheses by performing functional enrichment analysis. It also incorporates findings from recent, large published GWAS to test for shared genetic components across different phenotypes. We illustrate the applicability of this approach through the analysis of several published GWAS results.

676W

Sex specific prediction of genomic segments shared IBD by family members, with applications in disease mapping by family based sequencing. *M. Vigeland.* Department of Medical Genetics, Oslo University Hospital, Oslo, Norway.

We present an R package - IBDsim - for simulation and statistical processing of segments shared identically by descent (IBD) by pedigree members. The package provides flexible IBD simulation imposing few restrictions on the user, as well as offering specialized functionality for family based disease mapping. The recent advances in sequencing technology have made possible mapping of disease-causing mutations in families too small for traditional linkage analysis. By comparing sequence data from several family members, genomic regions not compatible with the observed segregation pattern are excluded to reduce the number of false positive variants. A well known example of this approach is autozygosity mapping, in which non-homozygous regions of inbred patients are excluded in search for recessive disease loci. Power assessment in exclusion mapping involves predicting how much of the genome that can be excluded. While expected values are easy to compute from Mendel's laws, one can go much further using simulations. In particular the distributions of the number and size of random IBD segments, and the size of the disease segment, are important tools both for power computations and in evaluating identified segments. Compared with existing IBD predicting software, IBDsim has several advantages. Building on an inhomogeneous χ^2 model for recombination, it incorporates up-to-date sex specific recombination maps of the human genome (Decode, 2010). The sizes of IBD regions are reported in physical length rather than genetic, giving researchers a direct indication of how much sequence will remain after the exclusion process. The use of male/female specific maps instead of averaged maps has a large impact on the distribution of IBD segments. For example, in a family with one parent and three offspring affected by an autosomal dominant disease, the expected length of the shared disease-containing haplotype is 65 Mb if the father is the affected parent, but only 37 Mb if the mother is affected. By taking advantage of the R environment, users of IBDsim can investigate the simulated IBD distributions in great detail. As an example we take a modern look at some classical results concerning relatedness, like the probability that two related individuals have no segments in common IBD. While the theoretical results agree well with the simulations for average pedigrees, the probabilities are shown to be highly dependent on the gender distributions within the pedigree.

677W

An algorithm for splitting and trimming large pedigrees. *Q. Yang[1,2], M. Chen[1,2,3].* 1) Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA; 2) The NHLBI's Framingham Heart Study, Framingham, MA, USA; 3) Department of Neurology, Boston University School of Medicine, Boston, MA, USA.

In genome-wide association studies (GWAS) with family data, it is possible to impute missing genotypes for un-genotyped individuals who have genotyped relatives. Including these additional samples with phenotypes and imputed genotypes in GWAS can substantially increase the power to detect an association. Large pedigrees however pose particular difficulty because the computation load in imputation increases exponentially with family size. To enable using as much familial genotype information as possible in imputing genotypes for each un-genotyped individual given computational limitations, we propose an algorithm for splitting and trimming large pedigrees into smaller pedigrees that maximize the genotyped relatives for each un-genotyped individual while keeping the new pedigree size within user-specified limit. The algorithm is described as follows: First we form clusters of related un-genotyped individuals within a pedigree. We then include genotyped blood relatives of each cluster member to construct a sub-pedigree for each cluster. If the bit size of the sub-pedigree is not close to the user-specified limit, we further include genotyped blood relatives of spouse and genotyped offspring of each cluster member. If the bit size of a sub-pedigree is greater than the user specified limit, we compute the numbers of closest, 2nd, 3rd, 4th, and 5th closest cluster members as scores for each genotyped person. From the bottom level of the sub-pedigree, we remove persons by the rank of the scores and if the bit size is still greater than the limit, we move up to the next level. The procedure repeats until the bit size is less than the limit. If the bit size is not close to the limit, we add back genotyped persons so that the bit size is as close to the limit as possible. We apply our algorithm to the Framingham Heart Study sample. Among about 1,200 selected un- or ill- genotyped persons, the products of our algorithm, the split sub-pedigrees keep 99.7% and 82% of their genotyped 1st and 2nd degree relatives, respectively. These split sub-pedigrees are used to impute the genotypes of the 1,200 persons which increases the sample size and thus the statistical power to identify genetic variants. We will use a GWAS to compare the results with and without using the imputed sample.

678W

Copy number variations detection using family data. *J. Chu[1], I. Ionita-Laza[2], A. Rogers[1], K. Darvishi[1], R. Mills[1], G. Maier[1], B. Klanderman[1], C. Lee[1], B. Raby[1].* 1) Brigham & Women's Hosp, Boston, MA; 2) Columbia University, New York, NY.

In recent years there has been a growing interest in the role of copy number variations (CNV) in genetic diseases, which leads to the rapid development in technologies and statistical methods devoted to detection in CNVs from array data. To establish association between CNVs and diseases one would still have to overcome the noisy nature of the data, which remains a challenging problem. To help address the data quality issue we introduce a statistical framework for the intensity-based array data that takes into account the family information in detection of the CNVs. The method is based on the traditional methods for SNP genotyping in which the distribution of intensity data is modeled with a Gaussian mixture model and the samples are clustered into different copy number groups. In our method we perform the CNV calling for the whole family simultaneously, incorporating family information to reduce CNVs that are incompatible with Mendelian inheritance while still allowing de-novo CNVs. We applied this method to 403 asthmatic trios who were genotyped using a 180K Agilent CNV array. We found that our method substantially reduced Mendelian inconsistency in this dataset. In particular, in 50 CNV regions near or overlapped with an asthma candidate gene our method eliminated 68% of the CNVs calls with Mendelian inconsistencies, which are likely false positive. In conclusion, we have demonstrated that the use of family information can improve the quality of CNV calling and hopefully give more powerful association test of CNVs.

679W

Cheek swabs, SNP chips, and CNVs: Assessing the quality of copy number variant calls generated with subject-collected mail-in buccal brush DNA samples on a high-density genotyping microarray. *S.W. Erickson, S.L. MacLeod, C.A. Hobbs.* Pediatrics, Univ Ark Med Sci, Little Rock, AR.

Multiple investigators have established the feasibility of using buccal cytobrush samples to genotype single nucleotide polymorphisms (SNPs) with high-density genome-wide microarrays, but there is currently no consensus on the accuracy of copy number variants (CNVs) inferred from these data. To demonstrate the quality of CNVs generated from these samples and instruments, we evaluated the concordance of CNV calls from DNA extracted from blood to DNA extracted from subject-collected mail-in buccal cytobrushes (i.e. cheek swabs). The Illumina Human660W-Quad BeadChip was used to determine CNVs of 39 Arkansas participants in the National Birth Defects Prevention Study (NBDPS) who provided both whole blood and buccal brush DNA samples. Analysis of these genotypes revealed a 99.9% concordance rate of SNP calls in the 39 blood-buccal pairs. From the same dataset, we performed a similar analysis of CNVs. Each of the 78 samples was independently segmented into regions of like copy number using the Optimal Segmentation algorithm of Golden Helix SNP & Variation Suite 7. Across 640,663 loci on 22 autosomal chromosomes, segment-mean Log R ratios had an average correlation of 0.90 between blood-buccal pairs, while the correlation between unrelated pairs averaged 0.32. An independent analysis using the QuantiSNP algorithm produced average correlations of 0.94 between blood-buccal pairs versus 0.33 between unrelated pairs. These results show that DNA derived from subject-collected mail-in buccal-brush samples can be used for genome-wide scans of both SNPs and CNVs, and that high rates of CNV concordance were achieved whether using a change-point-based algorithm or one based on a hidden Markov model (HMM).

680W

A Likelihood-Based Framework for Association Analysis of Allele-Specific Copy Numbers. *Y.J. Hu[1], W. Sun[2], D.Y. Lin[2].* 1) Biostatistics, Emory University, Atlanta, GA; 2) Biostatistics, University of North Carolina, Chapel Hill, NC.

Copy number variants (CNVs) and single nucleotide polymorphisms (SNPs) co-exist throughout the human genome and jointly contribute to phenotypic variations. Thus, it is desirable to consider both types of variations, as characterized by allele-specific copy numbers (ASCNs), in association studies of complex human diseases. Current SNP genotyping technologies can simultaneously capture the CNV and SNP information. The common practice of first calling ASCNs from the SNP array data and then using the ASCN calls in downstream association analysis has important limitations. First, the association analysis may not be robust to the differential errors between cases and controls caused by the differences in DNA quality or handling. Second, the phenotypic information is not used in the calling process and the uncertainties in the ASCN calls are ignored. We present a general framework for the integrated analysis of CNVs and SNPs in association studies, including analysis of total copy numbers as a special case. Our approach combines the ASCN calling and association analysis into a single step while allowing for differential errors. We construct likelihood functions that properly account for case-control sampling and measurement errors. We establish the asymptotic properties of the maximum likelihood estimators and develop EM algorithms to implement the proposed inference procedures. The advantages of the proposed methods over the existing ones are demonstrated through realistic simulation studies and an application to a genome-wide association study of schizophrenia. Extensions to next-generation sequencing data are discussed.

681W

Detection of Copy Number Variation in Whole Exome Sequencing Data Using Read-Depth of Coverage. *J. Lihm[1,2,7], V. Makarov[1,2], T. O'Grady[6], G. Cai[1,2], N. Takahashi[2], E. Parkhomenko[1,2], M. Gazdoiu[2], J. Buxbaum[1,2,3,4,5], S. Yoon[1,2].* 1) The Seaver Autism Center for Research and Treatment, Mount Sinai School of Medicine, New York, NY; 2) Department of Psychiatry, Mount Sinai School of Medicine, New York, NY; 3) Department of Neuroscience, Mount Sinai School of Medicine, New York, NY; 4) Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 5) The Friedman Brain Institute, Mount Sinai School of Medicine, New York, NY; 6) Gustave L. and Janet W. Levy Library, Mount Sinai School of Medicine, New York, NY; 7) Stony Brook University, Stony Brook, NY.

In order to elucidate genetic mechanisms of disease in a cost effective way, a large number of individuals are being sequenced in various studies using whole-exome sequencing (WES) technology, which enables researchers to perform a survey of the coding regions in a thorough and exhaustive search. To detect copy number variation (CNV) in WES data, we developed a robust and scalable method based on read-depth of coverage. Our method relies on read-depth calculation using 10bp-non-overlapping windows with separation of uniquely and multiply mapped reads, GC-correction, normalization and segmentation of the log-ratio of read-depths of a sample to read-depths of the selected reference. For a data-driven approach to optimizing the callset, we used as comparison validated CNV calls from individuals who were whole genome-sequenced in high coverage in the 1000 Genomes Project. Our analyses, and validation studies, show that both common and rare CNV can be captured by calls derived from our algorithm. More generally, calls based on read-depth can complement and/or confirm calls from other methods based on such information as paired-end and split-read data, for the comprehensive analysis of WES data. In a disease association study, the CNV calls as well as the log-ratio itself can be used for comparing cases to controls regardless of the size of samples used in the study. The application code “exome-cnv” is freely available for academic use from http://exome-cnv.sourceforge.net/.

682W

Detecting chromosomal inversion polymorphisms using principal components analysis. *J. Ma, C. Amos.* University of Texas, M.D. Anderson Cancer Center, Houston, TX.

An inversion is a chromosome rearrangement in which a segment of a chromosome is reversed. In contrast to other types of structural variations, fewer inversion polymorphisms have been detected and characterized, because, unlike copy number variants, the allele intensity is unaltered and the alleles are mapped to their location on the reference genome. Inversions are usually detected using sequence-based and cytogenetic approaches, or unusual linkage disequilibrium (LD) patterns from high-density SNP data. Here, we propose to use principal components analysis (PCA) to detect inversion using genotype data. Because inversions suppress recombination when heterozygous, chromosomes with the inverted segment in different orientations represent two distinct lineages that have been diverging for many generations. Using genotype data in the inversion region, individuals can be classified as different "populations" according to their inversion genotypes, and this population structure can be readily detected using PCA. The pattern of the principal components scatter plot for an inversion region can be explained as a special admixture of "populations". Using the results of PCA, we can assign inversion genotypes to each individual in the sample. We used simulated data generated using the invertFREGENE software to demonstrate our method. We also applied our method to some real data sets and confirmed some of known inversion regions, such as the 8p23.1 and 17q21.31 inversions. Our method provides not only simple way to test association between disease and inversion polymorphisms, but also tool for correcting inversion-induced stratifications for association scan in an inversion region.

683W

Performances of copy number variant detection algorithms applied to SNP-array and impact on GWAS results. *G. Marennne[1,2], SJ. Chanock[3], L. Pérez-Jurado[4], N. Rothman[3], B. Rodríguez[4], M. Kogevinas[5], M. García-Closas[3], DT. Silverman[3], FX. Real[2], N. Malats[2], E. Génin[1].* 1) Inserm UMR-S946, Univ. Paris Diderot, Institut Universitaire d'Hématologie, Paris, France; 2) Centro Nacional de Investigaciones Oncológicas (CNIO) Madrid, Spain; 3) Division of Cancer Epidemiology and Genetics, National Cancer Institute, Department of Health and Human Services, Bethesda, MD, USA; 4) Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain; 5) Institut Municipal d'Investigació Mèdica (IMIM-Hospital del Mar), Barcelona, Spain.

SNP-arrays developed to perform genome-wide association studies are also applied to detect copy-number variants (CNV) and, to this end, several CNV calling algorithms have been proposed. However, previous studies have highlighted their low sensitivity [Dellinger, Nucleic Acids Research 2010; Marenne, Human Mutation 2011]. Results from CNV-association studies strongly depend on the accuracy of CNV assessment mainly if they are affected by differences between cases and controls. It is thus important to identify characteristics influencing sensitivity and false positive rate (FPR) of CNV detection algorithms. In this study, we used data on 21 HapMap samples genotyped on Illumina Human 1M array as part of the Spanish Bladder Cancer / EPICURO Study [Rothman, Nature Genetics 2010] to evaluate the performance of four CNV detection algorithms: cnvPartition, PennCNV [Wang, Genome Res 2007], QuantiSNP [Collela, Nucleic Acids Res 2007] and cnvHap [Coin, Nat Methods 2010]. The callings obtained with these algorithms in 3109 autosomal CNV regions were compared to those reported in the Sanger public database [Conrad, Nature 2010]. The overall sensitivities for the four algorithms were 0.05, 0.09, 0.07 and 0.21 and the FPR were 0.28, 0.33, 0.37 and 0.31, respectively. These figures varied depending on the CNV regions (length, number or density of probes). To assess the impact on association tests of differences in the accuracy of CNV detection in cases and controls, we performed some simulations of CNVs in cases and controls under different models of correlation between calling performance and disease status. We evaluated type one error and power rates and studied whether accounting for CNV region characteristics related to sensitivity and FPR reduces false positive association signals.

684W

Genome-wide analysis shows enrichment of copy-number variations in preeclampsia patients. *L. Zhao[1], E. Triche[2], A. Saftlas[3], J. Hoh[1], A. DeWan[1].* 1) Yale University, New Haven, CT; 2) Brown University, Providence, RI; 3) University of Iowa, Iowa City, IA.

Background: Preeclampsia (PE) is a life-threatening, multi-system disorder of pregnancy characterized by hypertension and proteinuria. Specific genetic contributions for PE, particularly copy-number variations (CNVs), are currently unknown. This study aims to identify distinct maternal CNVs involved in the etiology of PE. **Methods:** A genome-wide CNV scan using SNP arrays was performed on 177 PE case and 117 normotensive control subjects. All subjects were of Caucasian ethnicity and genotyped on Affymetrix SNP 6.0 arrays with some 906,600 SNP and 946,000 copy number probes. CNV calls were made using a combination of Birdsuite, PennCNV, and QuantiSNP detection algorithms and merged using Combined_CNV. Merged regions were screened based on either the effect size of association between PE cases and controls or calls present in ⁄ 5 cases and absent in controls. Further, the same criteria was applied to the shortlisted regions comparing PE cases and an independent set of population-based controls (n=774). Regions were selected to be assayed on the entire case-control dataset using RT-qPCR based on the presence of genes at or near (≤100kb) site, the availability of DNA for samples displaying the CNV, and having the majority of samples without the CNV type to be copy normal. Finally, $\log_2$ratio and B allele frequency plots were visually inspected for a clear change in probe hybridization intensity and zygosity, respectively, to ensure patterns consistent with calls. **Results:** Putative CNVs across ⁄ 10 contiguous probes were merged into 4271 and 25,408 regions of deletion and duplication, respectively, as defined by minimum region of overlap for sample calls. Among these, 11 deletions and 41 duplications met the pre-specified screening criteria. Several merged regions of recurrent rare CNVs were detected in multiple cases, but were singular or undetected in controls. Loci of particular interest include enrichment of case deletions among regions within 14q21.1 (5 case, 1 control, and 6 population-based control deletions) and 19q13.31 (5 case, 1 control, and 2 population-based control deletions). **Conclusions:** These results suggest that several CNVs confer risk for pathogenicity in patients with PE. Rare CNVs, particularly deletions which disrupt genes, enriched in patients represent potentially interesting regions for PE that warrant further investigation.

685W

Admixture Analyses of Lipoprotein (a) Levels. *M. de Andrade[1], M. Matsumoto[1], T. Lesnick[1], E. Boerwinkle[2], S. Kardia[3], I. Kullo[4].* 1) Health Sciences Research, Mayo Clinic, Rochester, MN; 2) Health Sciences Center, University of Texas, Houston, TX; 3) Epidemiology, University of Michigan, Ann Arbor, MI; 4) Cardiovascular Diseases, Mayo Clinic, Rochester, MN.

Background: Levels of lipoprotein(a) (Lp(a)), a circulating lipoprotein that resembles low-density lipoprotein cholesterol, are higher in African Americans than European Americans. To better understand the basis of ethnic differences in Lp(a) levels, we conducted admixture analyses of Lp(a) levels in the Genetic Epidemiology Network of Arteriopathy (GENOA) cohorts. Methods: The GENOA cohorts comprise of sibships with least two individuals with essential hypertension diagnosed before age 60 years, enrolled in Jackson MS (African Americans, n =xxx) and in Rochester MN (European-Americans, n =xxx). Genome-wide admixture analysis was performed to test whether local ancestry at each site was associated with log transformed Lp(a) levels using a linear mixed model adjusted for age and sex and taking into account relatedness in the two cohorts. Results: Admixture analysis was performed using HapMix and LAMP. HapMix bases its local ancestry estimates on haplotypes. It returns a local ancestry estimate for any locus in the reference population. We used HapMap Phase 3 CEU and YRI samples as the reference with HapMix. LAMP bases its local ancestry estimates on allele frequency. It only estimates local ancestry at loci genotyped in both the study sample and the reference population. We used the HapMap Phase 2 CEU and YRI founder samples to calculate the reference population allele frequencies for LAMP. We observed average local ancestry to be ~0.87 (the average STRUCTURE-estimated global ancestry) across the genome with an estimated 6 admixture generations. Results were consistent for the estimates from HapMix and LAMP. There was a peak on Chrm 6, between LPA and PLG for both sets of estimates. We observed that the average percent European Ancestry across the LPA/PLG locus and a 500 kb flanking region was associated with Lp(a) levels, and the average local ancestry estimates within these regions were significantly associated with Lp(a) more than the global ancestry estimates. Furthermore, subjects with lower and higher Lp(a) levels (1st and 4th quartiles) had, on average, lower and higher African ancestry across the LPA/PLG locus , respectively. Conclusions: Greater African ancestry across the LPA/PLG locus was associated with higher Lp(a) levels, suggesting that increased transcription of LPA might be the basis of increased Lp(a) levels in African Americans.

686W

The associasion between genetic polymorphism of TGF-) 1 and obesity in Nan-tou County in Taiwan. *P. Lin[1], Y. Hsiao[1], R. Wang[1], F. Wu[1], C. Chen[1], T. Wu[1,2].* 1) Department of Public Health, China medical University, PhD; 2) Graduate Institute of Biostatistics, China Medical University, PhD.

Dietary, social economic and lifestyles change in recent years have lead to an increasing prevalence of obesity worldwide. In developed countries, obesity is a growing public health concern and also is a major risk factor for chronic diseases such as cardiovascular disease, diabetes, hyperlipidemia, metabolic syndrome and cancer. Transforming growth factor - beta 1 (TGF-) 1) is a candidate gene for the development of obesity. TGF-) 1 expression is associated with body mass index (BMI) and abdominal adipose tissue in morbid obesity. Obesity is not only affected by environmental factors but also caused by TGF-) 1 gene mutation. The purpose of this study was to evaluate the role of TGF-) 1 C-509T genotypes as genetic indicator of susceptibility to obesity. In this cross-sectional study 300 subjects was collected from adult health examination at Nan-tou County in Taiwan. BMI as a standard, the subjects separated into obese (n=131, BM⁄ 27 kg/m^2) and non-obese (n=169) two groups. Each subject completed the demographic and lifestyle questionnaire. Blood samples were collected for DNA extraction to analyze TGF-) 1 genotype by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The chi-square analysis was used to assess differences in genotypes and allele frequencies. Logistic and linear regression were performed to examine whether TGF-) 1 genetic polymorphism associated with obesity. Data analysis showed that TGF-) 1 gene polymorphism C-509T CC, CT and TT was no significant (p = 0.588) between obese and non-obese group. After adjusting risk factors, the logistic regression showed subjects with CT and TT genotypes compared with CC genotype have a higher risk of obesity, but not significant (OR = 1.05 and 1.13, 95% CI = 0.54-2.05 and 0.54- 2.36). In non-obese group TGF-) 1 genotypes were associated with BMI (p = 0.002) and waist circumference (p = 0.018) respectively. In the obese group TGF-) 1 genotypes were significantly associated with waist circumference (p = 0.031). The C-509T variant of TGF-) 1 was not associated with obesity, but results showed that subjects with the TT genotype expressed higher obesity-related biochemical indicators than with the CC genotype. This study may be due to sampling bias, smaller sample size and other environmental factors were not considered. The study may provide a suggestion for obesity prevention. In future, we need more extensive samples to confirm that TGF-) 1 C-509T plays an important role in obesity.

687W

The effect of a family history of myocardial infarction on the risk of venous thromboembolism among White and Black Americans. *F.D. Mili[1], W.C. Hooper[1], C. Lally[2], H. Austin[2].* 1) Research Laboratory Branch, Division of Blood Disorders, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; 2) Department of Epidemiology, Emory University Rollins School of Public Health, Atlanta, Georgia, USA.

There is evidence that venous thrombosis and arterial thrombosis are part of a continuous spectrum of the same disease and might have a common pathogenesis, risk factors and clinical manifestations. Family history of venous thromboembolism (VTE) is a risk factor for VTE, and family history of myocardial infarction (MI) is a risk factor for MI. Findings from the TromsØ study, a prospective study conducted in Norway, indicated that a family history of MI was a risk factor both for MI and VTE, supporting the existence of a link between arterial and venous thrombosis. To confirm and extend the TromsØ study's findings to different populations, we analyzed data from the Genetic Attributes and Thrombosis Epidemiology (GATE) study, a matched, case-control study of VTE that enrolled 1,123 Blacks and 1,235 Whites aged 18-70 years residing in Atlanta, Georgia, United States. A total of 1,094 case patients with a first or recurrent episode of deep vein thrombosis or pulmonary embolism and 1,264 control patients were interviewed about their family history of thrombosis. We found that a positive family history of MI was associated with VTE among Whites only (odds ratio [OR] = 1.3, 95% confidence interval [CI] 1.03-1.8; P = 0.03). In addition, a family history of MI was associated more strongly with VTE among Whites who had diabetes (OR = 3.1, 95% CI 1.2-8.0; P = 0.017) compared with Whites who did not have diabetes (OR = 1.2, 95% CI 0.92-1.6; P = 0.2) (P for interaction = 0.004). Among Whites with plasma fibrinogen levels⁄ 3 grams per liter, we also found that the association between VTE and a family history of MI was stronger among Whites with diabetes than those without diabetes (OR = 2.3, 95% CI 1.2-4.5; P = 0.01 vs. OR = 1.1, 95% CI 0.86-1.5; P = 0.4, respectively) (P for interaction = 0.02). Furthermore, the association between a family history of MI was statistically significant among Blacks aged 18-44 years who experienced a recurrent episode of VTE compared with Blacks with a first VTE event (OR = 2.3, 95% CI 1.1-4.7; P = 0.02). From a public health perspective, our study showed that the attributable fraction of VTE due to family history of MI (adjusted for covariates) was 4.5% among Blacks and 11% among Whites. We concluded that a family history of MI is a risk factor for VTE among certain populations stratified by race, age, and co-morbid conditions.

688W

Previously identified obesity loci display longitudinal differences in their effect on BMI through adolescence. *S.D. Bailey[1], J. O'Loughlin[2], N.C. Low[3], E. Dugas[7], N. Rudzicz[4], K. Desbiens[4], M.-H. Roy-Gagnon[2,5], M. Lambert[5], J.C. Engert[1,4,6].* 1) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Department of Social and Preventive Medicine, University of Montreal, Montreal, Quebec, Canada; 3) Department of Psychiatry, McGill University, Montreal, Quebec, Canada; 4) The Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada; 5) Sainte-Justine Hospital Research Centre, Montreal, Quebec, Canada; 6) Cardiology Division, Department of Medicine, McGill University Health Centre, Montreal, Quebec, Canada; 7) University of Montreal Hospital Research Center, Montreal, Quebec, Canada.

Several genetic loci are known to contribute to obesity and recent genome-wide association (GWA) studies have identified additional loci that are associated with obesity-related traits in adults. Relatively few studies have examined these variants in childhood or in adolescence. From the Quebec based Nicotine Dependence in Teens (NDIT study), a prospective cohort recruited from schools beginning at grade 7 and followed until age 22, we genotyped 571 Quebec adolescents of European origin for 35 SNPs in from 35 obesity genes. Body mass index (BMI) was available from each participant for up to three time points through adolescence (mean age of at each time point: 13, 15, 17 years). Using linear regression adjusting for age and sex the variants in FTO and in LYPLAL1 were significantly associated with BMI in all three age groups with similar effect sizes. Eight SNPs demonstrated significant longitudinal trends. Interestingly, a SNP in TNFA (rs1800629) demonstrated a trend of increasing BMI effect size and only became significant in the oldest age group. Conversely, (rs1778323) a SNP in MC4R was significant in the youngest age group and displayed a significant trend of decreasing BMI effect size. Thus, we have identified several genetic variants that may display differential effects on BMI through adolescence.

689W

The developmental signature of steroid response genes in the human fetal lung. *S. Sharma[1], A. Kho[1], W. Qiu[1], R. Gaedigk[2], J.S. Leeder[2], S.T. Weiss[1], K.G. Tantisira[1].* 1) Channing Laboratory, Brigham and Women's Hospital, Boston, MA; 2) Children's Mercy Hospital and Clinics, Kansas City, MO.

Rationale: Asthma is the most common chronic respiratory disease of childhood. Inhaled corticosteroids (ICS) are an essential treatment for asthma and decrease exacerbation rates in children. While corticosteroids enhance lung maturation, it is unclear whether steroid response genes are important during human lung development. **Objective**: To determine whether steroid response genes are important in lung development and influence early response to asthma therapy. **Methods**: Immortalized lymphoblastoid cell lines were obtained from 164 adolescent subjects with asthma participating in the Childhood Asthma Management Program (CAMP). Cells were cultured, split, and treated with either 10-6 M dexamethasone or vehicle in a paired fashion. RNA was run on Illumina HumanRef8 v2 microarrays. Differential expression by steroid exposure was performed using t-tests with multiple comparison adjustment. Using gene expression profiles from 36 human fetal lung tissue samples, we next tested whether steroid response genes were enriched during lung development. Principal components analysis (PCA) was performed to identify the principal component (PC) most highly correlated with gestational age. A chi square test was used to determine whether the top 5% of genes contributing to this PC were enriched for genes in the steroid pathway. We then tested the association between variants in the most differentially expressed gene with asthma susceptibility and severity in 403 CAMP trios using family-based association tests under a dominant model with covariate adjustment. **Results**: Treatment of cell lines with dexamethasone resulted in differential expression of 6,385 genes. PCA of the fetal lung data demonstrated that PC1 was associated with age. Of the differentially expressed genes during lung development 903 of these genes were also involved in steroid response(p=3.12 x10-18). Gene ontology demonstrates involvement of these genes in cell cycle and DNA repair (p<0.05). *SPRY2* was the most differentially expressed gene, therefore, we tested four variants in *SPRY2* for association with asthma and disease severity. SNP rs9545409 was associated with asthma(p=0.003) and rs504122 with airway responsiveness (p=0.009) and lower FEV_1 (p=0.02). **Conclusions**: Genes involved in human lung development are enriched for elements of the steroid response pathway. These genes may influence ICS response in asthmatics and may provide further insight into novel therapeutic targets for asthma.

690W

FTO genotype independently and strongly influences both adiposity and insulin resistance in the Mapuche of Chile. *M.E.S. Bailey[1], C.A. Celis-Morales[1,2], N. Ulloa[3], C. Calvo[3], F. Pérez-Bravo[4], J.M.R. Gill[2].* 1) School of Life Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom; 2) Institute of Cardiovascular and Medical Sciences, CMVLS, University of Glasgow, Glasgow, UK; 3) Department of Clinical Biochemistry and Immunology, Faculty of Pharmacy, University of Concepcion, Chile; 4) Laboratory of Nutritional Genomics, Department of Nutrition, Faculty of Medicine, University of Chile, Santiago, Chile.

FTO is the gene with the strongest influence on adiposity/BMI yet discovered. In populations of European ethnicity, *FTO* influences diabetes risk only via this effect on adiposity, but recent studies of other ethnic groups have challenged this conclusion. We present here evidence that *FTO* strongly influences insulin resistance in a non-European population. We conducted a cross-sectional study of a population sample (n=472) from Chile, recruiting adults from four population groups - a native American ethnic group, the Mapuche, living in traditional rural environments or Westernised urban environments, and rural and urban citizens of European origin. All participants underwent comprehensive assessment in the field (Celis-Morales et al., submitted) of a wide range of physical, metabolic, social and environmental variables, including insulin resistance (HOMA-IR). rs17817449, a SNP in intron 1, was used to assess the influence of variation in *FTO* on BMI and HOMA-IR in the whole cohort. The G allele was associated with higher BMI and with greater insulin resistance (GLM tests: $p \leq 0.000001$). Adding ethnicity as a factor in a regression model of additive allelic effects revealed a significant genotype x ethnicity interaction ($p = 0.0002$ for HOMA-IR). In this model genotype explained 18.2% of the variance in HOMA-IR in the Mapuches. After adjustment for multiple factors in a full model with HOMA-IR as the outcome variable (76% of variance explained; urban/rural environment, smoking status and sedentary time were significant as main effects, $p < 0.0003$; age, sex, socioeconomic status, BMI and adiposity variables, activity and fitness variables and dietary intake variables were not significant), the genotype x ethnicity interaction remained ($p = 0.00014$). After stratification, *FTO* genotype explained only 0.6% of the variance in HOMA-IR in Europeans, but 6.7% in Mapuches ($p=0.000003$), with a per allele effect size in this group of 0.26 S.D., or 1.46 HOMA-IR units. Our results reveal that *FTO* has a large effect on insulin resistance in this small sample of Chilean Mapuches, independent of its influence on adiposity/BMI and after adjustment for many other factors. If generalizable to other non-European populations, this may point to the existence of modifier genes that reduce the influence of *FTO* on insulin resistance in Europeans. Such genetic differences may contribute to the larger increase in diabetes risk on urbanisation observed in non-European populations.

691W

TCF7L2 polymorphisms/haplotypes association with T2D in an Italian population and correlation with some complications. *C. Ciccacci[1], D. Di Fusco[1], L. Cacciotti[2], V. Spallone[2], G. Novelli[1,3], P. Borgiani[1].* 1) Department of Biopathology and Diagnostic Imaging, Medical Genetics Section, School of Medicine, Tor Vergata University, Rome, Italy; 2) Endocrinology, Department of Internal Medicine, Tor Vergata University, Rome, Italy; 3) Medical Genetics Unit, San Pietro FBF Hospital , Rome, Italy.

Type 2 diabetes (T2D) is a complex disease resulting by the contribution of both environmental and genetic factors. Recently the list of genes implicated in the susceptibility with T2D has grown substantially due to the great development of the genome wide association studies (GWAs). Among these genes, the TCF7L2 genes, identified by GWAs in 2007, has shown the strongest effect. TCF7L2 is a transcription factor which regulates embryogenesis, cell proliferation, myogenesis and adipogenesis via the Wnt signaling pathway. Several studies have demonstrated that TCF7L2 influences the rate of progression from impaired glucose tolerance to T2D. The aim of our study is to confirm the TCF7L2 role in the susceptibility to T2D in the Italian population. At this purpose we have perfomed a case-control association study in an Italian sample of 150 T2D patients and 170 healthy controls and a genotype/phenotype correlation analysis. We investigated three TCF7L2 polymorphisms (rs7903146, rs7901695 and rs12255372) by allelic discrimination assays (TaqMan technology). Differences in allelic, genotypic and haplotypic frequencies between cases and control were evaluated by Pearson $\chi 2$ test. All three SNPs resulted highly associated with susceptibility to T2D both at allelic and genotypic level. Rs7901695 resulted the mostly associated SNP: patients carry the C allele in heterozygote status has an OR=1.6 (P= 0.057), and patients carry the C allele in homozygote status has an OR=3.03 (P=0.0009) respect to the TT carriers. From the haplotypic analysis, the CTT haplotype (carrying all three variant alleles) resulted to be highly associated with the T2D risk (OR= 1.86 with P=0.0019), while the TCG haplotype (carrying the wildtype alleles) resulted to have a protective effect (OR= 0.55 with P=0.0003). The phenotype/genotype analysis revealed a significant correlation with T2D complications. In particular the rs7901695 and rs7903146 resulted associated with the presence of cardiovascular disease (P=0.033 and P=0.18 respectively). Moreover, the rs7903146 and rs12255372 resulted associated with the presence of retinopathy (P=0.048 and P=0.014). In conclusion, we confirmed that polymorphisms/haplotypes in TCF7L2 gene play a significant role in the T2D pathology in the Italian population. Moreover, we observed a significant association with the risk to develop cardiovascular disease and retinopathy in the T2D affected patients carrying the variant TCF7L2 alleles.

692W

Investigating the *PARL* / *ABCC5* gene region as a susceptibility locus for type 2 diabetes. *K. Direk[1], W. Lau[2], K. Small[1,3], H. Elding[2], N. Maniatis[2], T. Andrew[1].* 1) Department of Twin Research & Genetic Epidemiology, King's College London, London, United Kingdom; 2) Genetics, Evolution & Environment, University College London, Gower Street, UCL, London; 3) MuTHER Consortium, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, United Kingdom.

The *PARL* gene coding for a mitochondrial protease, has recently been identified as a potential candidate gene for type 2 diabetes (Walder *et al*., 2005; Civitarese *et al*., 2010). There is conflicting evidence regarding the association between the non-synonymous Leu262Val (rs3732581) polymorphism in *PARL* and fasting insulin serum levels - the initial observation (Walder *et al*., 2005) has failed to replicate in subsequent studies (Fawcett *et al*., 2006; Powell *et al*., 2008; Hatunic *et al*., 2009). However, functional evidence (Walder *et al*., 2005; Civitarese *et al*., 2010) exists that empirically demonstrates down-regulation of *PARL* expression in diabetic subjects. Mitochondrial dysfunction is thought to be a major etiological component of insulin resistance (Turner & Heilbronn, 2008; Patti & Corvera, 2010). Using TwinsUK cohort data, approximately 3000 twins were genotyped for rs3732581. No association was observed between fasting serum insulin and glucose levels and any genotyped *PARL* SNPs, including Leu262Val. Analyses were conducted using individual SNP association and a multi-locus Malecot model for the *PARL*/*ABCC5* gene region. Using the Wellcome Trust Case Control Consortium (WTCCC) type 2 diabetes data, we also observed no evidence of genotypic association with *PARL*. However, strong evidence of association for type 2 diabetes was observed in the neighbouring gene *ABCC5*, immediately upstream of *PARL* (Malecot model χ^2 = 21.7, p = 1.54 x 10^{-6}). In addition, despite no evidence of association with fasting insulin levels in the TwinsUK cohort, *ABCC5* and *PARL* expression levels for subcutaneous adipose tissue samples measured for 776 twins from the same cohort, did show evidence of association (p =< 10^{-3}) between *ABCC5* expression and *ABCC5* SNPs, but not between *PARL* expression and *PARL* SNPs. As *PARL* and *ABCC5* are situated in a region of substantial linkage disequilibrium (LD), we tested the hypothesis that the association between rs3732581 and insulin may be the result of rs3732581 being in LD with intra-genic *ABCC5* SNPs, particularly those associated with *ABCC5* expression. We also used *in silco* analyses to examine this region for evidence of regulatory elements. Given the two independent lines of evidence implicating *ABCC5*, we will test whether there are either two co-located, but independent susceptibility genes for type 2 diabetes in this region, or that the contradictory reports of genotypic association with *PARL* may just reflect LD with *ABCC5*.

693W

Comprehensive evaluation of type 2 diabetes susceptibility in the Japanese population by genome-wide imputation analysis using the 1000 Genomes Project data. *K. Hara[1], H. Fujita[1], T.A. Johnson[2], M. Horikoshi[3], S. Maeda[2], T. Tsunoda[2], M. Kubo[2], Y. Nakamura[2], T. Kadowaki[1].* 1) Department of Metabolic Diseases, University of Tokyo, Tokyo, Japan; 2) Center for Genomic Medicine, RIKEN, Yokohama, Japan; 3) The Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom.

We had previously identified several type 2 diabetes (T2D)-associated polymorphisms in *UBE2E2* (rs7612463) and *C2CD4A-C2CD4B* (rs7172432) using genome-wide association analysis of 4,470 cases and 3,071 control cases that were genotyped for 459,359 single-nucleotide polymorphisms (SNPs). To identify additional T2D susceptibility loci and to fine-map the location of functional variants for the previously identified T2D loci, we imputed genotypes of 10,524,368 single-nucleotide polymorphisms (SNPs) from the 1000 Genomes Project data set (388 Asian haplotypes) using MACH software. We then performed genome-wide association tests for T2D before and after adjusting for possible confounding factors such as age, sex, and BMI (body mass index). In addition to variants in or near the previously reported loci, we found that SNPs in *CTBP1* (C-terminal-binding protein 1) - *MAEA* (macrophage erythroblast attacher) region (rs730831, rs4974585, and rs4974586) were possibly associated with T2D. Subsequent genotyping of these SNPs in an independent sample set of 1,175 cases and 839 controls showed evidence for replication of association (P values between 1.7×10^{-2} and 2×10^{-3}), and combined P values reached genome-wide significance (ranging from $P = 5 \times 10^{-8}$, OR = 1.18 [1.03 - 1.35] to $P = 1 \times 10^{-10}$, OR = 1.25 [1.08 - 1.44]). Conditional logistic regression analyses were performed to assess the independence between SNPs, with results identifying rs730831 in *CTBP1* as the best significant SNP in this region. The CTBP1 expression level was higher in visceral adipose tissue (V) than in paired subcutaneous tissue (S)(P = 0.0061), while there were no differences in expression levels between V and S for genes surrounding *CTBP1*. Subjects with the risk allele of rs730831 tended to have a higher expression level of *CTBP1* (P = 0.043). These results indicate that *CTBP1* is a plausible candidate for a novel T2D gene. The association with T2D was no stronger in the present lead SNPs than in the previous lead SNPs detected in previously reported loci in our population except in *CDC123/CAMK1D* and *ZFAND6*. Our study highlights the benefit of mapping T2D variants in combination with data derived from next-generation sequencing of the human genome. We are currently conducting a replication study to see if the present results would be confirmed in another sample of 1,634 T2D cases and 21,697 controls.

694W

Modeling HLA epistatic interactions using a unified GWAS and linkage analytical method maps new putative genes for Type 1 Diabetes. *Y. Huang[1], Y. Tomer[2], VJ. Vieland[1].* 1) Battelle Ctr Math Med, the Res Inst at Nationwide Childrens Hospital, Columbus, OH; 2) Division of Endocrinology, Department of Medicine, Mount Sinai Medical Center, and James J. Peters VA Medical Center, New York, N.Y.

The PPLD is an association statistic developed under the PPL framework. It is tailored to detection of association due to linkage disequilibrium (LD) for complex disorders, and can uniquely combine different types of genetic information obtained from disparate data structures for single-locus and two-locus (2L) epistasis analyses. Here we combine publicly available Type 1 Diabetes (T1D) case-control GWAS data from the Wellcome Trust Case Control Consortium (WTCCC) with a separate family dataset, for combinined linkage and LD analyses, taking advantage of strong LD to the HLA region to search for novel HLA-interacting genes. The PPLD agreed with the primary previously reported findings for the WTCC data and identified some new regions as well. In at least one case combining the GWAS and family data provided evidence of LD to a known candidate gene that did not show LD on its own (CTLA4). Novel PPLD findings included a 67% probability of LD between T1D and ACOXL (rs10187034) and 55% probability of LD with ADAMTSL1 (rs949682). Allowing for 2L epistasis involving HLA-DQA1 boosted these PPLDs to 99% and 82%, respectively. Epistasis analysis also implicated association to ADAMTS1 (2L PPLD = 21%), a member of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motif) protein family found in the extracellular matrix, which showed no evidence of LD in single-locus analysis. Both ADAMTSL1 (ADAMTS -like protein 1) found in the single locus analysis and ADAMTS1 from the 2L epistasis analysis may be associated with inflammation processes. Finding two genes with similar putative functions in non-syntenic chromosomal regions supports the power of epistasis analyses to reveal gene families underlying complex disorders.

695W

Admixture Mapping Reveals Potential Novel Loci for Type II Diabetes in African Americans Using Electronic Medical Records as a Tool for Genome Science. *J. Jeff[1], L. Armstrong[7], M. Ritchie[1], J. Denny[2,3], A. Kho[6], M. Basford[4], W. Wolf[9], J. Pacheco[8], K. Doheny[11], D. Mirel[12], E. Pugh[11], A. Crenshaw[12], R. Li[10], T. Manolio[10], R. Chisholm[8], D. Roden[2,4,5], G. Hayes[7,8], D. Crawford[1].* 1) Center for Human Genetics, Vanderbilt Univ, Nashville, TN; 2) Department of Medicine, Division of Clinical Pharmacology,Vanderbilt Univ, Nashville, TN; 3) Department of Biomedical Informatics,Vanderbilt Univ, Nashville, TN; 4) Office of Personalized Medicine,Vanderbilt University, Nashville, TN; 5) Department of Pharmacology,Vanderbilt University, Nashville, TN; 6) Division of General Internal Medicine,Northwestern University, Chicago, IL; 7) Division of Endocrinology, Metabolism, and Molecular Medicine, Northwestern University, Chicago, IL; 8) Center for Genetic Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL; 9) Division of Genetics, Children's Hospital Boston, Boston, MA; 10) Office of Population Genomics, National Human Genome Research Institute, Bethesda, MD; 11) Center for Inherited Disease Research, Johns Hopkins University, Baltimore, MD, USA; 12) Genetic Analysis Platform and Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, USA.

Type 2 diabetes (T2D) is a complex metabolic disease that disproportionately affects African Americans compared to European Americans. Obesity is a major risk factor for T2D, and it is postulated that chronic inflammation possibly stemming from adipose tissue macrophages and T cells plays a key role. Genome-wide association studies (GWAS) have identified over 20 disease loci that contribute to T2D in European American but few studies have been performed in diverse or admixed populations. Admixture mapping disease loci in recent mixed populations, such as African Americans is potentially a more powerful alternative to GWAS. As part of the electronic Medical Records and Genomics (eMERGE) network, we performed an admixture scan in 1,563 African Americans from the Vanderbilt Genome-Electronic Records Project and Northwestern University NUgene Project. T2D cases were identified from the electronic medical record (EMR) using a combination of billing codes, T2D medications, and abnormal glucose or glycated hemoglobin (HbA1c) labs. Exclusions included patients diagnosed with T1D and patients who were undertaking insulin only with no records on T2D medications, abnormal glucose and/or HbA1c were excluded. Controls had to have ⁄ 2 clinical visits, ⁄ 1 normal glucose labs, no diabetes-related billing codes, no diabetes or insulin-related medications, and no family history of T2D. With these criteria, we identified 736 cases and 827 controls for Illumina 1M-Duo BeadChip genotyping. Using ANCESTRYMAP, we performed an admixture scan with 4,208 autosomal ancestry informative markers (AIMs) derived from the 1M dataset, assuming a 1.2 relative risk score for T2D. The average genome-wide European ancestry was similar between cases (20.3%) and controls (20.6%; p= 0.72). The average genome-wide LOD score, the likelihood of being a disease locus, was 0.55, and scores ranged from -0.89 to 2.30. Of the 22 autosomes tested, chromosome 11 was the only chromosome with a significant genome-wide LOD score (LOD >2). The admixture peak encompassed multiple genes, including TCIRG1, a T-cell immune regulator expressed in the pancreas and liver and not previously implicated in T2D.Several isoforms of TCIRG1 have been described, including the short isoform b (TIRC7) required for normal T cell activation. Our results suggest a novel region on chromosome 11 identified by admixture mapping associated with T2D in African Americans and warrants additional fine-mapping in these regions.

696W

A MAGIC study of genome-wide joint meta-analysis of SNP by BMI interaction identifies 14 common variants associated with fasting insulin and glucose homeostasis. *A. Manning[1], R.A. Scott[2], M.-F. Hivert[3,4], J. Grimsby[4,9], C.-T. Liu[5], H. Chen[5], L. Bielak[6], L. Rasmussen-Torvik[7], J. Dupuis[5, 8], J.C. Florez[1, 4, 9], R.M. Watanabe[10, 11], C. Langenberg[2], J.B. Meigs[4, 9], MAGIC.* 1) Broad Institute, Cambridge, MA; 2) Medical Research Council (MRC), Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK; 3) Centre de Recherche Medicale de l'Universite de Sherbrooke, Sherbrooke, Quebec, Canada; 4) Massachusetts General Hospital, Boston, MA; 5) Biostatistics Department, Boston University, Boston, MA; 6) Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI; 7) Department of Preventive Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL; 8) National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, MA; 9) Harvard Medical School, Boston, MA; 10) Department of Physiology and Biophysics, Keck School of Medicine, University of Southern California, Los Angeles, CA; 11) National Institute of Health and Welfare, Oulu, Finland.

Recent genetic studies have identified many loci implicated in type 2 diabetes (T2D) pathophysiology, most of which are associated with beta-cell dysfunction rather than insulin resistance. We hypothesize that accounting for (1) heterogeneity in body mass index (BMI) and (2) potential SNP×BMI interactions, will increase our ability to identify genetic loci associated with traits associated with insulin resistance. We developed the joint meta-analysis (JMA) approach, which tests both the main genetic effect and any potential interaction between a SNP and BMI, and provides increased power for detecting the association signal when underlying interaction effects are suspected but unknown. Here, we present the results of a genome-wide study by the Meta-Analysis of Glucose and Insulin-related traits Consortium (MAGIC) wherein we jointly meta-analyze SNP and SNP×BMI effects to screen for genetic loci associated with log(fasting insulin) and fasting glucose in 47 cohorts comprising up to 88,771 individuals. After discovery analyses, we promoted 51 loci (JMA $P<10^{-6}$) to a follow-up phase (31 for fasting insulin, 21 for fasting glucose, one overlap). In addition to loci previously associated with T2D or glycemic related traits, 14 new loci reached genomewide significance in combined discovery and follow-up analysis in either JMA or main effects models. The nearest genes and JMA P values for fasting insulin are *COBLL1*/*GRB14* ($P=8\times10^{-14}$), *IRS1* ($P=7\times10^{-14}$), *PPP1R3B* ($P=7\times10^{-14}$), *PEPD* ($P=8\times10^{-8}$), *UHRF1BP1* ($P=1.4\times10^{-7}$), *PDGFC* ($P=7.2\times10^{-10}$), *LYPLAL1* ($P=2.1\times10^{-8}$), and for fasting glucose are *PCSK1* ($P=2\times10^{-9}$), *OR4S1* ($P=1\times10^{-8}$), *ARAP1* ($P=1\times10^{-12}$), *FOXA2* ($P=3\times10^{-11}$), *GRB10* ($P=6\times10^{-8}$), *PPP1R3B* ($P=1.8\times10^{-9}$ in main effects models adjusting for BMI), *DPYSL5* ($P=1\times10^{-12}$), *CREB3L1* ($P=3.4\times10^{-8}$ in main effects models without adjustment for BMI). Four loci have nominally significant interaction tests: *COBLL1*/*GRB14* ($P=0.0005$) and *IRS1* ($P=0.02$) for fasting insulin and *PCSK1* ($P=0.04$) and *OR4S1* ($P=0.009$) for fasting glucose. Our study demonstrated that by allowing for interaction with adiposity using the JMA method, we can more readily detect loci associated with insulin resistance traits at genome-wide significance levels. The identification of these new loci offers the potential to further characterize the role of insulin resistance in T2D pathophysiology.

697W

Impact of loci contributing to type 2 diabetes susceptibility on variation in physiologic glycaemic traits in healthy individuals. *M. McCarthy[1,2,3], A.S Dimas[2,4], V. Lagou[2], R. Mägi[2], A. Barker[5], D. Rybin[6], M.-F. Hivert[7,8], T. Assimes[9], T. Quertermous[9], M. Walker[10], I. Barroso[11], C. Langenberg[5], J.C. Florez[12,13,14], R.M. Watanabe[15,16], J. Knowles[9], J. Dupuis[17,18], E. Ingelsson[19], I. Prokopenko[1,2], GENESIS, DIAGRAM, MAGIC.* 1) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford OX3 7LJ, UK; 2) University of Oxford, Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford OX3 7BN, UK; 3) Oxford NIHR Biomedical Research Centre, Churchill Hospital, Oxford OX3 7LJ, UK; 4) Alexander Fleming Biomedical Sciences Research Center, 34 Fleming Street, Vari 16672, Athens, Greece; 5) MRC Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge CB2 0SL, UK; 6) Boston University Data Coordinating Center, Boston, MA 02118, USA; 7) Department of Medicine, Université de Sherbrooke, Sherbrooke, J1H 5N4 (Quebec), Canada; 8) General Medicine Division, Massachusetts General Hospital, Boston, MA 02114, USA; 9) Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA; 10) Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne NE2 4HH, UK|; 11) Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK; 12) Center for Human Genetic Research and Diabetes Research Center (Diabetes Unit), Massachusetts General Hospital, Boston MA 02114, USA; 13) Program in Medical and Population Genetics, Broad Institute, Cambridge, MA 02142, USA; 14) Department of Medicine, Harvard Medical School, Boston, MA 02115, USA; 15) Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA; 16) Department of Physiology & Biophysics, Keck School of Medicine, University of Southern California, Los Angeles, California 90033, USA; 17) Department of Biostatistics, Boston University School of Public Health, Boston, MA 02118, USA; 18) The National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, MA, USA; 19) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden.

Genome-wide association studies (GWAS) have identified multiple genetic loci contributing to risk of type 2 diabetes (T2D), a condition characterized by beta-cell dysfunction and insulin resistance. The aim of this study was to uncover the mechanistic basis of these T2D associations by exploring the effects of T2D-risk alleles on intermediary metabolic phenotypes in healthy individuals. We considered 38 established T2D susceptibility variants and their effects on validated indices of insulin processing, secretion and sensitivity gathered from both basal (fasting glucose, insulin, HOMA-B, HOMA-IR, proinsulin) and dynamic measures (insulin sensitivity [ISI] and insulinogenic indices derived from oral glucose tolerance tests [OGTTs]). An additive genetic model with adjustment for sex, age and BMI was applied for cohort analyses followed by fixed effects inverse variance meta-analysis. Sample sizes ranged from 11,282 to 58,614 for basal and from 11,889 to 13,829 for dynamic tests. Based on a cluster analysis of these data, the 38 loci fell into 5 major groups. Variants near *PPARG*, *KLF14*, *IRS1* and *GCKR* loci were characterized by a primary effect on insulin resistance (HOMA-IR, ISI) with compensatory improvements in beta-cell function (insulinogenic index, HOMA-B). Risk-alleles at the *ARAP1* locus demonstrated a pronounced impact on proinsulin and insulinogenic index indicating a defect in insulin processing. Variants near *MTNR1B* and *GCK* displayed elevations in fasting glucose and concomitant reductions in HOMA-B and insulinogenic index. *TCF7L2*, *SCL30A8* and *HHEX/IDE* risk-alleles combined defects in insulin processing and secretion (insulinogenic index). For all other loci, there were, despite the sample sizes, no clear-cut associations with either basal or OGTT-derived measures. Data were also available for up to 4,169 individuals from 7 cohorts with more detailed measures of insulin sensitivity derived from euglycemic clamps (M-value), insulin suppression tests (SSPG) or intravenous glucose tolerance tests (Si). Meta-analysis of these three highly correlated intravenous assessments were broadly consistent with the ISI and HOMA-IR data; though reduced sample size compromised power at many loci. The T2D-risk allele was associated with reduced insulin sensitivity at *IRS1* ($p=7x10^{-4}$) and at *ADCY5* (p=0.006). Our findings indicate that T2D-susceptibility variants exert their effects on glucose homeostasis through a variety of mechanisms.

698W

Large-scale sex-differentiated meta-analysis reveals four novel susceptibility loci for type 2 diabetes. *A.P. Morris[1], T. Ferreira[1], T.M. Teslovich[2], A. Mahajan[1], B.F. Voight[3], DIAGRAM Consortium.* 1) Wellcome Trust Centre for Human Genetics, Univ Oxford, Oxford, United Kingdom; 2) Center for Statistical Genetics, University of Michigan, Ann Arbor, MI, USA; 3) Broad Institute of Harvard and MIT, Cambridge, MA, USA.

Despite the success of genome-wide association studies (GWAS), much of the genetic component of the T2D is unexplained. One source of genetic variation which may contribute to this “missing heritability” is that with effects that differ between sexes. We have undertaken the first large-scale meta-analysis of sex-specific T2D GWAS to: (i) assess the evidence of heterogeneity of allelic effects between males and females at confirmed disease loci; and (ii) identify novel sex-differentiated associations with the disease.

We performed sex-specific meta-analysis of association studies of T2D (total effective sample size of 47,917 males and 39,454 females) incorporating: (i) 12 GWAS of European descent, imputed for up to 2.5 million SNPs; and (ii) 25 additional cohorts of European descent and 1 cohort of Pakistani descent, genotyped using the Metabochip. Each SNP was tested for association, separately for each sex, under an allelic-dose model. For each sex, allelic odds ratios (OR) were then combined across cohorts through fixed-effects meta-analysis. A sex-differentiated test of association, allowing for heterogeneity in allelic effects between sexes, was then performed. Heterogeneity in allelic OR between sexes was assessed by Cochran's Q-statistic.

Among confirmed T2D loci, there was nominal evidence of heterogeneity in allelic effects between the sexes at 3 loci, all showing stronger association in males: KCNQ1 (p=1.1x10-3, male OR = 1.12 [1.09-1.16], female OR = 1.05 [1.01-1.08]), DGKB (p=6.1x10-3, male OR = 1.15 [1.11-1.19], female OR = 1.06 [1.02-1.11]) and BCL11A (p=1.1x10-2, male OR = 1.10 [1.06-1.13], female OR = 1.04 [1.00-1.07]). Our sex-differentiated meta-analysis revealed genome-wide significant evidence of association ($p<5x10-8$) at 4 novel loci. The strongest signal of association was observed at CCND2 (p= 6.6x10-10), which also is much stronger in males (male OR = 1.12 [1.08-1.16], female OR = 1.04 [1.00-1.09]). However, the other 3 loci show stronger effects in females: GIPR (p=1.3x10-8), RREB1 (p=3.5x10-8) and TMEM154 (p=3.9x10-8). These loci would not have been identified through traditional sex-combined meta-analysis, and thus highlight the importance of allowing for heterogeneity in allelic effects between males and females in sex-differentiated analyses in searching for complex trait loci.

699W

Fine-mapping of Type 2 Diabetes Risk Loci in African Americans Using the Metabochip: The PAGE Study. *F.R. Schumacher[1], K.E. North[2], J. Haessler[3], K.L. Spencer[4], N. Franceschini[5], K.R. Monroe[1], B.V. Howard[6], R.D. Jackson[7], W.H.L. Kao[8], L.N. Kolonel[9], S. Liu[10], V. Aroda[6], L.H. Kuller[11], L.R. Wilkens[9], L.A. Hindorff[12], J.L. Ambite[13], L. Le Marchand[9], D.C. Crawford[14], S. Buyske[15], J.S. Pankow[16], U. Peters[3], C.A. Haiman[1], Population Architecture using Genomics and Epidemiology.* 1) Preventive Medicine, Univerisity of Southern California, Los Angeles, CA; 2) Department of Epidemiology and Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, NC, USA; 3) Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; 4) Center for Human Genetics Research, Vanderbilt University, Nashville, TN, USA; 5) Department of Epidemiology, University of North Carolina, Chapel Hill, NC, USA; 6) MedStar Research Institute, Washington, DC, USA; 7) Department of Internal Medicine, Ohio State Medical Center, Columbus, OH, USA; 8) Department of Epidemiology, John Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; 9) University of Hawaii Cancer Center, Honolulu, HI, USA; 10) Program on Genomics and Nutrition, Department of Epidemiology, University of California, Los Angeles, Los Angeles, CA, USA; 11) Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA; 12) Office of Population Genomics, NHGRI, NIH, Bethesda, MD, USA; 13) Information Sciences Institute, University of Southern California, Los Angeles, CA, USA; 14) Department of Molecular Physiology and Biophysics; Center for Human Genetics Research, Vanderbilt University, Nashville, TN, USA; 15) Department of Genetics, Department of Statistics, Rutgers University, Piscataway, NJ, USA; 16) Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN, USA.

Recently, numerous genotype-phenotype associations have been identified for a variety of traits through genome-wide association (GWA) studies. The majority of these, and subsequent replications, were discovered primarily in individuals of European (EU) ancestry. These EU genotype-phenotype associations may fail to replicate in populations of African ancestry due to differences in effect sizes, allele frequencies, and linkage disequilibrium with the true underlying functional SNP. The Metabochip is a high-density 200K SNP array with content selected from HapMap and the 1,000 Genomes Project across several ancestral populations to fine-map GWA regions found in association with metabolic traits including type 2 diabetes (T2D). We, as part of the Population Architecture using Genomics and Epidemiology (PAGE) Study, have utilized this high-density array to characterize genetic risk for T2D in an African American sample of 1,233 T2D cases and 3,896 population-based controls from the Atherosclerosis Risk in Communities Study and Women's Health Initiative. Overall, 161,098 polymorphic SNPs passed quality control and were tested using an additive genetic model adjusting for age, sex, BMI, recruitment center, and population structure with unconditional logistic regression in a pooled analysis. Uncorrected p-values are presented. We reproduced significant (P<0.01) associations with several EU T2D index SNPs, such as *TCF7L2* rs7903146 [OR=1.3; P= $4.5x10^{-7}$] and *NOTCH2* rs10923931 [OR= 1.2; P=$4.6x10^{-3}$] in our African American T2D sample. Within several T2D risk loci we observed stronger associations for SNPs correlated with the EU index SNP (CEU r^2>0.3), such as *CDNK2A/2B* rs10757283 (P=$3.3x10^{-3}$) vs EU SNP rs2383208 (P=0.8), as well as associations that appear to be independent of the EU index SNP (CEU r^2<0.01), such as *JAZF1* rs73297579 (P=$3.4x10^{-4}$) vs EU SNP rs864745 (P=0.04). Other suggestive associations (P<$5.0x10^{-5}$) for novel loci were observed and require further evaluation. Additional analyses of T2D-related traits (e.g. baseline glucose and insulin levels) are ongoing. Overall, we demonstrate utilizing ancestral populations differing from the discovery population may be informative for discovering secondary signals independent of the index SNP, localizing biologically relevant variants and prioritizing SNPs for functional evaluation in known T2D risk loci.

700W

Genetic variants associated with diabetes related circulating metabolite levels and their role in type 2 diabetes and insulin sensitivity. *W. XIE[1], A.R. Wood[1], M.N. Weedon[1], J.W. Knowles[2], T.L. Assimes[2], T. Quertermous[2], F. Abbasi[2], J. Paananen[3], H. Häring[4], T. Hansen[5,10], O. Pedersen[5,11,12,13], U. Smith[6], M. Laakso[3], E. Ferrannini[7], W.E. Gall[8], T.M. Frayling[1], M. Walker[9], the MAGIC investigators, the DIAGRAM consortium, the GENESIS consortium, the RISC consortium.* 1) Genetics of Complex Traits, Peninsula School of Medicine, University of Exeter, UK; 2) Department of Medicine, Stanford University School of Medicine, Stanford, California, USA; 3) Department of Medicine, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland; 4) Department of Internal Medicine, Division of Endocrinology, Diabetology, Nephrology, Vascular Medicine and Clinical Chemistry, University of Tübingen, Tübingen, Germany; 5) Novo Nordisk Foundation Center for Basic Metabolic Research Faculty of Health Science, University of Copenhagen, Copenhagen, Denmark; 6) The Lundberg Laboratory for Diabetes Research, Department of Molecular and Clinical Medicine, Sahlgrenska Academy, Gothenburg, Sweden; 7) Department of Internal Medicine, University of Pisa, Pisa, Italy; 8) Metabolon, Inc., Research Triangle Park, North Carolina, US; 9) Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK; 10) Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark; 11) Hagedorn Research Institute, Copenhagen, Denmark; 12) Institute of Biomedical Science, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark; 13) Faculty of Health Sciences, University of Aarhus, Aarhus, Denmark.

Objective: Circulating metabolites may represent useful biomarkers, but their causal role in diabetes disease processes is less certain. We aimed to identify common genetic variants associated with 14 insulin sensitivity related metabolites, and assess whether these metabolites have causal effects on diabetes and insulin sensitivity. Research Design and Methods: Using 1,004 non-diabetic individuals from the RISC study we performed a genome wide association study on 14 insulin sensitivity related metabolites. We assessed the association of these variants with diabetes related traits in meta-analyses (MAGIC, DIAGRAM and GENESIS) and using Mendelian randomisation approaches. Results: We identified eight association signals with four metabolites: adrenate (rs174541 at *FADs* (*P*=2.9×10^{-9})); betaine (rs499368 at *SLC6A12*(*P*= 8.10×10^{-9}), rs17823642 at *BHMT* (*P*=1.40×10^{-6})); glycine (rs715 at *CPS1* (*P*=5.30×10^{-30}), rs1107366 at *ALDH1L1* (*P*=8.00×10^{-5})); serine (rs478093 at *PHGDH* (*P*=1.50×10^{-9}), rs4275190 at *PSPH* (*P*=4.00×10^{-4}), rs13233754 at *PSPH* (*P*=2.00×10^{-5})). The association between rs715 and glycine levels has a highly sex-specific effect (P=2.7×10^{-13} for female-specific effect). The allele in the *ALDH1L1* gene associated with raised glycine levels was associated with increased clamp based measures of insulin sensitivity (*P*=5.50×10^{-3}, (+0.09 umol.min^{-1}kgFFM^{-1}, 95%CIs 0.03, 0.15)), an effect consistent with a causal effect of glycine levels on insulin sensitivity, although further studies are needed to confirm this association. The associations observed between metabolite SNPs and fasting insulin were lower than those expected based on the SNP-metabolite and metabolit×10fasting insulin associations. Conclusions: Genetic variants influence insulin sensitivity related metabolites and can help understand the role of metabolites in diabetes related traits.

701W

Assessing RNA-seq differential expression levels with low-confidence mapped reads. *M. Pongpanich, J.Y. Tzeng, D. Nielsen.* North Carolina State University, Raleigh, NC.

RNA-seq is a promising approach for understanding transcriptomes due to its accuracy, large dynamic range of expression level and ability to detect novel transcripts. In RNA-seq experiment, millions of reads are generated from high-throughput sequencer. Before these reads can be used to estimate the expression level of a gene, they have to be mapped against a reference genome or transcript set using a certain alignment tool. It is often that a fair amount of reads cannot be mapped by the aligners and will be excluded from down-streaming analyses (e.g., 40%-50% reads unmapped using TopHat with defaults parameters in our datasets). To maximize the potential utility of sequenced reads, we study the characteristics of the unmapped reads and identify the major causes of reads fail to be aligned. Specifically, we use BLAST to align the unmapped reads, which allows a read to be aligned even if a sub-segment of the read is perfectly or partially consistent with the reference sequence. From the loosely mapped results of BLAST, we find that one key feature of the reads that are mapped by BLAST but not TopHat is the high level of dissimilarity occurring in the head region (>10 bp), or the tail region (>10bp), or both regions of a read. We then construct a statistical method to incorporate these low-confidence mapped reads by assigning weights that reflect the mapping confidence of a read. We investigate the pros and cons of incorporating the low-confidence mapped reads in detecting genes with differential expressions.

702W

Quantitative trait analysis for next-generation sequencing. *Y. Zhu[1], L. Luo[2], M. Xiong[1].* 1) University of TexasSchool of Public Health, Houston, TX; 2) University of New Mexico School of Medicine.

Next-generation sequencing (NGS) technologies will generate unprecedented massive, high-dimensional, and nonlinearly-structured data which combined with the biological, genetic and environmental heterogeneity of the populations present formidable challenges to data analysis. The current platform for quantitative trait analysis is single-variant regression (SVR) in which a quantitative trait is regressed on a single variant at a time. This platform provides useful tools for identifying the association of common variants with quantitative traits, but has limitations in analyzing thousands of sequences collected for very large population-based studies of humans. It is increasingly realized that a single polymorphic site is not the unit of genetic analysis (UGA), but that variation in a particular phenotype is influenced by variation in combinations of multiple common and rare variants in a genomic region. Simple linear regression which tests the genetic effect of an individual variant has limited power to detect association of rare variants. The classical multiple regressions also suffer from two serious problems: presence of multicolinearity and overfitting. To overcome these limitations, we propose smoothed functional linear models (FLM) for sequence-based quantitative trait analysis in which all variants in the genomic region are are collectively incorporated into the analysis as a UGA. The smoothed FLM can substantially reduce the multicolinearity in the genomic data and effectively control overfitting. We develop a novel statistic for testing the association of UGA with a quantitative trait in FLM. Extensive simulations are conducted to evaluate its type 1 error rates and compare its power with regressions. To further evaluate its performance, the proposed FLM is applied to two datasets of genetic studies of expressions which consists of 60 CEU and 57 YRI with expressions generated by RNA-seq and SNPs generated in 1000 Genomes Project. A number of genomic regions that contributes to the variation of overall expression levels of the genes is identified. We also observed that large proportion of identified genomic regions is overlapped in two datasets. Our preliminary results show that the smoothed FLM has much higher power to detect genomic regions that include entire spectrum of variants than the simple and multiple regressions. The smoothed FLM might emerged as a major tool for sequence-based quantitative trait analysis.

703W

Heritability of densitometric, structural and strength properties of bones in extended pedigrees from Spain: data from the GAO Project. *G. Athanasiadis[1], J. Malouf[2], A. Laiz-Alonso[2], A. Marin[2], A. Martinez-Perez[1], L. Rib[1], R. Perez[1], A. Buil[3], J. Casademont[2], J. Farrerons[2], JM. Soria[1].* 1) Unitat de Genòmica de Malalties Complexes, Institut de Recerca, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; 2) Servei de Medicina Interna, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; 3) Department of Genetics and Development, University of Geneva, Geneva, Switzerland.

The genetic basis of primary osteoporosis is complex, with multiple genes and environmental factors acting jointly to determine its risk. In order to shed more light in the genetics of osteoporosis, we designed the Genetic Analysis of Osteoporosis (GAO), a study based on extended pedigrees from Spain. For the purpose of the present analysis, a total of 110 individuals from five extended families were considered. All families were recruited on the basis of a proband with osteoporosis and the participants signed an informed consent statement. Spine, femur and whole body densitometry was performed using a Discovery densitometer with the Apex 2.3 software from HOLOGICH. Hip strength and geometrical properties were analyzed using the HSAH software included in Apex. Maximum likelihood-based covariance decomposition analysis was used to assess heritabilities (h2) and the genetic and environmental correlations (rG and rE) between phenotypes. This work presents the heritability found among the phenotypes. This is the first study that quantifies the genetic component of several important parameters involved in osteoporosis and fracture risk in extended pedigrees. The high heritability found for most of them indicates that genetic effects account for a significant proportion of the observed phenotypic variation of these parameters. Thus, a family-based strategy through a genome scan can be used to identify the genes involved in osteoporosis and fracture risk, hopefully leading to clinical applications.

704W

Estimating causal effects in Mendelian Randomization studies: how different is that from Randomized Controlled Trials? *K. Fischer[1,2], M. Kals[1,2], A. Metspalu[1,2].* 1) Estonian Genome Center, University of Tartu, Tartu, Estonia; 2) Center of Translational Genomics, University of Tartu, Estonia.

The aim of many studies in epidemiology is to estimate causal effects of an exposure phenotype on an outcome phenotype. However, even the best possible study designs cannot completely exclude the possibility of unobserved confounding, leading to biased estimates of the effects of interest. One way out is so-called Mendelian Randomization, provided there is a genetic marker (or a set of markers) that is predictive for the exposure, but does not have any effect on the outcome (except the possible indirect effect via the exposure). Now the genetic marker resembles a random assignment, creating groups of individuals that are comparable with respect to all possible confounding factors influencing the outcome. The causal effect is then estimated using Instrumental Variables (IV) estimation techniques, where the marker is used as an instrument. Such estimation techniques resemble the estimation of the effect of non-compliance (some patients not receiving their assigned treatment) in Randomized Controlled Trials (RCTs). Therefore the IV analysis methods that are used for the analysis of RCTs, have recently been implemented in the context of Mendelian Randomization. However, the interpretation of the resulting estimates in Mendelian Randomization studies is usually not the same in the context of RCTs. We will discuss three possible causal parameters in the context of RCTs - Complier Average Causal Effect (CACE), Average exposure Effect in the Exposed (AEE) and the marginal causal effect. We will show that the same IV estimator can be interpreted as an estimate for any of the three parameters, depending on the level of untestable assumptions one is ready to make. Using an example based on the data of the Estonian Genome Center, we will discuss the meaning of the same parameters in the context of Mendelian Randomization. We will argue that in this context, AEE is possibly the most natural parameter to aim for, whereas CACE is most difficult to interpret. We will also show that in order to achieve the interpretation of AEE for the IV estimate, a set of untestable assumptions is needed, such as the assumption of no-treatment-effect-heterogeneity. We will argue that the latter may often be implausible. As an alternative, we propose a sensitivity analysis approach, where possible lowest and highest values for the causal parameter are found. This idea will be implemented using the data of the Estonian Genome Center.

705W

Novel genetic locus for kidney stone using diverse ancestry populations: the Women's Health Initiative study. *N. Franceschini[1], A. Reiner[2,3], T. Chi[4], M.L. Stroller[4], A. Kahn[5], C. Carty[2], Y. Li[6], T.L. Edwards[7], R. Jackson[8].* 1) Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 3) University of Washington, Seattle, WA; 4) Department of Urology, University of California, San Francisco, San Francisco, CA; 5) San Francisco Coordinating Center, California Pacific Medical Center, San Francisco, CA; 6) Department of Biostatistics and Department of Genetics, University of North Carolina, Chapel Hill, NC; 7) Department of Medicine, Center for Human Genetics Research, Institute for Medicine and Public Health,Vanderbilt University, Nasville, TN; 8) Division of Endocrinology and Center for Clinical and Translational Science The Ohio State University, Columbus, OH.

Background. Kidney stones are highly prevalent in the US and have a known genetic component. Ethnic variation appears to play an important role in determining the risk for stone disease, as prevalence varies by ethnicity. **Methods.** To search for sequence variants accounting for kidney stone risk across populations of diverse ancestry, we conducted genome-wide association analyses in 8,400 African American and 3,587 US Hispanic women in the Women's Health Initiative SHARe cohort. Kidney stone was self-reported at study recruitment. Genotyping was performed using Affymetrix 6.0 and standard quality control was applied. Genome-wide ancestry estimates were calculated using Frappe. Imputation to 2.5 million single nucleotide polymorphisms (SNPs) was done in African Americans using a 1:1 ratio of HapMap Phase II CEU and YRI haplotypes as the reference population. Analyses used logistic regression and additive genetic models adjusted for age, body mass index and ancestry. **Results.** Overall mean age was 62 years, and 49 percent of women lived in Southern US. There was little evidence for genomic inflation in both African American and Hispanic samples (lambda 1.02) after adjusting for ancestry. Among 8,067 African American women with genotypes and phenotype data (cases=267), we identified several SNPs associated with kidney stones (lowest P=4.3 x 10^{-8}, minor allele frequency [MAF]= 0.37, imputation rsq 0.99) in a novel locus on 10p15.3. The SNPs have low frequency in CEU samples (MAF < 0.02). We then explored associations at this locus in our Hispanic genotyped sample (180 cases) and identified an associated proxy SNP (P=0.004, MAF= 0.05, D'=1.0 in HapMap CEU). Replication in additional African Americans is currently underway. For loci previously reported to be associated with kidney stones, we identified associations near the calcitonin receptor gene (*CALCR*, rs2106432, P=0.0001, African Americans), which has been implicated in kidney stone in children, but not 21q22.13 locus identified in Iceland. **Conclusions.** We identified a new locus for kidney stones in African Americans. The implicated SNPs have low MAF in both our Hispanic sample and in the HapMap CEU, suggesting that this effect may be relatively population-specific to persons of recent African Ancestry, who also experience a lower incidence of urinary stones. Our findings, if confirmed, demonstrate the utility of using samples of subjects with diverse ancestry in gene discovery.

706W

G80A polymorphism in the RFC-1 gene in rheumatoid arthritis Mexican mestizo patients treated with methotrexate. *M.G. González-Mercado[1,2], M.P. Gallegos-Arreola[2], M.C. Morán-Moguel[2], M. Salazar-Páramo[3], J.F. Muñoz-Valle[4], A. Moreno-Andrade[5], G. Martínez[6], J.I. Gámez-Nava[3], L. González-López[3], I.P. Dávalos[1,2].* 1) Doctorado en Genética Humana, IGH, CUCS, Universidad de Guadalajara; 2) División de Genética y Medicina Molecular, CIBO, Instituto Mexicano del Seguro Social; 3) División de Investigación, UMAE, HE, CMNO, Instituto Mexicano del Seguro Social; 4) Doctorado en Ciencias Biomédicas, IIRSME, CUCS, Universidad de Guadalajara; 5) Universidad Autónoma de Guadalajara; 6) Hospital Civil “Fray Antonio Alcalde”, OPD, Guadalajara, Jalisco, México.

Introduction. Rheumatoid arthritis (RA) is a common rheumatic disease. Methotrexate (MTX), a folic acid antagonist, is a disease modifying antirheumatic drug (DMARD) commonly used in AR. MTX is introduced to cell by the reduced folate carrier (RFC-1). Several studies have associated RFC-1 G80A polymorphism with a different response to MTX in RA patients. There are few studies in Mexican RA patients related to RFC-1 G80A polymorphism. Objective. To determine the frequency of RFC-1 G80A polymorphism in patients with RA treated with MTX (RA-MTX). Methods: Group RA-MTX consisted in 30 RA mestizo Mexican patients diagnosed according to the American College of Rheumatology 1987 and Group M in 121 normal Mexican mestizo individuals (Rodarte et al 2007). RFC-1 G80A genotypification by PCR/RFLP's HhaI method. Results. Genotype frequencies (GF) for RFC-1 G80A in group M (%,n) were: GG 28% (34), GA 54% (65), AA 18% (22). In group RA-MTX: GG 10%(3), GA 77% (23), AA 13% (4). Allelic frequency (AF) in group M for allele A was 45% (109) and in group RA-MTX was 52% (31). Statistically differences (p=0.04) was observed in the genotype GG with OR 0.28 (CI 95% 0.05-1.03), and GA genotype with OR 2.83 (CI 95% 1.07-8.36); p=0.03. Conclusions. The genotypic and allelic distribution between both groups (group AR-MTX and group M) were similar. However the analysis with codominant (RFC-1 GA) and dominant (RFC-1 GG) models showed statistically difference between both groups.

707W

Genotype imputation in African Americans: an evaluation for selecting an optimal reference panel. *D.B. Hancock, J.L. Levy, G. Page, E.O. Johnson.* Research Triangle Institute International, Research Triangle Park, NC and Atlanta, GA.

Genotype imputation is often conducted to expand coverage of single nucleotide polymorphisms (SNPs) in genome-wide association studies by using reference haplotype panels at a dense set of SNPs. In African Americans, imputation has relied on HapMap phase II reference populations of Africans (YRI), European Americans (CEU), and Asians (CHB/JPT). HapMap phase III now offers admixed populations that provide a closer genetic match, such as ASW (African ancestry in Southwest USA). There has been limited evaluation of imputation with phase III samples. Here, we compared imputation results in African Americans using 15 different reference panel combinations with 117 YRI, 121 CEU, 170 CHB/JPT, and 68 ASW population samples, by imputing with a single reference panel from one population, imputing with a combined panel of two or more populations, or sequentially imputing with single panels and then merging the imputed genotypes. After applying standard quality control, we imputed chromosome 22 SNPs using MACH in 1,070 African Americans genotyped on the Illumina HumanHap550.v1 BeadChip, available from Illumina's iControl database. We evaluated 20,085 genotyped and imputed SNPs with the following metrics: (1) number of SNPs with minor allele frequency (MAF)>1%; (2) efficiency rate defined as the percentage of SNPs with r^2>0.3 between imputed and true genotypes; and (3) concordance rate based on masking 2% of the genotyped SNPs and comparing imputed and true genotypes (averaged over 10 sets of randomly masked SNPs). When using one reference population, YRI and ASW had similar rates of concordance (~90%) and efficiency (96-98%), but the smaller ASW panel captured more phase III SNPs with MAF>1% [17,961 SNPs (89%) for YRI and 19,118 SNPs (95%) for ASW]. When using two or more reference populations, sequential imputation with YRI, CEU, and ASW provided the largest number of SNPs with MAF>1% [19,540 SNPs (97%)], but combining YRI, CEU, and ASW into one reference panel gave the highest rates of concordance (93%) and efficiency (99%). The imputation patterns were similar in 595 African Americans genotyped on HumanHap550.v3 from the iControl database. Our findings show that imputation performance in African Americans was optimized by using reference panels which include ASW. Selection of the best procedure for including different reference population samples appears to require the investigator to balance maximal SNP coverage with imputation quality.

708W

FCGR2A is the first Ulcerative Colitis susceptibility gene in African Americans. *C. Huang[1], S.E. Ellis[1], B. Kuhr[1], K.L. Isaacs[2], A.L. Silverman[3], J.D. Lewis[4], D.T. Smoot[5], J.F. Valentine[6], H.A. Kader[7], J.H. Cho[8], R.H. Duerr[9], J.D. Rioux[10], M. Silverberg[11], K.D. Taylor[12], Y. Wu[1], S. Hooker[13], R.A. Kittles[14], L.W. Datta[1], S.R. Brant[1], NIDDK IBD Genetics Consortium.* 1) Johns Hopkins University, Baltimore, MD; 2) University of North Carolina, Chapel Hill, NC; 3) Henry Ford Health System, Detroit, MI; 4) University of Pennsylvania, Philadelphia, PA; 5) Howard University College of Medicine, Washington, DC; 6) University of Florida , Gainesville, FL; 7) University of Maryland School of Medicine, Baltimore, MD; 8) Yale University, New Haven, CT; 9) University of Pittsburgh, Pittsburgh, PA; 10) Université de Montréal and the Montreal Heart Institute, Research Center, Montreal, QC, Canada; 11) University of Toronto, Samuel Lunenfeld Research Institute and Mount Sinai Hospital, Toronto, ON, Canada; 12) Cedars-Sinai Medical Center, Los Angeles, CA; 13) University of Chicago, Chicago, IL; 14) University of Illinois at Chicago, Chicago, IL.

BACKGROUND Ulcerative Colitis (UC) is a disease of chronic inflammation of the colon. It occurs in 0.2% of Americans, but is less frequent in African Americans (AA). Multiple genetic loci have been identified for UC in whites and in East Asian populations. Three UC susceptibility loci (FCGR2A, SLC26A3, and a locus at 13q12) were previously identified in a genome-wide association (GWA) study in Japanese samples. We evaluated these GWA signals in the under-studied AA population. METHODS A case-control study was carried out in 104 UC patients and 268 healthy controls. These samples were genotyped by either Taqman® SNP Genotyping Assay or direct sequencing. Loci that showed significant association were also genotyped in 293 samples of Crohn's disease, the other major inflammatory bowel disease phenotype. Allele-based and haplotype-based statistical analyses of the genotyping data were performed in PLINK. 97 Admixture Informative Markers (AIM) were genotyped to control for variation in admixture in 76% of study subjects. RESULTS The non-synonymous SNP (rs1801272, H131R) demonstrated significant association with UC (OR=1.638 (1.169-2.300) p-value=0.003). West African and European admixture did not differ between cases and controls (p=0.398), and UC association (in AIM genotyped samples) with H131R adjusted for admixture was similar (OR= 1.815 (1.248-2.642) p=0.002). Association for CD did not reach 0.05 alpha level (OR= 1.223 (0.960-1.559) p-value=0.094). To better localize the association at this gene five more SNPs in the haplotype block were genotyped. Only one SNP upstream of rs1801274, rs10800309, showed marginal association (OR=1.382 (0.970-1.963) p-value=0.065). The haplotype analysis of these two SNPs demonstrated that the haplotype consisted of the two major alleles was protective (=0.605 (0.434-0.844) p-value=0.003). This association was driven by the known functional SNP, rs1801274. The p-value of independent effect test was 0.062. Our results in the AA population were consistent with the previous findings in the Japanese population and in the recent UC GWA meta-analysis in whites. CONCLUSION UC risk in AA has molecular genetic causes. FCGR2A H131R was associated with UC in AA's. It is a gene polymorphism that alters function of a receptor for the Fc portion of IgG, and also has been found associated with lupus, rheumatoid arthritis and susceptibility to pseudomonas infection. Further research on genetic causes of UC in AA's are greatly anticipated.

709W

Association of osteoarthritis and serum bilirubin level -- a Framingham Heart Study. *J. Lin[1], N. Jeffries[1], F. Kronenbery[2], S. Hwany[3], L. Vitek[4], H. Schwertner[5].* 1) Office ob Biostatistics Research, Division of Cardiobascular Science, National Heart, Lung and Blood Institutes, National Institutes of Hearth, Bethesda, MD; 2) Division of Genetic Epimiology, Innsbruck Medical University, Innsbruck, Austria; 3) Framingham Heart Study, National Heart Lung and Blood Institute, National Institutes of Health, Framingham MA; 4) Department of Internal Medicine and Institute of Clinical Biochemistry and Laboratory Diagnostics, 1st Faculty of Medicine, Charles Universtiy In Pragur, Czech Republic; 5) Clinical Research, Wilford Hall Medical Center, San Antonio, TX.

The anti-oxidant properties of bilirubin exert a physiologic anti-inflammatory effect which might provide protection against rheumatoid arthritis. On the other hand, experimental data on mouse collagen-included arthritis exhibited a retarded elimination of bilirubin and was therefore associated with high serum bilirubin levels. Arthralgias after quinupristin-dalfopristin therapy demonstrated a strong association with elevated bilirubin levels at baseline. To date, no association study between human osteoarthritis and serum bilirubin levels has been reported. We investigated in the Framingham Heart Study population whether serum bilirubin levels are associated with osteoarthritis. The study population included 1597 individuals of Framingham Offspring cohort, mean age 53 years, 46.8% male. Bilirubin levels were measured during Offspring exam 1, in the early 1970's, and osteoarthritis was diagnosied in the exam 5 between 1993 and 1994. Of 1597 individuals, 456 had osteoarthritis, having a baseline age of 29-81 years, and 46.8% male. Logistic regression was used to test the association between osteoarthritis and bilirubin levels, adjusting for age, gender and BMI. Bilirubin levels are positively associated with osteoarthritis (p=0.001), with a doubling bilirubin associated with a 33% increase in the odds of osteoarthritis. Osteoarthritis has an opposite association with bilirubin levels as rheumatoid arthritis, but has the same positive association with mouse collagen-included arthritis and arthralgias after quinupristin-dalfopristin therapy.

710W

Distribution of Mitochondrial Haplogroups in the National Health and Nutrition Examination Surveys. *S. Mitchell, K.D. Brown-Gentry, P. Mayo, M. Allen, N. Schnetz-Boutaud, D. Murdock, D.C. Crawford.* Center for Human Genetics Research, Department of Molecular Physiology and Biophysics, Vanderbilt Univ, Nashville, TN.

Mitochondrial haplogroups are defined by combinations of mitochondrial DNA (mtDNA) polymorphisms inherited from a common ancestor. A number of studies report that mitochondrial haplogroups confer disease risk or even protection from disease. The National Health and Nutrition Examination Surveys (NHANES) are large, population-based surveys conducted in the United States by the National Center for Health Statistics at the Centers for Disease Control and Prevention (CDC). Each NHANES collects demographic, lifestyle, and health data from participants using surveys, laboratory measures, and medical examinations. For participants consenting to Genetic NHANES, DNA samples were collected for NHANES III (1991-1994), NHANES 1999-2000, and NHANES 2001-2002. Approximately 15,000 DNA samples representing 6,605 non-Hispanic whites (NHW), 3,477 non-Hispanic blacks (NHB), 3,950 Mexican Americans (MA), and 1,004 participants of other race/ethnicity are available for research purposes. The goal of the present work is to classify and describe the distribution of mitochondrial haplogroups in the NHANES samples. Ten SNPs were genotyped in all NHANES samples and 360 samples from the International HapMap Project using Sequenom. These ten SNPs were selected to determine major European haplogroups (H, I, J, K, T, U, V, W, and X) and are located throughout the coding and control regions of the mitochondrial genome. The major mitochondrial haplogroups were determined for 90% of NHW, 12% of NHB and 13.5% of MA. Additionally, 22% of participants of other self-declared racial/ethnic groups were successfully classified. As expected, among the NHW, haplogroup H was the most common classification (42%). The frequency of haplogroup H observed in our NHW population is similar to the frequency in the European descent (CEU) HapMap samples (44%). Other haplogroups identified among NHW included: U (15%), J (10%), T (9%), and K (7%), with the remaining haplogroups (I, V, W, X) found among fewer than 5% of NHW participants. With these ten SNPs, we were able to classify mitochondrial haplogroups for the majority of NHW in the combined NHANES sample. As expected, we were able to assign mitochondrial haplogroups for only a small fraction of NHB and MA individuals based on the SNPs genotyped. Additional SNPs are required to resolve haplogroups for NHB and MA participants, and full haplogroup resolution is ideal for downstream genotype-phenotype correlations in NHANES.

711W

Identifying Independent Genetic Associations in Complex Disease using Penalised Multivariate Logistic Regression and Hierarchical Modelling: A Case Study on Systemic Lupus Erythematosus. *D.L. Morris[1], J. Bentham[1], M.E. Alarcón-Riquelme[2], J.D. Rioux[3], T.J. Vyse[1], BIOLUPUS.* 1) Department of Medical and Molecular Genetics, King's College London, London, SE1 9RT. United Kingdom; 2) GENYO. Centro de Genómica e Investigación Oncológica Pfizer. Universidad de Granada, Junta de Andalucía, Avda de la Ilustración 114, Granada, Spain. 18007. +34 958 637 078; 3) Université de Montréal, Montreal Heart Institute, 5000 rue Belanger, Suite S-6300, Montreal, Quebec, Canada. H1T 1C8.

Delineating the genetic contribution to the risk of developing a complex disease is complicated by the likelihood that disease is caused by multiple genes. The search for disease causing loci will therefore require the identification of multiple markers, each contributing individually perhaps a small increase in risk. Rather than performing multiple individual tests for single marker association, we propose that information should be shared between markers in Genome Wide Association Studies (GWAS). This can be achieved using multivariate penalised regression or hierarchical modelling with hyper-parameters that are shared across all markers. In this setting, the size of the data is a strength rather than a weakness. In this study we compare the use of stepwise regression, penalised logistic regression (PLR) and hierarchical modelling, applied to the largest GWAS in Systemic Lupus Erythematosus (SLE) (4,254 Cases and 8,578 Controls typed on the Illumina Omni-Quad chip) in a European population. SLE is one of the many complex diseases that are still without an adequate explanation for the known heritability (>66% in the case of SLE), and many studies have analysed genetic variation within the genome with the aim of identifying multiple loci independently associated with SLE. The results from the SLE GWAS study show that PLR is more robust against false positives than stepwise regression, but without loss of power. We demonstrate the benefits of PLR with an analysis of the MHC region. Stepwise regression was sensitive to the metric used for including variables and identified more than ten loci in some cases (depending on the metric) while PLR using the Hyper Lasso (and one SR approach with the FDR) selects five, two of which were previously known to be associated and three which are novel. We also show, analysing each chromosome separately (Number of markers: 20k-60k), that hierarchical modelling accounts for multiple testing (MT) naturally, without user specification of hyper-parameters or pre-defined adjustments. We argue therefore that there is enough information in current GWAS to estimate hyper-parameters shared across markers. These hyper-parameters act as a penalty against the inclusion of multiple correlated markers and avoid the requirement for arbitrary adjustments for MT. We conclude that GWAS should exploit the data size by sharing information between markers in the search for multiple disease associated loci.

712W

Prevalence of Spinocerebellar ataxia type15 in Japan screened with Taqman PCR assay. *M. Obayashi[1], M. Takahashi[1], Y. Niimi[1], N. Sato[1], O. Onodera[2], K. Ishikawa[1], M. Nishizawa[2], H. Mizusawa[1].* 1) Department of Neurology and Neuroscience, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo-to, Japan; 2) Department of Molecular Neuroscience, Niigata University.

Spinocerebellar ataxia type 15 (SCA15) is a slowly progressive neurodegenerative disorder characterized by almost pure cerebellar ataxia. The cause of SCA15 is mutation of the ITPR1 gene (inositol 1,4,5-triphosphate receptor, type 1). Many SCA15 families have partial deletions and one family has a missense mutation. prevalence of SCA15 were reported from Europe. In Japan some SCA15 families were reported. We perfomed Taqman PCR assay to search for ITPR1 deletions in all 225 patients' samples which were gathered to SCA genetic diagnosis but were negative for mutations in SCA1, 2, 3, 6, 31, and DRPLA genes. We identified only one patient with an ITPR1 deletion. She developed gait disturbance at 51 years old, and progressed to show pure cerebellar ataxia. Her two brothers have ataxic symptoms. In our study, ITPR1 deletions were rare in Japan, comprised 0.03% in all SCA in our cohort. This indicates that SCA15 is much rare in Japan than in Europe.

713W

Impact of the Amerindian genetic ancestry on the clinical and socio-demographic expression of systemic lupus erythematosus in a European-Amerindian Admixed population. *E. SANCHEZ[1], L. RIBA[2], A. RASMUSSEN[1], J.A. KELLY[1], K.M. KAUFMAN[1,3,4], E. ACEVEDO[5], M.H. CARDIEL[6], E.E. BROWN[7], G.S. ALARCON[7], J.C. EDBERG[7], J.M. ANAYA[8], J.F. MOCTEZUMA[9], J.A. JAMES[1,3], J.D. REVEILLE[10], M. PETRI[11], R. RAMSEY-GOLDMAN[12], P.M. GAFFNEY[1], I. GARCIA DE LA TORRE[13], C. PERANDONES[14], P. ALBA[15], J. MUSURANA[16], A. GOECKE[17], J.A. ESQUIVEL-VALERIO[18], M.A. MARADIAGA-CECEñA[19], T. TUSIE-LUNA[2], J.B. HARLEY[20], B.A. PONS-ESTEL[21], C.O. JACOB[22], M.E. ALARCON-RIQUELME[1,23], SLEGEN and GENLES.* 1) OKLAHOMA MEDICAL RESEARCH FOUNDATION, OKLAHOMA CITY, OK; 2) Instituto de Investigaciones Biomédicas de la Universidad Nacional Autónoma de México, Mexico City, Mexico; 3) University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA; 4) Department of Veterans Affairs Medical Center, Oklahoma City, Oklahoma, USA; 5) Hospital Nacional Guillermo Almenara Irigoyen, Lima, Peru; 6) Hospital General Dr. Miguel Silva, Morelia, Mexico; 7) Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA; 8) Center for Autoimmune Diseases Research (CREA), Universidad del Rosario, Bogotá, Colombia; 9) Hospital General de Mexico, Mexico City, Mexico; 10) Department of Medicine, University of Texas-Houston Health Science Center, Houston, TX, USA; 11) Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA; 12) Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; 13) Hospital General de Occidente, Zapopan, Mexico; 14) Centro de Educación Médica e Investigaciones Clínicas (CEMIC), Buenos Aires, Argentina; 15) Hospital Córdoba: Patria y Libertad, Córdoba, Argentina; 16) Hospital José Bernardo Iturraspe, Santa Fe, Argentina; 17) Hospital Clinico, Universidad de Chile, Santiago de Chile, Chile; 18) Hospital Universitario, Nuevo León, Mexico; 19) Hospital General de Culiacan, Culiacan, Mexico; 20) Rheuamtology Division and Autoimmune Genomics Center, Cincinnati Children's Hospital Medical Center; and US Department of Veterans Affairs Medical Center, Cincinnati, OH, USA; 21) Sanatorio Parque, Rosario, Argentina; 22) Department of Medicine, University of Southern California, Los Angeles, CA, USA; 23) GENYO. Centro de Genómica e Investigacion Oncológica Pfizer-Universidad de Granada-Junta de Andalucía.

Background: Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease characterized by the presence of auto-antibodies and immune-complexes and with diverse clinical manifestations. The prevalence and severity of SLE has a wide variation among different ethnic groups. SLE occurrence is three to four times higher amongst Asian and African-American women. In addition, Hispanics, Asians and African-Americans have an excess morbidity from SLE and higher prevalence of lupus nephritis than Caucasians. Probably both genetic and environmental factors are involved in these disparities. Objective: To analyze the relationship between genetic ancestry and socio-demographic characteristics and clinical features in a large cohort of Hispanics SLE patients. Material and methods: A total of 922 SLE patients and healthy controls of Hispanic origin with socio-demographic data and a total of 1274 SLE patients with clinical data were used in the study. Genotyping of 347 continental ancestry informative markers (AIMs) was performed on the Illumina platform. The STRUCTURE software was used to determine genetic ancestry of each individual. Correlation between ancestry and socio-demographic and clinical data were analyzed using logistic regression. Results: The total Amerindian genetic ancestry for 1274 SLE patients is 36.4% ± 0.20. After logistic regression analysis we found a correlation between increased Amerindian ancestry and low medical coverage (p= 0.002) and less years of education (p= 0.0003). However, there were no significant differences between a low socio-economic level using the Graffar scale and Amerindian ancestry. We found no differences between age of onset and Amerindian ancestry. We found a decrease in Amerindian ancestry in SLE patients with photosensitivity (p= 0.004) and an increase in Amerindian ancestry in patients with renal involvement (p= 0.0012). Renal involvement did correlate with early age of onset, independently of the ancestry (p< 0.0001). Conclusion: In general, genetic Amerindian ancestry correlates with lower educational level, worse medical coverage and renal disease than genetic European ancestry, which correlates with photosensitivity. Age of onset correlated with renal disease independently of the ancestry.

714W

Using Multiple Measures for Quantitative Trait Association Analyses : Application to Estimated Glomerular Filtration Rate (eGFR). *A. Tin[1], B.C. Astor[1], E. Boerwinkle[2], E. Colantuoni[1], J. Coresh[1], W.H. Kao[1].* 1) 1. Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe Street, Baltimore, MD; 2) 2. Human Genetics Center, University of Texas School of Public Health, Houston, TX 77030, USA.

Background: Although non-genetic studies have shown that multiple measures of a quantitative trait can improve the efficiency (i.e. reduce standard error) of subject level parameter estimates, little is known about the improvement in efficiency in genome-wide association studies (GWAS) that utilize multiple measures of a trait. Methods: We conducted a simulation study to examine how efficiency, for a fixed sample and genetic effect size, is affected by the number of measures, the variance of and the correlations between measures, and the missing data rate. We used linear regression for single measure and mixed effect model for multiple measure model. For ease of comparison, the gain in efficiency in the multiple measures model versus the single measure model was expressed as a gain in equivalent sample size. We also compared the empirical results from 3 GWAS of renal function in 9049 European American participants of the Atherosclerosis Risk in Communities (ARIC) study: a single measure of eGFR, a 3-measure model of eGFR based on serum creatinine and a 6-measure model using six outcomes derived from four biomarkers (serum creatinine, cystatin C, beta trace protein, and beta-2 microglobulin). Results: Given similar effect sizes across measures, the gain in efficiency increased as the correlation or variance of outcome measures decreased. When the variances and effect sizes were the same from two different measures, the equivalent sample size gain in using two measures was approximately 30% for a correlation of 0.5 between measures, but only 9% for a correlation of 0.8. The addition of a third measure brought further gain albeit smaller. When the variance of the additional measures exceeded the variance of the first measure given similar effect sizes, the gain in equivalent sample size was much reduced. When the variance of the second measure was 70% larger than the first measure, the gain in equivalent sample size dropped to 16% for a correlation of 0.5 between the two measures. In the GWAS of renal function, the single measure model detected one genome-wide significant (p<5x10-8) locus while the 3-measure model detected three, and the 6-measure model detected two. Conclusion: Multiple measures of a quantitative trait can increase the efficiency, thus the power, of a study without additional recruitment. However, careful attention must be paid to the effect sizes and variances of and the underlying correlations between the measures.

715W

A Comparison of Approaches to Control for Confounding Factors by Regression Models. *C. Xing[1], G. Xing[2].* 1) McDermott Ctr, Univ Texas SW Med Ctr, Dallas, TX; 2) 1Bristol-Myers Squibb Company, Pennington, NJ.

A common technique to control for confounding factors in practice is by regression adjustment. There are various versions of regression modeling in the literature, and in this paper we considered four approaches often seen in genetic association studies. We carried out both analytical analyses and simulation studies comparing the bias of effect size estimates and examining the test sizes under the null hypothesis of no association between an outcome and an exposure. Further, we compared the methods in a genome-wide scan for plasma lipoprotein(a) levels in the Dallas Heart Study. We found that a widely employed approach that models the covariate-adjusted outcome and the exposure leads to an infranominal test size and underestimation of the exposure effect size. In conclusion, we recommend either using multiple regression models or modeling the covariate-adjusted outcome and the covariate-adjusted exposure to control for confounding factors.

716W

Association between 11q23-25 region and triglyceride in metabolic syndrome. *M.S. Fallah[2], M.S. Daneshpour[1], S. Alfadhli3[3], A. Rebai[4], M. Hedayati[1], M. Zarkesh[1], F. Azizi[5].* 1) 1. Obesity Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti MC, Tehran, Iran; 2) 2. Kawsar Human Genetics Research Center (KHGRC), Tehran, Iran; 3) 3. Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Kuwait University, Kuwait; 4) 4. Centre of Biotechnology of Sfax, Tunisia; 5) 5. Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti MC, Tehran, Iran.

Introduction: Metabolic syndrome is common and has a rising prevalence worldwide. Raised serum triglyceride is one of the risk factors of metabolic syndrome. Here we tried to find the association between triglyceride level and metabolic syndrome using micro-satellite as genetic markers. Materials and methods: Twelve microsatellites in 4 different chromosomes were selected to investigate possible genes linked to the serum triglyceride level. 65 families with metabolic syndrome pattern who were participated in Tehran lipid and glucose study included in the study. Family based association test (FBAT) used to test the association and linkage. LOD score was calculated by Merlin. Result: Family Based Association Tests (FBAT) under the bi-allelic mode showed that serum triglyceride is linked with D11S1304 after bonferroni correction for multiple testing (p<0.05). Max LOD score calculated by non-parametric linkage analysis in dominant model was 0.96 in 16q23-24 region (D16S3096). Conclusions: Serum triglyceride is one of the main components of metabolic syndrome and the result of this study showed that genetic variation could play a significant role in this regard. To find a candidate gene responsible for triglyceride change in metabolic syndrome, these associated regions should be investigated further.

717W

Using linkage data to prioritize analysis of data from whole exome sequencing. *T. Foroud[1], D. Koller[1], D. Lai[1], H. Lin[1], N. Pankratz[1], Y. Liu[1], R. Deka[2], L. Sauerbeck[2], H. Ling[3], K. Doheny[3], E. Pugh[3], J. Broderick[2], Center for Inherited Disease Research and The FIA Study.* 1) Indiana Univ Sch Med, Indianapolis, IN; 2) University of Cincinnati Sch Med, Cincinnati, OH; 3) Center for Inherited Disease Research, Johns Hopkins University School of Medicine, Baltimore, MD.

Whole exome sequencing is becoming a relatively common approach to identify genes contributing to Mendelian disorders. It can also be used to identify genes contributing to diseases with complex genetic etiology, particularly in subsets of families that might segregate a highly penetrant rare variant(s). In these families, prior linkage data is often available and can be used to prioritize chromosomal regions for initial review of sequencing data. We are currently performing whole exome sequencing in 7 families selected because of the high density of affected members with intracranial aneurysms (IA). In each family we had at least 3 affected individuals, all with either a ruptured or unruptured IA. In one family, there is an additional member with an abdominal aortic aneurysm. Prior to performing whole exome sequencing (Agilent© SureSelect™ 50Mb Human All Exon Kit), an Illumina® OmniExpress array was genotyped for each affected family member. In addition, both affected and unaffected family members were previously genotyped using the Illlumina 6K linkage screen. Some families were also genotyped with microsatellite markers. We performed linkage analysis using both dominant and recessive models employing all available marker data. We assumed that each family might have a unique genetic etiology and reviewed LOD scores across the genome for each family. We identified chromosomal regions providing LOD scores greater than 1.5 with support from multiple markers. We reviewed each interval in detail to identify SNPs that could delimit the region of linkage and provided definitive evidence of recombination. Five of the seven families generated LOD scores greater than 1.5. We also identified chromosomal regions with more modest evidence of linkage but which overlapped across families. Preliminary next generation sequencing data in six of the seven families was recently generated. Comparison of genotypes from the OmniExpress and sequencing data had greater than 99.9% concordance. Average per sample exon coverage (at > 8x) was 89.4% and the average missing exon coverage (no coverage) was only 3%. These measures support the high quality of the sequencing. The preliminary linkage results are currently being used as an early filter to identify promising variants and genes. This approach may be very helpful, particularly in families with autosomal dominant inheritance in which many variants will be identified and prioritization will be essential.

718W

Age-related macular degeneration in an Amish population: the reliability of self reporting disease. *J.L. Haines[1], A.C. Cummings[1], D. Fuzzell[1], A. Agarwal[2], J. Gauthier[1], R. Laux[1], W.K. Scott[3], M.A. Pericak-Vance[3].* 1) Ctr Human Genetics, Vanderbilt Univ Medical Ctr, Nashville, TN; 2) Vanderbilt eye Institute, Vanderbilt Univ Medical Ctr, Nashville, TN; 3) Dr. John T. Macdonald Foundation Department of Human Genetics, Univ of Miami, Miami, FL.

Age-related macular degeneration (AMD) is a complex disorder that attacks the central retina, causes drusen, atrophy, and choroidal neovascularization, and causes debilitating vision loss in approximately 1.5 million Americans affected by advanced forms of the disease. Genes known to increase risk for AMD are *CFH*, *ARMS2*, and *C3*. Cigarette smoking also increases risk. The gene *CFB* decreases AMD risk. We have been working with the elderly population (age 65+) of the well-defined and genetically isolated Amish communities of Holmes County and surrounding counties in Ohio as part of a larger study of other elderly phenotypes. Seventy three individuals, who connect into a single 1505-member pedigree obtained from the Anabaptist Genealogy Database, have been examined by an ophthalmologist and self reported to have been or to not have been given a previous diagnosis of AMD. Forty two of those individuals were diagnosed with AMD, and thirty nine of the 42 diagnosed with AMD had previously self reported to have ever been diagnosed with AMD. Thirty one individuals were determined to not have AMD, and only five of those individuals self reported to have ever been diagnosed with AMD. We found the false positive rate of reporting AMD when AMD diagnosis was denied by an ophthalmologist to be 16%, and the false negative rate of not reporting previous AMD affection when an ophthalmologist confirmed AMD to be 7%. The corresponding positive predictive value for those that truly had AMD out of those that self reported to be affected is 89%, and the negative predictive value for those determined to not have AMD out of those reporting no previous diagnosis of AMD is 90%. Therefore, self report of AMD can be used as a reliable proxy for actual AMD affection status. This information paves the way for future studies of AMD genetic risk factors in the Amish population, through which we can determine if currently known risk genes have an effect on this population. We have already determined that a small subset of the affected individuals do not harbor *CFH* risk alleles. This unique genetically and environmentally homogeneous population also provides the opportunity for discovering novel risk variants for AMD.

719W

Identification of loci enriched for nuclear-encoded mitochondrial proteins underlying mobility in oldest-old Amish. *J.E. Hicks[1], J.R. Gilbert[1], L. Jiang[1], A.C. Cummings[2], L. Caywood[1], L. Reinhart-Mercer[1], D. Fuzzekk[2], C. Knebusch[2], R. Laux[2], C.E. Jackson[3], M.A. Pericak-Vance[1], J.L. Haines[2], W.K Scott[1].* 1) Hussman Institute for Human Genomics University of Miami, USA; 2) Center for Human Genetics Research Vanderbilt University, USA; 3) Scott & White Temple, TX.

Gait speed is heritable and strongly predicts incipient disability and mortality. To examine genetic influences on aging-related traits such as gait speed, adults over 80 were enrolled in a population based survey of Amish communities in Indiana and Ohio. The Anabaptist Genealogy Database was used to connect all 407 participants in a 13-generation pedigree. Genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 6.0. Gait speed was measured by timing subjects on a 10ft walk. The large pedigree was cut down into 46 computationally feasible families, containing 273 mobile individuals (those with gait speeds greater than 0.52 m/s, representing the top 2/3 of all individuals). These were then analyzed using multipoint non-parametric linkage (LOD*) analysis implemented in Merlin. PLINK was used to select a set of 4966 SNPs in linkage equilibrium (pairwise r2 < 0.16 for all markers). Sex equal inter-marker map distances (in Kosambi cM) were obtained from the Rutgers Combined Physical-Linkage Map. Associations within linkage signals were tested using the MQLS test for correlated data. Linkage analysis identified 7 regions significantly linked to mobility: two regions on chromosome 1 (LOD*=3.62, 1 LOD unit support interval (SI): 9.25cM to 21.14cM; LOD*=3.15, SI: 36.54cM to 48.45cM), and regions on chromosome 4 (LOD*=4.20, SI: 182.30cM to 192.06cM), chromosome 5 (LOD*=3.08, SI: 114.77cM to 146.73cM), chromosome 10 (LOD*=3.181, SI: 122.93cM to 146.73cM), chromosome 17 (LOD*= 3.19, SI: 120.68cM to 131.38cM) and chromosome 19 (LOD*=4.01, SI: 46.58cM to 52.69cM). No SNPs in the seven SI were associated with mobility after correcting for multiple comparisons. The chromosome 4 interval overlaps just one gene, ODZ3. The interval on chromosome 19 overlaps a previously observed osteoarthritis locus and contains UQCRFS1, a protein in the mitochondrial electron transport chain. Indeed, six of the seven intervals contain excellent candidate genes that localize to the mitochondrial membrane, suggesting that maintenance of mitochondrial function may be important in preserving mobility, and lending support to theories of mitochondrial involvement in aging processes.

720W

Pedigree-based Imputation Infers Pure *in-silico* Genotypes and Improves Power for Genetic Associations: the Framingham Heart Study. *J. Huang[1, 2], MH. Chen[1,3,4], WM. Chen[5], C.J. O'Donnell[1,2,6], Q. Yang[1,4].* 1) Framingham Heart Study, National Heart Lung & Blood Institute, framingham, MA; 2) Division of Intramural Research, National Heart, Lung and Blood Institute, Bethesda, MD; 3) Department of Neurology, Boston University School of Medicine, Boston, MA; 4) Department of Biostatistics, Boston University School of Public Health, Boston, MA; 5) Center for Public Health Genomics, University of Virginia, Charlottesville, VA; 6) Cardiology Division, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Background: The Framingham Heart Study started in 1948 and has recruited 3 generations of participants over more than 60 years. The majority of participants are in multigenerational families. 9,247 out of 14,531 participants were genotyped with Affymetrix 550K panel while the rest do not have genome-wide genotypes mainly due to lack of DNA samples. Our objective is to examine validity and potential power gain from imputing completely un-genotyped individuals using genotyped relatives in genome-wide association studies in this large family cohort. **Methods**: Large pedigrees over 20 bits were split into sub-pedigrees equal to or less than 20 bits, using a strategy to maximize the number of genotyped relatives for each un-genotyped individual. With the input of 8,520 individuals with high quality genome-wide genotyping and 150 individuals with sparse genotyping (N SNPs=5,749), we imputed genome-wide genotype data for the 150 sparse and 2,971 un-genotyped participants who have one or more genotyped relatives. For these 2,971 un-genotyped individuals, 1990, 931, and 50 belong to the first, second, and third generations, respectively. A SNP was defined as "well imputed" when the most likely genotype could be estimated with over 95% certainty. The imputed dosage was used in association analysis. We used an established association (*FGB* with fibrinogen) as a positive control to evaluate the power for association analysis when purely inferred genotype dosage is included. **Results**: For the 150 individuals with sparse genotype, 98 were imputed attaining more than 75% of well imputed SNPs genome-wide. For the 2,971 un-genotyped individuals, 271 have more than 75% well imputed SNPs, and the number increases to 738 and 1,440, respectively, for 50% and 25%. For the SNP rs2227426 within *FGB*, previously found to be associated with fibrinogen ($p=1.66x10^{-10}$) with genotyped individuals only, the significance improves to $p=5.38x10^{-11}$ when the imputed samples are included. We will present genome-wide association results for several phenotypes, where a significant number of cases have only imputed genotypes. **Conclusion**: This is the first study that demonstrated pedigree structure could be used to impute genome-wide genotype data for samples originally without any genotype. Our findings suggest that purely imputed *in-silico* genotypes based on informative family structures can be used to increase power in genome-wide association studies.

721W

Integrating sparse and dense marker data to interrogate a linkage signal. *E. Marchani[1], E. Wijsman[1, 2, 3].* 1) Div Med Gen, University of Washington, Seattle, WA; 2) Dept Biostat, University of Washington, Seattle, WA; 3) Dept Genome Sci, University of Washington, Seattle, WA.

Linkage analysis is an important step toward disease gene identification, but it is rarely the final one. Here, we combine a genome-scan panel of microsatellites (STRs) with dense SNPchip data to explore a linkage region in a large Alzheimer's disease pedigree. We defined a linkage region to encompass all STRs with single-marker evidence of linkage, plus two flanking markers on either side. We then combined the underlying STRs with SNPs selected for high heterozygosity and low missingness to create a 1cM marker map across the linkage region. Our primary goals were validate and narrow our linkage region and to identify probable carriers of the segregating trait locus.

We found that dense SNP data reduced the uncertainty caused by missing genotype data by increasing the constraints on ancestral genotypes. We observed a dramatic reduction in the number of equivalent sets of inheritance vectors at a given locus, estimated by the MORGAN programs gl_auto and IBDgraph, when using a dense rather than sparse marker panel. We also found that dense marker data replicated our linkage signal and improved its strength, likely because of this reduction in uncertainty. The denser marker map consistently increased the posterior probability of linkage relative to the original genome scan when analyzed using Markov chain Monte Carlo (MCMC) methods. We finally identified SNPs to tag the chromosomal segment co-segregating with the underlying trait locus. We investigated 11,313 SNPs across the linkage region using a measured genotype approach, and examined in greater detail those SNPs explaining the greatest amount of genetic variance in the trait. The SNPs falling within the 99th percentile spanned the entire linkage region, had minor allele frequencies ranging from 0.1 to 0.5 at the population level, and reduced the linkage signal when included as a covariate in the trait model. This highlights the importance of examining an entire linkage region, rather than the linkage peak alone, and not focusing too heavily on rare variants. In the future, we will combine genotypes at these influential SNPs with the inheritance vectors estimated from the 1cM marker panel to build a SNP haplotype tagging the chromosomal segment co-segregating with the trait. Relatives sharing that haplotype are most likely to share an underlying trait variant, and will be targeted for additional sequencing and functional analyses.

722W

The Role of Rare Genetic Variation in Asperger Syndrome in Finnish Families. *M. Rossi[1,2], K. Rehnström[3], H. Kilpinen[3], I. Hovatta[1,4,5], S. Ripatti[1], A. Palotie[1,3].* 1) Institute for Molecular Medicine Finland (FIMM), Helsinki, Finland; 2) Unit of Public Health Genomics, National Institute for Health and Welfare, Helsinki, Finland; 3) Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom; 4) Research Program of Molecular Neurology, University of Helsinki, Helsinki, Finland; 5) Department of Medical Genetics, University of Helsinki, Helsinki, Finland.

Background: Asperger syndrome (AS) belongs to autism spectrum disorders (ASD). AS is differentiated from autism by later onset of symptoms, lack of mental retardation and lack of language development delay. Recent studies have estimated the prevalence of AS as 2.5-2.9 per 1000. The etiology of AS has remained unknown but it is generally considered as a multifactorial disorder with several predisposing genes. However, gene mapping of AS candidate genes has been hindered by significant clinical and genetic heterogeneity, whereupon the search for common variants has not been successful. Aim of the study: The aims of the study are to 1) Identify genomic regions shared identity-by-descent in the Finnish AS families, 2) Identify rare functional variants within localized regions, and 3) Identify CNVs modifying the risk for AS. Materials and methods: The AS family sample consists of 175 individuals from 25 large families (naffected=123) genotyped with Illumina HumanOmni1-Quad BeadChip and Illumina HumanHap550 BeadChip. Multipoint linkage was performed with 19 000 SNPs using Merlin, and singlepoint linkage with 150 000 SNPs using ANALYZE 1.9.4.beta. Exome seguencing has been performed in Wellcome Trust Sanger Institute, Cambridge, UK. Results: Two loci in the whole AS study sample result in NPL>1.5; 3p22-3p14 (NPLall=1.93) and 20q12-20q13 (NPLall=1.52). This region in 3p22-3p14 overlaps with the region identified in a previous microsatellite linkage scan by our group, although NPLall=3.83 was observed using microsatellites. The region on chromosome 20 did not show evidence for linkage in the original microsatellite scan. Conclusions: Our preliminary results reveal several IBD shared regions within families, which suggests there are familial effects contributing to AS risk. Exome sequencing data will be followed up for these regions with an initial focus on families that show limited number of IBD shared regions in all together 15 genomic loci. The aim is to identify functional variants contributing to AS risk in each family.

723W

Individuals with both type 1 diabetes and celiac disease autoimmunity carry private risk variants for both diseases. *J. Romanos[1], G. Trynka[1], L. Franke[1], M. Platteel[1], S.A. Medema-Jankipersadsing[1], A.K. Steck[2], J.M Norris[3], G. Eisenbarth[2], M. Rewers[2], E. Liu[2], C. Wijmenga[1].* 1) Department of Genetics, University Medical Center Groningen, Groningen, Netherlands; 2) Barbara Davis Center for Childhood Diabetes, University of Colorado Denver, Aurora, Colorado, USA; 3) Epidemiology Department, Colorado School of Public Health, Aurora, Colorado, USA.

Type 1 diabetes (T1D) and celiac disease (CD) cluster in families and co-occur in patients. Some of the genetic regions and even some SNPs have been associated with both diseases. Our aim was to determine the genetic differences between individuals developing both diseases and those having only T1D or only CD. In a pilot study, we genotyped 48 established CD and/or T1D associated SNPs in 1115 non-Hispanic white American (NHWA) T1D patients, in 803 Dutch CD patients (confirmed by biopsy) and in 260 NHWA T1D patients with CD-autoimmunity (CDA&T1D group), defined as persistent tissue-transglutaminase positivity on two consecutive visits. Our analysis showed that individuals with both diseases accumulate private T1D and CD risk variants. To improve the study, we genotyped 196,524 SNPs present on Immunochip in 257 American and 4 Dutch CDA&T1D patients, and 1146 Dutch CD patients. The immunochip (Illumina iSelect custom array) includes variants from the 1000 Genomes pilot CEU and re-sequencing study variants for 183 loci associated to 12 immune-mediated diseases. Association analysis was performed using logistic regression, adjusting for gender and the first two components from the multidimensional scaling to adjust for the different ethnic backgrounds of our samples. We saw an enrichment of association signals in the autoimmune disease regions, but not for 1753 SNPs associated to bipolar disorder. This indicates that the association signals are true and our data set shows no stratification. We calculated a platform-specific significance threshold to be 1.9e-6 at 5% false discovery rate and identified two regions, IRF4 locus (OR=2.6 [1.9-3.7]) and a new signal on chromosome 11 (OR=1.7 [1.4-2.1]), reaching this threshold. Another three loci were suggestive at p-value <1e-5. These regions include PTPN22 (OR=2.0 [1.5-2.7]), CTLA4 (OR=1.7 [1.4-2.1]) and INS (OR=2.0 [1.5-2.7]) loci, which are known to be associated to T1D. We acknowledge that the CDA&T1D group was small so we are now expanding it by adding cases from the Type 1 Diabetes Genetics Consortium samples. We are also performing the same analysis to compare the genetic background of individuals with only T1D to those who develop both T1D and CDA. Finally, despite its limitations, this study suggests that individuals with both T1D and CDA do carry the celiac risk variants as well as private T1D risk variants but do not have enrichment for those that are shared between both diseases.

724W

Genome-wide interaction analysis of exogenous estrogen in age-related macular degeneration (AMD): Novel association of Retinitis Pigmentosa and Retinoblastoma loci. *MD. Courtenay[1], AC. Naj[2], WH. Cade[2], PL. Whitehead[2], I. Konidari[2], SG. Schwartz[3], JL. Kovach[3], A. Agarwal[4], G. Wang[2], JL. Haines[5], MA. Pericak-Vance[1,2], WK. Scott[1,2].* 1) Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL; 2) John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 3) Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL; 4) Vanderbilt Eye Institute, Vanderbilt University Medical Center, Nashville, TN; 5) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

AMD is the leading cause of irreversible vision loss in developed countries. Its multifactorial etiology includes both genetic and environmental risk factors. Previous studies have shown that women who take exogenous estrogen in the form of hormone replacement therapy (HRT) or birth control pills (BCPs) have reduced risk of developing AMD. The purpose of this study was to detect novel gene-environment interactions with these established risk factors using case-only analysis (CO). Affymetrix 6.0 chipsets were used to genotype 668238 SNPs in 540 Caucasian females with early and late AMD. After quality control checks, imputation against the HapMap CEU reference panel was used to generate genotypes for a total of 2.5 million SNPs with imputation quality R^2>0.3. History of ever taking HRT or BCPs was collected with a self-administered questionnaire. Next, CO tests were conducted for association between each SNP and HRT (278 Ever, 262 Never) or BCPs (108 Ever, 426 Never) using logistic regression models adjusted for age and smoking. Tests were conducted overall and in the neovascular AMD subset. Although no results met Bonferroni-corrected genome wide significance, there were 33 SNPs with $p<10^{-5}$ and several were in genes associated with retinal diseases. For all AMD cases, a variant in PCDH19 had suggestive association with ever taking BCPs (rs12861320: interaction odds ratio (ORi)=6.78, $p=4.7x10^{-6}$). For neovascular AMD (150 Ever HRT, 173 Never), an imputed SNP in PCDH15 was inversely suggestively associated with ever taking HRT (rs10763126: ORi=0.45, $p=7.1x10^{-6}$). Mutations in PCDH15, which is expressed in retina, are associated with retinitis pigmentosa (RP) in individuals with Usher Syndrome type 1F, and PCDH19 is from the same family of cell-cell adhesion molecules. Also, an imputed variant in RB1CC1 (rs10504143) was suggestively associated with BCPs in all (ORi=5.25, $p=2.2x10^{-6}$) and neovascular AMD cases (ORi=8.95, $p=2.3x10^{-6}$). RB1CC1 is a transcription factor associated with retinoblastoma (RB) gene expression and its function in regulating cell growth, proliferation, and migration also make it a promising gene for the development of neovascularization. Also, progesterone, a key component of BCPs, was previously shown to increase the expression of RB1CC1 mRNA. These results demonstrate that genome-wide environmental interaction studies can implicate novel loci for complex traits and suggest a novel role for other retinal disease genes in AMD.

725W

Entropy-Based Information Gain Approaches to Detect and to Characterize Gene-Gene and Gene-Environment Interactions/Correlations of Complex Diseases. *R. Fan[1], Z. Zhong[2], S. Wang[3], Y. Zhang[1], A. Andrew[4], M. Karagas[4], J. Moore[4], S. Chen[5], C. Amos[6], M. Xiong[7].* 1) Dept Statistics, Texas A&M Univ, College Station, TX; 2) Global Pharmaceutical Research and Development, Abbott Laboratories, 100 Abbott Park Rd, R436 AP9A-1, Abbott Park, IL 60064; 3) School of Information Science and Engineering, Yunnan University, Kunming 650091, P. R. China; 4) Department of Community and family Medicine, Department of Genetics, Dartmouth Medical School, Lebanon, NH 03756; 5) Surveillance Research Program, National Cancer Institute, 6116 Executive Blvd. 5016, Rockville, Maryland 20852; 6) Department of Epidemiology, MD Anderson Cancer Center, University of Texas, Houston, TX 77030; 7) Human Genetics Center, University of Texas, P. O. Box 20334, Houston, Texas 77225.

For complex diseases, the relationship between genotypes, environment factors and phenotype is usually complex and nonlinear. Our understanding of the genetic architecture of diseases has considerably increased over the last years. However, both conceptually and methodologically, detecting gene-gene and gene-environment interactions remains a challenge, despite the existence of a number of efficient methods. One method that offers great promises but has not yet been widely applied to genomic data is the entropy-based approach of information theory. In this paper we first develop entropy-based test statistics to identify 2-way and higher order gene-gene and gene-environment interactions. We then apply these methods to a bladder cancer data set and thereby test their power and identify strengths and weaknesses. For two-way interactions, we propose an information-gain approach based on mutual information. For three-ways and higher order interactions, an interaction-information-gain approach is used. In both case we develop one-dimensional test statistics to analyze sparse data. Compared to the naive chi-square test, the test statistics we develop have similar or higher power and is robust. Applying it to the bladder cancer data set allowed to investigate the complex interactions between DNA repair gene SNPs, smoking status, and bladder cancer susceptibility. Although not yet widely applied, entropy-based approaches appear as a useful tool for detecting gene-gene and gene-environment interactions. The test statistics we develop add to a growing body methodologies that will gradually shed light on the complex architecture of common diseases.

726W

Genotype x Sex Interactions in Asthma: Genome-Wide Studies in the EVE Consortium Data. *R. Myers[1], N. Scott[1], C. Ober[1], D. Nicolae[1,2], The Eve Consortium.* 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Department of Statistics, University of Chicago, Chicago, IL.

Asthma is a complex disease with contributions from genes, environment, and interactions between them. Sex, which can be considered an `environmental' exposure, affects asthma risk: the prevalence is highest among boys in early childhood but is highest among women in adulthood. Here, we investigate genotype x sex interactions using nearly all of the North American genome-wide association study data sets making up the EVE Consortium. Combining these ethnically diverse datasets allows us to increase sample size and, subsequently, increase the power to detect interactions that may be undetectable at the single study level. We performed case-only genome-wide interaction studies (GWIS) for all EVE component studies, using approximately 2.5 million SNPs. Meta-analysis was used to combine individual study results, yielding a total sample size of 2513 asthmatic males and 2671 asthmatic females. We observe a significant enrichment (penrichment = 0.023) of SNPs with association p-values < 10-5. Among the most significant associations is a series of non-coding SNPs, including rs10117983 (T/C, p = 3.2 x 10-8), on chromosome 9 ~200 KB upstream of the gene SMARCA2. Further analysis confirmed that the allele frequency differences between the males and females are specific to affected individuals; there is no difference in allele frequency between male and female controls. The interaction model is a `flip-flop': the C allele is associated with protection from asthma in males (odds ratio (OR) = 0.76; 95% CI: 0.65 - 0.88) but with risk for asthma in females (OR = 1.12; 95% CI: 0.97-1.3). This result suggests the presence of sex-specific asthma risk alleles in the human genome that can be discovered through the use of existing data sets. Supported by RC2 HL101651.

727W

The association between genetic polymorphism of LEP and obesity in Remote area in Taiwan. *R. Wang[1], L. Ke[1], C. Chen[1], F. Wu[1], T. Wu[1,2].* 1) Department of Public Health, China Medical Univ, PhD; 2) Graduate Institute of Biostatistics, China Medical Univ, PhD.

The prevalence of obese and overweight was surveyed by Nutrition and Health Survey in Taiwan, NAHSIT,2005-2008 is more than half in male and larger than 1/3 in female. However, obesity is an important risk factor for cardiovascular disease, chronic disease and is an important issue in public health. Obesity is regulated by genetic effects, environmental factors and interaction. The -2548 polymorphism of LEP gene was suggested to have a genetic effect on obesity. This is a cross-sectional study, and the aim of this study is to evaluate the association between G-2548A polymorphism of LEP gene and obesity in remote area at Sinyi downtown, Nantou County in Taiwan in 2008. Our study aim is to investigate the association between LEP -2548 polymorphism and obesity. A total of 281 individuals were recruited. Those subjects with a BMI ⁄ 27 (kg/m^2) are classified as obese (n=77) and 204 normal subjects. We collected peripheral blood, determined the biochemical data, and also administrated a questionnaire with interview for demographic information. The genotypes of LEP G-2548A were carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The result showed that G allele frequency of G-2548A polymorphism in obesity patients was more significantly than normal subjects (p=0.5099). The results demonstrated that obese had a higher mean BMI (30.1±2.9) than non- obese (23.0±2.2).The odds ratio of obesity who carried GA and AA genotype are 2.0 (95% confidence interval, 1.35-7.92) than GG genotype were analysed by logistic regression model after adjusting age and gender. In conclusion, our study suggested that LEP gene G-2548A polymorphism is associated with obesity in remote area in Taiwan. This study might provide a suggestion for obesity prevention. In the future, extensive studies to reconfirm the association will be warranted.

728W

A unified Bayesian framework for analyzing heterogeneous genetic association data: meta-analysis and GxE interaction. *X. Wen[1], M. Stephens[1,2].* 1) Dept Statistics, Univ Chicago, Chicago, IL; 2) Dept Human Genetics, Univ Chicago, Chicago, IL.

In genetic association analysis it is often desired to analyze data from multiple potentially-heterogeneous subgroups. For example, 1. To detect modest genetic association signals that are too weak to be detected in smaller individual studies, meta-analysis of multiple studies are often required. Th ese studies are typically carried out by different investigators, at different centers, which might be expected to exhibit heterogeneity of genetic effects. 2. In analysis of a single study, genuine environmental interactions may cause some genetic variants to have different effects on individuals in different su bgroups. We propose a unified Bayesian framework to deal with potentially heterogeneous genetic association data. Within this framework, We address the problems of WHETHER and HOW a particular genetic variant acts on the phenotype of interest by Bayesian testing and model comparison approaches in a systematic way. We propose Bayesian models, derive easy-to-compute Bayes Factors for this purpose and discuss the general strategy for exploratory analysis in these settings. To demonstrate our methods, we apply our Bayesian approach in two different real data examples. The first example is the meta-analysis of global lipids originally repor ted by Teslovich et al (2010, Nature). In the second example, we analyze the data from Dimas et al (2010, Science) to map tissue-specific eQTLs.

729W

Genome-wide interaction analysis of two independent schizophrenia datasets. *D. Xie[1], Y. Zhu[1], J. Chen[2], M. Xiong[1], X. Chen[2].* 1) Department of Biostatistics, University of Texas Health Science Center, Houston, TX; 2) Department of Psychiatry and Virginia Institute for Psychiatric and Behavioral Genetic, Virginia Commonwealth University, Richmond, VA 23219.

The essential problems in performing genome-wide interaction analysis are the power of test statistics, feasibility of computations, and efficient methods for P-value correction of multiple tests. Due to the lack of power of the widely used statistics for testing interaction between loci and its computational intensity, exploration of genome-wide gene-gene interaction has been limited. Many geneticists question the universe presence of significant gene-gene interaction. In this report, we develop a novel LD-based statistic for testing interaction between linked or unlinked loci and apply this statistic and another recently developed haplotype odds-ratio based statistic for testing interaction to two independent GWA datasets of SZ (MGS_GAIN and MGS_nonGAIN) from dbGaP. The MGS_GAIN dataset included 1,135 individuals with SZ and 1,362 controls with 727,479 typed SNPs. The MGS_nonGAIN dataset included 1,089 individuals with SZ and 1,273 controls with typed 696,510 SNPs. The total number of SNPs matched in two studies was 693,772. We identified 7,697 interacting pairs of SNPs (P-value < 1.89E-13) in the MGS_GAIN dataset, and 5,268 pairs in the MGS-nonGAIN dataset. Among them, 17 pairs were shared in the two datasets. We also identified 3,576 and 2,481 pairs of interacting genes in MGS_GAIN and MGS_nonGAIN respectively, and a total of 848 pairs of shared genes in the two datasets. These genes have at least one common pair of significantly interacting SNPs or more than one pair of independent significantly interacting SNPs in the two datasets. It is interesting that these gene pairs form gene interaction networks. The largest connected interaction network involves 122 genes that were shared in two datasets. Of the 11 hub genes (defined as interacting with at least 5 genes) in the network, 8 of them have been shown to have significant functions in neuronal cells/neurons. KCNIP4, CTBP2 and RORA have been shown to play a role in increasing suicide ideation, development of Alzheimer's disease and synaptic actions, all of these conditions are directly related to schizophrenia.

730W

Characterization of the Associations between rs2231142 (Q141K) in *ABCG2* and Serum Uric Acid and Gout in Four U.S. Populations: the PAGE Study. *L. Zhang[1], K.L. Spencer[2], V.S. Voruganti[3], N. Jorgensen[4], M. Fornage[5], L. Best[6], K.D. Brown-Gentry[2], S. Cole[3], D.C. Crawford[2], N. Franceschini[7], A. Gaffo[8], K.R. Glenn[2], G. Heiss[7], N.S. Jenny[9], A. Köttgen[10], Q. Li[2], K. Liu[11], K.E. North[7], J.G. Umans[12], W.H. Kao[1].* 1) Department of Epidemiology, Johns Hopkins School of Public Health, Baltimore, MD; 2) Center for Human Genetics Research, Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN; 3) Department of Genetics, SW Foundation for BioMedical Research, San Antonio, TX; 4) Department of Biostatistics, University of Washington, Seattle, WA; 5) Health Science Center, University of Texas, Austin, TX; 6) Missouri Breaks Industries Research, Inc., Timber Lake, SD; 7) Department of Epidemiology, University of North Carolina, Chapel Hill, NC; 8) University of Alabama, Birmingham Division of Clinical Immunology and Rheumatology, Birmingham, AL; 9) Department of Pathology, University of Vermont College of Medicine, Burlington, VT; 10) Renal Division, University Hospital Freiburg, Freiburg, Germany; 11) Northwestern University, Department of Preventive Medicine, Chicago, IL; 12) Penn Medical Laboratory, Hyattsville, MD.

The *ABCG2* gene encodes the protein ATP-binding cassette sub-family G member 2, which has been shown to be a urate efflux transporter in the brush broader membrane of kidney proximal tubule cells. Associations between a missense coding variant rs2231142 (Q141K) in *ABCG2* and serum uric acid concentration and risk of gout in populations of European descent are well established; however, less is known about the magnitude of these associations in other ethnicities or whether these associations are modified by non-genetic factors. *ABCG2* rs2231142 was genotyped in 39,853 individuals from four different populations from the U.S. as part of the Population Architecture using Genomics and Epidemiology (PAGE) study. Multivariate linear and logistic regressions were performed to assess the associations between rs2231142 and serum uric acid levels and gout prevalence, respectively. Meta-analysis was conducted to obtain joint estimates, and Cochran's Q test was used to assess heterogeneity across subgroups (P_{het}). A total of 22,734 European Americans (EA), 9,720 African Americans (AA), 3,849 Mexican Americans (MA) and 3,550 American Indians (AI) were included. Data on prevalence of gout was not available in AI. Mean uric acid concentration (and gout prevalence) in EA, AA, MA, AI, was 5.7 mg/dL (4.4%), 5.7mg/dL (5.9%), 5.2mg/dL (1%), and 5.1mg/dL. The Frequency of the T allele of rs2231142 was 0.11, 0.03, 0.19 and 0.20 in EA, AA, MA, AI, and it was associated with higher serum uric acid levels ($P=2.37\times10^{-67}$, 3.98×10^{-5}, 6.97×10^{-9}, and 5.3×10^{-4}) and prevalent gout ($P=2.83\times10^{-10}$, 0.01 and 0.01) in EA, AA, MA, and AI. After meta-analysis across populations, the T allele was associated with a 0.24 mg/dL increase in uric acid levels (95%CI 0.17-0.31, $P=1.37\times10^{-80}$) and a 1.75-fold increase in odds of gout (95%CI 1.50-2.04, $P=1.09\times10^{-12}$). Significantly stronger association was observed in men than in women for both uric acid ($P_{het}=1.64\times10^{-5}$) and gout ($P_{het}$= 0.03). The association between rs2231142 and uric acid was stronger in postmenopausal women compared to premenopausal women (P_{het}=0.02) and in hormone replacement therapy users compared to non-users (P_{het}= 0.03). In conclusion, rs2231142 of *ABCG2* is significantly associated with serum uric acid and gout in four U.S. populations, with stronger association observed in men than in women. In addition, menopausal status and hormone replacement therapy may modify the association between rs2231142 and serum uric acid levels.

731W

Prostate Cancer Risk Prediction using a genetic profile in an international consortium (PRACTICAL). *A. Amin Al Olama[1], S. Benlloch[1], D.A. Leongamornlert[2], E.J. Saunders[2], M. Tymrakiewicz[2], M. Guy[2], K. Govindasami[2], Z. Kote-Jarai[2], R.A. Eeles[2,3], D.F. Easton[1], PRACTICAL.* 1) Public Health & Primary Care, University of Cambridge, Strangways Laboratory, Cambridge, Cambridgeshire, United Kingdom; 2) The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, SM2 5NG, UK; 3) Royal Marsden NHS Foundation Trust, Fulham and Sutton, London and Surrey, UK.

Genome-wide association studies have identified multiple genetic variants associated with prostate cancer risk, opening up the possibility for using genetic profiling in risk prediction. To evaluate the potential for risk prediction, we genotyped 25 known prostate cancer susceptibility SNPs in 31 case-control studies in an international consortium (PRACTICAL), and analysed data from 20,132 prostate cancer cases and 20,234 male controls of European ancestry. Odds ratios (OR) associated with each SNP genotype, and genotypes for pairs of SNPs, were estimated using unconditional logistic regression. We found some evidence for weak departures from a multiplicative model for pairs of loci (10 pairs significant at P<0.01 compared with 3 expected by chance), but no individual pair was significant after correction for multiple testing., Based on the assumption of a log-additive model, we constructed a risk score from the summed genotypes weighted by the per-allele log-odds ratios. Using this score, the top 1% of the population had an estimated increased risk of 40 fold compared with the bottom 1% of the population, and 4.2 fold compared with the average population risk, while the bottom 1% of the population had an estimated risk of 11% of the population risk. These results demonstrate that genetic risk profiling may play an important role in targeted prevention.

732W

A simulation pipeline for genetic disease models. *H. Baurecht[1,2], T. Augustin[3], S. Wagenpfeil[4], K. Strauch[1], P.A. Scheet[5].* 1) Institute of Genetic Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany; 2) Department of Dermatology, Allergology and Venerology, University Hospital Schleswig-Holstein, Kiel, Germany; 3) Department of Statistics, Ludwig-Maximilians Universität München, Munich, Germany; 4) Institute of Medical Statistics and Epidemiology, Technische Universität München, Munich, Germany; 5) Department of Epidemiology, University of Texas, MD Anderson Cancer Center, Houston, USA.

The advent of next-generation sequencing and generation of large data sets of population genetic data have allowed interrogations of association between phenotype and increasingly rare forms of genetic variation. To detect association with extremely rare variants some form of aggregation is necessary to combine the effects of variants from multiple affected individuals; for example, one way this is done is on the genic level. Evaluation of these procedures may involve simulated data, and these simulations typically assume independence among alleles at variant sites within genes. While this may be a reasonable approximation for some extremely rare variants, LD at moderate frequencies may affect association detection. Here we aim to address this phenomenon in data simulation. We develop an R package to simulate case-control data using externally generated haplotype data, such as those from a coalescent simulator. We explore various ways to partition population attributable risk (PAR), which is used for specifying the disease model, among non-independent variant sites to maintain an overall PAR. Our simulation pipeline provides a flexible tool for simulating a varierty of scenarios for validating statistical methods in the context of next-generation sequencing data, GWAS or even candidate genes.

733W

***APOL1* variant modifies the HDL-kidney function association in populations of African Ancestry.** *A.R. Bentley, A. Doumatey, H. Huang, J. Zhou, D. Shriner, A. Adeyemo, C. Rotimi.* Center for Research in Genomics and Global Health, National Human Genome Research Institute, Bethesda, MD.

Low levels of high-density cholesterol (HDL) are known to accompany chronic kidney disease (CKD), but the association of HDL and the estimated glomerular filtration rate (eGFR, an index of kidney function) in the general population is unclear. We investigated the relationship between HDL and eGFR in Han Chinese (Ch, n=1100), West Africans (Af, n=1497), and African Americans (AA, n=1539). Generalizability of observed associations was tested in European Americans (NHEA, n=859) and African Americans (NHAA, n=326) of NHANES, a national population-based survey. The HDL-eGFR association varied by population: in Ch, increased HDL was associated with improved eGFR (1.2 ml/min/1.73 m^2 for a 10 mg/dl increase in HDL [same units throughout], p<0.0001), but with worse eGFR among Af (-2.1, p<0.0001) and AA (-0.8, p=0.04). In NHANES, the association also differed by African ancestry: NHEA (0.8, p=0.05) and NHAA (-1.3, p=0.1). Given this surprising but consistent inverse relationship between HDL and eGFR in African-ancestry populations, we investigated the potential role of a newly-identified risk variant for kidney disease (rs73885319 in the *apolipoprotein L 1* (*APOL1*) gene) that is absent in East Asians and Europeans. Data show that this risk variant likely rose to high frequency in Africa because it confers resistance to trypanosomal infection and protects against African sleeping sickness. Although rs73885319 did not predict eGFR in AA or Af, HDL was 4-5 mg/dl higher among those with the CKD risk genotype (GG vs. GA/AA) in AA women (p=0.04) and in Af men and women (p=0.03). There was an interaction between rs73885319 and HDL in AA (p=0.04), with a stronger negative HDL-eGFR association with the risk genotype (-3.5 vs. -0.6); a directionally similar but not significant (p=0.1) result was observed in Af. These findings support a role of HDL in kidney function in the general population. Among non-African ancestry populations, HDL was positively associated with eGFR, consistent with expectations of the broadly protective role of HDL in a variety of chronic diseases. The inverse association between HDL and eGFR in African-ancestry populations is unexpected. The discovery of an interaction between *APOL1* genotype and HDL in AA suggests that genetic factors contribute to this paradoxical association. Notably, these findings suggest that the unexplained mechanism by which the *APOL1* locus affects the risk of kidney disease involves HDL.

734W

Fine-mapping *CASP8* risk variants in Breast Cancer. *N.J. Camp[1], M. Parry[2], S. Knight[1], R. Abo[3], L.A. Cannon-Albright[1], G. Elliot[4], S. Rigas[2], S.P. Balasubramanian[5], A. Cox[2].* 1) Division of Genetic Epidemiology, University of Utah, Salt Lake City, UT; 2) Institute for Cancer Studies, Department of Oncology, University of Sheffield, Sheffield, UK; 3) Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA; 4) University of Manchester, Manchester, UK; 5) Academic Unit of Surgical Oncology, Department of Oncology, University of Sheffield, Sheffield, UK.

Multiple genomewide and candidate gene association studies have been performed in search of common risk variants for breast cancer. Recent large meta analyses, consolidating evidence from these studies, have been consistent in highlighting the caspase-8 (*CASP8*) gene as important in this regard. Previously, we described a breast cancer risk haplotype across variants in *CASP8* identified using genotype data for 14 tagging-SNPs (tSNPs) from a single data set. In order to further refine a risk haplotype and map the *CASP8* gene with respect to underlying susceptibility variant/s, we broadened our search to four genes in the 2q33-q34 region based on their physical proximity to *CASP8* and/or role in apoptosis. Here, we have studied two independent data sets from the United Kingdom and the United States, including 3,888 breast cancer cases and controls for 45 tSNPs in the expanded *CASP8* region. We identified a three-SNP haplotype significantly associated with breast cancer ($p<5\times10^{-6}$), with a dominant risk ratio and 95% confidence interval of 1.28 (1.21-1.36) and a frequency of 0.29 in controls. Evidence for this risk haplotype was extremely consistent across the two study sites and also consistent with prior single SNP associations and our prior risk haplotype. This three-SNP risk haplotype represents the best characterization so far of the chromosome upon which the susceptibility variant resides and provides a strong foundation for re-sequencing efforts. Identification of the underlying risk variant may prove useful for individual-level risk prediction, provide novel insights into breast carcinogenesis, and potential for improved prevention and treatment.

735W

A Weighted Fisher's Method to Detect Rare-Variant Complex Trait Associations using Next Generation Sequence Data. *Y.H. Cheung[1], G. Wang[2,3], S.M. Leal[2,3], S. Wang[1].* 1) Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, NY; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Department of Statistics, Rice University, Houston, TX.

Next generation sequencing technology has enabled the paradigm shift in genetic association studies from the common disease/common variant to common disease/rare variant hypothesis. It has been shown that analyzing individual rare variants is underpowered and therefore methods have been developed that aggregate variants across a genetic region. For exome sequence data, the number of variants which are analyzed jointly can range from dozens within a gene to hundreds in a biological pathway. The foreseeable wide-spread use of whole genome sequencing, despite opening up unprecedented opportunities, poses challenges in statistical analysis. One particular problem is the attenuation of signal due to inclusion of high proportions of non-causal variants because of difficulties in determining region boundaries and inclusion of non-coding regions. These obstacles call for new rare variant association methods that are statistically powerful, robust against high levels of noise due to inclusion of non-causal variants, and yet computationally efficient. Here we proposed a new statistic that combines the disease-associated p values of individual variants using a weighted Fisher's approach, with the weights being the inverse of the expected standard deviation of the allele frequencies under the null. The Weighted Fisher's method is extremely robust to the presence of a high proportion of non-causal variants (e.g. >80%). It is also demonstrated that this method is powerful in the situation where both detrimental and protective variants are present within a genetic region. We tested the performance of the Weighted Fisher's method using realistic population demographic and disease models to simulate data and the power is compared to several previously published methods. The results demonstrate that the Weighted Fisher's method generally outperforms other rare variant association methods over a wide range of models. Additionally, sequence data on the ANGPTL family of genes from the Dallas Heart Study were tested for associations with eight metabolic traits and both known and novel associations were uncovered using the Weighted Fisher's method.

736W

How can genotype imputations contribute to the identification of disease causal variants in genome-wide association studies (GWAS)? *E. Corda[1,2,3], M. Brossard[1,2,3], M.M Iles[4], J.H. Barrett[4], A.M. Goldstein[5], P. Kanetsky[6], E.M. Gillanders[7], B. Bakker[8], N. Gruis[9], J.A. Newton-Bishop[4], D.T. Bishop[4], F. Demenais[1,2,3], Melanoma Genetics Consortium (GenoMEL).* 1) Fondation Jean Dausset-CEPH, Paris, France; 2) INSERM U946,Paris, France; 3) Université Paris Diderot, Paris, France; 4) Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, Leeds, UK; 5) Genetic Epidemiology Br., DCEG, National Cancer Institute, NIH, Bethesda, MD; 6) Dept of Biostatistics and Epidemiology, University of Pennsylvania, PA; 7) Inherited Disease Research Br., National Human Genome Research Institute, NIH, Baltimore, MD; 8) Dept. of Clinical Genetics, Leiden University Medical Centre, Leiden, The Netherlands; 9) Dept. of Dermatology, Leiden University Medical Centre, Leiden, The Netherlands.

GWAS have been successful in uncovering many loci associated with multifactorial diseases. However, the causal variants within these loci are unknown for the most part. Our goal was to investigate to what extent imputations based on Hapmap3 and/or 1000 Genomes reference panels were able to identify disease-causing variants. We used as a paradigm *MC1R*, a gene known to be involved in melanoma, located in the 16q24 region strongly associated with this cancer in a previous GWAS from the GenoMEL consortium, but with no functional variant present on the genotyping chips. We conducted genotype imputations over 1.9 Mb on 16q24 in 10,772 GenoMEL subjects (2,985 cases, 7,787 controls) using the IMPUTE2 software and either Hapmap3 or Hapmap3+1000 Genomes reference panels. After applying stringent quality control criteria, 351 SNPs (Hapmap3) and 1,543 SNPs (Hapmap3+1000G) were available for analysis. We carried out univariate population-adjusted logistic regression with each SNP followed by joint analysis of all SNPs using a Bayesian-inspired penalised maximum likelihood approach (HyperLasso software). Four known functional *MC1R* variants (R151C, R160W, V92M, R163Q) were imputed using either reference panel and passed QC filtering. Univariate analysis of all imputed SNPs showed the strongest association signal with R151C ($p=3.6x10^{-30}$) while R160W had $p=9.3x10^{-10}$ and from 22 (Hapmap3) to 70 (Hapmap3+1000G) SNPs in other genes reached the genome-wide threshold ($p\leq10^{-8}$). Joint analysis of all Hapmap3 imputed SNPs, using 100 iterations of HyperLasso, showed significant effects of *MC1R* variants (R151C and/or R160W) in all models which converged plus the effects of SNPs in other genes. Similar conclusions were reached when using imputed SNPs from Hapmap3+1000G. We also sequenced the whole *MC1R* gene in 1,844 subjects (937 cases, 907 controls). Stepwise regression of 8 sequenced *MC1R* non-synonymous variants (MAF／ 1%) alone or together with imputed SNPs associated with melanoma showed significant effects for R151C ($p=4.0x10^{-15}$), R160W ($p=1.2x10^{-9}$) and D294H ($p=2.0x10^{-4}$), a rare variant (MAF=2%) absent from the imputation reference panels. In conclusion, this study shows that imputations can be of great use in pinpointing a gene that has a functional role in disease but may not be identified through genotyping alone. However, a full picture of the effect of causal variants on disease risk is revealed only by resequencing.

737W

A novel pairwise shared genomic segment statistic comparing cases and controls: application to polycythemia vera. *K. Curtin[1], S. Swierczek[2], F. Lorenzo[2], A. Thomas[1], K. Wang[3], H. Hakonarson[4], J. Prchal[2], N. Camp[1].* 1) Internal Medicine Div. Genetic Epidemiology, Univ Utah Health Sci Ctr, Salt Lake City, UT; 2) Intern Medicine Div. Hematology, Univ Utah Health Sci Ctr, Salt lake City, UT; 3) Zilkha Neurogenetic Inst., Univ Southern California, Los Angeles, CA; 4) Chilren's Hospital of Pennsylvania, Div. Human Genetics, Univ of Pennsylvania, Philadelphia, PA.

Dense SNP genotyping has been used to identify identity-by-state (IBS) genomic regions shared between distantly-related cases in large pedigrees. Excessively long regions shared IBS likely contain identity-by-descent regions that can be used for localization of disease-susceptibility genes. For diseases with substantial genetic heterogeneity it is unlikely that all cases will be attributable to the same underlying causal variant. Examining sharing among pairs, rather than across all affected individuals, will likely have more power. We previously developed a novel method that examines paired shared genomic segments in related individuals in pedigrees using a mean sharing test statistic. Here we describe a method that compares homozygous sharing (pHGS) in cases and controls; significance is empirically assessed conditional on local linkage disequilibrium (LD) patterns. Using this pairwise statistic, we studied high-density SNP genotypes (Illumina 610Q) from granulocyte (somatic) and T-cell (germline) DNA in 34 unrelated individuals with polycythemia vera (PV), a myeloproliferative neoplasm (MPN). Genotypes of 60 unrelated HapMap CEU founders on the same platform were used as controls to provide an LD model and expected sharing. Pairwise(pHGS) sharing across all possible [n(n-1)/2] pairs of PV cases were examined for evidence of a deletion/acquired uniparental disomy across chromosomes 1, 7, and 9 which contain previously-identified MPN and leukemia candidate regions. Nominal and chromosome-wide significance were derived from 10,000 null simulations. No suggestive regions were identified on chromosomes 1 and 7 for either granulocyte or T-cell samples. On chromosome 9, we observed three broad regions of pHGS sharing in granulocyte samples which reached suggestive significance. The longest runs (average sharing 15-20 SNPs, 4.3 Mb) occurred upstream of the miR-101-2 and *JAK2* locus. Additionally, slightly shorter runs from 8.2-12.4 Mb and 16.7-17.4 Mb occurred further downstream. In contrast, no homozygous sharing was observed in T-cell samples. Using a new case-control method of assessing pairwise genomic sharing, we detected regions of homozygosity in somatic DNA on chr. 9 (up- and downstream of *JAK2* not evident in germline DNA. This illustrates the potential utility of shared segment techniques for independent individuals and case-control designs and suggests if germline susceptibility variants exist in *JAK2* they are unlikely to be homozygous in nature.

738W

Gene set analysis of GWAS data for human longevity highlights the relevance of the insulin/IGF-1 signaling and telomere maintenance pathway. *J. Deelen[1,2], H.W. Uh[2,3], R. Monajemi[3], D. van Heemst[4], P.E. Thijssen[1,5], S. Böhringer[3], E.B. van den Akker[1,6], A.J.M. de Craen[4], F. Rivadeneira[7,8], A.G. Uitterlinden[2,7,8], R.G.J. Westendorp[2,4], J.J. Goeman[3], P.E. Slagboom[1,2], J.J. Houwing-Duistermaat[3], M. Beekman[1,2].* 1) Section of Molecular Epidemiology, Leiden University Medical Center, Leiden, The Netherlands; 2) Netherlands Consortium for Healthy Ageing, Leiden University Medical Center, Leiden, The Netherlands; 3) Section of Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands; 4) Department of Gerontology and Geriatrics, Leiden University Medical Center, Leiden, The Netherlands; 5) Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands; 6) Department of Mediamatics, Delft Bioinformatics Lab, Delft University of Technology, Delft, The Netherlands; 7) Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands; 8) Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands.

Genome-wide association studies (GWAS) using single SNP analysis have been very successful in identifying disease loci for various traits. However, this approach has been less successful for complex traits, like human longevity, limited by the small sample sizes that can be achieved. Analyzing the combined effect of a SNP set grouped per pathway or gene region, instead of single SNPs, is a way to overcome this limitation. We used this approach to study the joint effect on human longevity of genetic variation in two candidate pathways, the insulin/insulin-like growth factor (IGF-1) signaling (IIS) and telomere maintenance (TM) pathway. For the analyses we used genotyped GWAS data of unrelated nonagenarian cases (n = 403, mean age 94 years) from long-lived families from the Leiden Longevity Study and a younger set of population controls (n = 1670, mean age 58 years) from the Rotterdam Study. We analyzed SNPs within a 10 kb window around genes encoding proteins that belonged to the IIS and TM pathway. In total, 1021 SNPs in 68 IIS pathway genes and 88 SNPs in 13 TM pathway genes were analyzed. To investigate the association of the SNP sets from the IIS and TM pathway with longevity, three self-contained (PLINK set-based test, Global test, GRASS) and one competitive (SNP ratio test) pathway test were used. Although we observed small differences between the results of the pathway tests, they all showed consistent nominal significant association ($P < 0.05$) of the IIS and TM pathway with longevity. To determine which genes were mainly responsible for the observed association of the pathway SNP sets with longevity, we also investigated the association of gene SNP sets from these pathways. Seven of the IIS pathway gene SNP sets (*AKT1, FOXO4, IGF2, INS, PIK3CA, SGK1* and *SGK2*) and one of the TM pathway gene SNP sets (*POT1*) showed a nominal significant association ($P < 0.05$) with longevity. In conclusion, this study shows that genetic variation in genes involved in the IIS and TM pathway is associated with human longevity. In addition, since self-contained tests, as compared to competitive tests, are less computational elaborate and provide comparable results, we recommend self-contained tests for candidate-based pathway analysis.

739W

THE ANO3/MUC15 LOCUS IS ASSOCIATED WITH ECZEMA IN FAMILY SAMPLES ASCERTAINED THROUGH ASTHMATICS. *M.H. Dizier[1], P. Jeannin[1], A.M Madore[2], J. Esparza[3,4], M. Moffatt[5], E. Corda[1], F. Monier[1], I. Annesi[6], J. Jsut[7], I. Pin[8], F. Kauffmann[9], W. Cookson[5], Y.A. Lee[3,4], C. Laprise[2], M. Lathrop[10], E. Bouzigon[1], F. Demenais[1].* 1) U946, INSERM, Paris, France; 2) Université du Québec, Chicoutimi, Canada; 3) Max-Delbrück-Center for Molecular Medicine (MDC), Berlin, Germany; 4) Charité Universitätsmedizin Berlin, Germany; 5) National Heart Lung Institute, Imperial College, London, UK; 6) Inserm U707, Paris, France; 7) Centre de diagnostic et traitement de l'asthme, Hôpital Trousseau, Paris, France; 8) Département de Médecine Aigüe Spécialisée, CHU Michallon, Grenoble, France; 9) Inserm U1018, Paris, France; 10) CEA-CNG, Evry, France.

A previous genome-wide linkage scan in 295 families of the French Epidemiological study on the Genetics and Environment of Asthma (EGEA) reported strong evidence of linkage of 11p14 to eczema. Our purpose was to conduct fine-scale mapping of the 11p14 region to identify the genetic variants associated with eczema and to investigate the influence of the mode of ascertainment of the data on the association outcomes. Association analyses were conducted in the EGEA discovery dataset using two statistical methods for internal validation: the family based association method (FBAT) and logistic regression. Replication of the EGEA findings was sought in French Canadian (SLSJ study) and UK (MRCA study) family samples, which similarly to EGEA, were ascertained through asthmatic subjects. We also tested for association in two German samples ascertained through subjects affected with eczema. We found significant association of eczema with 11p14 SNPs in the vicinity of the linkage peak in EGEA (p=10-4 for rs1050153 using FBAT, that reached the multiple testing-corrected threshold of 1.3x10-4; p=0.003 using logistic regression). Pooled analysis of the three asthma-ascertained samples (EGEA, SLSJ, MRCA) showed strong improvement in the evidence for association (p=6x10-6 for rs293974 (OR= 0.66), p=3x10-5 for rs1050153 (OR=0.68), p=6x10-5 for rs15783 (OR=0.69). No association was observed in the eczema-ascertained samples. The significant SNPs are located within the overlapping ANO3 and MUC15 genes. Several lines of evidence suggest that MUC15 is a strong candidate for eczema. Further investigation is needed to confirm and better understand the role of ANO3/MUC15 locus in eczema and its relationship with respect to asthma.

740W

Distinguishing gene-gene interaction from heterogeneity. *E.N. Drill[1], R.L. Subaran[1,2], D.A. Greenberg[1,2].* 1) Division of Statistical Genetics/Biostatistics, Columbia Univ Sch Pub Hlth, New York, NY; 2) Dept. of Psychiatry, New York State Psychiatric Institute, Columbia-Presbyterian Medical Center, New York, NY.

Introduction: Identification of gene-gene interaction in complex human disease has remained elusive and has been implicated as a factor in the heritability not explained by common variants. Here, we develop binary classification tests to distinguish between gene-gene interaction and genetic heterogeneity using multipoint linkage analysis. Our goal is to use the information from linkage analyses to distinguish epistasis from heterogeneity. Multiple analyses of the same dataset but assuming different genetic models result in differing degrees of evidence for linkage (e.g. maxLODs determined assuming recessive and dominant modes of inheritance, assuming heterogeneity, and using maxNPL). We hypothesize that, given the presence of two disease loci, these statistics taken together can yield information about the way the two loci interact to influence disease.

Methods: We simulated dense SNP data using a number of 2-locus disease models. Generating models had either heterogeneity or gene-gene (epistatic) interaction. Performing multipoint linkage analysis, we used the MOD score, the HLOD and the NPL to develop binary classification tests to identify heterogeneity or epistatic gene-gene interaction where there was at least suggestive evidence for linkage at the two loci. Our interaction test uses the difference between the MOD score and the NPL expressed on a LOD scale (Δ_I) to indicate presence or absence of interaction: $\Delta_I > t_I$, where t_I is a threshold determined through simulation. Our heterogeneity test compares the difference between the HLOD and the MOD score (Δ_h) to a threshold, t_h, to indicate presence or absence of heterogeneity: $\Delta_h > t_h$.

Results: Our test for interaction had a sensitivity of 90% and a specificity of 84% for t_I=1. When t_I=1.5, sensitivity is 82% and specificity is 90%, while t_I=2 gives a sensitivity of 73% and a specificity of 94%. For the heterogeneity test, when t_h=0, the sensitivity is 97% and the specificity is 34%. For t_h=1 the sensitivity decreased to 65% and specificity increased to 99%. An in-between threshold value of 0.5 resulted in a sensitivity of 83% and a specificity of 96%. For both tests, the area under the curve (AUC) representing the trade-off between sensitivity and specificity (the Receiver Operating Characteristic curve) is .94, indicating high performance as binary classifiers.

741W

Using known genotype-phenotype associations to detect sample mix-up. *C.T. Ekstrom[1], B. Feenstra[2].* 1) Statistics, Basic Sci & Environment, Univ Copenhagen, Copenhagen, Denmark; 2) Statens Serum Institut , Copenhagen, Denmark.

Genome-wide association studies (GWAS) have rapidly become a standard method for identification of disease genes and genes influencing quantitative traits. GWAS typically uses unrelated individuals which makes detection of sample mix-up errors more problematic than for studies where related individuals are available.

We present a method for identifying potential sample mix-ups in GWAS (and other genetic studies) building on ideas from forensic sciences. A widely used ad-hoc method for error detection is to check if the sex of an individual matches its X-linked genotype. This idea can be generalized to less stringent associations between known genotypes and phenotypes, and if several known association are combined then the power to detect sample mixups is increased substantially. Individuals with an unlikely set of phenotypes given their genotypes are more likely to be flagged as potential errors.

Analytical and simulation results show that only a few (around 10) highly informative genotype-phenotype associations are needed to have a sensitivity and specificity above 95%. The odds that the genotype and phenotype are both from the same individual are compared for different information content and for different number of available genotype-phenotype associations. The method is applied to data from a larger Danish GWAS study where random errors are introduced to evaluate the effectiveness in a real dataset.

742W

Fine mapping in over 10,000 Rheumatoid arthritis cases and 14,000 controls refine associations to known loci, indicate multiple independent affects and reveal novel associations. *S. Eyre[1], J. Bowes[1], A. Barton[1], S. Raychaudhuri[2,6,7], C. Amos[5], D. Diogo[2], A. Lee[3], L. Klareskog[4], L. Padyukov[4], E. Stahl[2,6], P.K. Gregersen[3], R. Plenge[2,6], J. Worthington[1].* 1) Arthritis Research UK Epidemiology Unit, Stopford Building, The University of Manchester, Manchester, UK; 2) Division of Rheumatology, Immunology, and Allergy, Brigham and Women's Hospital, Boston, MA, USA; 3) The Feinstein Institute for Medical Research, North Shore-Long Island Jewish Health System, Manhasset, NY, USA; 4) Rheumatology Unit, Department of Medicine, Karolinska Institutet at Karolinska University Hospital Solna, Stockholm, Sweden; 5) University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA; 6) Broad Institute, Cambridge, Massachusetts, USA; 7) Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts, USA.

Genome wide association studies (GWAS) have been tremendously successful in identifying loci associated with a range of traits and disorders. Indeed, there are over 240 confirmed susceptibility loci reported for nine autoimmune diseases alone. One task now is to take these findings and translate them into clinical utility. The first step in this is to elucidate the genetic architecture of the loci in each disease with fine mapping experiments. These are expected to localise the association signal, indicate whether multiple, independent genetic affects are present and may point to the casual gene. The aim of this study was to fine map loci significantly associated with autoimmune disease in a large cohort of 10,000 rheumatoid arthritis (RA) cases and 14,000 controls using the custom Illumina Immunochip. The chip was designed by a consortium of researchers investigating 12 autoimmune diseases and represents all known genetic variation from dbSNP, 1kG and sequencing projects for approximately 200 validated loci. The genotyping for the RA samples was performed in multiple centres and therefore all raw genotyping data was collated centrally for combined clustering and analysis. The data was first re-clustered and after applying strict QC metrics (99% SNP and 98% sample) the samples were subjected to further pruning for relatedness and ancestral outliers. In the initial analysis we have examples of re-focusing of the strongest association signal(e.g.2q11), evidence for multiple independent associations within a locus (e.g.PADI4) and associations to previously unconfirmed RA loci (e.g.TMEM170). Within the 2q11 locus the peak of associations has moved 76kb to now lie within the AFF3 gene. The PADI4 gene has, for the first time, been robustly associated with RA in a Caucasian population (p=3x10-7). There is also evidence for two independent affects in this region, one of which is correlated with the variant previously reported to be associated with RA in Japanese populations. TMEM170, a type 1 diabetes susceptibility locus, is an example of a novel region robustly associated with RA (p=1x10-5) and adds to the growing number of overlapping autoimmune susceptibility genes. Acknowledgments:We kindly acknowledge the Wellcome Trust Sanger Institute for the generation of RA case and control data, the WTCCC for the provision of control data and the Steve Rich laboratories at the University of Virginia for the generation of control data for this project.

743W

Re-ranking sequencing variants in the post-GWAS era. *L.L. Faye[1,2], S.B. Bull[1,2], L. Sun[2,3].* 1) Biostatistics Division, Dalla Lana School of Public Health, University of Toronto; 2) Samuel Lunenfeld Research Institute, Mount Sinai Hospital; 3) Department of Statistics, University of Toronto, Toronto, ON.

Whole-genome next-generation sequencing (NGS) dramatically increases our ability to pin-point disease-causing variants by providing information at all variant SNPs. To reduce cost and heterogeneity, among other reasons, GWAS samples are often used for NGS. To increase power, associated regions identified by significance at GWAS tag SNPs are often prioritized for sequencing analysis and the locations of causal variants are refined using the sequencing data. This analytic strategy introduces bias into the ranking of sequencing SNPs, increasing the probability that non-causal SNPs will be top-ranked. This bias is most pronounced when the tag and causal SNPs are in moderate LD. We quantify this bias analytically and show that selection at the tag alters the joint distribution of the sequencing association statistics. Simulation studies comparing the ranking of SNPs using the combined GWAS sequencing analysis to the ranking of SNPs in an independent sequencing sample verify these effects. In the independent sequencing sample, the top-ranked SNPs tend to be the causal SNP or SNPs in highest LD with the causal SNP. In contrast, when associated regions are selected by GWAS, high LD with the tag tends to increase the rank of a SNP, even when it is not highly correlated with the causal SNP. When the tag and causal SNPs are in moderate LD, this increases the probability that SNPs in high LD with the tag will out-compete the causal SNP for the top ranks. To improve causal SNP identification, we develop a re-ranking procedure that re-calibrates the association evidence for all GWAS and sequencing variants in a given region. We use BR-squared (Faye et al, 2011) to estimate the amount of bias induced by selection, that is the difference between the observed test statistic at the GWAS tag SNP and the mean of its underlying distribution. The degree of this bias depends on GWAS power. The amount of GWAS tag bias that carries through to the sequencing SNPs depends on the LD between the GWAS tag and sequencing SNPs. Therefore, we adjust each sequencing SNP test statistic by the estimated bias at the tag, scaled by the LD between the tag and sequencing SNPs. The sequencing SNPs are then re-ranked using the adjusted test statistics. Simulation studies show that when bias is detrimental to the rank of the causal SNP, the re-ranking procedure tends to improve the rank of the causal SNP and SNPs in high LD with the causal SNP.

744W

DNA methylation involved in chemobrain. *R. He[1], J. Eggert[1], P. Tate[1], L. Larcom[1], C. Chen[2].* 1) Clemson University, Clemson, SC; 2) Greenwood Genetic Center, Greenwood, SC.

Chemotherapy may initiate or potentiate memory loss and other cognitive impairments experienced by cancer patients. Loss of memory may persist following treatment and can significantly impact the quality of life for a subset of cancer survivors. Many studies have linked epigenetic mechanisms to memory formation postulating that transient reprogramming of epigenetic codes such as DNA methylation and histone modifications is required for memory consolidation. This poster will share results of a preliminary in vitro study designed to determine if doxorubicin chemotherapy causes morphological and epigenetic changes in the PC12 neuron cells. Experiments were conducted separately on a group of non-treated PC12 neuron cells and a group of PC12 neuron cells treated with doxorubicin chemotherapy. The cell morphology, cell viability and epigenetic changes are compared before and after treatments. Results suggest that minute amounts of doxorubicin chemotherapy decrease metabolic activity and neurite outgrowth of PC12 neurons, and DNA methylation is a mechanism that is associated with this process.

745W

Advanced methods for genome-wide methylation detection reveal novel epigenetic dynamics of leukemia. *A. Akalin[1,2], M. Figueroa[1], F. Garrett-Bakelman[1], M. Kormaksson[1], J. Busuttil[1], A. Melnick[1], CE. Mason[1,2].* 1) Weill Cornell Medical College, Cornell University, New York, NY 10065, USA; 2) HRH Prince Alwaleed Bin Talal Bin Abdulaziz Alsaud Institute for Computational Biomedicine, Weill Medical College, Cornell University, New York, NY 10065, USA.

DNA methylation is a critical epigenetic modification that regulates gene expression and mediates cellular differentiation states. Recently, methylation signatures have been utilized to stratify different sub-types of leukemia, which indicate their important relevance for clinical applications. Current high-throughput methods for examination of the epigenome, such as reduced representation bisulfite sequencing (RRBS), ameliorate the cost of sequencing after bisulfite treatment but limit the number of regions that can be examined. Here, we report biochemical and bioinformatics improvements in the RRBS method, creating a new technique called enhanced RRBS (eRRBS). We show extremely high reproducibility with this technique (R>0.96), even with very-low input levels (5ng vs. 1ug). Our method also shows an improved conversation efficiency of the non-methylated cytosines (>99.5%). We also find an increased number (11-61%) of CpG sites that can be detected and quantified in all areas: CpG islands, promoters, CpG shores, introns, and exons. Moreover, we can use this method to effectively determine differentially methylated regions (DMRs) and shores (DMSs) that reveal new regions of the genome that are altered in acute lymphoblastic leukemia.

746W

Epigenomic dysregulation of enhancer elements in cancer. *B. Akhtar-Zaidi[1], A. Saiakhova[1], J. Willis[2,3], S. Markowitz[2,4,5], P. Scacheri[1].* 1) Genetics, Case Western Reserve University, Cleveland, OH; 2) Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH; 3) Department of Pathology, Case Western Reserve University, Cleveland, OH; 4) Department of Medicine, Case Western Reserve University, Cleveland, OH; 5) Howard Hughes Medical Institute, Cleveland, OH.

Recent studies have highlighted a central role for enhancer elements in establishing gene expression programs that drive development and differentiation. Far less is known about enhancer dysregulation in human disease processes. To investigate the role of enhancer activity in carcinogenesis, we used the method of ChIP-seq to map the genome-wide distribution of mono-methylated lysine 4 of histone H3 (H3K4me1), the epigenetic signature of gene enhancer elements, in primary cell lines derived from human colorectal tumors. We have identified thousands of loci that have either gained or lost the H3K4me1 signal in colon cancer compared to normal colon crypts. We call these regions variant enhancer loci, or VELs. The data further reveal a strong association between VELs and gene expression patterns that distinguish individual colon cancers from each other and collectively from normal colon epithelium. Based on our finding that approximately 200 VELs are shared between all colon cancer samples, we propose that the epigenetic landscape at distal enhancers is far more homogeneous than the mutational landscape of protein coding sequences in colon cancer. High-frequency VELs are robustly associated with genes aberrantly expressed across multiple colon tumors, and are enriched near genes and pathways extensively implicated in colon cancer. Lastly, we describe a strong association between lost VELs and DNA hypermethylation that we subsequently validated in an independent set of primary colon tumors. Taken together the data are consistent with a model of colon cancer in which targeted dysregulation of enhancers by a mechanism involving both H3K4me1 and DNA methylation is a major feature of carcinogenesis.

747W

Methylation Signature of Breast Cancer Metastasis. *Z. Barekati, R. Radpour, XY. Zhong.* Laboratory for Gynecologic Oncology, Women's Hospital/ Department of Biomedicine, University of Basel.

Invasion and metastasis are two important hallmarks of malignant tumors that followed by complex of genetic and epigenetic alterations. Present study investigated the contribution of aberrant methylation profile of twelve cancer related genes in paired metastasis and non-metastasis axillary lymph nodes in comparison to the primary tumor tissue and the adjacent normal tissue from the same breast cancer patients. The quantitative methylation analysis of the candidate genes showed higher methylation proportion for the primary tumor tissue versus matched normal tissue and the differences were significant for APC, BIN, BMP6, BRCA1, CST6, ESR-b, P16, PTEN and TIMP3 promoter regions (P<0.05). Among the significant methylated genes, APC, BMP6 and BRCA1 represented high methylation proportion in paired metastasis and non-metastasis lymph nodes compared to the normal tissue (P<0.05) whereas the P16 promoter was methylated only in the metastasis lymph node (P<0.05). We identified even greater hypermethylation proportion of BMP6 in the metastasis lymph node than the primary tumor tissue (P<0.05). Conversely, the promoter region of BIN1, GSTP1 and P14 significantly showed less methylation proportion in both the lymph node metastasis and non- metastasis compared to the matched normal tissue (P<0.05). Taken together present study showed methylation heterogeneity between primary tumors and metastatic lesion. Contribution of aberrant methylation alterations of APC, BMP6, BRCA1 and P16 genes in metastasis lymph node suggests more investigation for the pathways and networks related to these genes which might improve knowledge of mechanism underlying metastasis and might improve prognosis and therapeutic strategies for the breast cancer patients.

748W

Epigenetic pattern, mRNA and protein expression of E-cadherin and Caveolin-1 in gastric adenocarcinoma. *C.O. Gigek[1], M.F. Leal[1], P.N.O. Silva[1], L.C.F. Lisboa[1], E.S. Chen[1], D.Q. Calcagno[1], E.M. Lima[2], P.P. Assumpção[3], R.R. Burbano[4], M.A.C. Smith[1].* 1) Disciplina de Genética, Departamento de Morfologia e Genética, Universidade Federal de São Paulo, SP, Brasil; 2) Laboratório de Genética Humana e Oncogenética, Departamento de Biologia Molecular, Universidade Federal do Piauí, PI, Brasil; 3) Serviço de Cirurgia, Hospital Universitário João de Barros Barreto, Universidade Federal do Pará, PA, Brasil; 4) Laborátorio de Citogenética Humana e Genética Toxicológica, Departamento de Biologia, Centro de Ciências Biológicas, Universidade Federal do Pará, PA, Brasil.

Plasma membrane proteins play a role in cell signalling, adaptation to environment and cell-matrix interactions, and are also involved in the acquisition and maintenance of invasive and metastatic properties of tumour cells. E-cadherin is a member of calcium-dependent adherins and contributes to cell-cell adhesion. Caveolin-1 is an integral membrane protein and it has been implicated in diverse cellular processes such as cholesterol homeostasis, vesicular transport, cell migration, regulation of cell transformation and signal transduction. Their respective genes have potential sites embedded within CpG islands, being potential sites for epigenetic regulation by DNA methylation. Epigenetic modifications have a central role in several types of cancer, including gastric adenocarcinoma. In the present study, E-cadherin and Caveolin-1 immunostaining were analyzed in about 50 gastric neoplastic and 20 non-neoplastic gastric mucosa samples. DNA methylation and mRNA were also investigated through methylation specific PCR and qRT-PCR. Statistical analyses were performed to assess associations between protein and mRNA expression and methylation status with clinicopathological characteristics. Absence of E-cadherin immunostaining was associated with gastric carcinogenesis (p<0.0001) and with metastasis (p= 0.0035). 84.2% of neoplastic samples had positive immunostaining for Caveolin-1 (p<0.0001) and this protein was also more frequently observed in intestinal than in diffuse type gastric cancer (1 vs 0.6786, p=0.0008) and in H. pylori infected samples (p=0.0196). Our data showed an inverse relationship between E-cadherin and Caveolin-1 expression. CAV1 mRNA levels of 37-paired samples showed a significant increase in tumors samples (5.3781 ± 1.6950) when compared to non-neoplastic gastric mucosa (4.2630 ± 1.5015) (p=0.0004). We observed an association between hypermethylated tumor samples status and lack of Caveolin-1 expression (p=0.001). Therefore, we originally demonstrated epigenetic regulation of CAV1 by DNA methylation in gastric cancer. This event has been previously reported in different tumors, such as breast and sporadic colorectal cancer and hetapatocellular carcinoma. In conclusion, E-cadherin was associated with gastric cancer and with a metastatic phenotype and Caveolin-1 seems to have a role as a pro-tumorigenic factor and might be a good marker for gastric cancer. Financial Support: FAPESP and CAPES.

749W

ASSOCIATION OF DNA METHYLATION PATTERN WITH PARENTAL SMOKING IN ACUTE LYMPHOBLASTIC LEUKEMIA CASES. *L. Hsu[1], J. Xiao[2], A. Chokkalingam[1], C. Metayer[1], P. Buffler[1], J. Wiemels[2].* 1) School of Public Health, University of California Berkeley, Berkeley, CA; 2) Department of Epidemiology and Biostatistics, University of California, San Francisco, CA.

PURPOSE: Childhood acute lymphoblastic leukemia (ALL) is a multifactorial disease. Evidence has suggested that environmental exposures may affect DNA methylation at specific genomic loci in certain smoking-related cancers, which could serve as an important intermediate between exposure and cancer. GoldenGate DNA methylation profiling on a series of leukemia bone marrows demonstrated that DNA methylation patterns are associated with parental smoking. Here we attempt to validate methylation at individual gene loci identified through pilot profiling as markers of parental smoking in leukemic cells. METHODS: Leukemia bone marrow DNA was isolated and bisulfite-treated prior to Sequenom methylation analysis. We examined the association between parental smoking (yes/no) during several critical developmental periods (pre-pregnancy, during pregnancy and postnatal) and methylation at 38 individual CpG loci among 206 B-cell leukemia cases. The association between methylation levels at specific loci and parental smoking variables assessed using Student's T-test (parametric) or the Wilcoxon rank-sum test (non-parametric). RESULTS: Among the 38 CpG units, methylation at two CpG loci in two genes (TSP50, C4B) were significantly associated with maternal smoking during pregnancy (p=0.003) and maternal smoking after giving birth (p=0.04) respectively, but only TSP50 remains significant after adjustment for multiple testing. CONCLUSIONS: The results from our study suggest that individual methylated CpG loci are associated with maternal smoking, and that maternal smoking may affect childhood leukemia risk via its impact on DNA methylation processes.

750W

The difference in methylation status of the p53 gene in smokers versus non-smokers. *M. Simkin[1,2], M. Abdalla[2], M. Elmogy[2], Y. Haj-Ahmad[1,2].* 1) Brock University, St. Catharines, Ontario, Canada; 2) Norgen Biotek Corp, Thorold, Ontario, Canada.

DNA methylation is a valuable epigenetic alteration that could be used for cancer screening, prevention and therapeutics. DNA methylation is easier to detect than mutations, and methylation changes usually occur early in tumour progression. DNA methylation is also reversible; allowing potential treatments to begin before cancer even develops. The tumour-suppressor protein p53 is responsible for cell cycle arrest and DNA repair. Mutations in the p53 gene have been found in more than half of all cancers, and hypermethylation of the promoter sequence adjacent to p53 has been found to lead to cancer. Smoking has been demonstrated to lead to hypermethylation of tumour-suppressor genes in many tissues, including both cancerous and non-cancerous lung biopsy tissues. This association has never been explored in young, healthy individuals, with no clinical signs of cancer. The present study looked at DNA isolated from urine and saliva samples taken from 56 healthy participants, male and female smokers and non-smokers, aged 18-25. Using GAPDH as a negative control, p53 gene promoter methylation was assessed using methylation-sensitive restriction enzymes HpaII and HhaI followed by end-point PCR and agarose gel electrophoresis. This study demonstrated that qualitatively, DNA derived from both the saliva and urine of smokers and non-smokers were equivalent in that none of the samples obtained were found to show p53 gene promoter hypermethylation. Quantitatively, however we found that the DNA concentration is greater in the smoker samples (both urine and saliva) than the non-smokers. Saliva and urine have been recognized as a non-invasive method of obtaining a blood sample, with both representing total systemic cell apoptosis. The findings from this study indicate that urine and saliva represent a good source of total systemic DNA for downstream molecular biological analyses, and that both samples are robust enough to study epigenetic alterations in human DNA.

751W

The role of MBD2 in hypomethylation and activation of pro-metastatic genes in liver cancer. *B. Stefanska[1], B. Bhattacharyya[1], M. Suderman[1], J. Huang[2], M. Hallett[3], Z.G. Han[2], M. Szyf[1].* 1) Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada; 2) Shanghai-MOST Key Laboratory for Disease and Health Genomics, Chinese National Human Genome Center at Shanghai, Shanghai, China; 3) McGill Centre for Bioinformatics, McGill University, Montreal, Canada.

DNA hypomethylation, a process of losing methyl marks, may play an important role in cancer, especially through activating genes that promote metastasis. Using whole genome promoter methylation microarrays, we previously delineated the landscape of DNA hypomethylation in liver cancer, which is one of the most common cancers worldwide. Our studies revealed that 50% of differentially methylated promoters are hypomethylated in tumors, compared to adjacent normal tissue. In these studies, using liver cancer cell lines as a model system, we evaluated the importance of the identified hypomethylated genes in liver cancer development and progression. We tested whether the hypomethylation observed in liver cancer is driven by MBD2, a protein reported previously to be implicated in DNA demethylation, which we found to be overexpressed in liver cancer patients. Following siRNA MBD2 depletion in HepG2 liver cancer cells, we used methylated DNA immunoprecipitation, chromatin immunoprecipitation (ChIP) and 60K custom microarrays covering 12K bp upstream and 10K bp downstream of transcription start site to determine changes in methylation as well as MBD2 binding and RNA polymerase II binding in response to MBD2 depletion in 306 top genes that were found to be hypomethylated and/or induced in liver cancer patients and/or HepG2 cells. The array data were validated by quantitative ChIP and/or pyrosequencing. We discovered MBD2 binding peaks in 172 genes that are located mostly within their promoter regions and overlap or are in the vicinity of RNA polymerase II binding peaks that mark transcription start sites. Using bioinformatic tools, we identified MBD2 binding motif and established putative transcription factors recognizing this sequence which may play a key role in recruiting MBD2 to target genes. 41% of those genes were hypermethylated in HepG2 cells after MBD2 depletion in the region corresponding to the identified MBD2 binding site. In addition, expression analysis for some of the genes showed a reduction in response to MBD2 depletion. The genes that are regulated by MBD2 in liver cancer are important for the cancer phenotype since siRNA depletion of these genes reverses the transformed phenotype. Our results establish a role for MBD2 in coordinating inhibition of a panel of genes involved in cancer growth and metastasis. This study was supported by a grant from the MDEIE program of the government of Quebec and National Cancer Institute of Canada to MS.

752W

OxLDL causes both epigenetic modification and signaling regulation on the microRNA-29b gene: novel mechanisms for cardiovascular diseases. *K.C. Chen[1], I.C. Hsieh[2], Y.S. Wang[1], Y.C. Liao[3,4,5], C.Y. Hu[1], S.H.H. Juo[1,2].* 1) Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 2) Department of Medical Genetics, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 3) Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 4) Section of Neurology, Taichung Veterans General Hospital, Taichung, Taiwan; 5) Department of Neurology, National Yang-Ming University School of Medicine, Taipei, Taiwan.

We previously reported that microRNA-29b can epigenetically regulate proatherogenic genes in response to oxLDL stimulation. However, the mechanisms underneath the transcriptional regulation of miR-29b gene remain to be explored. The present study aims to investigate whether transcription factor activation or histone modification is involved in oxLDL-induced miR-29b upregulation. We first demonstrated that mice fed with a high-fat diet had an increased miR-29b expression, which was consistent with our previous findings in the cellular studies. We found that oxLDL enhanced miR-29b expression through activating the transcription of miR-29b-1/miR-29a cluster gene. We used the promoter assay and chromatin immunoprecipitation to confirm the binding between miR-29b-1 promoter and activator protein-1 (AP-1), a transcriptional activator. By using the MAPTAM (Ca^{2+} chelator), NAC (ROS scavenger), U0126 (ERK inhibitor) and c-Fos shRNA, we found the signal pathway of LOX-1/Ca^{2+}/ROS/ERK/c-Fos was involved in oxLDL-mediated miR-29b overexpression. We also showed that down-regulation of HDAC1 and the modifications on histone 3 lysine 4 (H3K4) and H3K9 significantly affected miR-29b expression. Furthermore, knockdown of c-Fos expression attenuated oxLDL-induced histone modifications on miR-29 gene. To conclude, transcription factors activation and histone modifications are both important regulatory mechanisms of oxLDL-induced atherogenic process.

753W

Epigenetics and genomics of constitutional trisomy 8: a uniqe model system for chromosome gain. *J. Davidsson[1], S. Veerla[2], B. Johansson[1].* 1) Dept. of Clinical Genetics, Skåne University Hospital, Lund, Sweden; 2) Dept. of Oncology/SCIBLU DNA Microarray Resource Centre, Lund University, Lund, Sweden.

Aneuploidy is frequently seen in cancer cells and constitutional genetic disorders, but how chromosomal imbalances produce pathophenotypes is currently not well understood. Gene expression and epigenetic patterns of gained chromosomes have previously only been studied *in vivo* by comparing triploid cells with unrelated disomic cells from healthy individuals. Our novel approach aimed to characterize cells with and without trisomy, derived from the same cell type and individual. Using fibroblasts from a carrier of trisomy 8 mosaicism, we by single cell suspension assays cloned cultures purely 46,XY or 47,XY,+8, as confirmed by FISH. Utilizing microarrays and methylated DNA immunoprecipitation we profiled gene and miRNA expression, as well as DNA methylation and hydroxymethylation patterns in three 47,XY,+8 cultures, three 46,XY cultures, as well as in two reference fibroblast cultures. Unsupervised clustering and principal component analysis of the methylation, hydroxymethylation, miRNA and gene expression data demonstrated that the 47,XY,+8 cells in each individual dataset clearly separated from the 46,XY cells, with the latter clustering together with the two reference fibroblast cultures. Genes on chromosome 8 were generally overexpressed in the 47,XY,+8 cells, but significantly altered expression patterns were detected also on other chromosomes, supporting that downstream effects of trisomy is not restricted solely to the gained chromosome. A number of differentially expressed miRNAs and genes were identified, which could be candidate contributors to both the phenotype and increased risk for malignant disorder seen in patients with the trisomy 8 mosaicism syndrome. Performing pathway analysis of genes differentially expressed in the 47,XY,+8 cells, cancer and genetic syndromes were the most significant diseases, whereas cell morphology and cellular development were the top scoring pathways. Several genes and miRNAs were differentially hypermethylated and underexpressed in 47,XY,+8 compared to 46,XY cells, indicating that methylation and hydroxymethylation of DNA is a functional downstream response to trisomy 8 in human cells. Moreover, on the additional chromosome 8 in the 47,XY,+8 cells, a global hypomethylation of gene-poor regions could be detected, something that is generally seen as a hallmark of X-chromosome inactivation and have previously only been reported in association with chromosome gain in neoplasia, not in constitutional disorders.

754W

EFFECT OF HISTONE 4 METHYLATION AT *BDNF* EXPRESSION AFTER ANTIDEPRESSANT TREATMENT ON HUMAN PREFRONTAL CORTEX. *E.S. Chen[1], C. Ersnt[2], M.A.C. Smith[2], G. Turecki[1].* 1) Morfologia e Genética, UNIFESP, São Paulo, São Paulo, Brazil; 2) Department of Psychiatry, McGill University, Montreal, Quebec, Canada.

Antidepressant drugs have been reported to increase BDNF expression levels in brain and might reverse the depression-associated decrease in BDNF expression both in human and rat, suggesting a relationship between BDNF expression and antidepressant action. One of the variants of BDNF, transcript IV, has been related to psychiatric disorders in some animal models. To date, the mechanisms underlying this event are poorly understood. Our group has previously showed that antidepressants are associated with decreased methylation at H3K27 and an increase in BDNF IV expression. In this study, we aimed to investigate whether methylation at H4K20 lead to altered BDNF IV expression in prefrontal cortex of control subjects with no psychiatric history (N=9; C), depressed patients without positive toxicology for antidepressants or history of antidepressants use (N=11; AD-), and depressed patients with a history of antidepressant use and with antidepressant detected in blood post-mortem, using MS (N=7; AD+). Antidepressants used included: fluoxetine (1/7), venlafaxine (2/7), clomipramine (1/7), amytriptyline (1/7), citalopram (1/7) and doxepine (1/7). qRT-PCR and ChIP were performed. All analyses were carried out using SPSS v18.0. ANOVA analysis showed a significant higher levels of BNDF promoter IV expression in AD+ group when compared to AD- (F=4.395; p=0.024). We found a significant difference in H4K20 methylation levels among groups (F=8.419, p=0.001). These results suggest a relationship between antidepressant use, methylation at H4K20, and BDNF IV expression, with similar results to that obtained previously by our group when another histone residue was analyzed. Thus, we may conclude that antidepressants treatment may lead to gene expression alterations in prefrontal cortex of suicide patients by chromatin remodelling. Financial support: CAPES, FAPESP.

755W

Epigenetic regulation of polyamine biosynthetic genes in suicide. *J. Gross, L. Fiori, B. Labonté, G. Turecki.* Douglas Mental Health University Institute, Montreal, Quebec, Canada.

Suicide is among the leading causes of death worldwide. Recently, the polyamine system has become an interesting target of research aimed towards understanding the neurobiological alterations associated with suicide. Previous research has linked suicide behavior with genetic variations in the promoter of spermidine/spermine N1-acetyl transferase, the rate-limiting enzyme in polyamine catabolism. However, the epigenetic regulation of other polyamine biosynthetic genes has yet to be elucidated. Using quantitative real time polymerase chain reaction (qRT-PCR), expression of ornithine decarboxylase antizymes 1 and 2 (OAZ1 and OAZ2), S-adenosylmethionine decarboxylase (AMD1), and arginase, type 2 (ARG2) was assessed in Brodmann area 44 of 80 French-Canadian males comprising 40 suicide completers and 40 non-suicide control subjects. Using chromatin immunoprecipitation (nChIP) and antibodies targeting histone H3 trimethylated at lysine 4 (H3K4me3) and at lysine 27 (H3K27me3), markers of open and closed chromatin respectively, DNA nucleosomes were extracted from a group of the above subject in which expression of each of the four genes was significantly higher in suicide completers compared to controls. The resulting products were analyzed by qRT-PCR. In addition, Sequenom's EpiTYPER was used to characterize differentially methylated CpGs in the promoter region of each gene mentioned above. We found significant increase in H3K4me3 in suicide completers for OAZ1, and the levels of this modification were significantly correlated with expression of this gene. Levels of H3K27me3 in OAZ1 were not nominally significant, however, our results indicated a trend of increased H3K27me3 in the suicide group as compared to the control subjects. Finally, we found site-specific differences in methylation in the promoter regions of each gene between suicide completers and controls, however only those of AMD1 and ARG2 correlated significantly with gene expression. These preliminary findings suggest that epigenetic modifications are involved in regulation of genes associated with polyamine biosynthesis and that this might contribute to the complexity of suicide behavior.

756W

Impact of genetic variation on chromatin state and genome-wide gene expression phenotypes. *H. Kilpinen[1], S. Waszak[2], R.M. Witwicki[3], A. Orioli[3], S. Raghav[2], M. Gutierrez-Arcelus[1], L. Romano-Palumbo[1], N. Hernandez[3], B. Deplancke[2], A. Reymond[3], E.T. Dermitzakis[1].* 1) Department of Genetic Medicine and Development, University of Geneva Medical School; Geneva, Switzerland; 2) Laboratory of Systems Biology and Genetics, Institute of Bioengineering, École Polytechnique Fédérale de Lausanne (EPFL); Lausanne, Switzerland; 3) Center for Integrative Genomics, University of Lausanne; Lausanne, Switzerland.

During the last few years, genome-wide chromatin state and histone modification profiling has generated information about specific chromatin signatures related to different functional elements of the genome in multiple cell types. However, the degree of stability and the genetic basis of such signatures across individuals remain largely unknown. We have used ChIP-seq to produce genome-wide enrichment profiles of three transcription factors, RNA Pol II, MYC, and SPI1, as well as histone modifications H3K4me1, H4K20me1, H3K27me3, and H3K4me3, from lymphoblastoid cell lines of two trios sequenced as part of the 1000 Genomes project. In the second phase of the study these marks will be profiled in 100 individuals from the 1000 Genomes CEU population for which low coverage genome sequence and genotype data is available. We will also produce genome-wide mRNA and miRNA sequencing data from the same 100 individuals. For the two trios, additional functional data such as global DNA methylation profiles will be produced, which allows us to overlay multiple layers of genomic information for a comprehensive picture of the transcriptional state of the cells. With this dataset we will (i) compare the enrichment patterns of chromatin and transcription factor signatures across individuals and around key sequence elements such as expression quantitative trait loci (eQTLs), shown to affect transcript levels in these individuals (ii) estimate the degree of heritability of such signatures (iii) analyze the allele-specific effects of DNA sequence variation on chromatin structure and correlate this information with allele-specific gene and miRNA expression levels from the same individuals (iv) explore the factors which affect the general transcriptional state of the cell. This study will significantly improve our understanding of the biological landscape around regulatory and other functional elements of the genome, and provide better means to address the cellular basis of phenotypic diversity, such as disease susceptibility, in humans.

757W

Analysis of DNA methylation supports variable spread of X-chromosome inactivation into X;Autosome translocations. *A. Cotton[1,2], L. Lam[1,3,4], M. Kobor[1,3,4], C. Brown[1,2].* 1) Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada; 2) Molecular Epigenetics Group, Life Sciences Institute, Vancouver, BC, Canada; 3) Child & Family Research Institute, Vancouver, BC, Canada; 4) Centre for Molecular Medicine and Therapeutics, Vancouver, BC, Canada.

X-chromosome inactivation (XCI), the mammalian dosage compensation process, is normally random, with either X being transcriptionally silenced early in development. In unbalanced X;autosome (X;A) translocation carriers the translocated chromosome is usually inactivated; however, the spread of XCI into the autosomal portion of the translocated chromosome is variable. At X-linked CpG island promoters the inactive X is hypermethylated compared to the active X, while autosomal CpG island promoters generally show low levels of methylation on both chromosomes. Having previously shown that the methylation of CpG island promoters can be used to predict the XCI status of X-linked genes, we investigated if methylation could be used to determine the extent of spread of XCI into the autosomal portion of unbalanced X;A translocations. Using the Illumina 450K array to examine the methylation of unbalanced X;A translocations we designated CpG island promoters on the autosomal portion of the X;A translocation that showed hypermethylation as being subject to XCI, while those which remained unmethylated were predicted to escape XCI. For five unbalanced X;A translocation cell lines we observed an average of 19% of autosomal genes showing evidence for being subject to XCI, with the frequency of genes subject to XCI decreasing with distance from the translocation breakpoint. Genes located in a region of autosomal trisomy showed a higher frequency of inactivation (27%) than those in regions of disomy (6%), likely reflecting an ascertainment bias for translocations in which the spread of XCI has minimized the impact of aneuploid regions. DNA methylation was also used to refine the previous determined breakpoint regions from an average of 12 Mb to an average of 3.6 Mb. The spread of XCI into the autosomal portions of X;A translocations provides a unique opportunity to study chromosomal features involved in XCI and we used methylation in these cells as a means to refine models of the elements involved in the spread of silencing distinct from the evolutionary history of the sex chromosomes. The clustering of autosomal genes subject to XCI on X;A translocations supports a model in which elements or `waystations' capable of spreading silencing are also present on the autosomes. That autosomal genes which escape XCI are found in close proximity to genes subject to XCI further supports the presence of boundary elements which confer the ability to avoid inactivation signals.

758W

Neuron-specific impairment of inter-chromosomal pairing and transcription in a novel model of human 15q-duplication syndrome. *S. Horike[1], D.H. Yasui[2], W. Powell[2], D.I. Schroeder[2], M. Oshimura[3], J.M. LaSalle[2], M. Meguro-Horike[1].* 1) Frontier Science Organization, Kanazawa University, 13-1 Takaramachi, Kanazawa 920-0934, Japan; 2) Department of Medical Microbiology and Immunology, Genome Center, and M.I.N.D. Institute, University of California Davis School of Medicine, 1 Shields Avenue Davis, CA 95616; 3) Department of Biomedical Science, Tottori University, 86 Nishi-cho, Yonago, Tottori 683-8503, Japan.

Although the etiology of autism remains largely unknown, cytogenetic and genetic studies have implicated maternal copy number gains of 15q11-q13 in 1-3% of autism cases. In order to understand how maternal 15q duplication leads to dysregulation of gene expression and altered chromatin interactions, we used microcell-mediated chromosome transfer to generate a novel maternal 15q duplication model in a human neuronal cell line. Our 15q duplication neuronal model revealed that by quantitative RT-PCR transcript levels of *NDN*, *SNRPN*, *GABRB3*, and *CHRNA7* were reduced compared to expected levels despite having no detectable alteration in promoter DNA methylation. Since 15q11-q13 alleles have been previously shown to exhibit homologous pairing in mature human neurons, we assessed homologous pairing of 15q11-q13 by fluorescence in situ hybridization (FISH). Homologous pairing of 15q11-q13 was significantly disrupted by 15q duplication. To further understand the extent and mechanism of 15q11-q13 homologous pairing, we mapped the minimal region of homologous pairing to a ~500 kb region at the 3' end of *GABRB3* which contains multiple binding sites for chromatin regulators MeCP2 and CTCF as well as long-range interactions with the imprinting control region. Using RNA interference, we show that MeCP2 and CTCF are required for the homologous pairing of 15q11-q13 during neuronal maturational differentiation. These data support a model where 15q11-q13 genes are regulated epigenetically at the level of both inter- and intra-chromosomal associations and that chromosome imbalance disrupts the epigenetic regulation of genes in 15q11-q13.

759W

Copy number variation and chromatin structure. *R.M. Witwicki[1], E. Migliavacca[1], N. Gheldof[1], G. Didelot[1], A. Kurg[2], A. Reymond[1].* 1) Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland; 2) Institute of Molecular and Cell Biology, University of Tartu, Estonian Biocenter, Tartu, Estonia.

Copy number variation (CNV) was shown to influence the phenotype by modifying, in a somewhat dose-dependent manner, the expression of genes that map within them, as well as that of genes located on their flanks. To assess the possible mechanism(s) behind this neighboring effect, we compared the histone modification status of cell lines from patients affected by genomic disorders (WBS, WBRdup, DGS and SMS) and control individuals. Consistent with the changes in expression levels observed for multiple genes mapping on the entire length of chromosomes affected by structural variants, we detected regions with modified histone status between samples, up- and downstream from the critical regions, up to the end of the rearranged chromosome. Coherently, we pinpointed alteration of intrachromosomal interactions (chromosomal looping) between affected genes loci and the rearranged interval using an unbiased variant of chromosome conformation capture (3C-seq).

We conclude, that large genomic rearrangements can lead to changes in the state of the chromatin spreading far away from the critical region, thus possibly affecting expression globally. For example, we observe that the chromatin conformation of the *FoxP2* gene, mutations of which are associated with language and speech disorders, is modified in Williams-Beuren region duplication syndrome patients, who present language difficulties.

760W

Methlyation differences between discordant monozygotic twins: a role for cysteine sulfinic acid decarboxlase (CSAD) in type 1 diabetes susceptibility. *E. Elboudwarej[1], J. Jeffrey[2], P.R. Fain[2], P.P. Ramsay[1], H. Quach[1], J.A. Lane[3], G.S. Eisenbarth[2], L.F. Barcellos[1], J.A. Noble[3].* 1) Division of Epidemiology, Genetic Epidemiology and Genomics Laboratory, School of Public Health, University of California, Berkeley, CA; 2) University of Colorado Health Sciences Center, Barbara Davis Center for Childhood Diabetes, Denver, CO; 3) Children's Hospital Oakland Research Institute, Oakland, CA.

Type I diabetes (T1D) has a substantial genetic component; however, traditional genetics cannot completely explain susceptibility, as evidenced by varying levels of disease discordance among monozygotic (MZ) twin pairs. In this matched case-control study, four MZ twin pairs, selected for T1D discordance of at least 8 years, were examined for differences in DNA methylation at 27,578 CpG sites across the human genome using the Illumina Infinium HumanMethylation27 BeadChip. Although global methylation levels did not differ significantly between the affected and unaffected twin in a pair, 12 CpG sites were found to have greater than 10% difference in DNA methylation between the twins in a pair, including sites within: ABCC12, ATP2B4, C14orf48, C1orf107, CA2, CDC2L2, CSAD, CUEDC1, EED, PTRH2, SOAT2, and ZNF671. A site within CSAD (cg03933322) demonstrated > 20% difference in methylation for all four twin pairs (20.4%, 23.0%, 26.5%, 35.8%) and was consistently hypomethylated in the twin with diabetes. The trend toward hypomethylation of the affected twin at the CSAD locus was reproduced for three of the four original pairs in a repeat experiment using new samples, although the effect was less pronounced. Furthermore, five additional MZ twin pairs were tested, four of which were consistent with the initial result. CSAD encodes cysteine sulfinic acid decarboxlyase, an intracellular enzyme that controls the rate-limiting step in biosynthesis of taurine, an amino acid involved in glucose homeostasis. CSAD shares a 50% amino acid sequence identity with GAD65 (glutamic acid decarboxlyase), a pancreatic enzyme that is a major target of autoantibodies in T1D. The strong and reproducible methylation differences observed in the T1D discordant MZ twin pairs, combined with the biological role of CSAD and its sequence similarity to GAD65 support the notion that CSAD methylation may play a role in T1D susceptibility and/or progression. Our results indicate CSAD is a good candidate locus for targeted follow-up methylation studies in both discordant MZ twins and large case-control cohorts.

761W

Influence of DNA modification on INS gene expression in thymus and implications in Type I diabetes (T1D). *T. Khare[1], M. Pal[1], S.C Chong[2], A. Pugliese[3], A. Paterson[4], J. Mill[5], A. Petronis[1].* 1) Centre for Addiction and Mental Health, Toronto, Canada; 2) Institute of Systems Biology and Bioinformatics, National Central University, Taiwan; 3) Diabetes Research Institute University of Miami Miller School of Medicine ,USA; 4) Department of Medicine, University Health Network, University of Toronto, Toronto, Canada; 5) MRC Social, Genetic and Developmental Psychiatry Centre, King's College London, UK.

During embryogenesis, low abundance of insulin (INS) in thymus is linked to deficient thymic learning and to the higher risk of T1D. It is well established that VNTR at INS gene promoter has association with INS mRNA expression and also to the increased susceptibility to T1D, however the underlying mechanism is still not clear. Human insulin gene is mapped on Chr11p15.5, in the vicinity of an imprinting cluster and exhibits sporadic parent of origin dependent mono-allelic expression in thymus, spleen and pancreas. These observations make INS a viable target for epigenetic studies of T1D. In the present study an attempt is made to understand the influence of DNA methylation on INS expression in thymus tissue samples from human embryos and paediatric patients, followed by comprehensive analysis of peripheral blood tissue of T1D patients and matched controls. The working hypothesis is that mono versus biallelic INS gene expressing thymii share similar DNA regions for epigenetic aberrations as diabetic patients, and these epimutations can be detected on the unaffected tissues of the patients. Out of 52 thymus samples, we identified monoallelic (n= 14) and biallelic (n=15) INS expressing thymii. These were quantified for INS steady state mRNA expression and investigated for DNA methylation by a restriction enzyme based method developed at our lab. The DNA methylation profiling method allows enrichment of unmethylated genomic DNA fraction which is then hybridized to human tiling array (covering human chr8, 11 & 12). Our preliminary results indicate that INS expression is correlated to the VNTR type, consistent with earlier reports, but independent of the INS allelic state. We also identified a modest correlation (r2=0.53) in individuals homozygous for VNTR type III between DNA methylation to INS expression at a region ~500kb away from INS gene. Interestingly, diabetic patients homozygous for VNTR III also showed aberrant DNA methylation compared to matched controls. (~10% difference; p=0.001 after bonferroni correction). Aim of our ongoing analysis is to compile common genomic regions with alterations in DNA methylation in thymus and in the T1D patients' genomic DNA. T1D is a complex non-mendelian disorder with possibly multiple epigenetic risk factors. Our long term goal is to identify these risk factors by a comprehensive DNA methylome (5mC and 5hmC) study in multiple tissues from T1D patients and controls.

762W

Epigenetic alterations and stress among new mothers and infants in the Democratic Republic of Congo: A biocultural look at the intergenerational effects of war. *C.J. Mulligan, N. D'Errico, J. Stees, C.C Gravlee, T.P. Yang.* University of Florida, Gainesville, FL.

Adaptation is a complex process involving genetic, physiological, and behavioral mechanisms. There is growing evidence that epigenetic modifications may serve as an intermediate adaptive mechanism that mediates between the rapidly changing environment and our slowly evolving genome. The current proposal is one of the first to investigate epigenetic alterations as a possible mechanism for phenotypic plasticity during fetal development. We are testing the idea that epigenetic modifications may create heritable changes in response to extreme environmental stressors that affect infant health in a multigenerational manner. Maternal blood and umbilical cord blood samples were collected from 25 mother-infant dyads in the eastern Democratic Republic of Congo. Detailed ethnographic interviews and perinatal trauma surveys were administered to all mothers including questions designed to develop emic measures of stress. Medical histories of mothers were accessed and birth weights and gestational age of infants were recorded. DNA was extracted and treated with sodium bisulfite. A 321 bp promoter of NR3C1 with 39 CpG sites was amplified, cloned and sequenced. Twenty clones per sample were sequenced. NR3C1 is a glucocorticoid receptor that was previously implicated in methylation-mediated changes in gene expression associated with differences in childhood trauma. Our preliminary results show increased methylation in stressed mothers (material deprivation, emotional stress, recent rape), but infants show the opposite trend regardless of maternal stress exposure. When comparing mothers to their infants, no infants show an increase in methylation when compared to stressed mothers (emotional stress, recent rape) but a majority of infants show increased methylation when compared to less stressed mothers. Our results suggest that methylation patterns are different between mothers and infants and may correlate with specific maternal stress exposures.

763W

Role of CTCF protein in regulating FMR1 gene transcription. *G. Neri, S. Lanni, M. Goracci, L. Borrelli, E. Tabolacci.* Istituto di Genetica Medica, Università Cattolica , Rome, Italy.

Fragile X syndrome (FXS, OMIM #300624) is the commonest cause of inherited mental retardation, due to mutation of FMR1, an X-linked gene containing a CGG repeat in its promoter region. Expansion of this sequence beyond 200 repeats and consequent epigenetic changes (full mutation, FM) cause the transcriptional inactivation of FMR1 and lack of the FMRP protein. Rare individuals of normal intelligence, carrying an unmethylated full mutation (UFM), have been reported and the epigenetic modifications in their FMR1 gene were characterized [Pietrobono et al., 2005; Tabolacci et al., 2008]. Cell lines from these individuals may reflect the status of FXS cells before the silencing of FMR1 during development (approximately 11 weeks of gestation). CTCF (CCCCTC-binding factor), a zinc-finger protein which binds to the chromatin insulators, is an important regulator of the transcription of genes harbouring trinucleotide repeats, acting as insulator between different chromatin domains. We started to investigate the role of CTCF in regulating FMR1 gene expression and observed that the amount of CTCF bound to FMR1 was slightly higher in the UFM cell lines, compared to normal controls, while in the FXS cells this amount was low. On the other hand, knock-down of CTCF (around 85%) with anti-CTCF siRNA transfected into normal and UFM fibroblasts, resulted in a 50% reduction of FMR1 transcript, both sense and antisense (AS-FMR1), with respect to untreated controls. After CTCF knock-down, the epigenetic analysis of the FMR1 promoter region demonstrated a reduction of H3-K4 methylation and an increase of H3-K9 methylation, while the DNA methylation of the FMR1 promoter region and of the upstream methylation boundary [Naumann et al., 2009] remained unmodified. ChIP assays demonstrated that CTCF knock-down affected specifically its binding to the 5' UTR of the FMR1 gene. These results suggest that CTCF is a modulator of the FMR1 transcription, given that its depletion causes FMR1 transcript reduction and the transition to a heterochromatic configuration. The elucidation of the mechanism sparing UFM males from inactivating their full mutation is important for planning therapeutic attempts at converting methylated into unmethylated full mutations, restoring the expression of the FMR1 gene. Supported by FRAXA Foundation and Telethon Onlus.

764W

DNA methylation of multiple promoter-associated CpG islands in gastrointestinal stromal tumors (GIST). *M. Ravnik-Glavač, D. Glavač.* Department of Molecular Genetics, Faculty of Medicine, Vrazov trg 2, 1000, Ljubljana, Slovenia.

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms in the gastrointestinal tract. Important prognostic factors for estimating malignant potential are: tumor size and mitotic rate and, to a more variable degree, the proliferation index or tumor site. Other potential and promising markers of GIST malignancy are molecular alterations. GIST is primarily defined by activating mutations in the c-KIT or PDGFRA receptor tyrosine kinases. To identify additional alterations we investigated the methylation status of ten methylation-sensitive CpG islands of (hMLH1, RARB, CHFR, IGSF4, APC, RASSF1, p15, p27, p73, and PTEN) in 54 patients with GIST. Aberrant DNA methylation of these loci was found in 73 % of all GIST. The rates of DNA methylation at each locus were as follows: hMLH1, 60%; CHFR, 45% , IGSF4, 41%, RASSF1, 38%, APC, 32 %, TIMP3, 18%, p15, 8%, p27, 5%, p73, 48 % and PTEN 0,0%. CpG islands methylator phenotype, which was defined as methylation involving more than three gene promoters, was found in 75% of all GIST. According to the risk categories, CpG islands methylator phenotype was present in 45% of low-risk GIST, and in 65% of high-risk GIST. CIMP was found to be much lower 45% of GIST with c-KIT gene mutations. Our results suggested that, the aberrant methylation of CpG islands, especially of hMLH1, CHFR, IGSF4 and p73, may play an important role in the tumorigenesis of GIST.

765W

Epigenetic studies of a newborn twin cohort: insights into pre- and postnatal development. *J.M. Craig[1], E.J. Joo[1], R. Andronikos[1], M. Ollikainen[1,5], X. Li[1], Y-J. Loke[1], B. Novakovic[2], R. Morley[1], J. Carlin[3], L. Gordon[4], R. Saffery[2].* 1) Environment, Genes & Health, Murdoch Childrens Research Institute, Melbourne, Victoria, Australia; 2) Cell Biology, Development and Disease, Murdoch Childrens Research Institute, Melbourne, Victoria, Australia; 3) Clinical Epidemiology and Biostatistics Unit, Murdoch Childrens Research Institute, Melbourne, Victoria, Australia; 4) Bioinformatics Unit, Murdoch Childrens Research Institute, Melbourne, Victoria, Australia; 5) Twin Study, Hjelt Institute, Department of Public Health, P.O. Box 41, FI-00014 University of Helsinki, Finland.

The Peri/postnatal Epigenetic Twins Study (PETS) is a cohort of 250 mothers and their twins. Women were recruited from three Melbourne hospitals midway through their second trimester. Recruiting at this time enabled measurement of maternal and fetal factors at multiple time points and minimization of recall bias in dietary questionnaires. The aims of the PETS cohort are to study the plasticity of epigenetic marks and the genes they control during the intrauterine period and in early childhood. It also aims to apply the classical twins model to determine the genetic and environmental factors they are influenced by. We focused on obtaining extensive data on maternal factors during pregnancy, collection of multiple biological specimens (cord blood, cords, placenta and buccal samples at birth, blood and buccals at 18 months), and on state-of-the art technologies using both gene-specific and genome-wide approaches. Furthermore, because of the mitotic heritability of epigenetic marks, we are treating epigenetic state at birth as an intermediary between the intrauterine exposures that influence birth weight and the risk of chronic disease later in life. We measured DNA methylation on a genome scale in DNA from three cell types from 22 MZ and 11 DZ twin pairs using Illumina Infinium HumanMethylation27 BeadChip arrays. This platform interrogates 27,578 CpG dinucleotides covering 14,475 transcription start sites. Using a single, compound measurement of with-pair discordance we found that both MZ and DZ pairs exhibited a range of within-pair discordance greater than for technical replicates and that DZ twins were more discordant than MZ twins. Using gene-specific measurements we identified groups of genes consistently discordantly methylated within twin pairs and using regression analysis, identified genes whose methylation levels correlated with birth weight in MZ pairs. Many of these genes were linked with cardiovascular and metabolic function, providing a plausible biological mechanism for the previously described link between low birth weight and increased risk of later complex disease. We also measured DNA methylation on a genome scale in DNA from buccal cells at birth and 18 months using Illumina Infinium arrays interrogating >485,000 CpG dinucleotides and detected extensive postnatal age-associated epigenetic change. To our knowledge, this is the first study to demonstrate genome-wide epigenetic differences within newborn and very young twin pairs.

766W

Genome-wide DNA methylation in sib-pairs discordant for autism. *R.S. Alisch, P. Chopra, K.E. Szulwach, B.G. Barwick, B. Lynch, J. Mowrey, G.A. Satten, K.N. Conneely, P. Jin, S.T. Warren.* Dept Human Gen, Emory Univ Sch Med, Atlanta, GA. 30322.

The autism spectrum disorders (ASD) comprise a broad group of behaviorally related neurodevelopmental disorders affecting as many as 1 in 110 children. Family, twin and epidemiological studies reveal a strong familial component to ASD risk, suggesting a polygenetic and/or epistatic susceptibility model involving many genes. However, despite great effort only a few genes have been linked conclusively to ASD risk and, even then, only in a small minority of ASD cases suggesting that the etiology of ASD is likely to be complex and may also include both epigenetic and environmental factors. Here we investigate a potential epigenetic component to ASD by interrogating DNA methylation using an established high-throughput assay that interrogates DNA methylation (DNAm) at 27,578 highly informative CpG dinucleotides (spanning 14,495 genes). DNAm profiles from whole blood-extracted DNA of 448 male sib-pairs discordant for ASD do not reveal loci with significant DNAm differences associated with autism, suggesting that this epigenetic component does not play a role in ASD. However, this assay does not distinguish between 5-methylcytosine (5-mC) and the recently identified modification of cytosine, 5-hydroxymethylcytosine (5-hmC), which was identified in stem cells and post-mitotic neurons and raises new questions as to the role of cytosine modifications in mediating epigenetic effects. As a preliminary analysis, we profiled 5-hmC throughout the genomes of two dizygotic twin sib-pairs discordant for autism and find a consistent reduction in 5-hmC in the exonic sequences of the ASD individuals; this reduction is not present in intronic or intergenic genomic sequences. Consistent with this finding, we identify 114 ASD-associated loci that are deficient in the autistic boys as well as fourteen ASD-associated loci that are enriched for 5-hmC, both of which are more prevalent than would be expected by chance alone ($p < 10^{-6}$). A closer examination within these 128 ASD-associated 5-hmC loci in 403 sib-pairs discordant for ASD at 485,764 CpG dinucleotides again does not reveal DNA methylation differences associated with autism, suggesting that the total DNAm content in ASD individuals remains static and the ASD-associated epigenetic information is contained in the 5-hmC profile, which ultimately may serve as ASD peripheral biomarkers.

767W

Familial and environmental determinants of methyl quantitative trait loci genome-wide in nuclear families. *L. Liang[1], S.G. Willis-Owen[2], K.C.C. Wong[2], A. Binia[2], P.J. Farrell[3], G.M. Lathrop[4], G. Abecasis[5], M.F. Moffatt[2], W.O.C.M. Cookson[2].* 1) Epidemiology, Harvard Sch Public Health, Boston, MA; 2) National Heart and Lung Institute, Imperial College, London SW3 6LY, UK; 3) Section of Virology, Imperial College Faculty of Medicine, London W2 1PG, UK; 4) CEA / Centre National de Genotypage, 91057 Evry, France; 5) Center for Statistical Genetics, Dept. of Biostatistics, SPH II, Ann Arbor, MI 48109-2029, USA.

Epigenetic variation in the methylation patterns of DNA is thought to regulate gene expression. Familial transmission of methylation patterns is recognised for a few genes that exhibit genomic imprinting, but the extent of epigenetic inheritance in the genome and its relevance to common diseases is not known. We therefore studied the genome-wide distribution of methylation status at approximately 27,000 CpG islands in peripheral blood leukocytes (PBL) from a panel of 95 nuclear families recruited through an asthmatic proband. We found that 17.9% of the average variation in methylation across all loci (meQTL) was explained by age and 2.9% by sex. 25% of the residual familial variation in meQTL was attributable to the shared family environment. Only 10% appeared heritable and could be attributed to epigenetic or genetic effects. We observed true genetic influences on methylation at approximately 10% of loci, with significant SNP associations (meSNPs) (false discovery rate (FDR) <5%) present at 2079 loci in cis and at 522 loci in trans. The peak meSNP at these loci explained 22.3% of their variation. Individual variance components estimations identified loci primarily influenced by genetic, epigenetic or environmental factors. Methylation status in PBL (predominately T lymphocytes) was highly correlated (R2=0.92) with meQTL in Epstein-Barr virus (EBV) infected lymphoblastoid cell lines (LCL) derived from B lymphocytes, although LCL showed constant differences in methylation at distinct loci. Methylation status was related to gene expression, as 666 (25%) of the peak meSNPs were in linkage disequilibrium (R2>0.5) with expression quantitative trait loci (eQTL). We were able to map multiple meQTL associated with age (Pmin=1x10-24) and the total serum IgE concentration (Pmin=1.7x10-9). These results indicate that meQTL mapping in DNA derived from existing large-scale collections of PBL may be of value to understanding mechanisms underlying common diseases, but emphasise the necessity of analytical strategies that take into account the complexity of influences on methylation at individual loci.

768W

Alterations in DNA and Nuclear Lamina Interactions and Chromatin Organization in Hutchinson-Gilford progeria syndrome. *A. Nazario-Toole, K. Cao.* Molecular and Cellular Biology, University of Maryland, College Park, MD.

Hutchinson-Gilford progeria syndrome (HGPS) is a genetic premature aging disorder. The majority of HGPS cases are caused by a de novo point mutation at position 1824 of the LMNA gene. This nucleotide substitution activates a cryptic splice donor site and causes in frame deletion of 150 base pairs within the prelamin A mRNA. This is translated into a mutant lamin A protein, known as progerin, with 50 internally deleted amino acids near the C terminus. Recent studies have demonstrated that progerin accumulation leads to progressive alterations in epigenetic controls and the gradual loss of peripheral heterochromatin. The Polycomb repressive complex 2 (PRC2) catalyzes the tri-methylation of H3K27 (H3K27me3). Quantitative RT-PCR analysis showed down-regulation of the PRC2 core components EZH2, SUZ12 and EED in HGPS fibroblasts. To map the changes in H3K27me3 in HGPS fibroblasts, we performed ChIP-seq with antibodies against HeK27me3 and found that the reduction in H3K27me3 consistently occurred in the regions of low-gene density. To compare the regions of interaction between DNA and A-type lamins, we performed ChIP-seq using two anti-lamin A/C antibodies in normal and HGPS fibroblasts. Bioinformatics analysis demonstrated a loss of chromatin binding in gene-desert regions in HGPS fibroblast. Consistently, Hi-C analysis revealed a less defined compartmentalization in HGPS fibroblasts. Together, our results suggest a model that down-regulation of PRC2 leads to the global alterations in chromatin compartmentalization in HGPS fibroblasts, and implies that H3K27me3 plays a role in controlling the association of chromatin and nuclear lamins.

769W

Reversal of cocaine-conditioned placed preference through methyl supplementation: altering global DNA methylation in the prefrontal cortex. *M. Zhao[1], W.P. Tian[2], M. Li[5], T.B. Song[2], M.Z. Li[3], L. Quan[3], S.B. Li[2], Z.S. Sun[4,5].* 1) Institute of Psychology Chinese Academy of Science, Beijing, China; 2) Department of Forensic Science, School of Medicine, Xi'an Jiaotong University, Xi'an, Shaanxi, China; 3) Department of Molecular Immunology, Capital Institute of Pediatrics, Beijing 100020, China; 4) Beijing Institutes of Life Science, Chinese Academy of Sciences, Beijing 100101, China; 5) Institute of Genomic Medicine, Wenzhou medical college, Wenzhou 325000, China.

Purpose: Recent studies suggest that epigenetic regulation through DNA methylation is responsible for adaptation induced by addictive drugs. Examination of global methylation in cells has revealed correlations of overall DNA methylation status with some biological states. However, there is no investigation to determine the global DNA methylation status after exposure to addictive drugs. Methods: An unbiased conditioned place preference protocol was used to determine the rewarding effects of food, morphine and cocaine. To exam the effects of methionine treatment on rewarding responds to food, morphine and cocaine, mice were administrated systemically methinonine or saline twice a day for 15 consecutive days before and during CPP training. Global DNA methyaltion was analyzed on An Agilent 1200 series rapid resolution liquid chromatography-QQQ mass spectroscopy system. Real-time PCR was used to exam the expression of DNA methytransferase (Dnmt) and methylcytosine binding domain protein 2 (Mbd2). Results and conclusions: In the present study, we found that the global DNA methylation in the mice prefrontal cortex (PFC) was decreased after cocaine conditioned place preference (CPP) training. The expression of DNA Dnmt 3a was upregulated, whereas Dnmt3b and Mbd2 were downregulated following testing CPP. Global DNA hypomethylation in the PFC induce by cocaine CPP was reversed by repeated methionine, the precursor of S-adenosylmethionine (SAM) administration. The downregulation of Dnmt3b and Mbd2 were reversed, but the upregulation of DMNT3a was unchanged. Strikingly, chronic treatment with methionine specifically inhibited the expression of cocaine-CPP without affecting the expression of morphine- or food-CPP. Our study provides new evidence to suggest the causal role of DNA hypomethylation in certain aspects of drug addiction and reversal of DNA hypomethylation may have important therapeutic implication of cocaine addiction.

770W

Diverse neurodevelopmental abnormalities in a novel mouse model of Rett syndrome. *J. LaSalle[1], D. Yasui[2].* 1) Microbiol & Immunology, Genome Center, Univ California Davis Sch Med, Davis, CA; 2) Microbiol & Immunology, Univ California Davis Sch Med, Davis, CA.

Rett syndrome is a neurodevelopmental disorder which presents as loss of speech and motor skills along with breathing abnormalities and seizures with onset from 6-18 months of age. Mutations in MECP2 on Xq28 are observed in greater than 80% of Rett syndrome patients, the vast majority of which are heterozygous females. Two protein isoforms, MeCP2e1 and MeCP2e2 are created by translation of alternatively spliced exon 1 and exon 2 containing mRNAs respectively. Almost all MECP2 mutations in Rett patients occur in exons 3 and 4. Thus, previous attempts to model Rett syndrome in mice focused on genetic deletion of exons 3 and/or 4. These Mecp2 knockout mice recapitulate some features of the Rett syndrome phenotype but appear to have milder symptoms as Mecp2 hemizygous males are viable for up to 12 weeks and heterozygous females remain symptom free until 3 months of age. Recently, Saunders et al identified MECP2 exon 1 translational start site mutations in patients with Rett syndrome. As Mecp2e1 is the predominant isoform expressed in normally developing brain, our hypothesis was that MECP2 exon 1 mutations contribute to the Rett phenotype. To test this hypothesis we generated transgenic mice with a Mecp2 exon 1 translational start site mutation based on the human mutation. Heterozygous (-/+) and hemizygous (-/y) mice were born at the expected frequency from mating of heterozygous females and C57BL/6 wild-type males. Prior to weaning at 3 weeks, hemizygous males were indistinguishable from wild-type littermates. However, starting at 5 weeks post-natal, hemizygous males developed hind limb clasping, along with gait and grooming abnormalities similar to those observed in mice deficient in exons 3 and 4. Mecp2 exon 1 hemizygous males also have spontaneous trembling and increased anxiety compared to wild-type controls. Interestingly, some of the Mecp2 hemizygous males also developed an unusual forelimb hand washing motion in response to tail suspension and open skin lesions consistent with obsessive grooming. Such behavioral abnormalities had not been observed in previous Mecp2 knockout mice and may result from the selective loss of the Mecp2e1 isoform. Formal assessment of social behavior, anxiety and motor function in hemizygous male, and control littermates is currently underway. These studies will help define the overlap between Mecp2e1 and Mecp2e2 biologic function and provide a new and relevant system for testing potential Rett therapies.

771W

Involvement of genes encoding epigenetic regulators in genomic imbalance pathogenic for Intellectual Disability. *F.R. Zahir[1], T. Tucker[1], S. Adam[1], D. Chai[1], A.D. Delaney[2], P. Eydoux[3], M. Griffiths[2], S.L. Langlois[3], J.L. Michaud[4], E. Tsang[1], J.M. Friedman[1].* 1) Medical Genetics, Univ British Columbia, Vancouver, BC, Canada; 2) British Columbia Genome Sciences Center, Vancouver, BC, Canada; 3) Provincial Medical Genetics Program, Children's and Women's Health Center of British Columbia, Vancouver, BC, Canada; 4) Center for Excellence in Neuromics of Universite de Montreal, CHU Sainte-Justine Research Center, Montreal, Canada.

Intellectual Disability (ID) affects 1-3% individuals globally, and for ~50% of cases, the cause is unknown. Microarray genomic hybridization has shown that submicroscopic genomic imbalance causes ID in at least 10% of idiopathic cases. In previous microarray studies we have found a high incidence of genes encoding epigenetic regulators to be involved in potentially pathogenic imbalance. Genes belonging to this category are also known to be causative for well characterized ID syndromes, e.g., Rett Syndrome. Therefore we hypothesized that dosage change of epigenetic regulators would be frequently causative for ID.

We have designed a custom microarray that probes all known genes encoding epigenetic regulatory proteins with exonic resolution. Epigenetic regulatory proteins include those with DNA methylation, histone modifications and chromatin remodeling activity. We have conducted comparative genome hybridization on our cohort of 177 trios with idiopathic ID. and have found 16 de novo CNVs that involve epigenetic regulators in 15 patients (8.5%). Of these, the CNVs most likely to be pathogenic are five that produce SMARCA2 loss, MEF2C loss, CHD6 loss, JMJD1A loss and ARID2 gain. A high copy number gain for CHD7 is also likely to be pathogenic, while copy number gains for ARID1B, ARID4B, and JMJD1C may possibly be pathogenic. We observed 7 recurrent copy number losses and copy number gains of exon 5 of JARID2 that are unlikely to be pathogenic for ID. In contrast, only 19 de novo CNVs (in 19 patients, 10.7%) were found that included other candidate genes included in the design, viz., genes involved in synaptogenesis, genes known to be causative for ID syndromes or representative of larger reported pathogenic CNVs. Therefore, the rate of confirmed CNVs identified for genes in the epigenetic regulatory class (46% of the total from 36% of the probes on the array) is higher than that of all of the other classes put together (54% of the total CNVs from 64% of the probes on the array (chi square test = 4.30, degrees of freedom = 1, two tailed P = 0.0372). We discuss genotype-phenotype correlations for these cases and show that epigenetic perturbation as a whole is a significant cause for ID pathogenicity.

772W

Epigenetic induction of Fetal Hemoglobin in CD133+ Stem Cells in vitro. *D. Ali[1], M. Ahmadvand[1], M. Mahmoodinia Maymand[2], M. Soleimani[1], S. Abroun[1], S. kaviani[1], M. Noruzinia[3].* 1) Department of Hematology , TarbiatModares University, Tehran, Tehran, Select a Country; 2) Sarem Cell Research Center, Sarem Hospital, Tehran, Iran; 3) Department Of Medical Genetic, Tarbiat Modares University, Iran.

) -hemoglobinopathies are) -globin gene disorders that result in considerable morbidity and mortality throughout the world.Sickle cell disease (SCD) and) -thalassemia are two common types of) -hemoglibinopathies due to identified gene mutations. New therapies based on epigenetic patterns of globin genes are tried to increase the levels of fetal hemoglobin (HbF). In this regard, . -globin gene inducers including hydroxyurea, HDAC (Histone Deacetylase) inhibitor compounds such as sodium butyrate, azacytidine, decitabineas well as new immunomodulator drugs such as pomalidomide, lenalidomide and thalidomide can improve (/) chain imbalance which is considered as the most crucial complication in) thalassemia. In this research we set out to investigate the mechanism of regulation of thalidomide and sodium butyrate. CD133+stem cells were enriched using a magnetic activated cell sorting (MACS) CD133 isolation kit (Miltenyi Biotech,Germany), according to manufacturer's instruction. The cells were differentiated to erythroid lineage by IL3 and EPO then treated with combination of sodium butyrate and thalidomide. Total RNA was extracted from progenitor cells and RT-PCR was performed for the first strand cDNA synthesis. For analysis of . -globin gene expression, we performed real-time PCR using cDNA, . -globin gene specific primers and SYBR Green master mix. Flow cytometry analysis showed over 90% purity for CD133+ stem cell expanded. Interestingly, the results of RT-PCR and real-time PCR showed a remarkable increase in . -globin gene expression using these two agents in comparison with control group. In addition, real-time PCR confirmed synergistic effects of sodium butyrate and thalidomide. More effective and safer agents with capacity to induce higher levels of HbF are clearly needed for treatment of) -hemoglobinopathic patients. Hence, in vitro induction of . -globin gene expression by single and combination treatments with different agents could be a prospect for specific targeted therapies and in vivo applications.

773W

Investigation exon 1 in FRDA gene in Suspect Friedreich Ataxia patients. *m. naseroleslami[1], k. Parivar[1], s. sanjarian[1], m. houshmand[2].* 1) Science and Research Branch, Islamic Azad University ,Tehran-Iran; 2) Institute of Genetic Engineering.

Introduction:Friedreich ataxia (FA) is an autosomal recessive disorder that is typically associated with dysarthria, muscle weakness, spasticity in the lower limbs, scoliosis, bladder dysfunction, absent lower limb reflexes, and loss of position and vibration sense. Approximately two-thirds of this patient have cardiomyopathy; up to 30% have diabetes mellitus.Individuals with FRDA have identifiable mutations in the FXN gene. The most common type of mutation, which is observed on both alleles in more than 98% of individuals with FRDA, is a GAA triplet-repeat expansion in intron 1 of FXN. Approximately 2% of individuals with FRDA are compound heterozygotes for a GAA expansion in the disease-causing range in one FXN allele and another inactivating FXN mutation in the other allele. Aim of present study was to investigation exon 1 in FRDA gene in patients with Clinical symptomatic of Friedreich Ataxia that they havent GAA triplet-repeat expansion in intron 1 of FXN. Materials and Methods:we analyzed exone 1in7 patient suspect to FA with using PCR and sequencing.results: Change of nucleotide A to G was detected in exon 1` nt:815284 in our patients.discusion:we believed in this report because of consanguinity marriage in Iran some patients with homozygote mutation may show FA phenotype.

774W

Alternative splicing associated with unclassified variants in CFTR exon 3: implications for CFTR-related disorders. *A. AISSAT[1,2], A. de Becdelièvre[1,2,3], L. Golmard[1,3], C. Costa[1,3], A. Chaoui[1], N. Martin[1], B. Costes[1,3], M. Goossens[1,2,3], E. Girodon[1,3], P. Fanen[1,2,3], A. Hinzpeter[1].* 1) INSERM U955, CRETEIL, 94000, France; 2) Université Paris Est, CRETEIL, 94000, France; 3) AP-HP; CHU H. Mondor, Génétique, CRETEIL, France.

With the introduction of high-throughput sequencing, a great number of sequence variations have been identified. Most of them, not functionally characterized, are named unclassified variants (UVs). Among the 1800 mutations within the extensively analyzed *CFTR* gene responsible of cystic fibrosis (CF, MIM#219700), the p.Arg75Gln sequence variation is usually considered as a neutral polymorphism but is frequently identified in patients affected with CFTR-related diseases. As this amino-acid substitution has been shown to lead to a functional protein, we searched for another defect such as an associated splicing defect.

We identified an alternative exon 3 splicing (13%) in two heterozygotes carriers of the c.224G>A (p.Arg75Gln) sequence variation. This alternative splicing was reproduced *in vitro* in bronchial epithelial cells using a minigene model which produced a 39% skipping of exon 3. A small set of mutations located within exon 3 was tested with this assay and the c.220C>T (p.Arg74Trp) and c.224G>T (p.Arg75Leu) mutations also induced a partial skipping of exon 3. *In silico* predictions suggested a key role for the SF2/ASF splicing protein in the vicinity of the substituted nucleotides sites. Namely, some nucleotides induced a putative loss of the binding of this protein with the mRNA strand, directly emphasized with changes in its predicted local RNA 2D structure. Direct association was validated by EMSA experiments with recombinant SF2/ASF. Additionally, over-expression of SF2/ASF enabled to partially correct exon 3 exclusion for both c.224G>A and c.220C>T.

All these results led to the definition of an enhancer motif within CFTR exon 3. We demonstrated that c.224G>A (p.Arg75Gln), a common sequence variation that does not provoke cystic fibrosis *per se*, induced a decrease in full-length functional CFTR mRNA. This could explain in part its high frequency in CFTR-related disorders such as chronic pancreatitis (CP), congenital bilateral absence of vas deferens (CBAVD) or chronic obstructive pulmonary disease (COPD). Thus, variants that are often unnoticed in molecular diagnostic laboratories because of their apparent neutral effects on proteins, may induce pathogenic consequences on mRNAs through splicing.

775W

Identification and tissue distribution of alternative splice variants in the ATP-binding cassette transporter ABCA2. *R.M. Grimholt[1], C. Stormo[1], M.K. Kringen[2], J.P Berg[1], A.P Piehler[1].* 1) Department of Medical Biochemistry, Oslo University Hospital, Ulleval, Oslo, Norway; 2) Department of Pharmacology, Oslo University Hospital, Ulleval, Oslo, Norway.

Background ABCA2 is the second member of the A-subfamily of ABC transporters. It is predominantly expressed in the brain and may play a role in lipid transport in brain cells and neural development. ABCA2 shows a particularly compact structure with 48 exons within a genomic region of only 21 kb. ABC transporters, like most other human genes, undergo alternative splicing, which is an important post-transcriptional regulation mechanism. In the present study, we sought to identify novel splice variants in the ABCA2 gene and investigate their tissue distribution. **Methods** To identify alternative splice variants, primer pairs were designed to amplify overlapping cDNA fragments spanning from exon 1 to exon 48 in a cDNA pool of 44 different human tissues. PCR products were separated and analyzed by agarose gel electrophoresis. Bands of interest were excised and the purified DNA was sequenced. Alignment of the predicted ABCA2 splice variants with the reference sequence (NM_001606) was performed using the Smith-Waterman algorithm from the software package EMBOSS. To investigate the relative expression of the predicted splice variants, primer pairs specific to each splice variant and to the canonical ABCA2 transcript were designed. Quantitative real-time PCR was performed on an ABI 7900HT Real-Time PCR System using SYBR Green. **Results** Two novel splice variants of ABCA2 were identified in a variety of human tissues showing intron retention of intron 15 and intron 47, respectively (named ABCA2Δ+int15 and ABCA2Δ+int47). We were also able to confirm the existence of two other splice variants, ABCA2Δ+int6 and ABCA2Δ+int39. Interestingly, all the splice variants in our study, seems to derive from intron retention, which has proved to be the rarest type of alternative splicing in humans. RT-qPCR analysis demonstrated that ABCA2 and its predicted splice variants were abundantly expressed in spinal cord, cerebellum and whole brain. A distinct expression pattern of the splice variants were detected in 44 different human tissues and in tissues from different developmental stages and regions of the human brain for all the ABCA2 splice variants. **Conclusion** We identified two novel splice variants of ABCA2 and demonstrated their differential expression in a variety of human tissues. Further work will show whether these splice variants have a distinct physiological role or reflect the mere noise of the splicing machinery.

776W

Pharmacological inhibition of JNK/ERK MAP kinase pathways attenuates mutant DFNA5 induced apoptosis. *K. Op de Beeck[1], A. Schepers[1], N. Cools[2], VFI. Van Tendeloo[2], G. Van Camp[1].* 1) Center for medical genetics, University of Antwerp, Edegem, 2650 Antwerp, Belgium; 2) Vaccine & Infectious Disease Institute (VIDI), Laboratory of Experimental Hematology, University of Antwerp, 2610 Antwerp, Belgium.

DFNA5 was identified in 1998 as a gene responsible for an autosomal dominant form of inherited deafness. After the initial report, 4 additional DFNA5 mutations have been identified. DFNA5 mutations leading to hearing loss are in fact gain of function mutations, where only mutations leading to skipping of exon 8 result in hearing loss while mutations in other parts of the gene do not. At the time of its identification in 1998, it was unknown how mutations in this gene could lead to hearing loss. Now, it is clear that DFNA5 is able to induce apoptosis and, in line with its role in the apoptotic pathway, there is strong evidence that this gene is a tumor suppressor gene as it is frequently methylated in breast, colon and gastric cancer. However, the molecular mechanisms leading to DFNA5 induced apoptosis remain largely unknown. Using transcriptomics analysis, we show that specific JNK and ERK MAP kinase pathways are activated in vitro after transfections of mutant DFNA5 and that inhibition of this pathway is able to attenuate the resulting cell death. Furthermore, we report that specific upregulation of the EGR1 gene is involved in the molecular mechanisms leading to DFNA5 induced apoptosis. Moreover, we show that DFNA5 induced apoptosis is accompanied by an increased production of reactive oxygen species (ROS) and by depolarization of the mitochondrial membrane. We believe that these findings may yield possible therapeutic targets for DFNA5 induced hearing loss and shed more light on the role of DFNA5 in some of the most frequent forms of cancer.

777W

Quantification of chitinase transcripts in mouse tissues. *M. Ohno, K. Tsuda, M. Sakaguchi, Y. Sugahara, F. Oyama.* Kogakuin Univ, Hachioji, Tokyo, Japan.

Chitin, the second most abundant polysaccharide in nature, is a component of the exoskeletons of crustaceans and insect, and in the microfilarial sheaths of parasitic nematodes. Chitin is also an important structural polymer in fungal cell walls. Although mammals do not have chitin and chitin synthase, two active chitinolytic enzymes, chitotriosidase and acidic mammalian chitinase (AMCase), have been identified in mouse and human. Increased expression of both chitinases has observed in different pathological conditions: chitotriosidase in lysosomal lipid storage disorders like Gaucher disease and AMCase in allergic airway responses in mouse models of asthma. Little is known, however, about the pathophysiological functions of these chitinases in mammals. We determined the absolute expression levels of chitotriosidase and AMCase mRNAs in mouse tissues by means of real time RT-PCR. We found that mouse tissues express more AMCase than chitotriosidase. Stomach, lung, spleen predominantly express AMCase mRNA, whereas chitotriosidase was prevalent only in eye. Our study indicates that AMCase is the major chitinase in mouse tissues.

778W

Regulation of gene expression of mammalian chitinases. *F. Oyama, M. Ohno, K. Tsuda, M. Sakaguchi, Y. Sugahara.* Dept Applied Chemistry, Kogakuin Univ, Hachioji, Tokyo, Japan.

Chitin is the second abundant polysaccharide in nature. It is an integral component of the fungal cell walls, the exoskeletons of crustaceans and insects, and the microfilarial sheaths of parasites. Although mammals do not have chitin and its synthase, genes encoding chitotriosidase and acidic mammalian chitinase (AMCase) and their translation products have been found in both human and mouse. Marked elevation of plasma chitotriosidase activity was reported in Gaucher disease, an autosomal recessive lysosomal storage disorder, whereas significant increases in AMCase were detected in an induced asthma mouse model. We investigated gene expression of AMCase in mouse tissues by real-time PCR and found that AMCase mRNA is predominantly expressed in stomach and moderately in lung. We raised specific antibody against mouse AMCase and found that the antibody recognized a single protein band in the extracts of the stomach and lung. The relative protein expression levels between stomach and lung were comparable in their mRNA levels. We determined the chitinolytic activity at multiple pHs by using a synthetic substrates and found strong chitinase activity at acidic pH in the extract of stomach. These results indicate that the high level of chitinase activity in stomach results from high level expression of AMCase gene.

779W

Expression of mouse chitinase in Escherichia coli and characterization of its properties. *A. Kashimura, K. Ishikawa, K. Sekine, Y. Kida, M. Sakaguchi, Y. Sugahara, F. Oyama.* Applied Chemistry, Faculty of Engineering, Kogakuin University, Hachioji, Tokyo, Japan.

Chitinase hydrolyzes chitin, a polymer of N-acetyl-D-glucosamine, which is present in a wide range of organisms, including fungi, insects, and parasites. Although chitin is not present in mammals, chitinase genes are found in both human and mouse genomes. It has been known that chitinase activity is significantly increased in plasma from patients with Gaucher disease, an autosomal recessive lysosomal storage disorder. Recent researches have shown that elevated expression of chitinases is closely associated with progression of allergy and asthma. The pathophysiological role for the mammalian chitinases remains unproven. To understand how chitinase interacts with chitin and degrades it, we expressed the mouse chitinase in Escherichia coli as a fusion protein. The recombinant fusion protein with full length chitinase was capable of cleaving artificial chitin-like substrates and natural substrates, whereas fusion protein without it was not. Thus, this recombinant protein can be used to elucidate detailed biological functions of the mouse chitinase.

780W

Novel mutations in CYP21A2 gene and their influence on the enzyme activity and protein structure. *A.L.G. Lusa[1], F.C. Soardi[1], G. Guerra-Junior[2], S.H.V. Lemos-Marini[2], M.P. de-Mello[1].* 1) CBMEG, UNICAMP, Campinas, São Paulo, Brazil; 2) Department of Pediatrics, UNICAMP, Campinas, São Paulo, Brazil.

Mutations in CYP21A2 gene cause steroid 21-hydroxylase deficiency, an inherited disorder of adrenal steroidogenesis. It leads to the accumulation of metabolic intermediates, progesterone and 17-hydroxyprogesterone. The disease occurs in a wide spectrum of clinical forms, including a severe (salt-wasting) with impairment in both aldosterone and cortisol biosyntheses, a form with apparently normal aldosterone biosynthesis (simple virilizing) and a mild nonclassical form that may be asymptomatic or may be associated with androgen excess during childhood or at puberty. We describe four patients with 21-hydroxylase deficiency. A boy admitted to the hospital with dehydration and presenting hyponatremia (Na = 112 mEq/L) and hyperkalemia (K = 7.7 mEq/L). Salt-wasting CAH due to 21-hydroxylase deficiency, confirmed by elevated levels of 17-hydroxyprogesterone, was diagnosed. A female patient presenting ambiguous genitalia with no palpable gonads was diagnosed as salt-wasting indicated by serum sodium of 123 mEq/L and potassium of 9.1 mEq/L and elevated basal serum levels of 17-hydroxyprogesterone. The two other patients were female with the nonclassical form. Novel mutations were identified in the CYP21A2 gene: p.S113F, p.P267L, p.V358I, p.R426C, however the p.V358I and p.R426C occurred in the same allele with pI172N and p.V281L, respectively. Using site-direct mutagenesis, each nucleotide variant and both combinations were introduced into a cloned wild-type cDNA and the mutant proteins were expressed in COS-7 cells to compare the enzymatic activity between the wild-type and mutant proteins. The enzymatic conversion of 17-hydroxyprogesterone into 11-desoxycortisol was decreased in the CYP21A2 enzymatic activity for all mutants. The enzymatic activity for CYP21A2 mutant proteins bearing p.S113F, p.R426C, p.I172N+p.V358I and p.V281L+p.R426C mutations was null. Whereas the p.V358I mutation itself presented some residual enzymatic activity and the p.P267L mutation demonstrated a enzymatic activity similar to the wild-type. The putative pathogenic mechanisms for the novel protein variants were evaluated in silico. The mutations caused protein changes in the whole structure and altered intra-molecular amino acid interactions. The analysis of mutant enzymes also revealed changes either in protein stability or in the surface charge in addition to the decreased enzymatic activity, which can correlate the genotypes found for each patient to the clinical manifestation.

781W

New Features for the New SIFT Website (http://sift-dna.org). *P. Ng[1], J. Hu[2], N.L. Sim[1], G. Schneider[3].* 1) Genome Institute of Singapore, Singapore; 2) Franklin & Marshall College, Lancaster, PA; 3) Bioinformatics Institute, Singapore.

I have recently moved the SIFT website from JCVI to the Bioinformatics Institute in Singapore and its new url is http://sift-dna.org The updated features of the new SIFT website includes 1000 Genomes annotation, acceptance for many of the next-generation sequencing file formats, and improved indel prediction in both speed and accuracy. We have also enabled cloud computing for easier installation of SIFT software.

782W

Functional study of Peptidylarginine deiminase type 4 as genetic risk factor for RA. *A. Suzuki[1], Y. Kochi[1], H. Shoda[2], K. Fujio[2], M. Yamanaka[1], E. Kanno[1], T. Sawada[3], R. Yamada[1, 4], K. Yamamoto[1, 2].* 1) Ctr for Genomic medicine, Kanagawa, RIKEN, Yokohama City, Japan; 2) Department of Allergy and Rheumatology, Graduate School of Medicine, The University of Tokyo; 3) Department of Rheumatology, Tokyo Medical University; 4) Ctr for Genomic Medicine, Kyoto University.

Rheumatoid arthritis (RA) is well-known as an autoimmune disease and is a chronic inflammatory disorder characterized by the destruction of multiple joints. Many genome wide association studies were performed and multiple RA-susceptibility loci and autoimmune-susceptibility loci have been identified. These studies suggested that multiple genes and its functions were related with disease causing and development. Previouly, peptidylariginine deiminase type 4 (PADI4) was identified as a susceptibility gene for RA in a Japanese population by case-control association study (Ref 1). PADI4 is a member of the PADI gene family and converts arginine residue (peptidylarginine) to citrulline residue (peptidylcitrulline). PADI4 is highly expressed in bone marrow, macrophages, neutrophils and monocyte. Peptidylcitrulline is an interesting molecule in RA, because it is an antigen of ACPA and only PADs (translated protein from PADI genes) can provide peptidylcitrullines, via modification of protein substrates. To evaluate the importance of PADI4 gene in the progression of RA, we generated Padi4 -/- C57BL/6 (B6) mice by speed congenic method. We used Padi4-/- mice to show that PAD4 is affected to development and progression of collagen induced arthritis (CIA), well known as an RA model animal. Padi4-/- B6 and WT mice were immunized with Chicken type II collagen (CII) for CIA. Clinical disease score was reduced and the incidence of WT and Padi4-/-mice were 56% and 44%, respectively. Padi4-/- mice also significantly reduced concentrations of serum anti-CII IgM and IgG compared with WT mice. Resulting from these studies, we suggested that Padi4 enhanced collagen-initiated inflammatory responses. 1) Suzuki, A. et al Nat. Genet.34, 395-402 (2003).

783W

Models of , -Sarcoglycan knock-down and their Implications for the Pathology of Myoclonus Dystonia. *A. Given[1 2], D.E. Bulman[1 2], D.A. Grimes[1 3].* 1) Ottawa Hospital Research Institute 501 Smyth Road Ottawa, ON K1H 8L6, Canada; 2) University of Ottawa; 3) The Ottawa Hospital 501 Smyth Road Ottawa, ON K1H 8L6, Canada.

Myoclonus Dystonia (MD) is a movement disorder characterized by bilateral myclonic jerks paired with dystonia. Mutations have been mapped to the epsilon-sarcoglycan (SGCE) gene in about 40% of patients. The purpose of this project is to examine the properties of SGCE in the CNS and use this knowledge to elucidate the pathology of MD. Although Sgce forms known sarcoglycan complexes (SGC) in other tissues, little is known about its interactions in the CNS. Mutations found in MD patients produce a null SGCE protein therefore; Sgce knock out (KO) models should approximate MD conditions both in vivo and in vitro. Of the SGs, , -SG is the most widely expressed with high expression in the CNS. Sgce is known to form heterotetrameric SGCs with) -, > and +-SGs in smooth muscle, retina and the PNS but its pattern of interaction in the CNS has yet to be elucidated. In muscles where they are known to form, the SGCs are located at the sarcolemma within the dystrophin-glycoprotein complex (DGC), stabilizing it. The SGC maintains the presence of sarcospan at the sarcolemma and aids in anchoring signalling proteins, such as neuronal nitric oxide synthase (nNOS). To determine changes in neuronal cell functions due to loss of , -SG, siRNA was designed to knock-down the expression in N1E-115 neuroblastoma cells. A clone was isolated displaying a 77% decrease of Sgce mRNA. This clone also displayed a 50% decrease of nNOS protein and a 50% increase in proliferation. qRT-PCR was used to show that the decrease of nNOS protein resulted in approximately a 1.5 fold up-regulation of nNOS mRNA. Chemically inhibiting nNOS in N1E cells also results in increased proliferation as well as a decrease of NO metabolites. These results suggest that loss of SGCE may disrupt the SGC and destabilize nNOS. A previous mouse model was developed to KO exon 4 of Sgce. This model is currently unavailable. Therefore we will investigate the role of sgce in the brain through the establishment of two new transgenic mouse models. The first model will conditionally KO exon 4 of sgce and the second model will conditionally repair sgce by removing an artificial splice-stop site placed in intron 1. Embryonic stem cell clones have been isolated and are currently being screened for correct mutation inclusions. These mice will hopefully corroborate in vitro work and allow further insight into the importance of developmental time points and tissue specific effects.

784W

Functional characterization of the 2p15-16 microdeletion. *C. Harvard[1,2], R. Colnaghi[3], D. Alcantara[3], H. Hutter[4], C. Dunham[1], P. Pavlidis[5], J. Pan[4], R. Wildin[6], M. Nowaczyk[7], B. Maranda[8], C. Tyson[9], M. Hrynchak[9], S. Martell[1,2], Y. Qiao[1,2,10], J. Eichmeyer[6], J.J.A. Holden[11,12], M.E.S. Lewis[2,10,11], M. O'Driscoll[3], E. Rajcan-Separovic[1,2].* 1) Pathology & Laboratory Medicine, University of British Columbia, Vancouver; 2) Child & Family Research Institute, Vancouver; 3) Human DNA Damage Response Disorders Group, Genome Damage & Stability Centre, University of Sussex, Brighton, UK; 4) Department of Biological Sciences, Simon Fraser University, Burnaby; 5) Department of Psychiatry, UBC, Vancouver; 6) St. Luke's Health System, Boise; 7) Clinical Genetics, McMaster University Medical Centre, Hamilton; 8) Medical Genetics, Quebec City; 9) Cytogenetics Laboratory, Royal Columbian Hospital, New Westminster; 10) Department of Medical Genetics, UBC, Vancouver; 11) Department of Psychiatry, Queen's University, Kingston; 12) Genetics and Genomics Research Laboratory, Ongwanada, Kingston.

We have previously described a microdeletion syndrome involving chromosome 2p15-16 which includes congenital and neurodevelopmental abnormalities. Additional cases with similar phenotypes and overlapping microdeletions with variable breakpoints have been reported. **Methods:** We characterized 5 subjects with 2p15-16 deletions using high resolution whole genome (WG) SNP array and WG expression arrays to delineate a minimal deletion region (MDR) and to look for candidate genes affected by copy number (CN) change. Furthermore, we are investigating the function of candidate genes from the MDR in a) *C. elegans* by RNAi and transgenic studies b) in control mouse and human tissues as well as in patient lymphoblasts using immunohistochemistry (IHC) and Western blotting to determine protein expression. **Results:** The MDR for currently reported 2p15-16 deletion cases contains two known genes, XPO1 and USP34. Knock-down of XPO1 by RNAi in *C. elegans* is early embryonic lethal while transgenic XPO1::GFP expressing *C. elegans* strains show ubiquitous XPO1 expression throughout development and in the adult worm. XPO1 is expressed in adult mouse brain, gut and lung. In human fetal brain, mild positivity for XPO1 is seen in the immature ependyma and in Cajal-Retzius cells (cerebellar cortex). USP34 expression in human fetal brain is seen in grey matter but not white matter, and is strong in the Purkinje cell layer (cerebellar cortex). In human mature brain, positivity is visible both in white and grey matter remaining strong in the Purkinje cell layer. Whole genome expression shows expression fold changes corresponding to reduction of copy number for 4 genes (COMMD1, USP34, AHSA2, and REL), but not for XPO1. Preliminary studies confirm a decrease in COMMD1 protein levels in a subject's lymphoblast cell line. **Conclusions:** Our preliminary studies suggest functional involvement of COMMD1 in the 2p15-16 microdeletion. COMMD1 is essential for copper homeostasis and changes in copper levels are detrimental to brain and liver function. USP34 is also of interest as it is expressed strongly in Purkinje cell layer of human brain, which shows degeneration in neurodevelopmental disorders (e.g. fragile X, autism). Further functional investigations of all candidate genes are underway.

785W

Neuronal Differentiation Effect of Huntingtin Associate Protein 1. *A. Li, H. Yang, S.H Li, X.J Li.* Human Genetics, Emory University, Atlanta, GA.

Polyglutamine (PolyQ) expansion is the cause for 10 inherited neurogdegenerative diseases, including Huntington disease (HD); SCA-1. 2, 3, 6, 7, 17; DRPLA; SBMA and HDL2. Despite its widespread expression throughout the body and brain, the HD protein huntingtin causes selective neurodegeneration, which preferentially occurs in the striatum and extends to other brain regions as the disease progresses. It has been thought that mutant huntingtin abnormally affects the function of other neuronal proteins to mediate neuropathology. Huntingtin associate protein 1 (HAP1) is the first Huntingtin associated protein identified by the yeast two hybrid system and is a neuronal protein enriched in brain. The binding of HAP1 to huntingtin is enhanced by the length of the polyglutamine tract in huntingtin, suggesting that Hap1 dysfunction may be involved in HD neuropathology. Mice homozygous for the targeted deletion of HAP1 show decreased feeding, growth retardation, early postnatal death (P1-P3), and hypothalamic neurodegeneration. Examination of the brains from postnatal HAP1 null mice revealed a decrease in Brdu incorporation, suggesting that Hap1 is important for neuronal proliferation. Using Hap1 siRNA in PC12 cells, a neuronal cell line, we found that decreasing Hap1 can impair the proliferation of PC12 cells in response to various neurotrophic factors. In addition, differentiation signaling of neurotrophic factors is also affected when Hap1 expression is reduced. Moreover, overexpression of mutant huntingtin also suppresses neurite outgrowth of PC12 cells. These findings raise the possibility that the abnormal interaction of mutant Htt with HAP1 may impair HAP1 function to affect neuronal proliferation and differentiation. Supported by NIH grants NS036232 and AG019206.

786W

Sub-cellular localization of Y-Box Protein 1 regulates proliferation, invasion, and increased mesenchymal phenotype in astrocytomas. *X. Liu[1], D. Faury[2], C. Sollier[1], B. Meehan[2], N. Gerges[1], Z. Dong[3], P. Siegel[3], A. Korshunov[4], S. Pfister[4], J. Rak[2], N. Jabado[1,2].* 1) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Montreal Children's Hospital, McGill University Health Centre, Montreal, Quebec, Canada; 3) Rosalind and Morris Goodman Cancer Research Centre, McGill University, Montreal, Quebec, Canada; 4) Division of Molecular Genetics, German Cancer Research Centre, Heidelberg, Germany.

Y-Box-Protein-1 (YB1) is a transcriptional and translational regulator implicated in cancer progression. Previously, using gene expression microarray, we established elevated YB1 levels in pediatric Glioblastoma (GBM), an aggressive high-grade astrocytoma, possibly driving oncogenesis in this cancer. The purpose of this study is to investigate the role of YB1 in astrocytoma genesis and its possible association with poor prognosis. We overexpressed or silenced YB1 protein in pediatric GBM (SF188), adult GBM (U87) and normal human astrocytes cell lines (NHA). Proliferation, migration, soft agar colony formation assays were performed in vitro, accompanied by in vivo mice xenograft to assess the tumorigenic and metastatic ability of these cells. Meanwhile, tissue microarrays including 150 pediatric GBM and 70 Grade I Astrocytoma were IHC stained with YB1. YB1 expression and sub-cellular localization were scored and analyzed in association with patient prognosis and tumor grades. YB1 silencing using shRNA reduced YB1 level by 90%, and surprisingly increased cell proliferation. As YB1 is predominantly cytoplasmic in physiological conditions, and nuclear YB1 is known to associate with increased cell growth, sub-cellular localization of YB1 was investigated. In the YB1 silenced clones, YB1 was greatly reduced in cytoplasm, but enriched in nucleus, explaining the increase in cell proliferation. YB1 overexpression was mainly cytoplasmic; it decreased cell proliferation and increased mesenchymal phenotype including migration. Importantly, although YB1 overexpression decreased size of tumor formed in SCID mice, it increased tumor metastasis into the liver. IHC on tissue microarrays of patient tumors showed strong YB1 expression in 66% of pediatric GBM samples, but only 8% in Grade I astrocytoma, suggesting a role of YB1 overexpression in a more invasive tumor phenotype. Further, nuclear YB1 expression indicated worse progression free survival compared to cytoplasmic YB1 in 150 pediatric GBM tumor samples. This is the first time nuclear YB1 was associated with poor patient outcome in pediatric GBM. Our results suggest that YB1 modulates cellular proliferation and mesenchymal properties, based on its sub-cellular localization. Nuclear YB1 drives cell proliferation, whereas cytoplasmic YB1 promotes cell migration and metastasis. The association of nuclear YB1 with worse patient prognosis argues for caution in targeting YB1 for therapeutic intervention.

787W

Functional analysis of a SNP in TSLP associated with asthma. *L. Akhabir, A.J. Sandford.* UBC James Hogg Research centre, Vancouver, BC, Canada.

TSLP is a cytokine secreted by epithelial cells in response to different stimuli such as TLR ligands or viruses. TSLP has been shown to be sufficient to initiate experimental allergic airway inflammation and its gene expression has been shown to be increased in murine and human asthmatic lungs. The binding of TSLP to its receptor on mast cells, dendritic cells and activated T cells results in the promotion of a Th2-type inflammation. rs1837253 was identified as a putative causal SNP based on consistent association data both from candidate gene and genome-wide association studies; as well as the absence of significant linkage disequilibrium with other SNPs in the region. The aim of this work was to perform functional assays to uncover the mechanism underlying the involvement of rs1837253 in asthma pathogenesis. DNA samples from the lungs of asthmatics and controls were genotyped using TaqMan assays and quantitative PCR (qPCR) assays were performed to compare levels of gene expression between genotypes. qPCR experiments were conducted for both TSLP isoforms. In silico analysis was performed to predict binding of regulatory proteins. Electrophoretic mobility shift assays (EMSA) were performed to test for potential differential binding of a regulatory protein to the SNP. The TaqMan genotyping was successful with 100% call rate; the data were in Hardy Weinberg equilibrium. No homozygotes for the minor allele were present. qPCR did not demonstrate differential gene expression between phenotypes (fatal asthma or non asthma) or between genotypes. This was most likely due to a low N and the fact that these data were solely for the short isoform as we were unable to detect high enough levels of the long isoform. Efforts are underway to increase production of the long isoform by stimulation of airway epithelial cells, increase the number of samples and pursue samples from a different source (blood). Preliminary EMSA data showed binding of a nuclear protein derived from A549 cells to the T allele and little or no binding to the C allele. *In silico* analysis resulted in the identification of candidates for the binding as FOX family proteins, GR receptor alpha and HMG box group proteins. Cold completion experiments using consensus sequences of candidate factors are in progress. The objective of this research is to explain the asthma association of a singleton SNP 2.5 Kb from the TSLP gene and hopefully participate in the identification of novel therapeutic targets.

788W

DYX1C1 affects cell migration by regulating neuronal migration genes and by interacting with cytoskeleton proteins. *K. Tammimies[1], M. Vitezic[2], H. Matsson[1], S. Le Guyader[1], T. Burglin[1], T. Ohman[3], S. Stromblad[1], CO. Daub[2], TA. Nyman[3], J. Kere[1,4], I. Tapia-Paez[1].* 1) Biosciences & Nutrition, Karolinska Institutet, Huddinge, Sweden; 2) Omics Science Center, RIKEN Yokohama Institute, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045 Japan; 3) Protein Chemistry Research Group, Institute of Biotechnology, University of Helsinki, 00014 Helsinki, Finland; 4) Department of Medical Genetics, Haartman Institute, University of Helsinki, and Folkhälsan Institute of Genetics, Helsinki, Finland.

The Dyslexia susceptibility 1 candidate 1 (DYX1C1) gene has been implicated in developmental dyslexia and reading ability. It has been shown to affect neuronal migration and modulate estrogen receptor signaling. Developmental dyslexia is a complex neurodevelopmental disorder with a genetic basis. Disturbances in neuronal migration might be one of the mechanisms leading to this disorder. In addition to DYX1C1 three other susceptibility genes are involved in this process; DCDC2, KIAA0319 and ROBO1. Subtle disturbances in cortex organization have also been found in post-mortem brain of dyslexic individuals. The molecular pathways in which the dyslexia genes are involved during neuronal migration remain to be identified. Here, we study the role of DYX1C1 in migration using live cell imaging, transcriptome and interactome profiling. We demonstrate that upregulation of DYX1C1 increases random migration about 30% when comparing to control using neuroblastom SH-SY5Y cells which is mediated by TPR and a newly defined DYX domains. Interestingly, we could also detect some of DYX1C1 protein in the centrosome and for this localization the TPR-domains were also necessary. We also measured the changes at the transcriptome level after perturbation of DYX1C1. When DYX1C1 was overexpressed or silenced, 376 and 88 genes were differentially expressed (p-value <0.01), respectively. Gene ontology analysis revealed significant over-representation of cell migration, and neuron migration genes. Interestingly, Reelin and DCX, two of the most studied examples of neuronal migration genes, were regulated by DYX1C1. To find new interacting partners for DYX1C1, we used co-immunoprecipitations followed by protein identification by mass spectrometry. We found 65 new proteins interacting with DYX1C1. We also tested interactions by a candidate approach, and could show that DYX1C1 interacts with LIS1, DCDC2 and KIF3A. Among all the DYX1C1 interacting partners, there was an overrepresentation of cytoskeleton proteins. We conclude that DYX1C1 affects random migration via the TPRs and DYX domains. The migration phenotype produced by DYX1C1 appears to be mediated by the regulation of neuronal migration genes and its interaction with relevant cytoskeletal proteins. Our results contribute to the hypothesis that dyslexia is a neuronal migration disorder by linking DYX1C1 with genes involved in other neuronal migration disorders such as lissencephaly.

789W

NNK metabolism by CBR1 and HSD11B1. *A. Hull[1], J. Engle[2], N. Fredericksen[1], C. Gallagher[2, 3], G. Chen[2], P. Lazarus[2, 3], J. Muscat[2].* 1) Biol Dept, Lincoln University, Lincoln University, PA; 2) Dept of Public Health Sciences, Penn State College of Medicine, PA; 3) Department of Pharmacology,Penn State College of Medicine, PA.

One of the major carcinogens in tobacco smoke is the nicotine derived nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). NNK is reduced to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). Both NNK and NNAL are metabolically activated by cyotchrome P450s, whereas detoxification by glucuronidation occurs for NNAL only. Metabolism of NNK to NNAL is carried out by carbonyl reductase 1 (CBR1), hydroxysteroid dehydrogenase 11B1 (HSD11B1) and the aldo-keto reductase 1C1, 1C2 and 1C4 (AKR1C1, AKR1C2 and AKR1C4). We aim to determine whether variability in gene expression in each of these five genes affects the variability of enzymatic activity in NNK reduction and the risk of developing tobacco-related cancers. The current study focuses on the genes CBR1 and HSD11B1. We measured mRNA expression (n=106), protein levels (n=48) and enzymatic activity for each of these two enzymes using human liver microsomes (n=97) and cytosols(n=89). High performance liquid chromatography was used to measure carbonyl reductase activity in cytosol, which contains CBR1 and AKR, and in microsomal extracts which contain HSD11B1. Gene expression levels on the same samples were measured using real-time PCR. No correlation between mRNA levels and enzymatic activity was found for either CBR1 or HSD11B1. Protein levels of CBR1 and HSD11B1 were measured by Western Blots from the same samples. CBR1 was found to be correlated with NNK reduction in the cytosol (R= 0.49 , p=0.001). No correlation was observed for HSD11B1. The current data shows that variation in activity levels are not explained by either DNA sequence variation or mRNA expression. Interindividual variations in NNK reduction may be due to post-transcriptional control of CBR1.

790W

Identification of Novel Fatty Acid Desaturase 1 Isoforms: Localization and Differential Expression in Tissues and Mammalian Cells. *W.J. Park, K.S.D. Kothapalli, H.T. Reardon, P. Lawrence, J.T. Brenna.* Division of Nutritional Sciences, Cornell University, Ithaca, NY.

Omega-3 (=3 or n-3) and omega-6 (=6 or n-6) long chain polyunsaturated fatty acids (LCPUFA) are nutrients and bioactive metabolites that are associated with most of the human diseases and play important roles throughout development and aging. Desaturases (FADS1, FADS2 and FADS3) are key enzymes for LCPUFA biosynthesis and regulation. The enormous diversity that can be produced at the RNA level is increasingly recognized as a significant mechanism in the regulation of gene expression and in expanding the function of the proteome. Recently, we showed for the first time the existence of alternative transcripts (ATs) for FADS2 and FADS3 genes generated by alternative splicing events, as well as their conservation in several vertebrate species. Here we report identification of novel FADS1 transcripts generated by alternative selection of 5'untranslated regions, Poly A sites, and by internal exon deletions from neonatal baboon liver, using rapid amplification of cDNA ends (RACE) technique. Importantly, we show the detection of protein isoforms by immunoblot in neonate stage and mammalian cells, protein isoform expression and fatty acid changes in response to human neuronal cell differentiation, and isoform specific subcellular localization by live cell imaging using confocal microscopy with confirmation by purification of specific organelles. Our results indicate that FADS isoforms may have critical roles as mediators of LCPUFA biosynthesis and/or regulation depending on tissues, organelles and developmental stage.

791W

In vitro and in vivo functional analysis of ARHGAP31 Mutations, in Adams Oliver Syndrome. *D. Dafou[1], L. Southgate[1], R.D. Machado[1], K.M. Snape[1], M. Primeau[2], D.M. Ruddy[3], P.A. Branney[4], M. Fisher[4], G.J. Lee[1], M.A. Simpson[1], Y. He[2], T.Y. Bradshaw[1], B. Blaumeiser[5], W.S. Winship[6], W. Reardon[7], E.R. Maher[8], D.R. FitzPatrick[4], W. Wuyts[5], M. Zenker[9], N. Lamarche-Vane[2], R.C. Trembath[1,3].* 1) King's College London, Division of Genetics and Molecular Medicine, School of Medicine, Guy's Hospital, London, SE1 9RT, United Kingdom; 2) McGill University, Department of Anatomy and Cell Biology, 3640 University Street, Montreal, Quebec, Canada; 3) King's College London, Division of Genetics and Molecular Medicine, School of Medicine, and Department of Clinical Genetics, Guy's Hospital, London, SE1 9RT, United Kingdom;; 4) MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU, United Kingdom; 5) Department of Medical Genetics, University and University Hospital of Antwerp, Prins Boudewijnlaan 43, 2650 Edegem, Belgium; 6) Nelson R Mandela School of Medicine, Faculty of Health Sciences, Department of Paediatrics and Child Health, University of KwaZulu-Natal, Durban 4041, South Africa; 7) National Centre for Medical Genetics, Our Lady's Hospital for Sick Children, Crumlin, Dublin 12, Ireland; 8) Medical and Molecular Genetics, School of Clinical and Experimental Medicine, College of Medical and Dental Sciences, University of Birmingham, Institute of Biomedical Research (E.R.M.) and West Midlands Regional Genetics Service, Birmingham Women's Hospi; 9) Institute of Human Genetics, University Hospital Erlangen, University of Erlangen-Nuremberg, Schwabachanlage 10, 91054, Erlangen (M.Z.) and Institute of Human Genetics, University Hospital Magdeburg, Leipziger Str. 44, 39120 Magdeburg, Germany.

Adams-Oliver syndrome is a congenital condition characterized by aplasia cutis congenita (ACC, terminal transverse limb defects(TTLD) and associated features. Genome-wide linkage analysis has identified a locus for autosomal dominant ACC-TTLD on chromosome 3. Candidate-gene and exome-based sequencing identified premature truncating mutations in the terminal exon of the Rho GTPase-activating protein 31 gene(ARHGAP31). ARHGAP31 is a member of the RhoGAP family of proteins known to inactivate the Rho GTPases Cdc42 and Rac1. Mutant transcript stability was assessed by quantitative RT-PCR in patient lymphoblastoid cells, and localisation of the protein was mainly observed to the Golgi.Protein immunoprecipitation analysis has demonstrated an interaction between the C terminus of ARHGAP31 and the N-terminal RhoGAP domain indicating the exposure of a constitutively active RhoGAP catalytic site by the truncated ARHGAP31 C-terminal domain in mutant proteins. Whole-mount in situ hybridization in mouse embryos has detected Arhgap31 expression to the terminal limb buds and craniofacial processes during development indicating possible sites of impaired organogenesis characteristic of the disease phenotype. We sought to expand on our preliminary findings to functionally validate the putative role of Arhgap31 in the regulation of Cdc42 and/or Rac1 signalling, which is critical during development. Expression of mutant ARHGAP31 resulted in increased rates of cell migration and significantly decreased proliferation rates in mutant fibroblasts, suggesting unregulated suppression of Cdc42/Rac1 function. Importantly, time-lapsed microscopy of single-cell random migration showed that mutant fibroblasts exhibit increased speed of cell movement, but with disorganised direction and altered adhesion properties, supporting the notion that defective Cdc42 and/or Rac1 signalling alters directed migration, proliferation, and differentiation. To further elucidate the role of ARHGAP31 in vivo, we injected zebrafish embryos with mutant ARHGAP31 mRNA and examined its effect on embryonic development. We showed that mutant ARHGAP31 expression disturbs epiboly and convergent-extension cell movements during gastrulation, exhibiting distinct morphological defects in 24-48 hours post fertilization. To further understand how mutations in ARHGAP31 regulate convergence and extension cellular movements, we are currently analysing cellular migration in different regions of gastrulating embryos.

792W

The retinoic acid induced one gene (*RAI1*) is a positive regulator of *CLOCK* and an essential component of the circadian feedback loop of transcription. *S.R. Williams[1], D. Zies[2], S.H. Elsea[1,3].* 1) Dept of Human and Molecular Genetics, Virginia Commonwealth University, Richmond, VA; 2) Dept of Biology, University of Mary Washington, Fredericksburg, VA; 3) Dept of Pediatrics, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA.

Mutation or deletion of the retinoic acid induced one gene (*RAI1*) results in Smith-Magenis syndrome (OMIM#182290), an intellectual disability and multiple congenital anomalies syndrome characterized by obesity, neurobehavioral abnormalities and a disrupted circadian sleep-wake pattern. An inverted melatonin rhythm (peak melatonin in day instead of night time) and associated sleep phase disturbances in persons with SMS, as well as a short period circadian activity rhythm in mice with a chromosomal deletion of *Rai1*, support SMS as a circadian rhythm dysfunction disorder. Circadian rhythms are endogenous ~24 h cycles in physiology and behavior characterized by central and peripheral feedback loops. The circadian oscillator in mammals temporally orchestrates metabolism, physiology, and behavior largely through transcriptional modulation. Data support RAI1 as a transcriptional regulator, but its molecular function, including the genes it may regulate, co-factors, and temporal expression pattern, are still unclear. Molecular investigation into the role that RAI1 plays in gene regulation and circadian regulation revealed that RAI1 regulates transcription of *CLOCK*, a key component of the mammalian circadian oscillator that transcriptionally regulates many downstream genes via E-box enhancer elements. Additionally, heterozygous loss of *RAI1/Rai1* in SMS fibroblasts and mouse hypothalamus results in transcriptional dysregulation of the circadian clock, causing altered expression and regulation of multiple circadian genes, supporting the observed disruption of circadian activity in humans and mice with reduced expression of *RAI1/Rai1*. These data suggest that genetic disruption of *RAI1/Rai1* leads to a disrupted circadian oscillator, resulting in abnormal sleep-wake cycle, abnormal feeding pattern, and dependent learning and cognitive deficits. Finally, we conclude that RAI1 is a positive transcriptional regulator of the *CLOCK* gene, pinpointing a novel and important role for this gene in the circadian oscillator.

793W

Evaluation of maternal and fetal effects of polymorphisms involved in the folate metabolism on Down syndrome. *J.M. Biselli[1], F.A. Poletta[2], B.L. Zampieri[1], C.C. Mendes[1], F.B. Machado[3], A.F.A. Silva[4], E. Medina-Acosta[4], J.S. Lopez-Camelo[2], E.E. Castilla[2], E.M. Goloni-Bertollo[1], E.C. Pavarino[1].* 1) Genetics and Molecular Biology Research Unit, Sao Jose do Rio Preto Medical School, São José do Rio Preto, São Paulo, Brazil; 2) Clinical investigation and Medical Education Center, Genetic Epidemiology Laboratory, Buenos Aires, Argentina; 3) São Paulo University (USP), Ribeirão Preto, São Paulo, Brazil; 4) Alvaro Alvim School Hospital, Benedito Pereira Nunes Foundation, Campos dos Goytacazes, RJ, Brazil.

Down syndrome (DS) is resulting from a failure of chromosome 21 segregation during maternal meiosis in about 80% of cases. Although advanced maternal age is the only well-established risk factor for DS, many DS children are born to mothers aged less than 35 years-old, suggesting other factors influencing DS etiology. Several studies have showed that genetic polymorphisms involved in folate pathway can modulate the maternal risk for DS. In addition, studies from DS family triads have questioned if these polymorphisms could be involved in embryo viability or fetal survival. Folate metabolism is a complex pathway that involves multiple enzymes which play key roles in purine and pyrimidine synthesis; and DNA methylation, an epigenetic process that acts on the control of gene expression and genomic stability. The role of genetic polymorphisms involved in this pathway as maternal risk factors for DS is attributed to the association between some genetic variants with enzyme alterations that could result on DNA hypomethylation and, consequently, chromosome 21 nondisjunction. Regarding embryo viability or fetal survival hypothesis, these genetic variants could promote the increase of homocysteine concentration, an amino acid essential for folate-dependent DNA synthesis and for cellular methylation reactions, important for fetal development. DS individuals present disturbed folate metabolism due to the presence of the CBS gene in triplicate and it subsequent overexpression, leading to reduced concentrations of some metabolites. We used a multi-marker procedure for testing association between 10 genetic polymorphisms involved in the folate pathway, considered as “haplotypes”, and maternal or “fetal” effects in DS etiology. There were included in the study 86 family triads comprising the DS individual with free trisomy 21 and their parents (mother and father). The analyses were made using the TRIMM (TRIad Multi-Marker test) program. First, it was evaluated the effect of maternal genotypes, including only informative families for testing this association. Second, the effect of “fetal” genotypes was evaluated, also considering only informative families. There was no evidence of association (transmission disequilibrium) between maternal (P=0.61) or “fetal” (P=0.67) genotypes and DS considering the 10 SNPs evaluated. In conclusion, the folate-polymorphisms combinations evaluated as “haplotypes” by multi-marker test were not associated with DS.

794W

A novel BBSome interacting protein is an ADP/ATP translocase, SLC25A31. *X. Chamling[1], S. Seo[2], V.C. Sheffield[1,2,3].* 1) Pediatrics, University of Iowa, Iowa City, IA; 2) Ophthalmology and Visual Sciences, University of Iowa Carver College of Medicine, Iowa City, IA; 3) Howard Hughes Medical Institute, Iowa City, IA.

Bardet-Biedl syndrome (BBS) is a genetically heterogeneous autosomal recessive disorder characterized by obesity, retinal degeneration, polydactyly, hypogenitalism and renal defects. At least 15 different BBS genes have been reported. Recent findings have implicated BBS with primary cilia dysfunction, and BBS proteins are thought to be involved in the trafficking of various ciliary proteins. Specifically, the BBSome, a stable complex of seven highly conserved BBS proteins, BBS1, BBS2, BBS4, BBS5, BBS7, BBS8, BBS9 and a novel protein, BBBIP10, is believed to traffic to cilia bringing various cargoes along with it. In recent studies, various different interacter proteins including the leptin receptor, Rabin 8, PCM1 and few others have been associated with the BBSome. Due to the pleiotropic nature of the disease and the abundance of proteins in and around cilia where BBS proteins localize, we hypothesized that there are other proteins that interact with the BBSome which have not been identified. To investigate this hypothesis we generated a transgenic (LAP-BBS4) mouse line that expresses LAP tagged BBS4 in multiple different tissues including eye, brain and testis. Using lysates from brain and testis of the transgenic mouse, we pulled down BBSome interacters. Mass spectrometry confirmed one specific interacting protein to be SLC25A31 (solute carrier family 25 member 31), also known as ADP/ATP translocase 4 or Ant4.

795W

Cep290 function in localization of cilia proteins resulting in phenotypic heterogeneity of CEP290-associated disease. *Y. Zhang, Q. Zhang, C. Searby, S. Seo, V.C. Sheffield.* Howard Hughes Medical Institute and Department of Pediatrics, The University of Iowa, Iowa City, IA.

Nephronophthisis (NPHP) is a heterogeneous, autosomal recessive medullary cystic kidney disease affecting children. CEP290/NPHP6 can cause several cilia-related disorders (ciliopathies), including NPHP, Leber congenital amaurosis (LCA) and Meckel-Gruber syndrome (MKS). This suggests that Cep290 interacts with different proteins to accomplish different functions. CEP290 is known to localize to the centrosome and basal body of ciliated kidney cells and to the connecting cilium of retinal photoreceptors. The majority of NPHP and MKS proteins co-localize with CEP290. In this study, we examined cellular phenotypes in CEP290 knock down cells and in cells over-expressing parts of the CEP290 protein. We generated several truncated CEP290 protein fragments containing various domains of the whole protein. We tagged these fragments with GFP and examined their localization in both 293T and RPE cells. We also evaluated the physical interaction between these truncated proteins and other cilia proteins by co-immunoprecipitation. The results show that the N-terminal and C-terminal fragments of CEP290 both localize to the centrosome. The N-terminus interacts with NPHP2 and NPHP5, whereas the C-terminus interacts with MKS1. We used siRNA to knock down CEP290 in 293T and RPE cells, and then examined the expression level and localization of other related proteins. We found that the NPHP2 level is decreased in the CEP290 knock down cells, while the NPHP3 level is increased in knock down cells. In addition, we found that several proteins, including NPHP2 and NPHP5, are mislocalized in the CEP290 knock down cells. We conclude that CEP290 plays a role in regulating the protein stability and correct localization of NPHP related proteins, which plays a role in the phenotypic heterogeneity resulting from CEP290 mutations.

796W

Loss of SQSTM1 copy number in Paget's disease of bone. *S. Guay-Belanger[1,2], E. Gagnon[2], J. Morissette[2], J. P. Brown[1,2,3], L. Michou[1,2,3].* 1) Laval University, Quebec (Quebec) Canada; 2) CHUQ (CHUL) Research Centre, Quebec (Quebec) Canada; 3) Rheumatology Department, CHUQ (CHUL), Quebec (Quebec) Canada.

Purpose: Paget's disease of bone (PDB), a common disorder affecting up to 3% of the population over 55 years of age, has an autosomal-dominant mode of inheritance with incomplete penetrance in one-third of cases. PDB is characterized by unifocal or plurifocal hyper-remodelling secondary to activation of osteoclasts, resulting in weakened bone structure and strength. To date, only the SQSTM1 gene has been linked to PDB, with more than 20 reported heterozygous mutations. The SQSTM1 gene P392L mutation was the first described and remains the most common mutation in PDB. Recently, a new prevalent form of genomic variation has emerged. Copy number variation (CNV) is described as a segment of DNA that is 1 kb or larger and is present in variable copy numbers in comparison with a reference genome. Three CNV have already been reported in the SQSTM1 gene region. The objective of this study was to determine if CNV of the SQSTM1 gene was associated with PDB. Methods: This investigation focussed on the CNV_0068 since it covers the whole SQSTM1 gene, including exons 7 and 8, where SQSTM1 mutations are located. We analysed 298 healthy donors, 244 unrelated PDB-affected patients and 54 patients with a familial form of PDB, of whom 42 were carriers of a P392L mutation. We used the TaqMan Copy Number Assays according to the manufacturer's protocol. We performed case-control comparisons of individuals with loss of copy (ie less than two copies) of the SQSTM1 gene or with gain of copy (more than two copies), to individuals with two copies, who were considered the reference. Also, the subgroup of patient carriers of a P392L mutation was compared to healthy controls. Statistical analyses included Fisher's exact test, odds radio (OR) and 95% confidence interval calculations. Differences were considered statistically significant when $P < 0.05$. Results: The comparison of pagetic patients to healthy controls demonstrated that loss of SQSTM1 copy number was significantly more frequent in patients than in controls (14.5% versus 9.5%, P=0.035, OR=1.77 [1.01-3.12]), particularly in the subgroup of patient carriers of a P392L mutation, in whom loss of SQSTM1 copy number was even more frequent than in controls (20% versus 9.5%, P=0.014, OR=3.47 [1.14-10.03]). Conclusion: This study suggests that there is a loss of SQSTM1 copy number in PDB, particularly in the subgroup of patient carriers of the P392L mutation within the SQSTM1 gene.

797W

Functional Evaluation of CLEC16A: Role in Type 1 Diabetes. *M. Bakay[1], R. Pandey[1], L. Monaco-Shawver[2], J. Glessner[1], C. Kim[1], F. Mentch[1], J. Bradfield[1], S. Grant[1], C. Polychronakos[3], J. Orange[2], H. Hakonarson[1].* 1) The Center for Applied Genomics, Children's Hosp Philadelphia, Philadelphia, PA; 2) Division of Immunology, The Children's Hospital of Philadelphia. Philadelphia, PA 19104; 3) McGill University Health Centre. Montreal, Quebec H3H-1P3, Canada.

Type 1 Diabetes (T1D) is a multifactorial childhood disease with a strong genetic component. Several GWAS recently showed association of CLEC16A with T1D. CLEC16A has homology domains that identify it as a C-type lectin. Still, the protein has no known function. Other C-type lectins have been shown to powerfully influence growth, survival, and autoimmune attack of pancreatic)-cells. The highest levels of CLEC16A expression were identified in NK cells, which are required for development of T1D. Therefore, defining the role of CLEC16A in NK cells will provide insight into the pathogenesis of T1D. We reported previously that protective alleles of CLEC16A [A/A] are associated with higher levels of mRNA. Recent studies of CLEC16A orthologue have defined regulatory roles for the protein in regulating endosomal trafficking. Thus, we hypothesize that CLEC16A functions in NK cells to restrain secretory functions including cytokine release and cytotoxicity after activation. To address the role of CLEC16A in T1D we studied consequences of knockdown and overexpression of this protein in NK cell lines. Using a retroviral expression system we created NK cell lines stably overexpressing different levels the canonical form of CLEC16A with a GFP expression reporter. Expression has been validated by Western blot analysis and functional studies, such as cytotoxicity and cytokine release assays, of these cells are in progress. We also designed small-interfering (si) RNA directed against CLEC16A that should impact all isoforms. Optimized protocols using CLEC16A siRNA enabled a 70% reduction in Clec16A protein levels in NK cells. Interestingly, this resulted in a 35% increase in cytotoxicity compared to cells receiving control siRNA. Our results indicate that CLEC16A serves a role in restraining NK cell function. The mechanism for this effect and its impact upon other secretory functions such as the release of inflammatory mediators represents our immediate focus. We predict a role for CLEC16A in regulating the secretory pathway in NK cells. Our ongoing mechanistic studies will give unprecedented insight into in the immunopathogenesis of T1D and will ultimately link back to patient-derived cells. Improved understanding of this novel T1D-linked gene and the protein it encodes will likely suggest new therapeutic interventions relevant to regulating autoimmune reactivity in patients progressing toward T1D.

798W

Genotype-function Correlation of APOE Gene Promoter Polymorphism: in Transcription Control and in Type 2 Diabetes Susceptibility. *Y.Y. Ho[1,4], H. Geng[1], P. Law[1], M. Ng[2], T. Li[1], L.Y. Liang[1], T.F. Ge[1], K.B. Wong[1], C. Liang[3], R.C. Ma[2], W.Y. So[2], J.C. Chan[2].* 1) Department of Biochemistry, The Chinese University of Hong Kong, Hong Kong, China; 2) Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, China; 3) Department of Biochemistry, Hong Kong University of Science and Technology, Hong Kong, China; 4) Departments of Biostatistics and Psychiatry, Columbia University, New York, NY, USA.

The apolipoprotein E gene (APOE) encodes three major protein isoforms (apoE2, apoE3, apoE4) and is a risk modifier of Alzheimer's disease, type 2 diabetes (T2D), and coronary heart disease. Aside from the coding variants, single nucleotide polymorphism (SNP) of the APOE promoter has also been shown to modify the risk of Alzheimer's disease. Whether APOE promoter polymorphism is associated with the risk of T2D is unknown. In this study we aimed at investigating the genotype-function correlation of APOE promoter polymorphism at molecular level and at physiological level: i.e., in transcription control of the gene and in T2D susceptibility, respectively. In molecular studies, the effect of the APOE promoter -491A/T polymorphism on transcription was accessed by dual-luciferase reporter gene assays. Substitution of -491A to T decreased ($p<0.05$) the activity of the cloned APOE promoter (-1017 to +406). Using the -501 to -481 nucleotide sequence of the APOE promoter as a `bait' to screen the human brain cDNA library by yeast one-hybrid system yielded ATF4 (Activating Transcription Factor 4, an endoplasmic reticulum stress response gene encoding a regulator of lipid and glucose homeostasis in mammals) as one of the interacting factors. Electrophoretic-mobility-shift assays (EMSA) and chromatin immuno-precipitation (ChIP) analyses further substantiated the physical interaction between ATF4 and the APOE promoter. Over-expression of ATF4 stimulated APOE expression whereas siRNA blockage of ATF4 suppressed APOE expression. However, interaction between the APOE promoter and ATF4 was not -491A/T-specific. The genotype-function relationship of APOE promoter polymorphism at physiological level was studied in the context of T2D. In a case-control study (630 cases and 595 controls), three APOE promoter SNPs -491A/T (rs449647), -219G/T (rs405509), and +113G/C (rs440446) were genotyped and tested for association with T2D in Hong Kong Chinese. There was no association between these SNPs and T2D. In conclusion, at molecular level, -491A/T polymorphism and the ATF4 transcription factor elicit independent control of APOE gene expression. At physiological level, APOE promoter polymorphism is not associated with T2D susceptibility in Hong Kong Chinese. Acknowledgement: This study was supported by Direct Grant No. 2041183, The Chinese University of Hong Kong, Hong Kong, China and Grant No. T32-MH-65213, National Institute of Mental Health, USA.

799W

ITGAM coding variant, rs1143679 (R77H), is associated with systemic lupus erythematosus (SLE) susceptibility and affects its own expression in monocytes of SLE patients. *A.K. Maiti[1], P. Motghare[1], C. Sandel[1], X. Kim-Howard[1], J-M. Anaya[2], S.K. Nath[1].* 1) Gen Epidemiology Unit, A&I, OMRF, Oklahoma city, OK; 2) Universidad del Rosario-Corporación para Investigaciones Biológicas, Bogota, Colombia.

Background: We recently identified ITGAM (CD11b) as a novel SLE susceptibility gene. To date, ITGAM and its associated variant rs1143679 (R77H) is one of the few SLE susceptibility genes consistently replicated in multiple populations with European, African, Hispanic, and Asian ancestries. However, functional significance of this missense mutation is not known. CD11b mainly expresses in neutrophil, monocyte, and macrophages and it is known to have numerous biochemical functions. Though there are no prior indication about its involvement in SLE, its role in phagocytosis, aggregation, leukocyte adhesion, and cellular signaling are well established. Since this exonic SNP is located at the ninth base from exon-intron boundary and includes an enhancer/silencer sequences we hypothesize that it can change its own expression. Methods: We purified monocytes from patient's PBMC with fluorescent activated cell sorting (FACS) using fluorescent labeled CD14 antibody. Total RNA was isolated and cDNA from these cells were subjected to RT-QPCR. Surface expression of CD11b was measured with FACS using CD11b antibody. Allele specific expression was quantitated by sequencing either allele carrying 30 clones from each heterozygous (GA) patients. Splicing abnormalities were assessed with splicing specific PCR, and methylation of promoter was checked with pyrosequencing. Results: Expression analysis of mRNA and surface protein (CD11b) in SLE patient's monocyte showed that both expressions are significantly reduced in risk genotype (AA) patients in compare to non-risk (GG) genotype carrying patients. Furthermore, allelic expression suggests that risk allele (A) carrying mRNA is 4 to 10 fold reduction in monocytes of SLE heterozygous patients. Subsequent experiments suggest that risk allele specific mRNA degradation, or any aberrant splicing, are not observed in patient's monocytes Conclusion: Expression differences due to allelic differences indicate functional significance of rs1143679. Risk allele specific methylation or silencing of its own expression could explain reduced expression of this allelic mRNA. In conclusion, apart from the predicted misfunction of the mutated protein (Arg>his), G>A nucleotide transition in ITGAM also affects its expression leading to immune modulation in SLE pathogenesis.

800W

High-Fidelity Mapping Of Exome Sequences Improves Outcomes In Disease Studies. *T. Gaasterland[1], L. Edsall[1], A. Patel[1], S. Soares[1], R. Thompson[1], A. Wu[1], S. Head[2], P. Lee[2], D.E. Gaasterland[3], R. Ayyagari[4].* 1) Laboratory of Computational Genomics, Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA; 2) Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA; 3) Eye Doctors of Washington, Chevy Chase, MD; 4) Department of Ophthalmology, University of California San Diego, La Jolla, CA.

Purpose: Maximize discovery of real, disease-related genome variations while minimizing false positive variant calls through DNA capture of exons genome-wide ("exome capture"), applied to eye disease and focused on glaucoma and retinal degenerations, both of which are neurologic-related afflictions. The study of neurologic eye diseases will lay genetic foundations for wider understanding of neurodegenerative processes. Methods: After consent, DNA was extracted from blood of individuals affected, or not affected, with one of several eye diseases. Commercial exome capture kits were applied to hybridize probes to target DNA in solution. For each person, captured DNA was sequenced (Illumina Genome Analyzer IIx or HiSeq) generating at least ~80 million pairs of 100-base reads. Initially, reads were mapped to a reference human genome (hg18; hg19) and SNPs (single nucleotide polymorphisms) identified using published parameters and methods. To overcome deficiencies, we devised a new tiered strategy that maps perfectly matching reads and then iteratively relaxes match stringency. Exome SNPs were compared with known SNPs found independently with the more accurate, low throughput, Sanger sequencing of the same samples. Results: Some of the exome SNPs were from pseudogenes. The tiered strategy mapped reads correctly to pseudogenes rather than genes, thus ensuring each exome SNP mapped to its true location. Further, this new tiered read mapping strategy partitioned reads into those that matched uniquely to exactly one position in the genome, or to two, to 3-5, or to 6-10. This allowed not only SNPs, but also insertions and deletions, to be predicted iteratively with strict subsets of mapped reads, and labeled according to mapping quality. Conclusions: Tiered mapping and variant calling allows multi-mapped reads, which identify otherwise weakly supported genome variants. Such variations are identified as ambiguous and possibly resulting from gene conversion (a process by which pseudogene sequences are inserted into actual genes). With this method, in comparison with conventional mapping strategies, variant calls are ranked by the mapping quality of supporting reads. It also identifies possible gene conversion, increases sensitivity and specificity, and enhances potential to discover disease-related genes.

801W

mRNA diversity of genes by alternative splicing during neuronal differentiation of NT2 pluripotential human embryonal carcinoma cells by retinoic acid. *T. Isogai[1], A. Wakamatsu[1], H. Sasaki[1], J. Imai[2], S. Watanabe[2].* 1) Grad Sch Pharmaceutical Sci, Univ Tokyo, Tokyo, Japan; 2) Fukushima Med. Univ., Fukusima, Japan.

To elucidate cause of disease related to neuronal differentiation, we analyzed genes and the protein-coding transcripts by alternative splicing (AS) using NT2 pluripotential human embryonal carcinoma cells which differentiates toward a neuron by all-trans retinoic acid (RA) induction. Gene could produce multiple protein-coding transcripts (MPCTs) by AS. It was known that AS particularly occurs in the mammalian nervous system and plays an important role in neuronal differentiation in generating biological and functional diversity. We analyzed expression levels by NT2 cells treated with RA using DNA microarray, and then identified 358 RA-responsive genes. mRNA diversity analysis revealed that 274 genes (77%) produced MPCTs by AS. Among 274 genes, we chose 16 genes producing MPCTs using alternate N-terminus (Alt. N-term), 30 genes producing MPCTs using alternate C-terminus and 12 genes producing MPCTs using alternate cassette-exon for further analysis. Using transcript-specific primers, we performed quantitative real-time PCR analysis to examine the expression profiles of the protein-coding transcripts produced by each one of these genes. Furthermore, we previously analyzed expression profiles of the genes containing alternative TSSs (transcription start sites), multiple variable first exons, by AS, and then found that alternative TSSs were shown to be utilized for tissue-specific expression. We think that the same gene could code for proteins with diverse function in different tissues by the proper utilization of alternative TSS. Analyzing expression profiles of 10,069 cDNAs, which corresponded to 5,542 genes, out of our identified 11,769 cDNAs, we found that the TSSs of 263 cDNAs, which correspond to 156 genes, showed specific expression patterns that were different from those already obtained for the genes with alternative TSSs. Using transcript-specific primers designed for their TSS regions, we analyzed expression profiles of 51 out of 156 selected genes by NT2 cells treated with RA using real-time PCR. Consequently, we identified genes which showed different RA-induced changes in the expression of their protein-coding transcripts by using Alt. N-term. The mRNA diversity of genes was occurred by not only genetic risk factors but also environmental risk factors. To clarify which risk factor caused their mRNA diversity, it will be important to discover novel target genes of new drugs for disease.

802W

Global alternative splicing variation reflects ethnical diversity. *J.W. Li[1,2], T.F. Chan[1,2].* 1) School of Life Sciences, Biochemistry Program, The Chinese University of Hong Kong, Hong Kong; 2) Hong Kong Bioinformatics Center, The Chinese University of Hong Kong, Hong Kong.

Population structure based on single nucleotide polymorphism (SNPs) and micro-satellites has demonstrated drastic population stratification among human populations. Expression phenotypic variations among the 3 HapMap populations (CEU, YRI, JPT/CHB) were well characterized. Relatively less known is the extent of global splicing variations (ASV) in the context of ethnicity.

We are interested in human diversity in molecular level. We used RNA-Seq to characterize 20 transcriptomes of CEU and CHB individuals. Compared to Reference Sequences alone, we were able to identify 447 genes that show differential splicing ratios. Ethnical splicing ratios were validated in 10 healthy unrelated Chinese individuals sampled in Hong Kong. Besides, thousands of ethnic specific splice junctions were pinpointed. We further analyzed 5634838043 sequencing reads retrieved from NCBI SRA, revealing thousands of novel transcribed exons in each ethnic group. RT-PCR and northern blot validation are in progress. Interestingly, meta-analysis on YRI showed a largely different spectrum of baseline splicing variations. Such observations suggest ASV as another molecular phenotype that delineates ethnical diversity. In addition, we observed minimal overlap in genes that showed inter-ethnic variations in gene expression and splicing. This suggests a complementary role of gene expression variation and splicing in functional diversity between and among populations. This study uncovers natural ASV with implications in complex human traits.

While numerous studies had revealed the linkage of drug susceptibility and clinical outcomes to molecular phenotypes, under-representation of certain ethnic groups in clinical trials contributes significantly to worldwide healthcare disparities. Evaluation of ethnical diversity in additional of individuals' molecular and expression phenotype in clinical examination will assist physicians in practice of evidence-based medicine.

803W

Alternative exon 1 usage defines a novel human Hydroxymethylglutaryl-CoA reductase (HMGCR) gene splice variant. *C. Stormo[1], M.K. Kringen[2], R.M Grimholt[1], J.P Berg[1], A.P Piehler[1].* 1) Medical Biochemistry, Oslo University Hospital, Ullevål, Oslo, Norway; 2) Department of Pharmacology, Oslo University Hospital, Ullevål, Oslo, Norway.

Background: 3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) encodes the rate limiting enzyme in cholesterol biosynthesis and the main target of commonly used LDL lowering drugs (statins). Previous studies have shown that a HMGCR splice variant lacking exon 13 has decreased enzymatic activity and is less sensitive to statin inhibition. The aim of this study was to identify putative novel HMGCR splice variants and to study their expression patterns and responses to atorvastatin treatment. Methods: An alternative splice variant, amplified from a RNA pool of human skin by RT-PCR, was cloned into a TOPO vector and sequenced. Expression of the splice variant and the canonical form was investigated in 36 different human tissues and in HepG2 cells upon 24 h atorvastatin (10 μM) treatment. Relative mRNA levels were obtained by RT-qPCR using custom Taqman qene expression assays specific for either transcript. Results: We have cloned a novel alternative HMGCR transcript containing an alternative exon 1 (HMGCR1b) compared to the canonical transcript (HMGCR1a). The open reading frame of the novel transcript predicts a protein with additional 20 amino acids at the N-terminus compared to HMGCR1a. Sequencing of HMGCR1b clones revealed that HMGCR1b, like the original HMGCR1a transcript, can be subjected to exon 13 skipping (HMGCR1bΔex13). HMGCR1b was expressed at various levels in 36 different tissues. Unlike HMGCR1a, the alternative transcript showed highest expression levels in skin, esophagus and uterus. Furthermore, results from in vitro experiments revealed an upregulation of HMGCR1b upon atorvastatin treatment in HepG2 cells compared to non-treated cells. Conclusion: We have identified and cloned a novel transcript of the human HMGCR gene exhibiting alternative exon 1 usage. Both the differential expression of HMGCR1b in a wide variety of healthy tissues and the mRNA upregulation of HMGCR1b upon statin treatment indicate a distinct physiological function of the HMGCR1b gene transcript.

804W

Optimized Filtering of High Throughput Sequence Variants in Clinical Cases from the National Institutes of Health Undiagnosed Diseases Program. *D.R. Adams[1,3], M. Sincan[1], K. Fuentes Fajardo[1], C. Toro[1], C.F. Boerkoel[1], C.J. Tifft[2], W.A. Gahl[1,2,3], T.C Markello[1,3], NIH Intramural Sequencing Center.* 1) Undiagnosed Diseases Program, Office of the Director, NIH, Bethesda, MD; 2) Office of the Clinical Director, NHGRI, NIH, Bethesda, MD; 3) Medical Genetics Branch, NHGRI, NIH, Bethesda, MD.

High Throughput Sequencing (HTS) of exomes and genomes is rapidly becoming incorporated into the standard armamentarium of basic scientists and is making inroads into clinical molecular genetics. Several publications have discussed the data acquisition phase of HTS including alignments, base calling and annotation. The challenge of sorting through the resulting large list of candidate DNA variations is also well known, but has been evaluated to a lesser extent in the literature. We have explored the various means of filtering and sorting HTS variant lists using data from the NIH Undiagnosed Diseases Program (UDP). We present an analysis of the methodology and stringency considerations at each data filtering step, and discuss the integration of HTS data with genetic and clinical information from other sources. For this study, 30 probands plus additional family members were subjected to HTS of their exomes or genomes. In each case, extensive clinical evaluation at the NIH Clinical Center was used to attempt diagnosis and perform phenotyping. Analysis of the HTS variants generated for each individual or family was performed using both published and novel techniques, including the incorporation of data from high-density SNP arrays. The presence of four successful, verified molecular diagnoses provided a positive control for the efficacy of filtering methods and strategies. Filtering procedures at each step were tuned to match the clinical information available for the family or individual. Marked improvements in HTS variant filtering corresponded with improvements in filtering methodology and other resources such as successive dbSNP releases. Careful design of each filtering step allowed for optimization of the exclusion of false-positive variants. At the same time, flexible parameterization of each step allowed for systematic stringency adjustments. Laboratory confirmation of a DNA sequence variants of unknown significance can be time consuming and expensive. Therefore, careful filtering of HTS variant lists is essential for excluding candidates that can be either ruled out or de-emphasized prior to experimental validation.

805W

CIDRSeqSuite 2.0: An Automated Analysis Pipeline for Next-Generation Sequencing. *M. Barnhart, S. Griffith, K. Hetrick, J. Goldstein, B. Marosy, D. Mohr, B. Craig, L. Watkins, Jr., K. Doheny.* Center for Inherited Disease Research (CIDR), Johns Hopkins University, Baltimore, MD.

The Center for Inherited Disease Research (CIDR) provides high quality genotyping services and statistical genetics consultation to investigators working to discover genes that contribute to common disease. In January 2011, CIDR began offering sequencing services using the Illumina® HiSeq™ 2000 sequencing platform. CIDRSeqSuite, an early version of which was presented at the 2010 ASHG meeting, was developed to automate a variety of bioinformatic workflows and analyses. Over the past year, the primary analysis workflow of CIDRSeqSuite has been redesigned and new features have been added. Like its predecessor, CIDRSeqSuite 2.0 is designed to run on 64-bit Linux and is written in Java SE 6 and Perl 5. CIDRSeqSuite's new primary analysis workflow glues custom tools to numerous third-party secondary and tertiary analysis software including ANNOVAR, SAMtools, Picard, BWA, the Genome Analysis Toolkit (GATK), VCFtools, and tabix. Beginning with the qseq.txt files produced from BCL files by the Illumina BCL converter, CIDRSeqSuite demultiplexes samples based on their indexes, then combines and converts them into FASTQ files. From there, independent analyses by sample are launched: aligning the FASTQ files with BWA to a reference genome for a paired-end sequencing run; conversion to BAM with Picard; local realignment around indels using GATK; synchronizing mate-pair information and flagging of molecular duplicates with Picard; recalibration of base call quality scores with GATK; variant calling with SAMtools, producing VCF output; annotation of variants with ANNOVAR; and other QC metric reports. Current work involves parallelizing these independent sequencing analysis runs across an Oracle (formerly Sun) Grid Engine-managed cluster. The ability to resume an analysis is now included; following a crash or cancellation, the pipeline can resume from the last step completed. For a single sample, a complete analysis runs to completion in ~ 24-36 hours, depending on factors such as network traffic, amount of sequencing data produced per sample, and hardware factors. However, since samples are analyzed in parallel, it is theoretically possible to analyze a large number of samples in roughly the amount of time that it takes to analyze a single sample, provided that appropriate resources are available.

806W

DCAURS: a database for the identification of disease-causing regulatory SNPs. *P. Beaulieu[1], D. Sinnett[1,2].* 1) Centre de recherche, Hopital Ste-Justine, Montréal, QC, Canada; 2) Departement de Pédiatrie, Université de Montréal, Montreal, QC, Canada.

Previous efforts to identify functional disease-causing DNA variants were essentially oriented towards the coding regions of candidate genes since these variants have a direct impact on the structure and function of the affected proteins. However, abnormal expression of finely regulated genes can also lead to disequilibria in different metabolic pathways and/or biological processes. Thus, investigation of the functional impact of polymorphisms as well as the determination of the importance of evolutionary conservation in the regulatory regions of candidate genes should improve our knowledge of complex disease aetiologies. Towards this goal we have screened extended regions of DNA around disease-associated polymorphisms for potential regulatory hotspots. Analysed regions encompassed 250kb upstream and downstream of every SNP present in the NHGRI GWAS catalog. We integrated several layers of information such as gene structure, predicted TFBS binding sites, conservation, chromatine structure, CPG islands and known regulatory elements. We developed a detailed computational analysis of potential disease-associated regulatory regions and attempt to improve our understanding of their content. Here we present the Disease CAUsing Regulatory Snps (DCAURS) database, containing all known SNPs in the screened regions as primary data. Users can interrogate the database to find regulatory SNPs according to several search criterias, namely: genomic region, co-location with various types regulatory features, LD with disease-associated SNPs, proximity to disease-associated SNPs from a given GWAS study or phenotype. Such functional annotation provide a more effective selection of regulatory targets and thereby facilitates our efforts understand human complex diseases.

807W

Exome sequencing identifies CCDC8 mutations in 3-M syndrome, suggesting CCDC8 contributes in a pathway with CUL7 and OBSL1 to control human growth. *S. Bhaskar[1,3], D. Hanson[2,3], P.G. Murray[1,2,3], J. O'Sullivan[1,3], J. Urquhart[1,3], S. Daly[1,3], L.G. Biesecker[4,5], M. Skae[1], C. Smith[6], T. Cole[7], J. Kirk[8], K. Chandler[1,3], H. Kingston[1,3], D. Donnai[1,3], P.E. Clayton[1,3], G.C.M. Black[1,3].* 1) Central Manchester University Hospitals Foundation Trust, UK; 2) Endocrinology, Manchester Academic Health Sciences Centre (MAHSC), School of Biomedicine, University of Manchester, UK; 3) Genetic Medicine, Manchester Academic Health Sciences Centre (MAHSC), School of Biomedicine, University of Manchester, UK; 4) National Institutes of Health (NIH) Intramural Sequencing Center (NISC), National Institutes of Health, Bethesda, MD, USA; 5) National Human Genome Research Institute, NIH, Bethesda, MD USA; 6) East Lancashire Hospital NHS Trust, UK; 7) West Midlands Regional Genetics Service, Birmingham Women's Hospital, Birmingham, UK; 8) Department of Paediatric Endocrinology, Birmingham Children's Hospital, Birmingham, UK.

3-M syndrome is a primordial growth disorder characterised by postnatal growth restriction, dysmorphic facial appearance and prominent heels. Mutations in both CUL7 and OBSL1 are known to cause 3-M. CUL7 is an E3 ubiquitin ligase protein known to target the growth factor signalling molecule IRS-1 for degradation. OBSL1 is a cytoskeletal adaptor protein and we have previously shown that siRNA knockdown of OBSL1 leads to concomitant loss of CUL7 suggesting both are part of a common pathway. We now report a cohort of 3-M patients of South Asian descent who did not carry either CUL7 or OBSL1 mutations. Autozygoisty mapping using 3 such unrelated individuals identified a third 3-M syndrome locus on chromosome 19q13.2-q13.32. Exome sequencing capture revealed two different duplication mutations in CCDC8, coiled coil domain containing protein 8. Further screening by Sanger sequencing in additional patients revealed in total, 2 distinct mutations (c.84dup and c.612dup) in 5 families. Co-immunoprecipitation assays show that CUL7 and OBSL1 physically interact and that OBSL1 and CCDC8 also interact. This is first reported identification of CCDC8 mutations as a cause of 3-M and broadens the hypothesis that 3-M is a disorder of a common biochemical pathway comprising of at least CUL7, OBSL1 and CCDC8. The data presented here suggests that OBSL1 is likely to act as the adaptor protein linking CUL7 and CCDC8 and infers that all 3 proteins are required for the control of human growth.

808W

Integration of Transposable Elements Dependent on Genome Landscapes. *R. Campos-Sanchez[1], F. Chiaromonte[2], K. Makova[1].* 1) Biology Department, Pennsylvania State University, University Park, PA; 2) Department of Statistics, Pennsylvania State University, University Park, PA.

Transposable elements (TEs) constitute a large portion of every eukaryotic genome studied so far. Their insertions have been also associated with multiple human diseases. Recently, the integration preferences of Alu and L1 elements in primate genomes have been analyzed via a multiple linear regression framework (MLR, regressing the counts of these elements on a list of regional genome features and chromosome type). Here, we analyzed DNA transposons in the human genome in order to explain their genome densities as predicted from genomic landscape features. The responses for each MLR were the content within 1-Mb windows (fraction of a window in bp) of a group of DNA transposons classified by age of integration and family. A comprehensive literature search guided us into the selection of the most appropriate predictors (i.e. such genome landscape features as location on sex chromosomes vs. autosomes, gene content, GC content, replication timing, etc.). The genomic features were extracted from the UCSC Genome Browser or from primary publications and were localized into the same 1-Mb windows. In order to select the best set of predictors for all the analysis, we conducted a hierarchical cluster analysis on the correlations among predictors, and then applied the appropriate transformations for the data. Our preliminary results revealed that DNA transposons are underrepresented on sex chromosomes. Also, the distribution of certain DNA transposons (Tc-Mar Tigger and hAT-Charlie) appears to associate positively with L1 target primed reverse transcription, telomerase-dependent RNA retrotranscription, non-canonical L1 integrations, and with the presence of other transposable elements as short (SINE) and long (LINE) interspersed nuclear elements. These predictors highlight the molecular mechanism of DNA transposon integration. Our regressions explain up to 36% of variation in density of some DNA transposon groups and allow us to explain their distribution in the human genome. This study reveals some remarkable details about the integration preference of these transposable elements, knowledge of which is vital for understanding human genome evolution and origins of some genetic diseases.

809W

Resources for the 1000 Genomes Project. *L. Clarke, H. Zheng Bradley, R. Smith, E. Kulesha, I. Toneva, B. Vaughan, P. Flicek, The 1000 Genomes Project Consortium.* Vertebrate Genomics, EBI, Cambridge, United Kingdom.

The 1000 Genomes Project is producing a deep catalogue of human variation, to provide a better baseline to underpin human genetics. The main data resource for the 1000 Genomes project is the project ftp site (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp). This represents more than 120TB of data and 200,000 files and presents a challenge for the average user to navigate. New tools to notify users when new data appears and to assist users in finding the data that is relevant to their own enquires. Data releases are announced by email on announce@1000genomes.org and there is also an rss and twitter feed for the announcements as they are made on the 1000 genomes website. Variant calls are made available initially in vcf format on the ftp site and are subsequently loaded into the 1000 genomes Ensembl browser. The browser allows users to discover variants in their genomic context and shows the consequence of the variant with respect to Ensembl's gene annotation. The browser also holds tools to allow uses to interact with both bam and vcf files. Attachment of remote files allows uses to see remotely visible bam and vcf files along side the annotation within ensembl. Ensembl's variant effect predictor has been setup and this can give users functional consequences for their own variants including polyphen and sift scores for non synonymous changes. We have also provided a data slicing tool which gives users easy access to subsections of all our bam and vcf files. It will also work for other remotely accessible and indexed bams and vcfs. http://browser.1000genomes.org With more than 100Tbytes and 20000 files on the ftp site it can be difficult to find the precise information that you are looking for. Newly developed search tools for both the ftp site and the website have been created to help users find the information they are looking for. http://www.1000genomes.org/ftpsearch With these tools and many other developments should enable all users to get better access to the wealth of new information provided by the project.

810W

Expanding the annotation of the human genome: transcripts targeted for degradation and the noncoding genes. *G. Despacio-Reyes, M. Suner, M. Thomas, A. Frankish, L. Wilming, J. Mudge, D. Manthravadi, M. Kay, V. Boychenko, A. Bignell, J. Gilbert, E. Griffiths, J. Loveland, C. Steward, J. Harrow, T. Hubbard.* The Wellcome Trust Sanger Institute, Cambridge, Cambridgeshire, United Kingdom.

The Human And Vertebrate Analysis and Annotation (HAVANA) Team has been at the forefront of GENCODE, a sub-project of the ENCODE program (Encyclopaedia of DNA Elements), which aims to create an encyclopaedia of gene features in the human genome by evidence-based manual annotation. We use the in-house developed and maintained Otterlace annotation suite to produce a gold standard set of annotation for coding genes, noncoding genes, alternative splicing events, and pseudogenes. We routinely update our annotation guidelines to incorporate new data sources and new trends in transcriptomics, proteomics and genomics. Such examples include transcripts destined for degradation by regulatory mechanisms either via the nonsense-mediated decay (NMD) pathway or the non-stop mediated decay (NSD) pathway. As a basic rule, the presence of a premature termination codon upstream of a splice junction predisposes a transcript for degradation by NMD mechanism. However, more than 70 transcripts have been identified as coding despite containing a premature stop codon. These transcripts were able to escape the cellular quality control process and produce a viable protein. We have therefore had to strengthen our annotation guidelines, adding flexibility to account for the NMD exception . The presence of poly-adenylation at the end of an NSD transcript, without any prior termination codon, would signify degradation by NSD mechanism. It remains to be seen if this rule would be true across all known cases. For noncoding genes, we have developed initial annotation which will be further enriched as developments and interests in the noncoding genes continue to emerge. So far, we have created five biotypes for representing both novel and known noncoding loci, i.e., lincRNA, antisense, 3' overlapping ncRNA, sense overlapping and sense intronic. The current GENCODE (v.7) geneset has about 7000 non-coding RNA genes that we have manually annotated. As more evidence becomes available, the way we annotate noncoding genes will continue to evolve as well. The recent explosion in the production of transcriptomics data in the form of RNAseq means that our annotation procedures must adapt quickly. These data will be used to provide information on alternative splicing patterns, tissue / developmental specificity and transcription levels, all of which are presumably subject to variation.

811W

Connecting the genome with the metabolome: novel bioinformatic approaches for integration and visualization. *H.K. Dharuri[1], P. Henneman[1], K. Willems van Dijk[1,2], C.M. van Duijn[3], B.A. Oostra[3], P.A.C. `tHoen[1], G.J.B. van Ommen[1].* 1) Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands; 2) Department of Internal Medicine, Leiden University Medical Center, Leiden, Netherlands; 3) Department of Epidemiology, Erasmus Medical Center, Rotterdam, Netherlands.

GWAS studies explain only a minor part of the heritability of metabolic syndrome (MetS) and related clinical outcomes. In addition, the biological context of candidate genes detected by means of GWAS often remains unclear. The association of genetic polymorphisms with metabolite levels has recently sparked interest. These intermediate phenotypes generally demonstrate larger effect sizes and potentially point at pathways relevant to disease. However, association of all SNPs with all measured metabolites comes with considerable multiple testing problems. Our objective is to increase the power in the identification of loci associated with metabolite traits through the use of background knowledge on metabolic pathways. We have used NMR to measured metabolite levels in the serum of subjects in genotypically and genealogically well characterized Erasmus Rucphen Family (ERF) cohort. Preliminary genetic association analysis on just 20 metabolites already yielded considerable amount of data in the form of significant SNP-metabolite pairs (6 traits, 13 loci, P< 10-8). Therefore, it is imperative we build efficient, high throughput bioinformatic tools that help us to place these hits in a biological context. We have developed a software tool that automates the association of metabolites with SNPs near genes in the pathway relevant to the synthesis and degradation of the metabolite. An important aspect of this tool is the ability to handle a large amount of data and visualization techniques to help the biologist verify if the SNP-metabolite pair of interest does indeed have a convincing biological explanation. The software makes use of work flow management technologies like Taverna and Galaxy. This allows us to conduct our analysis across diverse datasets and applications like the UCSC Genome Browser, and metabolite and pathway databases like BioCyc, KEGG, and HMDB. Apart from curated entries in database, we have also used text mining to include biologically relevant connections between genes and metabolites that are not necessarily already present in pathway databases. The challenge here was to map metabolites to unique literature concepts, given the ambiguity surrounding metabolite names and their usage in the literature. The increased power of these tools to identify mechanistically relevant SNP-metabolite pairs will be demonstrated.

812W

Unbiased cataloging of novel variations across the complete genome. *S. Ghosh, J. Ebert, G. Nilsen, K. Pant, R. Drmanac.* Complete Genomics, Mountain View, CA.

A primary benefit of complete genome sequencing is the potential to identify rare variants implicated in complex diseases. The most challenging aspect of this is to discriminate causal variants from those that are neutral. One of the fundamental steps in this process is to catalog the frequency of variants in a normal population in an unbiased manner. This facilitates the identification of previously undiscovered/unstudied repertoire of genetic variants and the characterization of the causative disease variant. Complete Genomics has recently released complete genome sequence data from a diversity panel comprised of 69 normal samples representing 11 different populations. Samples are from the NHGRI (HapMap and 1000 Genomes Project) repositories. The purpose of sequencing the diversity panel is to provide a gender-balanced, high coverage - 50x median - complete genome data set across a spectrum of populations. This panel includes individuals of European, Asian, African descent and American admixture. Sequencing was performed on Complete Genomics DNA nanoarrays using a non-sequential, unchained read technology to generate 70mer paired end reads. Assembly was performed using the Complete Genomics pipeline, which performs an initial fast alignment to a reference followed by local *de novo* assembly in those regions of the genome that appear to contain variations. Both alleles were called in 95.03-97.43% of the genomes, with only one allele being called in an additional 0.25-1% of the genome. Median concordance with HapMap3 and 1000 Genomes Project were 99.73% and 99.83% respectively. A validation rate of greater than 93% was determined on loci absent from the 1000 Genomes Project data. The variations have been classified as SNPs, indels and substitutions. On a genome-wide scale, the median novelty rate(compared to dbSNP 130) is higher for indels at 25.49% (9=5.37%) compared to SNPs at 8.35% (9=4.11%). The median SNP novelty rate for Africans is 15.4%, Europeans is 6.78% and Asians is 8.31%. Similar trends are observed for the other variant types. The ratios of SNPs and indels in coding regions compared to the complete genome are 0.006 and 0.001 respectively. The median Ti/Tv ratios for genome-wide versus exome are 2.13 and 3.2 respectively, corroborating a negative selection pressure for variants at and proximal to coding regions. Detailed characterization of the population panel, including concordance with other published data will be discussed.

813W

Identity by descent in sequenced exomes. *A. Gusev, J. Zhuang, I. Pe'er.* Columbia University, New York, NY.

The identification of co-inherited genomic regions, referred to as being Identical by Descent (IBD) has traditionally been used to make inferences regarding population genetics, detect association, and correct errors in genotyping and phasing. In particular, recent family studies have used putative IBD segments detected from high-throughput sequence data to restrict the list of candidate disease linked variants by focusing on co-inherited regions. The distribution of such segments depends on population structure but scales quadratically with sample size, with standard techniques that rely on exhaustive search becoming intractable in large cohorts. However, discovering long IBD segments can be done efficiently by looking for short, identical matches between many individuals and extending from these “seeds” to long segments that are likely to be the products of recent co-inheritance. This approach depends significantly on dense SNP data with high-quality phasing to guide the initial seed process. We have previously presented GERMLINE, an algorithm to identify such segments in dense genotype array data; accurately detecting segments down to 3cM in length. Here we evaluate the effectiveness of this technique in whole-exome sequence data. We examine whole-exome sequence from a cohort of Ashkenazi Jewish origin, where we have previously observed an abundance of recent sharing, and compare detection accuracy to large-scale array data from the same samples. We find many long segments of 100% IBS identity in the exome data that are unsupported by analysis in the array. Such segments would be falsely identified as co-inherited in whole-exome data and could introduce biases into downstream analysis. Indeed, of such segments that are greater than 10cM, less than 10% are supported by array data. We explore various potential metrics for improving IBD detection in such data and find that the ratio of SNP density and recombination rate to be most effective. However, we find that such long false-positive segments cannot be entirely excluded from analysis without dramatically reducing sensitivity. We conclude that distant IBD detection in exome data must be performed with care and can still result in many spurious, seemingly identical segments.

814W

Using GWAs SNP arrays for quality control of DNA sequencing data. *K. Hetrick, H. Ling, E.W. Pugh, B.D. Craig, B.A. Marosy, J.M. Romm, K.F. Doheny.* Center for Inherited Disease Research (CIDR), Johns Hopkins University, Baltimore, MD.

The Center for Inherited Disease Research (CIDR) provides high quality next-gen sequencing (NGS), genotyping, and statistical genetics consultation to investigators working to discover genes that contribute to disease. Currently we offer NGS services using the Illumina® HiSeq™ 2000 sequencing platform and Agilent© SureSelect™ XT Library preparation and enrichment for whole exome (WES) and custom targeted sequencing. In order to ensure that there is not sample mis-identity issues, samples are run in a SNP assay prior to processing the sample for sequencing analysis (GWAs array for WES and 96 SNP barcode + 10% on GWAs arrays for custom targeted). This augments our ability to assess the DNA quality of the sample as well as identify sample misidentification (by checking gender and IBD). For WES, we identify large chromosomal anomalies prior to sequencing and allow the PI the option to replace the sample or provide us with a new aliquot of that subject. After sequencing, we utilize these genotypes to verify that the sequencing experiment is correctly identified as well as other QC assessments. The two most useful analyses for achieving this are; (1) the concordance between the sequencing variant calls for the experiment and the genotypes on the SNP array; (2) the proportion of heterozygous (het) calls on the SNP array that were detected from the sequence analysis. Having the GWAs genotypes for samples has allowed us to identify and later correct sample identification mis-mapping for the sequencing run and when two (or more) samples were run in the same flowcell lane with the same barcode index via concordance of variant calls between the two platforms. Calculating the proportion of het SNP calls on the array that were called as variant from the reference genome during the sequencing experiment helps us to assess whether there is enough sequencing coverage for a particular experiment. The proportion of het calls on the array detected as variants from the reference genome in the sequencing analysis is approximately linearly correlated with the percent of selected bases covered at a set minimum depth used to filter the variant calls. When experiments deviate from this trend line, we can then investigate. In a targeted sequencing experiment with much higher depth than WES, we were able to determine that a loss of heterozygote sensitivity was due to using our default parameters which resulted in over filtering variant calls.

815W

EpiCenter: a novel framework for accurately identifying genome-wide changes with next-generation sequencing data. *W. Huang[1], D.M. Umbach[1], N.V. Jordan[2], A.N. Abell[2], G.L. Johnson[2], L. Li[1].* 1) NIEHS, NIH, Res Triangle Pk, NC 27709, USA; 2) University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.

The next-gen sequencing based mRNA-seq and ChIP-seq are increasingly used for identifying genome-wide epigenetic/genetic changes. The new type and huge volume of data from these technologies, however, pose computational challenges unmet by existing methods. We propose a new and effective statistical framework for identifying differential epigenetic/genetic changes with these next-gen sequencing data. The key features of our framework are: 1) providing multiple normalization methods to achieve appropriate normalization under different scenarios, 2) using a sequence of three tests to eliminate background regions and to account for different sources of variation in determining statistical significance of changes, 3) allowing adjustment for multiple testing to control false discovery rate or family-wise type I error. We develop a new software tool EpiCenter that can efficiently use our framework to analyze both mRNA-seq and ChIP-seq data. EpiCenter can perform multiple data analytic tasks including: 1) detecting changes in epigenetic marks, 2) identifying differentially expressed genes, 3) finding transcription factor binding sites, 4) converting multiple-sample sequencing data into a single read-count data matrix. EpiCenter also supports major read alignment formats including SAM, BAM, ELAND export, and MAQ. By simulation, we show that our method robustly achieves a low false discovery rate in detecting differential change, and performs markedly better than some existing tools. Through two real examples, we demonstrate the features and effectiveness of our method and highlight the differences in analysis between mRNA-seq and histone ChIP-seq data. Our software EpiCenter is freely available at http://www.niehs.nih.gov/research/resources/software/epicenter.

816W

Joint genome assembly of populations, and potential impact on personal genomes. *Z. Iqbal[1], G. McVean[1,2].* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 2) Department of Statistics, University of Oxford, South Parks Road, Oxford, United Kingdom.

The need for high quality assemblies of individual human genomes from high throughput whole genome shotgun sequence data is a major challenge for the genetics community. Standard assembly tools use large amounts of sequencing data from a single individual. This approach is partially inherited from the time when sequencing multiple individuals was prohibitively expensive, but also reflects the computational complexity of existing algorithms, and the lack of data structures to handle multiple genomes. In the light of low cost sequencing, it is worthwhile revisiting the potential benefits of assembly in a population context. We have previously demonstrated that it is possible to incorporate the sequence data of up to ten human individuals in a single data structure (a de Bruijn graph) in which variants and paralogs produce similar motifs. We have also shown that using a probabilistic model based on the graph, it is possible to calculate genotype likelihoods even for variants that create highly complex and overlapping paths in the graph. We have now extended our methods in two directions. Firstly, we have made computational advances enabling joint assembly of 200 human genomes on a single large-memory (256Gb RAM) server. Secondly, we now incorporate population information into our models. We show with simulated data that using information on contig-sharing between samples allows one to to distinguish paralogs from variants, by investigating putative segregating sites and comparing likelihoods under a repeat-model and a polymorphism-model. This opens the door to integrating new constraints into genome assembly which should be informative even in circumstances when standard approaches (longer reads, longer insert libraries) struggle. For typical assemblers, contig generation is based on navigation through the graph structure of a single individual, and mistakes can occur when decisions need to be made at junctions. In that context, polymorphisms are not informative, but play the role of errors. However if one examines an individual in their population context, LD between SNPs on either side of a junction should allow the assembler to distinguish between local sequence and distant paralogs. Beyond this, more sophisticated incorporation of LD and haplotype structure promise even greater ability to resolve allelic structure. We will discuss quantitative aspects of the benefit of incorporating population information into personal genome assembly.

817W

Functional consequences of human genetic variant covariance. *M. Kaganovich, M. Snyder.* Genetics, Stanford University , Palo Alto, CA.

Much effort is currently being expended to document human genetic variation. As DNA sequencing technology continues to advance, an ever-increasing amount of genetic variation will be documented. We investigate whole genome sequencing data from the 1000 Genomes Project, and other sequencing efforts, to better understand covariance of rare variants. The ongoing advances in “phasing” (i.e. the identification of haplotypes; variants stringed together on the same chromosome), make it possible to calculate the frequency of correlation among many of the millions of variants identified so far. We utilized this to identify highly correlated or highly anti-correlated variants, which we term complements and substitutes, respectively. Substitutes are compensatory in relation to each other, whereas complements may work in concert as a compensatory allele relative to alternative alleles. To better understand the functional consequences of complement and substitute variants we intersected them with existing genomic annotation data. We found evidence of predicted Transcription Factor binding site loss, appearance, and turnover. Understanding the covariance matrix of human genetics is key to identifying the basis vectors of the space, which in turn is necessary for performing dimension reduction that is required to interpret phenotype-genotype association experiments. The presence of correlated predictor variables in regression analysis diminishes the contribution of any one predictor in determining the response. Hence, the recalculation and analysis of the covariance matrix of continuously updated publicly available phased genomic sequence variation is essential for understanding the relationship of genotype to phenotype. Our work provides a new approach for categorizing compensatory and non-compensatory mutation by calculating genomic covariance.

818W

Using LifeScope™ software through cloud computing to analyze targeted resequencing enrichment data. *D. Leon.* Biological Information Systems, Life Technologies, Foster City, CA.

Investigators who use second generation sequencing technologies for their research often need to analyze millions of sequence reads, which requires huge computing capabilities. Hence, purchasing powerful compute clusters and hiring additional system administrators can be costly for a small laboratory. An alternative for researchers who do not want to implement a computational infrastructure for their data analysis needs is cloud computing. To demonstrate the practical and reliable workflow of using cloud computing, we investigated the transferring and loading of data into a cloud environment, used the mapping and data analysis workflows of LifeScope™ Genomic Analysis Software and reviewed the final results for biologically relevant information. The targeted resequencing data from a human genomic sample that were analyzed contained 2.8 Million reads where each was 50 bases in length. The complete targeted resequencing workflow included pre-processing with SAET to improve mapping, mapping the sequencing reads to the human genome (Hg18 assembly), performing SNP calling (with the DiBayes algorithm), comparing against dbSNP with the targeted regions, and using annotation to identify genes associated with these SNPs. The mapping and the additional analyses required a processing time of less than 24 hours. Statistics were generated from the BAM file, and information about the target enrichment was reported through the graphical user interface. Heterozygous and homozygous SNPs were reported and were automatically correlated to known gene regions in the human genome. Analyzing this targeted resequencing data showed that using cloud computing for next generation sequence data analysis is a viable, reliable and efficient approach for focused, research projects. In terms of end-user experience, it was concluded that the cloud option was similar to running an on-site compute cluster.

819W

Common SNPs in Expression Microarray Probes Impact eQTL Studies. *C. Liu[1], C. Zhang[1], K. Zhang[2], c. Chen[1], E. Gamazon[3], X. Zhang[3], J.A. Badner[2].* 1) Dept of Psychiatry, Univ of Illinois at Chicago, Chicago, IL; 2) Dept of Psychiatry, Univ of Chicago, Chicago, IL; 3) Dept of Medicine, Univ of Chicago, Chicago, IL.

The hybridization of expression microarray probes to their targets can be affected by genetic variants within these probes, particularly single nucleotide polymorphisms (SNPs). Therefore, gene expression data produced by oligonucleotide microarrays with SNP-containing probes could be inaccurate. Previous reports that the Affymetrix Human Exon Array contained many SNPs did not differentiate between common and rare SNPs, and the impact of SNP-containing probes on analysis of expression quantitative trait locus (eQTL) is unknown. Using the latest HapMap and 1000 Genomes Project data, we evaluated common SNPs of three major populations (of European, Asian and African ancestry) in the three most recent Affymetrix expression arrays (Human Gene 1.0 ST, Human Exon 1.0 ST and Human Genome U133 Plus 2.0) and two Illumina BeadArrays (Human RefSeq-8 and HumanHT-12). We found that all five arrays had a small proportion of probes containing common SNPs. These probes are scattered across up to 50% of the transcripts that Affymetrix arrays target and up to 8% of genes that the Illumina arrays target. We further examined the effects of SNP-containing probes on eQTL results using two sets of Human Gene 1.0 ST data. Removal of SNP-containing probes reduced the numbers of eQTLs by 3-6% for transcript-level analysis and 20-64% for exon-level analysis. Therefore, removal of SNP-containing probes is a more critical step for exon-level eQTL analysis. To facilitate SNP-free microarray expression analysis, lists of affected probes in the five microarrays/Beadarrays and associated library files are provided at our website: http://bioprogramming.bsd.uchicago.edu/SNPsInProbes.jsp.

820W

dbNSFP 1.1: An integrated database of human non-synonymous substitutions and their functional predictions. *X. Liu, X. Jian, E. Boerwinkle.* Human Genetics Center, School of Public Health, The University of Texas Health Science Center at Houston, Houston, Texas.

dbNSFP (database for non-synonymous SNPs' functional predictions) is an integrated database of functional predictions from multiple algorithms for the comprehensive collection of human non-synonymous SNPs (nsSNPs). It compiles predictions and scores from four new and popular algorithms (SIFT, Polyphen2, LRT and MutationTaster), along with a conservation score (PhyloP) and other related information, for every potential nsSNP in the human genome (a total of 75,931,005). Functional prediction (of being deleterious) for nsSNPs plays an important role in exome sequencing based study of human diseases, especially rare Mendelian diseases. The main purpose of this database is to facilitate the tedious and time consuming process of querying predictions from different databases/web-servers for different prediction algorithms.

For each nsSNP, the original dbNSFP has entries of genomic coordinates based on human reference hg18 and hg19, gene name, gene Entrez ID, CCDS ID, reference codon, variant position on the codon (1, 2 or 3), degenerate type (0, 2 or 3), AA position as to the protein, coding sequence (CDS) strand, and estimated nonsynonymous-to-synonymous-rate ratio. Version 1.1 added the following entries: rs numbers from UniSNP (a cleaned version of dbSNP build 129), allele frequency recorded in dbSNP, allele frequency reported by 1000 Genomes Project, alternative gene names, descriptive gene name, and database cross references (gene IDs of HGNC, MIM, Ensembl and HPRD). This will further accelerate the filtering/prioritizing nsSNPs based on their novelty or allele frequencies as well as gene information. dbNSFP along with a companion search program written in Java is freely available for download at http://sites.google.com/site/jpopgen/dbNSFP.

821W

Novel Adipose and Blood Transcriptomic Signatures of Evoked Inflammation in a Healthy Human Subject. *Y. Liu[1], J.F. Ferguson[2], B. Keating[3], Yi. Guo[3,4], B.D. Gregory[5], D.J. Rader[2], M. Li[1], M.P. Reilly[2].* 1) Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA; 2) Cardivascular Institute, University of Pennsylvania, Philadelphia, PA; 3) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA; 4) Beijing Genomics Institute, Shenzhen, China; 5) Department of Biology, University of Pennsylvania, Philadelphia, PA.

Chronic activation of innate immunity and insulin resistance are major pathogenic pathways in diet-induced obesity, type 2 diabetes and atherosclerotic cardiovascular disease. Inappropriate inflammatory response to diet and lifestyle factors in genetically prone individuals may contribute to disease initiation and clinical complications. However, little is known for the modulation of the transcriptome by inflammatory atherogenic stress in disease relevant human tissues. Here we utilize a low dose endotoxemia model (LPS) to study inflammatory metabolic and atherogenic perturbations in human adipose and blood tissues. We applied RNA sequencing to a Caucasian female individual to identify novel adipose and blood transcriptomic signatures that may be directly relevant to human cardiometabolic diseases. On average, we generated ~10 million 75 bp paired-end reads for each of the four RNA-Seq samples (adipose and blood, before and after LPS). We aligned and processed sequence reads using programs Tophat, SAMTOOLs and Cufflinks, and then searched for tissue specific and LPS induced transcriptomic variations. First, we tested for differential expression before and after LPS and found 1,857 and 1,389 transcripts are differentially expressed at 5% FDR level in adipose and blood tissues following LPS. Second, we searched for transcripts with exon specific differential expression before/after LPS or between adipose and blood tissues to detect LPS induced or tissue specific alternative splicing. We recovered PRKAG, a gene that is well-known to be related to cardiometabolic diseases, to show LPS induced alternative splicing in adipose. Third, we identified potentially novel transcripts and segments that overlap with known non-coding RNA (ncRNA), including NEAT1 (suppressed by LPS in blood), MIR374AHG (induced by LPS in adipose) and PPP4R1L (induced by LPS in blood). We are currently undertaking wet-lab experiment to validate our findings. Our results show that RNA-Seq combined with human experimental models is a powerful tool for novel transcriptomic variation discovery of specific relevance to inflammatory and cardiometabolic diseases.

822W

Multifactorial differential expression analysis to account for technical and biological variability in RNA-Seq and digital gene expression data. *D. McCarthy[1], Y. Chen[1,2], E.J.C. de Geus[4], D.I. Boomsma[4], B.W.J.H. Penninx[5], G.B. van Ommen[3], P.A.C. `t Hoen[3], G.K. Smyth[1,2].* 1) Bioinformatics Division, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; 2) The University of Melbourne, Melbourne, Australia; 3) Center for Human and Clinical Genetics and Leiden Genome Technology Center, Leiden University Medical Center, Leiden, Netherlands; 4) Department of Biological Psychology, Netherlands Twin Registry, VU University, Amsterdam, Netherlands;; 5) Department of Psychiatry, Netherlands Study of Depression and Anxiety, VU University Medical Center, Amsterdam, Netherlands.

As the cost of sequencing continues to decrease, larger and more complex sequencing-based gene expression profiling studies become feasible. For many applications, on large and small scales, multifactorial experimental designs are desirable - from studying interactions between multiple treatments to accounting for batch effects. RNA-Seq and digital gene expression (DGE) data exhibit both technical and biological variability. Models for differential expression must account for biological variability and thus the negative binomial distribution has proven successful as a count-based model for differential expression (DE). Previous efforts have focused on two-group comparisons, inappropriate for analyzing multifactorial experiments.

To analyze general multifactorial experimental designs we implemented negative binomial generalized linear models (GLMs) in the R/Bioconductor package edgeR. Using Cox-Reid adjusted profile likelihood approaches, we obtain accurate estimates of the biological coefficient of variation (BCV), even in small samples. Our model separates technical from biological variation in the data. Inspired by empirical Bayes approaches to microarray analysis we share information between genes to improve inference, with smoothed genewise BCV values providing a flexible model for DE. Likelihood ratio tests are used to identify DE genes.

We applied our methods to a large deepSAGE study (94 samples) aimed at identifying possible risk factors for type II diabetes. We were interested to find differences in gene expression between individuals with high and low fasting glucose levels. Distinct sources of variation in the data included sizable biological variability between samples, confounders such as gender and body mass index, and batch effects revealed by novel visualization techniques. Whereas existing analytical tools would be inadequate, our versatile and efficient framework allowed us to account for these sources of variability and enabled a robust DE analysis.

Taking into account technical and biological variability, the edgeR software represents a powerful and flexible modular pipeline for multifactorial statistical analysis of small- and large-scale comparative RNA-Seq and DGE experiments.

823W

Effective Detection of Rare Variants in Pooled DNA Samples using Cross-Pool Tailcurve Analysis. *T. Niranjan[1], A. Adamczyk[1], H. Bravo[2,3], M. Taub[2], S. Wheelan[2,4], R. Irizarry[2], T. Wang[1,5].* 1) The Institute of Genetic Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Biostatistics, Bloomberg School of Public Health, The Johns Hopkins University, Baltimore, MD; 3) Center for Bioinformatics and Computational Biology, Department of Computer Science, University of Maryland, College Park, MD; 4) Department of Oncology, The Johns Hopkins University School of Medicine, Baltimore, MD; 5) Department of Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, MD.

Rare genetic variants of large effect may confer a substantial genetic risk to common diseases and complex traits. There is a considerable interest in sequencing limited genomic regions such as candidate genes and target regions identified by genetic linkage and/or association studies. Next-generation sequencing of pooled DNA samples is an efficient way to identify rare variants in large sample sets. While sample pooling can reduce labor and costs for sequencing, it also reduces the sensitivity and specificity for effective and reliable identification of rare variants. It remains a challenge to solve these problems using currently available computational genomics tools. We developed an effective Illumina-based sequencing strategy using pooled samples and have optimized a novel base-calling algorithm (SRFIM) and variant-calling algorithm, SERVIC^{4}E (Sensitive Rare Variant Identification by Cross-pool, Cluster, Continuity & Tail-Curve Evaluation). SERVIC^{4}E analyzes base composition by cycle (tail-curves) across sample pools and employs multiple filtering strategies, including quality and continuity cluster analysis, average quality filtering, tail-curve filtering, and proximity filtering to accurately identify rare sequence variants. We validated this algorithm using different pool sizes, sequence read lengths, and sequencing chemistries. In one large sample cohort (n=480), we successfully identified 32 coding variants including 14 rare variants across 24 exons present only once. Validation of these variants by Sanger sequencing revealed an excellent detection sensitivity (96.6%) and specificity (92.8%). These algorithms are compared favorably with currently available sequence analysis programs including SAMTools, SNPSeeker, and CRISP for a reliable detection of rare variants in pooled samples.

824W

Transcriptome and epigenome analysis in pancreatic cancer. *H. Parikh[1], J. Jia[1], W. Xiao[2], X. Liu[2,3], I. Collins[1], G. Petersen[4], J. Powell[2], S. Thorgeirsson[5], L. Amundadottir[1].* 1) Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD 20877, USA; 2) Bioinformatics and Molecular Analysis Section, Division of Computational Bioscience, Center for Information Technology, National Institutes of Health, Bethesda, MD 20892, USA; 3) SRA International, Fairfax, VA 22033, USA; 4) Department of Health Sciences Research, Mayo Clinic, Rochester, MN 55905, USA; 5) Laboratory of Experimental Carcinogenesis, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA.

Although pancreatic cancer is the tenth most commonly diagnosed cancer in the U.S., it is the fourth most common cause of cancer mortality with close to 37,000 deaths per year in the U.S. To enhance our understanding of the pancreatic cancer genome we catalogued functional elements and transcribed regions in cell lines derived from normal and neoplastic pancreatic tissues by next generation sequencing analysis. We conducted high-throughput genome-wide transcriptome sequencing (mRNA-seq and miRNA-Seq) for two human cell lines derived from normal pancreatic tissues (hTERT-HPNE) and pancreatic carcinoma (PANC-1). We aligned raw reads to the RefSeq, Ensembl and Human Genome (hg19 build) databases to quantify RNA abundance (using BWA and miRNAkey) and identified a total of 11,248 genes and 216 miRNAs expressed at 1 RPKM or higher in these cell lines. A total of 1,983 genes were differentially expressed at ⁄ 3-fold change (and a FDR < 0.05) in the PANC-1 as compared to the hTERT-HPNE cells. Of these, 971 were expressed at higher levels in PANC-1 cells and 1,012 at lower levels. In addition, we identified 131 miRNAs with increased and 35 miRNAs with decreased expression in the PANC-1 compared to hTERT-HPNE cells. KEGG Pathway enrichment analysis of the differentially expressed genes in the normal vs. cancer derived cell lines identified the following enriched pathways: pathways in cancer, focal adhesion, cell adhesion and Wnt signaling. Moreover, we performed chromatin immunoprecipitation (ChIP) combined with ultra high-throughput sequencing (ChIP-seq) experiments for mono- and tri-methylation of histone H3 at lysine 4 (H3K4me1 and H3K4me3), tri-methylation of histone H3 at lysine 27 (H3K27me3) and RNA polymerase II (Pol II) using chromatin prepared from the same cell lines. The highest density of reads surrounding the TSS for H3K4me1 and H3K4me3 were seen in highly expressed genes and lower levels were seen in genes expressed at medium or low levels. The opposite was seen for H3K27me3 where the highest number of reads was seen at genes expressed at low levels or not at all .Our genome wide transcriptome and epigenome profiling of pancreatic cell lines facilitates integrative analysis of the pancreatic cancer genome and provides understanding of the regulatory mechanisms, genes and pathways involved in this disease.

825W

Phenome-Wide Association Study (PheWAS) for Exploration of Novel Genotype-Phenotype Associations and Pleiotropy using MetaboChip in the PAGE Network. *S. Pendergrass[1,2], E.S. Torstenson[1], J.L. Ambite[3], C.L. Avery[4], C. Cai[3], M.D. Fesinmeyer[6], C. Haiman[7], G. Heiss[4], L.A. Hindorff[8], C.-N. Hsu[3], C. Kooperberg[6], L. Le Marchand[9], Y. Lin[6], T.C. Matise[11], K. Monroe[7], K.E. North[4,5], L.R. Wilkens[9], S. Buyske[10,11], D.C. Crawford[1,2], M.D. Ritchie[1,2].* 1) CHGR, Vanderbilt University, Nashville, TN, USA; 2) Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville TN, USA; 3) Information Sciences Institute; University of Southern California, Marina del Rey, CA, USA; 4) Department of Epidemiology, University of North Carolina, Chapel Hill, NC, USA; 5) Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, NC, USA; 6) Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; 7) Department of Preventive Medicine, Keck School of Medicine, University of Southern California/Norris Comprehensive Cancer Center, Los Angeles, CA, USA; 8) National Human Genome Research Institute, National Institutes of Health, Bethesda, MD USA; 9) Epidemiology Program, University of Hawaii Cancer Center, Honolulu, HI, USA; 10) Department of Statistics, Rutgers University, Piscataway, NJ, USA; 11) Department of Genetics, Rutgers University, Piscataway, NJ, USA.

A novel approach has emerged that combines both the exploration of phenotypic structure and genotypic variation, known as a phenome-wide association study (PheWAS). The Population Architecture using Genomics and Epidemiology (PAGE) network is a collection of diverse, population-based studies with a wealth of phenotypic and genotypic data for characterization and discovery, ideal for pursuing PheWAS. We describe here two initial results of a PAGE PheWAS utilizing the MetaboChip custom genotyping array and PAGE phenotypic data. The MetaboChip contains single nucleotide polymorphisms (SNPs) identified in genome-wide association studies up to 2009 (33.2% of the SNPs on the array) as well as fine mapping SNPs for select metabolic GWAS-identified variants (62.2%) drawn from multiple ethnic groups and the 1000 Genomes Project. Thus far, three groups of the PAGE network, Atherosclerosis Risk in Communities (ARIC), Multiethnic Cohort (MEC), and the Women's Health Initiative (WHI) have genotyped a total of 6,359 African-American individuals with the MetaboChip. The numbers of phenotypes available varied by study: 88 in ARIC, 26 in MEC, and 116 in WHI. After genotyping quality control, tests of association using linear/logistic regression assuming an additive genetic model were performed for all phenotypes and all SNPs on the MetaboChip (161,098 SNPs). All models were adjusted for the first two principal components of ancestry, associations not stratified by gender were adjusted for sex. An example of a potentially novel association identified in our study is the association between fibrin D-dimer and rs79266590 (p=5.10E-13) and rs74022438 (p=4.24E-12), within ARIC for the women only analysis with a sample size of 486. Fibrin D-dimer is typically used to diagnose conditions such as deep vein thrombosis. These two SNPs are in regions near an index SNP previously associated with HDL levels in European descent populations, and rs79266590 is in a proposed regulatory region. Future efforts include expanding this PheWAS to include PAGE populations of different ancestry and incorporation of additional phenotypes. A PheWAS utilizing the unique MetaboChip data has the potential to elucidate a more comprehensive picture of the associations between phenotype and genotype, help discover novel relationships between SNPs, phenotypes, and networks of interrelated phenotypes; identify pleiotropy; provide novel mechanistic insights; and foster hypothesis generation.

826W

Validation and Comparison of Variant Calling Pipelines for Next Generation Sequence. *M. Pirooznia[1], F. Goes[1], J. Parla[2], I. Iossifov[2], R. McCombie[2], J. Potash[1], P. Zandi[3].* 1) Department of Psychiatry and Behavioral Sciences, Johns Hopkins University, Baltimore, MD, USA; 2) Cold Spring Harbor, Woodbury, NY, USA; 3) Department of Mental Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA.

Background: The processing and analysis of the enormous amounts of data generated by Next-generation sequencing (NGS) experiments is challenging and is a burgeoning area of new methods development. Several new bioinformatics tools have been developed for calling variants from NGS data. Here, we validate the variant calling of these tools and compare their relative accuracy to determine which data processing pipeline is optimal. Methods: We developed an efficient pipeline which connects all necessary analysis steps into a unified application to address different tasks in the processing of NGS data. The pipeline uses BWA for mapping sequence reads and then incorporates two parallel approaches for further data processing and calling variants for downstream analyses. One approach uses SAMTools for duplicate removal and calling variants, while the other approach uses PICARD and GATK for duplicate removal, re-aligning reads around indels, re-calibrating quality scores and then calling variants. We processed NGS data generated on 30 bipolar subjects from an on-going whole exome sequencing project using both approaches built into our pipeline. We compared the variant calls between the two approaches and against gold-standard Sanger sequencing of 400 strategically selected variants. Results: The cleaned reads were mapped to the targeted exome sequence of human genome obtaining mean coverage of 97% at 1X, 91% at 10X and 62% at 40X coverage. Using SAMTools, a total of 12,859 variants per subject were identified, including 1,044 novel variants, of which 16 were nonsense and 634 were missense. Using GATK, a total of 13,796 variants were identified, including 1,120 novel variants, of which 17 were nonsense and 680 were missense. A total of 12,700 variants (91%) were called in common between the two approaches, while 159 (1.2%) were called unique to SAMTools and 1096 (7.8%) were called unique to GATK. We selected 400 variants that were called in common and uniquely to the two approaches and validated these in the same subjects using Sanger sequencing. The results of this validation will be reported. Conclusions: We have built a pipeline that incorporates multiple approaches for processing NGS data and calling sequence variants. We compared the results of the variant calls between two commonly used approaches, and against “gold standard” sequencing. The findings will help to determine the best approach to confidently call variants for downstream analyses.

827W

An integrative genomics approach to detect and classify unusual patterns in glioblastoma multiforme. *C. Rangel-Escareño, K. Baca-Lopez, MD. Correa-Rodriguez, R. Flores-Espinosa, R. Garcia-Herrera, CI. Hernandez-Armenta, E. Hernandez-Lemus, A. Hidalgo-Miranda, AJ. Huerta-Verde, I. Imaz-Rosshandler, AV. Mart'inez-Rubio, A. Medina-Escareno, R. Mendoza-Smith, M. Rodriguez-Dorantes, I. Salido-Guadarrama.* INMEGEN, Mexico City, Mexico.

Computational tools and mathematical algorithms to integrate, organize and mine the wealth of information generated from modern high-throughput genomic technologies are constantly created. A 3-state model for multidimensional data integration, which is a data driven approach that not only captures the usual and meaningful hypotheses in cancer biology but also a set of unusual scenarios that could lead to new discoveries is presented. TCGA Glioblastoma multiforme data classified into 4 levels based on the degree of processing were used for this study. A multidimensional integration of 5-Technology platforms (*mRNA,methylation,miRNAs,S.Mutations*, and *CNVs*) as well as clinical data generated genes for our analysis. The N-platform integrative analysis is based on a 3-state model applied to statistically significant genes from each platform. Each state can take values in {-1,0,1} interpreted as {Down,NoChange,Up}. A list of all possible scenarios is generated through a combinatorial approach where each scenario has as many states as platforms are integrated. This list represents the universe of hypotheses that may describe structural variations in the genome as well as transcription activity in coding and non-coding regions. Hypotheses can be chosen for their biological relevance or for their quantitative importance **Results:** Single platform analysis matched results reported in the literature. A 3-platform analysis {Som.Mut, DNAmethylation, mRNA} resulted in 16 possible scenarios. We focused on scenarios with interactions that cannot be easily explained from a biological perspective such as {0,1,1} the case of a 30-gene set with a particular pattern of promoter hypermethylation and over-expression with no mutations present. Mapping those genes to CNVs and miRNA targeting genes showed significant correlation of over-expression with CNV amplification, emerging as a possible explanation for the overexpression seen even in the context of promoter hypermetylation. No miRNAs active for them. Some genes for which no CNV was detected were targeted by differentially expressed miRNAs suggesting a kind of regulation of gene expression mainly directed by miRNAs. More complex scenarios arise from this analysis, like the one in which deletions are detected, yet genes are upregulated and hypermethylated. Nevertheless, this small set of genes though possible false positives should help to define a better threshold for differential expression improving classification accuracy.

828W

Robust statistical methods for genome-wide eQTL analysis. *M. Rantalainen[1,2], C. Holmes[1,3].* 1) Department of Statistics, University of Oxford, Oxford, United Kingdom; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 3) MRC Mammalian Genetics Unit, MRC Harwell, Harwell, OX11 0RD, United Kingdom.

Expression Quantitative Trait Loci (eQTL) analysis enable characterization of how common genetic variants influence the expression of individual genes. EQTLs are commonly analysed using a linear regression model including relevant covariates, an additive genetic effect and assuming a Gaussian error term. However, Gaussianity may not hold in noisy biological data (e.g. gene expression), which may be more heavy-tailed or have outliers present. Such departures from model assumptions may result in an increased rate of both type I and type II errors. Careful model checking can reduce the risk of spurious findings, however, this may be prohibitively expensive in in the case of genome-wide eQTL analysis with a high number of models. Therefore, robust statistical methods, which are less sensitive to departures from assumption of Gaussianity and presence of outliers, provide an attractive alternative for eQTL analysis. In this study we compare eQTL results between the conventional linear model and a robust alternative (M-estimator) in two publicly available eQTL data sets and in a simulation study in respect to bias in estimates, concordance of hypothesis test results and statistical power. Our results indicate that robust statistical models provide more reliable eQTL results over conventional linear models with reduced number of both type I and type II errors. This suggests that unless careful model checking can be carried out on each evaluated model, robust alternatives provide a valuable alternative to conventional linear models in genome-wide eQTL analysis.

829W

Meta-analysis of multiple expression quantitative trait locus (eQTL) datasets results in large gains in regulatory variant identification. *A.J. Rogers[1,3], J. Lasky-Su[1,3], B.E Himes[1,3], T. Raj[2,3], N. Morar[4], M.F. Moffatt[4], V.J. Carey[1,3], B. Stranger[2,3], L. Liang[5], B.A. Raby[1,3].* 1) Channing Laboratory, Brigham & Women's Hospital; 2) Department of Genetics and Genomics; 3) Harvard Medical School, Boston MA; 4) National Heart and Lung Institute, Imperial College London, London, UK; 5) Departments of Epidemiology and Biostatistics, Harvard School of Public Health, Boston, MA.

Rationale: Whole genome association studies for gene expression quantitative trait locus (eQTL) mapping have identified genetic variants related to thousands of genes on the genome, but a complete genetic map for most genes remains far from clear, especially for trans effect with modest effect size. Meta-analytic approaches offer an opportunity to identify additional important regulatory variants from existing data.

Methods: We performed an eQTL meta-analysis of 3 Caucasian datasets: expression profiles derived from lymphoblastoid cell lines (LCLs) from CEU founders from HapMap (N=110), LCL from 550 siblings identified from a asthma proband (MRCE), and primary peripheral blood CD4+ lymphocytes from 200 asthmatics in the Childhood Asthma Management Program (CAMP). With MaCH, we imputed SNP genotypes for ~8 million SNP using 1000 Genomes Project data. EQTL testing was performed for a subset of 8080 genes that were represented on both the Illumina Human Ref8-v1 and v2 arrays. Expression data was adjusted for PCA-derived eigenvectors to diminish the influence of non-genetic technical biases. For each population, regression models were adjusted for population stratification using Eigenstrat when appropriate. We then tested for eQTLs by combining $\chi 2$ statistics across all 3 populations and account for multiple testing using FDR < 1%.

Results: Meta-analysis resulted in substantial gains in the regulatory variants identified. In the individual cohorts, we identified cis eQTLs for 67 to 2981 genes within 50kb (at an FDR cut-off of 1% estimated within each dataset). In contrast, by combining eQTL measurements over 3 populations, we found cis-eQTL for 3969 unique genes (nearly 50% of the 8080 genes tested). eQTLs for 1419 genes were shared between CD4 and LCL, 378 are seen only in meta-analysis, and 2127 (53%) appear to be tissue specific, with 1307 in CD4 only, 865 in LCL.

Conclusion: eQTL analyses are increasingly recognized as a way of identifying important intermediate phenotypes in disease pathogenesis. We demonstrate a novel methodology of efficiently combining data across multiple populations to improve power to identify associations. These tools will be available as a Bioconductor package.

830W

A visualization platform for interpretation of structural genomic data. *T. Sante, S. Vergult, B. Menten.* Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium.

Working with next generation sequencing technology such as mate-pair sequencing, is a challenge. These new high-throughput sequencing techniques allow the detection of large and small CNVs, inversions, translocations and complex rearrangements at unprecedented resolutions. The growth in data size is accelerating greatly as the sequencing technology is becoming the tool of the future in genetic diagnostics and research, generating an ever increasing amount of sequence reads. The ability to visualize this data, to aid analysis, is a challenging but essential problem. We present a visualization platform which tackles that problem by developing a graphic web interface that allows the researcher to visually explore genomic microarray data and mate-paired data, and turn abstract data files into useful information. The platform provides a genome browser to inspect the copy number profile, supporting both CNV information from genomic microarrays, sequencing depth of coverage data and MatePair/PairedEnd-data. The browser uses a zoomable sliding window to explore the data at different resolution levels, and allows easy comparison of multiple samples. A circular view is available to facilitate comprehension of intra- and interchromosomal translocations. Experimental data can be complimented with a multitude of annotation tracks, to guide the interpretation. Segmental duplications and human chained self-alignments provide information on genomic architecture, RefSeq genes track place the data in its local genetic context, OMIM morbid/gene-map and database of genomic variants track provide a biological/clinical context. The custom tracks can be used to compare individual results with existing data collections of experiments to find correlations in your internal datasets. The platform employs the latest web technologies so it can be used in any modern web browser. The visualizations use the scalable vector graphics format for optimal image quality. Selected regions of interest can be downloaded as SVG or PNG files. The platform supports genomic microarrays (Agilent, Affymetrix, Illumina, Nimblen) and MatePair/PairedEnd sequencing experiment data (BAM/SAM, read clusters) input. Our experiences as a medical genetics center, enabled us to deliver a comprehensive visualization platform for genomic microarray experiments complemented with MatePair/PairedEnd sequencing data, and can be of great value in the toolbox of any investigator, research or clinical diagnostics.

831W

Twins, tissue and time: A comparison of genomic inference across monozygotic twins, DNA sources and longitudinal samples. *P. Scheet[1], E.A. Ehli[2,3], X. Xiao[1], A. Abdellaoui[4], R.R. Althoff[5], J.J. Hottenga[4], K.A. Nelson[2], P. Huizenga[2], Y. Hu[2], M. Bartels[4], M.M. Goen-Blokhis[4], E. de Geus[4], J. Hudziak[5], G.E. Davies[2,3], D.I. Boomsma[4].* 1) Dept. of Epidemiology, University of Texas M. D. Anderson Cancer Ctr, Houston, TX; 2) Avera Institute for Human Behavioral Genetics, Avera McKennan Hospital and University Health Center, Sioux Falls, SD; 3) Dept. of Psychiatry, University of South Dakota, Sanford School of Medicine, Division of Basic Biomedical Sciences, Sioux Falls, SD; 4) Dept. of Biological Psychology, Vrije Universieit, Amsterdam, The Netherlands; 5) Dept. of Psychiatry, University of Vermont College of Medicine, Burlington, VT.

With the desire to assess genetic variation with conveniently-obtained biological sources in large scale collaborative projects, one question is whether inference of copy number (CN) is overly sensitive to the source of material for DNA analysis (e.g. blood, buccal) or whether CN is stable over time. Here, we address these questions by analyzing data from the Affymetrix SNP 6.0 microarray applied to multiple samples from 705 individuals of the Netherlands Twin Register. We applied Birdsuite v.2 to make genome-wide CN calls, then assessed CN (and genotype) correspondence. To assess biological source consistency, we compared results from blood and buccal derived DNA in 371 individuals, including 43 monozygotic (MZ) twins. For each pair of samples, we calculated the square of the Pearson correlation coefficient (R^2), which is sensitive to deviations in CN calls in the presence of the large number of copy-neutral calls made across each individual. We found no greater discordance in CN or genotype inference between samples from different sources (blood, buccal) than within (e.g. both samples from blood). We also evaluated evidence of a temporal effect in 389 individuals that were sampled at 2 time points (over 1-12 years), and found no trend; ie. replicates collected 7 or 8 years apart were no more different than were samples collected at 2-year intervals. Copy number estimates between MZ twins are highly consistent for both deletions (R^2 _ 90%) and duplications (R^2 _ 86%), mirroring results from within-individual duplicate samples. We also assessed SNP genotype concordance between MZ twins and sample duplicates and found these to be highly consistent, (R^2 > 99%). Our experiment suggests that buccal-derived DNA is suitable for the current generation of microarray technologies, facilitating greater participation in genetic studies in a cost-effective manner.

832W

The Importance of Variation Databases in Interpretation of Exome Sequencing. *T.E. Scheetz[1,2], A.P. DeLuca[2], T.A. Braun[1,2], L.M. Streb[1], L.M. Affatigato[1], V.C. Sheffield[1,3,4], E.M. Stone[1,4].* 1) Dept Ophthalmology, Univ Iowa, Iowa City, IA; 2) Dept Biomedical Engineering, Univ Iowa, Iowa City, IA; 3) Dept Pediatrics, Univ Iowa, Iowa City, IA; 4) Howard Hughes Medical Institute, Iowa City, IA.

Determining which of the thousands of variations identified in exome sequencing projects represent the bona fide disease-causing mutations is a challenging process. Experiment design can aid in refining this list of variations by exploiting population structure (e.g. consanguinity) or familial segregation. However, experiment design alone cannot refine the set of variations to the disease-causing mutation(s). To supplement such designs, we have developed an analysis pipeline to exclude from consideration those variations that are inconsistent with ability to cause the disease. Disease prevalence can be used to place an upper limit on the minor allele frequency of a disease causing variation. For an autosomal recessive disease with a prevalence of 1:1000, any variant present at greater than 1% frequency is incompatible with a Mendelian cause of the disease. If the disease under investigation is genetically heterogeneous, the critical allele frequency would be even lower. This practice is critical, as many disease-causing variations are present in dbSNP - making exclusion of all dbSNP variations inappropriate. Multiple exomes were sequenced as part a disease gene identification project. Analysis of these exomes using BWA and GATK identified approximately 20,000 variations per sample. We sequentially applied multiple variations sets, to remove variations observed at population frequencies incompatible with Mendelian disease. These variation sets included dbSNP as well as sets derived from previous exome capture datasets (1000 genomes, LuCamp) that we reprocessed locally to avoid bias due to differing analysis pipelines. The reduction in the number of variations is dramatic, but does exhibit diminishing returns as additional filters are applied. The removal of incompatible variants based upon dbSNP, 1000 genomes and LuCamp reduced the average number of compatible variants from 20,000 to 3,000. Interestingly, each data set contributed substantially to the process, with a shared core of common SNPs shared in all three resources, as well as hundreds of novel SNPs in each. A further comparison to exomes sequenced locally was also performed, resulting in a final set of 2,000 variations. In conclusion, a combination of intelligent experimental design, identification of SNPs compatible with the disease model, and variation prioritization methods are required to make full use of exome and genome scale data.

833W

Comprehensive genomic analysis of tandemly repeated genes. *A. Sharp, M. Brahmachary, A. Guilmatre, C. Borel, F. Cheung, P. Warburton.* Genetics & Genomics Sci, Mount Sinai Sch Med, New York, NY.

Tandem repeats (TRs) represent a highly variable fraction of the human genome, but due to their multi-copy nature are poorly assayed by most genome-wide technologies. The human genome contains many entire genes that occur in highly polymorphic tandem arrays, and CNV of several TR genes, such as) -*defensin*, *CCL3L1* and salivary amylase, has already been implicated in human phenotypes. We identified 180 true multicopy genes by performing a self-join operation using Refseq to identify genes with >1 copy at a non-overlapping position on the same chromosome. We designed a custom Nanostring probeset to perform digital genotyping of copy number for these 180 genes, and 12 non-coding large tandem repeats, in 165 HapMap individuals and 5 primate species. We observed extreme variability in copy number of TR genes: 66% showed CNV in the HapMap, with many genes showing 5-10 fold variation in copy number between different individuals. In the most extreme case we observed 100-250 copies of *REXO1L2P* in humans, and >900 copies in Gorilla. However, this gene is only present in 4 copies in hg18. Comparison of TR copy number with flanking SNPs showed that the vast majority of TR genes show little or low LD with nearby SNPs, and are therefore invisible to standard GWAS approaches. We also identified 51 genes whose expression levels correlated with the size of nearby TRs. In several cases we observed an inverse correlation between TR size and the expression level of adjacent genes. We hypothesize that this represents an epigenetic mechanism termed repeat-induced silencing in which expanded TRs become heterochromatic, repressing flanking genes. Consistent with this we observed highly variable DNA methylation at these TRs. We also observe multiple signatures of rapid evolution in TR genes, suggesting important biological function, including i) highly divergent copy numbers between different ethnicities; ii) extreme evolutionary changes in copy number, with many TR genes showing >3-fold change in copy number versus other primate species, and iii) a significant excess of non-synonymous amino-acid changes in recent primate evolution compared to single copy genes. We conclude that TR genes represent a highly variable and biologically important fraction of the genome. We hypothesize that TR genes will be highly enriched for phenotypic effects, potentially explaining some of the `missing heritability' of the genome. Our studies provide many novel insights into the biology of TRs.

834W

Extensive evolutionary changes in regulatory element activity during human origins are closely associated with altered gene expression and positive selection. *Y. Shibata[1], N. Sheffield[1], O. Fedrigo[2], C.C. Babbitt[2], M.J. Wortham[1], D. London[1], L. Song[1], A.K. Tewari[1], S.C. Parker[3], E.H. Margulies[3], G.A. Wray[1,2], T.S. Furey[4,5], G.E. Crawford[1,6].* 1) Institute for Genome Sciences & Policy, Duke University, Durham, North Carolina, 27708, USA; 2) Biology Department, Duke University, Durham, USA; Institute for Genome Sciences & Policy, Duke University, Durham, USA; 3) Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland 20892, USA; 4) Department of Genetics, Carolina Center for Genome Sciences, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA; 5) Department of Biology, Carolina Center for Genome Sciences, and Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA; 6) Department of Pediatrics, Duke University, Durham, North Carolina, 27708, USA.

Understanding the molecular basis for phenotypic differences between humans and other primates remains an outstanding challenge. Mutations in non-coding regulatory DNA that alter gene expression have been hypothesized as a key driver of these phenotypic differences. Differential gene expression analyses support this hypothesis but have failed to pinpoint specific regulatory elements responsible for changes in transcription. To identify the genetic source of these regulatory differences, we mapped DNaseI hypersensitive sites (DHS), which mark all types of active gene regulatory elements, genome-wide in the same cell type isolated from human, chimpanzee, and macaque. Most DHS sites were conserved among all three species, as expected based on their central role in regulating transcription. Strikingly, however, several hundred DHS sites were apparently gained and lost on the lineages leading to human and chimpanzee using macaque as an outgroup. Species-specific DHS site gains are enriched near differentially expressed genes and are positively correlated with transcription, consistent with the hypothesis that they have functional consequences. In addition, newly evolved DHS sites showed evidence of branch-specific positive selection, as expected of regions that have acquired novel regulatory functions. These results implicate a set of several hundred regulatory elements as contributing to the genetic basis for phenotypic differences among primate species.

835W

Limitations of the human reference genome sequence. *T. Smith[1], N. Olson[1], J. Rosenfeld[2], C. Mason[3].* 1) Geospiza ((PerkinElmer) Inc. Seattle, WA 98119, USA; 2) 2. Sackler Institute for Comparative Genomics, American Museum of Natural History, New York, NY 10024, USA; 3) Weill Cornell Medical College, New York, NY 10021, USA.

The human genome reference sequence is well characterized, highly annotated, and its development represents a considerable investment of time and money. This sequence is the foundation for genotyping microarrays and DNA sequencing analysis. Yet, in several critical aspects it remains incomplete. Further, when the reference is reexamined with data available from the 1000 Genomes Project (1Kg) and Complete Genomics (CGI) genomes, it is clear that previous assumptions, based on tools and analyses guided by a single reference, about human genomics and genetics are incorrect.

We have used the 1Kg and CGI genome datasets to evaluate the increased knowledge of human variation. By analyzing identified variants within the interrogated regions of microarray probes, we have found that about 50% of probes on commonly used arrays contain confounding variation, which impact the results of 37% of GWAS studies to date. This confounding variation includes unknown variants in close proximity to the probed variant and alleles previously assumed to be di-allelic that are poly-allelic. These data also change previous assumptions regarding linkage disequilibrium (LD) between variants, which have been used to impute variation in incomplete data. When HapMap data are compared to 1Kg data, mean LD decreases from 16.4 Kb to 7.0 Kb within common samples and decreases to 5.4 Kb when random samples are compared. While many of the observations have been anecdotally understood, quantitative assessments of resources based on the reference sequence have been lacking. These findings have implications for the study of human variation and medical genetics, and ameliorating these discrepancies will be essential for ushering in the era of personalized medicine.

We investigate various solutions to these discrepancies regarding the reference genome. Some have suggested the use of either a graphical representation of the genome to allow for variation, or for population-based reference sequences, yet neither of these approaches are sufficient to encapsulate the variation with the human population. We suggest that the for loci of clinical importance, strong effort should be undertake to determine all forms of variation and evaluate whether the current genotyping techniques produce accurate results.

836W

***In silico* analyses of promoter regulatory targets in the iron metabolism pathway.** *N.J. Strickland, M.G. Zaahl.* Genetics, Stellenbosch University, Stellenbosch, Western Cape, South Africa.

AIM To investigate the promoter region of genes involved in the iron metabolism pathway by employing comprehensive bioinformatic analyses, in order to elucidate specific mechanisms of gene regulation. INTRODUCTION The human genome is a system regulated at many different levels. Transcriptional regulation is the first, and arguably the most important, step in the process of gene expression. This process is governed by the presence of specific *cis*-regulatory regions (*cis*-motifs) residing within the promoter region of genes and the functional interactions between the products of specific regulatory genes (transcription factors-TFs) and these *cis*-motifs. Bioinformatic tools can be utilised to formulate putative predictions on how specific *cis*-motifs may influence the expression patterns of specific genes or groups of genes (e.g. iron metabolism pathway). METHOD •Retrieve the DNA sequence of the promoter region (±2 kb) of 16 genes known to be involved in the iron metabolism pathway from the human Ensembl database. •Conserved Nucleotide Sequence (CNS) analysis of promoter regions using specific software tools (e.g. VISTA). •Computational analysis and *in silico* design of promoter models using probabilistic detection methods such as expectation maximization (MEME). •Refined analysis and identification of detected motifs analyzed using TRANSFAC and JASPAR. RESULTS The promoter sequences of nine of the 16 genes when examined were found to contain a genomic region that demonstrated over 75% sequence identity between the genes of interest. This conserved region (CNS) is approximately 140 bp in size and is common to each of the promoters of the nine genes. This finding adds strength to the hypothesis that genes with similar promoter architecture, and involved in a common pathway, may be co-regulated. Within these motifs, relevant tissue- and pathway-specific TF-binding sites were predicted to occur. Several TFs of interest were selected on the basis of their known (and previously described) functions in transcriptional regulation, tissue specificity and involvement in iron metabolism in order to assess their proposed functionality in vitro. CONCLUSION Accurate analyses of *cis*-motif architecture combined with integrative *in silico* modelling (once validated by experimental analysis) could offer insights into complex mechanisms governing transcriptional regulation, serving as a refined approach for prediction and study of regulatory targets.

837W

The eXtensible SeQuence file format: a better standard for sequencing reads. *D. Thomas, C. Yang, M. Muller, S. Utiramerur, J. Zhang, P. Suri, A. Siddiqui.* Life Technologies, Foster City, CA.

The growth of sequencing technologies has put significant pressure on data management and analysis, reinforcing the need for standards to support workflows in genomics. The primary concerns are currently i) file size required to store large datasets, ii) access to content within large files, and iii) support for associations between data elements. We have developed the eXtensible SeQuence (XSQ) file format to address these issues and are developing tools to support its use. The space saving advantage is derived from a binary encoding scheme that uses a single byte per color or base space call. Two bits are used for the call and the remainder for storing Phred scale quality values and flags for error states. This produces up to a 75% reduction in file sizes, which benefits storage and transmission needs and improves analysis times. The XSQ file format is based on the stable Hierarchical Data Format (HDF), which provides structures and tools for storing and accessing data stored in an extensible hierarchy. The HDF platform allows metadata to be stored in the same file as the data itself, and allows multiple data vectors to be present for a given fragment. Sets of data from a single fragment, such as forward and reverse reads, can be stored in parallel datasets in separate groups so that pairing is eliminated subsequent to mapping. For a single tag, reads of multiple encodings - e.g. 2 base color encoding, 3 base color encoding, and base space encoding - can be read in parallel to support Exact Call Chemistry datasets. To drive adoption, a set of utilities - XSQ Tools - is provided to ease transition to the new format. Standalone tools have been developed i) to convert legacy data (csfasta/qual) into XSQ for analysis in new pipelines, and ii) to convert XSQ into those same formats or fastq to support in older pipelines. We are also working with other platforms to expand this format into a community standard, focusing initially on flowspace data and later to include arbitrary basespace data. File format descriptions and converters are available for download here: http://solidsoftwaretools.com/gf/project/xsq/.

838W

Yet another pipeline for the next generation sequencing analysis. *L. Tian, H. Hakonarson.* Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA.

We have designed a bioinformatics pipeline to process next generation sequencing data from (1) whole genome; (2) whole exome; (3) targeted resequencing with multiple barcoded or non-barcoded samples; (4) multi-sample low-pass whole genome; (5) RNA-Seq transcriptome. Our pipeline integrates open source programs, including Bowtie, BWA, BLAT, RUM, TopHat, SpliceMap, GATK, SNVer, ANNOVAR, VAAST, PLINKSEQ, dbNSFP, into 5 stages: (1) multi-pass aligning of NGS sequencing data to the reference genome; (2) refinement of the alignment, including indel realignment, base quality recalibration; (3) genetic variant calling, including SNV, indel, and CNV; (4) variant functional annotation; (5) variant selection and prioritization based on predicted functions, family structure, rare variant test score, expression abundance, or RNA-editing events. We describe the development of the pipeline and discuss the bioinformatics challenges posed by the NGS, including data storage, variant calling, and the application of the pipeline in several Mendelian and complex diseases projects.

839W

The importance of reproducible research and genome geography. *L.K. Vaughan, V. Srinivasasainagendra.* Section on Statistical Genetics, Dept Biostatistics, Univ Alabama, Birmingham, Birmingham, AL.

With the advent of high throughput data technologies such as expression microarrays, SNP chips and genome wide sequencing, the volume and breadth of data available has become staggering. In addition, databases such as dbSNP, GEO (Gene Expression Omnibus), KEGG (Kyoto Encyclopedia of Genes and Genomes), GO (Gene Ontology) and Entrez Gene, which provide resources to annotate, translate and connect biological data, have grown exponentially in content. The availability of such data emphasizes the importance of bioinformatics and computational biology in genetics research and has led to the development of thousands of tools that have been (and are being) developed to integrate and utilize these databases. An important concept in bioinformatics is the idea of reproducible research, which is defined by the uniform Guidelines of the International Committee of Medical Journal Editors as the responsibility of authors to “identify the methods, apparatus and procedures in sufficient detail to allow other workers to reproduce the results”. One detail that is often neglected, and has the potential to have a serious impact, is the version of different databases used for a project. This can be particularly important when exploring genome geography, identifying SNPs in candidate genes or mapping genes to markers. Here we present a timeline for version releases of the Human Genome/NCBI, dbSNP and UCSC genome databases. We also provide selected case studies identified while 1) identifying SNPs in candidate genes and 2) mapping SNPs identified in a GWAs to genes which illustrate potential sources of error which can be introduced when non-concurrent databases are employed. We also show examples using popular bioinformatic tools of a lack of transparency where these databases are concerned. These examples include changes in gene symbols/synonyms, changing gene boundaries and inconsistent mapping across databases. Through these examples we show that resources are constantly evolving and in order to provide reproducible results, researchers should be aware of and connected to the correct version or release of the data, particularly when implementing computational tools.

840W

The NCBO Annotator and enrichment analysis with the Human Disease Ontology. *P. Whetzel, P. LePendu, R. Fergerson, M. Musen, N. Shah.* Stanford University, Stanford, CA.

Researchers have turned to the Semantic Web to annotate and integrate disparate knowledge. Ontologies provide the domain knowledge to drive these processes and the successful creation of semantic applications in the healthcare and life sciences require Web services that provide access to ontologies. The National Center for Biomedical Ontology (NCBO), one of the eight National Centers for Biomedical Computing created under the NIH Roadmap, developed BioPortal (http://bioportal.bioontology.org), which provides access to one of the largest repositories of biomedical ontologies. This ontology content is programmatically accessible via a suite of Web services for use in applications, such as data annotation to natural language processing. The NCBO Annotator Web service (http://www.bioontology.org/wiki/index.php/Annotator_Web_service) tags textual metadata submitted through the Web service (e.g., text of interest to the user) with ontology terms from BioPortal. Users can customize the Web service to limit results to a particular ontology (e.g. the Gene Ontology or SNOMED CT) or to a certain UMLS semantic type (e.g. T017 for `Anatomical Structure') as well as many other parameters. The ease of access to this annotation functionality makes the task of creating ontology-based annotations accessible for any biomedical researcher. Existing uses of the NCBO Annotator include triaging literature to prioritize curation of publications and annotating free text data descriptions from databases and clinical records to enhance information retrieval by tagging text with a variety of labels (preferred name, synonyms, etc. as found in the ontology). We have recently used the NCBO Annotator to enable enrichment analysis with the Human Disease Ontology. Usually, enrichment analysis refers to identifying over- or under-represented Gene Ontology (GO) terms associated with a set of genes of interest. Our goal is to develop and apply general enrichment analysis methods to profile other sets of interest, such as patient cohorts from the electronic medical record, using a variety of ontologies including SNOMED CT, MedDRA, RxNorm, and others. Through use of Gene Ontology Annotation files annotated with disease related GO terms, we were able to identify and analyze Human Disease Ontology annotations from PubMed abstracts, demonstrating the ability to generate disease-based enrichment.

841W

Analysis of data from whole genome sequencing in clinical practice. *e.a. Worthey[1,2], g. Kowalski[2], m. Tutaj[2], r. Lopez[2], w. Liu[2], w. Jin[2], p. Jayaraman[2], j. De Pons[2], j. Smith[2], d. Schauer[1], g. McQuestion[2,3], b. Taylor[2], d.p. Bick[1,2], h.j. Jacob[1,2], d.p. Dimmock[1,2,3].* 1) Pediatrics, The Medical College of Wisconsin, Milwaukee, WI, United States; 2) Human Molecular Genetics Center, The Medical College of Wisconsin, Milwaukee, WI, United States; 3) Physiology, The Medical College of Wisconsin, Milwaukee, WI, United States.

Rapid technological advances in sequencing have led to a dramatic increase in the number of whole genome sequencing (WGS) projects thereby furthering understanding of human traits, diversity, and disease. It is clear that WGS will transform clinical diagnostics. Over the last year, our institution has established a clinical WGS program initially focused on Mendelian disease with defined protocols for patient selection, data analysis and evaluation, informed consent and results disclosure. The major bottleneck and greatest cost is associated with data analyses; identified variants must be annotated and prioritized in order to support identification of likely disease associations. Significant IT and bioinformatics effort is required; whilst the costs associated with sequencing have dropped to $5,000, costs associated with analysis have remained constant. Tools are required to support the clinical geneticist or other clinical provider in their interpretation of whole genome data. At the Medical College of Wisconsin and the Children's Hospital of Wisconsin, we have developed just such a tool; it is certified for use in a College of American Pathologists accredited laboratory, and is being used in our regional medical center to realize the promise of personalized genomic medicine through diagnostic whole genome sequencing. The tool provides the geneticist with the ability to interprete the data in light of the clinical presentation and family history. It can be used to assign variants to pathogenic, unknown significance and benign variant not associated with disease categories using a wealth of data including; mutation type, conservation score, genic/genomic location, Polyphen/SIFT prediction, splice site association, amino acid properties, known disease association, novelty, and population frequency. Queries can be based on the hypothesized mode of inheritance, and variant report pages browsed by position or by gene or gene set allowing focus on variants in a particular set of genes of interest, or target region. Development of this system has significantly reduced both the analysis cost and time to diagnosis. Six clinical cases (in additional to over two dozen research cases) have been analyzed using the tool. To our knowledge this is the first tool to yield clinical diagnoses in a CAP/Clinical Laboratory Improvement Amendments Lab setting. We will present specific clinical cases to demonstrate the functionality of this tool in changing healthcare.

842W

An integrative variant analysis suite for whole exome next-generation sequencing data. *F. Yu[1, 2], D. Challis[1], J. Yu[1], U. Evani[1], A. Jackson[2], S. Paithankar[2], C. Coarfa[2], R. Gibbs[1,2], A. Milosavljevic[2].* 1) Human Genome Sequence Ctr, Baylor College Med, Houston, TX; 2) Department of Molecular and Human Genetics, Baylor College Med, Houston, TX.

We present the Atlas2 Suite, an integrative collection of variant analysis tools optimized for high-throughput whole-exome data. The Atlas2 Suite makes use of logistic regression models trained on validated exon-capture data in conjunction with user-defined heuristic filters to separate true genetic variants from sequencing and mapping errors. It is accomodating for all three different NextGen platforms (SOLiD, Illumina and Roche 454). We applied the Atlas2 Suite to 38 whole-exome samples from the 1000 Genomes Project to demonstrate its ability to call both SNPs and INDELs with high sensitivity and specificity. In addition to the command line version of the suite we integrated the tools into the Genboree Workbench, allowing scientists to remotely call, view and further analyze variants through a simple web interface. Non-bioinformatician users can easily carry out personal genomics analysis using the Atlas2 provided by the Genboree resources, by simple mouse clicks.

843W

Improved methods for filtering putative rare functional mutations for whole genome/exome-sequencing data analysis. *G.Y. Yu[1,2], X. Zhang[1,2], Z. Wang[1,2], S.J. Chanock[2], M. Yeager[1,2], K.B. Jacobs[1,2].* 1) Core Genotyping Facility, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD 21702, USA; 2) Division of Cancer Epidemiology and Genetics, NCI, NIH, Bethesda, MD 20892, USA.

Whole genome and exome-sequencing is becoming a common approach to identify uncommon genetic variants that contribute diseases and traits. Variants discovered by these sequencing approaches are often subjected to several progressive bioinformatic filters to determine those variants most likely to be functionally related to the disease or trait of interest. A commonly applied filtering approach uses the NCBI dbSNP database of known genetic variants. dbSNP is a general depository and it has been collecting data from thousands of submitters using many variant discovery technologies for over a decade. Many researchers used presence of a variant in dbSNP as a strong filter, with the rationale that such variants are fairly common and unlikely to be related to highly penetrant uncommon genetic diseases or traits. This is a potentially risky approach, as many databases containing catalogs of rare mutations have been submitted to dbSNP as well as variants observed in populations ascertained on having uncommon diseases or traits. We have created a new database that contains data from dbSNP where variants have been scored by two criteria: 1) those with evidence of being a common variant in one or more randomly ascertained population groups, and 2) those with evidence of functional or clinical relevance, disease links in the Online Mendelian Inheritance in Man (OMIM) or had been implicated in a prior association study. With these two scores, investigators will be able to apply more specific filters and ranking systems for variants discovered by whole genome/exome sequencing projects. This database will be available via a World Wide Web query interface, a UCSC Browser track file, and as an application that can annotate files in standard Variant Call File (VCF) file format.

844W

Comprehensive Study of *Cis* and *Trans*-acting eQTLs across 36 Gene Expression GWAS. *X. Zhang[1], V. Emilsson[2], D. Levy[1], C.J. O'Donnell[1], A.D. Johnson[1].* 1) Division of Intramural Research, National Heart, Lung and Blood Institute; the NHLBI's Framingham Heart Study; 2) The Age, Gene/Environment Susceptibility study, Reykjavik, Iceland.

Introduction: Genome-wide genetic analysis of thousands of transcript levels (eQTLs) has identified genetic variants affecting gene regulation, and may improve our understanding of disease risk and potential drug targets. We sought to standardize 36 heterogeneous datasets of many human tissues and cell types, and systematically characterize *cis* and *trans*-acting genetic variants across these studies. **Methods**: 36 eQTL datasets from distinct tissues including liver, monocytes, brain, and subcutaneous adipose tissue were collected from published results or via collaborators. Heterogeneous transcript identifiers were mapped to NCBI Entrez Gene IDs to create a common dataset for further analysis. The distance between expression-associated SNPs (eSNPs) and their associated transcripts was calculated (hg18 reference). Unsupervised hierarchical clustering was used to identify the consistency of regulatory variants' effects in different tissues and cell types. These eSNPs were cross-referenced with genome-wide significant SNPs in the NHGRI GWAS catalog, and were mapped to UCSC regulatory features to understand the wide spectrum of *cis/trans-acting* regulatory mechanisms across the human genome. **Results**: Of 60,397 significant eSNPs reported from the eQTL datasets, 38,867 are unique. Most (89.7%) of the unique eSNPs are *cis*-acting SNPs (<500kb), consistent with literature reports. Hierarchical clustering shows that several transcripts have strong genetic influences across a majority of studies (>80%), e.g., *OAS1, MTRR, PEX6, CHURC1*. eSNPs closer to their associated transcripts tend to have higher significance. Spearman correlation analysis reveals a significant correlation in strength of signal between reported eSNPs and associations with the same SNPs as described in the NHGRI GWAS catalog (P=1.3e-13). In bioinformatic analyses, binomial tests indicate that eSNPs are localized within several regulatory features in the genome more than expected by chance (P<0.001 for 11 out of a total of 17 regulatory features). **Conclusion**: These results demonstrate that many strong genetic variants coincide both with disease GWAS results and known regulatory features, suggesting that genetic analysis of gene expression can help identify functional alleles that modify disease risk. Our results imply that eQTLs will be useful in interpreting genetic associations, prioritizing replication candidates and designing follow-up experiments in the study of complex diseases.

845W

Peripheral Blood Monocyte-Expressed Anxa2 Gene Is Involved in Pathogenesis of Osteoporosis in Humans. *H.W. Deng[1,2,3,4,5], F.Y. Deng[1,2], S.F. Lei[1,3], Y. Zhang[6], Y.L. Zhang[2,4], Y.P. Zheng[2,5], L.S. Zhang[2,5], R. Pan[2,3], L.L. Wang[6], Q. Tian[1], H. Shen[1], M. Zhao[1], Y.X. Wang Lundberg[6], Y.Z. Liu[1], C.J. Papasian[2].* 1) Center for Bioinformatics and Genomics, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA 70112, U.S.A; 2) School of Medicine, University of Missouri-Kansas City, Kansas City, MO 64108, U.S.A; 3) College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, P. R. China; 4) Center of Systematic Biomedical Research, University of Shanghai for Science and Technology, Shanghai 200093, P. R. China; 5) College of Life Sciences and Bioengineering, Beijing Jiaotong University, Beijing 100044, P. R. China; 6) Department of Genetics, Boys Town National Research Hospital, Omaha, NE68131, U.S.A.

Low BMD is a risk factor of osteoporosis and has strong genetic determination. Genes influencing BMD and fundamental mechanisms leading to osteoporosis have yet to be fully determined. Peripheral blood monocytes (PBM) could access to bone resorption surfaces and differentiate into osteoclasts to resorb bone. Herein, we attempted to identify osteoporosis susceptibility genes and characterize their functions, through initial proteome profiling of PBM in vivo, and using a strategy of multi-disciplinary and integrative studies. We recruited three independent extreme-discordant sample sets consisting of Caucasians with extremely low vs. high hip BMD (N=28, 80, 44, respectively). First, we carried out quantitative proteomics analysis in PBMs from the first extreme-discordant sample set to discover proteins, thus candidate genes, functionally relevant to low BMD. Candidate genes were then assessed for differential expression at mRNA level in PBMs from the second extreme-discordant sample set. Furthermore, DNA polymorphisms in the replicated genes were tested for association with BMD variation in the third extreme-discordant sample set and an independent sample of 1,000 unrelated Caucasians. Based on integrative evidence, gene(s) of interest were selected and subjected to functional validation in vitro. Among 1,539 proteins identified in PBMs, Anxa2 was up-regulated 2.0-fold in subjects with low BMD. Up-regulation of Anxa2 expression in low BMD subjects were verified by western blotting, and further replicated at mRNA level. Three SNPs in Anxa2 were found to be associated with BMD variation ($p<0.05$). The above integrative evidence strongly supports that Anxa2 is involved in the pathogenesis of osteoporosis in humans. Furthermore, monocyte migration across endothelial barrier in vitro was elevated up to 4.9-fold by Anxa2 stimulation, suggesting that it may stimulate more PBM to migrate from blood to bone resorption surfaces to differentiate into osteoclasts in vivo, hence leading to increased bone resorption and decreased BMD. In conclusion, this study identified a novel osteoporosis susceptibility gene, and suggested a novel pathophysiological mechanism, mediated by Anxa2, for osteoporosis in humans.

846W

Interstitial microdeletion of chromosome 8q23-q24 in Langer-Giedion syndrome in association with maternal translocation. *B. Min[1], W. Park[1,2].* 1) Biomedical Science, Seoul National University, Seoul, Korea; 2) College of medicine, Seoul National University, Seoul, Korea, MD/Ph.D.

Langer-Giedion syndrome, also called trichorhinophalangeal syndrome type II (TRPS2) is an autosomal dominant genetic disorder characterized by skeletal abnormalities and dysmorphic facial features, including thinly-growing hair, large nose and ears, and malformed fingers and toes. We investigated family members including two Langer-Giedion syndrome patients and their parents with normal phenotype using array CGH to find chromosomal aberrations related to their clinical features. 7.3 Mb interstitial microdeletion in 8q23-q24 containing TRPS1 and EXT1 gene was detected in two patients and 1.3 Mb microdeletion in 8q24.1sharing distal breakpoint with the deletion in two patients was detected in the mother of two patients. Breakpoint and junction sequence for 2 patients were determined by using PCR and Sanger sequencing. But the microdeletion in their mother was not defined by using PCR and Sanger sequencing. Maternal translocation was confirmed by parental chromosomal analysis and the translocation might induce the 7.3 Mb interstitial microdeletion in the patients.

847W

Large-scale meta-analysis of Genome-Wide Association Studies (GWAS) for fracture risk: the GEFOS Consortium. *H.L. Oei[1], H.-F. Zheng[2], E. Ntzani[3], K. Estrada[1], P.M Ridker[4], M. Garcia[5], Y.-H Hsu[6], T. Lehtimäki[7], S. Trompet[8], S. Kaptoge[9], S. Wilson[10], Y. Liu[11], J. Eriksson[12], A. Kung[13], A. Vernon-Smith[14], C.-T. Liu[15], J. Viikari[16], R.L. Minster[17], N. Wareham[18], V. Aalto[19], S.A. Cummings[20], K.-T. Khaw[21], J.A. Cauley[22], P.C. Sham[23], T. Spector[24], J. Ioannidis[3], D.P. Kiel[6], D. Chasman[4], J.B. Richards[2,24], F. Rivadeneira[1].* 1) Departments of Internal Medicine and Epidemiology, Rotterdam, Netherlands; 2) Department of Human genetics and Epidemiology and Biostatistics, McGill University, Montréal, QC, Canada; 3) Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina, Greece; 4) Brigham and Women's Hospital, Boston, MA, USA; 5) National Institute on Aging (NIA), National Institutes of Health (NIH), Bethesda, MD, USA; 6) Hebrew SeniorLife, Harvard Medical School, Boston, MA, USA; 7) University of Tampere and Tampere University Hospital, Tampere, Finland; 8) Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands; 9) Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK; 10) University of Western Australia, Perth, Australia; 11) Department of Epidemiology and Prevention, Wake Forest University School of Medicine, Winsotn-Salem, USA; 12) Center for Bone and Arthritis Research, Institute of Medicin, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; 13) Department of Medicine, The University of Hong Kong, Hong Kong, China; 14) Icelandic Heart Association and University of Iceland, Kopavogur, Iceland; 15) Boston University School of Public Health, Boston, USA; 16) Department of Medicine, University of Turku and Turku University Hospital, Turku, Finland; 17) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA; 18) Medical Research Council (MRC) Epidemiology Unit, Cambridge, UK; 19) Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku and the Department of Clinical Physiology, Turku, Finland; 20) Research Institute, California Pacific Medical Center, San Francisco, CA, USA; 21) Department of Clinical Gerontology, University of Cambridge, Cambridge, UK; 22) ,Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA; 23) Department of Psychiatry, The University of Hong Kong, Hong Kong, China; 24) Department of Twin Research and Genetic Epidemiology, King's College London, London, UK.

Aim: While genetic factors appear to confer susceptibility to the risk of fracture, no large-scale GWAS meta-analysis consortia have been undertaken to identify the common variants associated with fracture within the framework of the Genetic Factors of OSteoporosis (GEFOS) consortium. We therefore sought the largest GWAS meta-analysis to date to identify the common genetic variants associated with fracture. Methods: We broadly defined fracture, as fracture of any bone occurring in individuals 18 years and older, confirmed through medical, radiological and/or questionnaire reports regardless of trauma type. This definition maximizes the case sample size, since common variants generally confer small risks for common disease. The GWAS discovery meta-analysis comprised 13 GWAS cohorts (n=53,016, of which, 9,185 were fracture cases). Additive association with fracture risk was tested for ~2.5 million imputed SNPs (HapMap CEU release 22, build 36) employing logistic regression models adjusted for gender, age, height, weight and population stratification when applicable. Results were meta-analyzed using inverse variance fixed-effects using METAL. Genome-wide significance (GWS) was set at $P<5x10-8$ while suggestive significance at $P<5x10-6$. Results: The strongest signal included six SNPs associated at GWS (lowest P=1.7x10-10, odds ratio = 1.13, 95% CI: 1.09 - 1.17) and maps to the novel 18p11 locus recently identified as a BMD locus by our parallel GEFOS BMD effort. This locus contains a gene coding for a protein of unknown function (C18orf19) with no other clear candidate gene on the vicinity. Other suggestive signals included a known variant in LRP5 (P= 3.1x10-6) and novel signals mapping in or near MDH2 (P=4.8x10-6), RGS9 (P=4.8x10-6), FAM20C (P=2.3x10-6) and MYO3B (P=9.0x10-7). Interestingly, mutations in FAM20C cause Rayne syndrome which presents with intracranial calcification, generalized osteosclerosis and enhanced periosteal bone formation. These variants will undergo further replication through GWAS and de-novo genotyping in > 80,000 individuals (~30,000 cases). Conclusion: In this first large-scale GWAS meta-analysis assessing fracture risk we identified the BMD locus on chromosome 18p11 as associated with fracture, albeit with modest effect size, and highlighted other loci in or near genes, some known to cause skeletal monogenetic disorders. These findings help to understand the genetic basis of fracture susceptibility.

848W

A susceptible haplotype within collagen 1 alpha 1(COL1A1) gene influences bone mineral density and exacerbates risk of postmenopausal osteoporosis in India. *M. Singh[1], P.P. Singh[1], S. Singh[2], P.K. Juneja[2], T.P Kaur[3].* 1) Human Biology, Punjabi University, Patiala, Punjab, India; 2) Aggarwal Orthopaedic hospital, Ludhiana, Punjab, India; 3) Amrit Polyclinic, Ferozepur, Punjab, India.

Purpose: The association of Collagen type 1 alpha 1 (COL1A1) with bone mineral density (BMD) and risk of osteoporosis have remained unclear. The influence of COL1A1 gene polymorphisms on BMD as genetic mediators of osteoporosis risk needs to be explored in Indian postmenopausal females where this disease is rising rampantly. Methods and Results: The present study investigated the role and relevance of two pertinent single nucleotide polymorphisms (SNPs): 1997G/T (rs1107946) and +1245G/T (rs1800012, Sp1) of the COL1A1 gene in DEXA verified 349 (145 osteoporotic, 87 osteopenic and 117 normal) postmenopausal women of India, who were not taking hormone replacement therapy. Minor allele frequencies of rs1107946 and rs1800012 were 0.15 and 0.20 in osteoporotic women, 0.18 and 0.18 in osteopenic and 0.20 and 0.17 in women having normal bone mass. An allele dose effect with BMD of lumbar spine has been exhibited by major allele G of rs1107946 (GG: 0.86g/cm^2, GT: 0.91g/cm^2 and TT: 0.93g/cm^2) and minor allele T of rs1800012 (GG: 0.91g/cm^2, GT: 0.87g/cm^2 and TT: 0.81g/cm^2). Disease association analysis revealed a haplotype GT which confers approximately three fold higher risk of osteoporosis in the carriers (OR 3.12, 95% CI 1.24-8.88, P=0.008) after adjusting the confounding effect of age, BMI and years since menopause. Conclusion: These results suggest that GT haplotype of COL1A1 gene is associated with a higher risk of postmenopausal osteoporosis in Northwest Indian women.

849W

Large-scale Meta-analysis of Genome-Wide Association Studies for Bone Mineral Density at Radius identified Susceptibility Locus at Wnt16. *H.-F. Zheng[1], L. Yerges-Armstrong[2], J. Eriksson[3], E. Duncan[4], B. Mitchell[2], C. Ohlsson[3], E. Streeten[2], M. Lorentzon[3], J.B. Richards[1,5].* 1) Dept. of Epidemiology & Statistic and Human Genetics, Lady Davis Institute, Jewish General Hospital, McGill University, Montreal, Quebec, Canada; 2) University of Maryland School of Medicine, Baltimore, MD, USA; 3) Center for Bone and Arthritis Research, Institute of Medicin, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; 4) Royal Brisbane and Women's Hospital and University of Queensland Diamantina Institute, Brisbane, Qld, Australia; 5) Department of Twin Research and Genetic Epidemiology, King's College, London, UK.

Backgrounds: Recent Genome-Wide Association Studies (GWASs) have identified multiple common variants associated with bone mineral density (BMD) at lumbar spine and femoral neck sites. However, while all BMD sites across the body are partially correlated, they separately provide additional information on risks of fracture, particularly, site-specific fractures. Aim: To identify common genetic variants associated with BMD at the forearm, we performed a GWAS meta-analysis within the Genetic Factors of OSteoporosis (GEFOS) consortium. Methods: This meta-analysis comprised 4 cohorts (n=4,777). Association with radius BMD was tested for ~2.5 million imputed SNPs (HapMap CEU release 22, build 36) using linear regression models adjusted for sex, age, height, weight, family-relatedness and population stratification when applicable. Genetic effect estimates across studies were combined using an inverse-variance fixed-effects meta-analysis. We also tested the genome-wide significant SNPs for forearm BMD in an independent cohort containing 273 forearm fracture cases and 1613 controls. Results: 60 SNPs in 7q31 showed genome-wide significance (P<5x10-8), the most significant SNP was rs2536189 (P=3.99x10-14, effect -0.16 standard deviations per risk allele). This locus maps to wingless-type MMTV integration site family member 16 (Wnt16). WNT16 is a member of the Wnt signaling pathway that is known to play an important role in the regulation of bone mass and bone turnover. It is worth to point out that rs2254595 in WNT16, which was strongly associated with BMD (P=8.04x10-13, BETA=-0.14), was also moderately associated with forearm fracture (P=9.92x10-3, OR=1.28). In addition, 37 SNPs showed suggestive association signals (P<5x10-6), including 6 variants in known locus 5q14.3 (MEF2C, P=2.34x10-6) and novel signals in or near 6q23 (RPS12, P=1.29x10-6), 10p15 (DIP2C, P=1.26x10-6), and 11p13 (PDHX and CD44, P=1.39x10-6). Conclusion: In this first large-scale GWAS meta-analysis for forearm BMD, we identified Wnt16 as an important genetic determinant of forearm BMD and fracture and highlighted other novel loci. These variants will soon undergo further replication in upcoming GWAS and by de-novo genotyping for BMD and fracture. These findings increase our understanding of the genetic basis of osteoporosis. Keywords: Genome-wide association study; Osteoporosis; Bone mineral density; Radius; Meta-analysis.

850W

Pluripotent human cells differ in their ability to accommodate retrotransposition of engineered LINE-1 elements. *J. Garcia-Perez[1], S. Heras[1], S. Morell[1], A. Macia[1], E. Blanco-Jimenez[1], M. Munoz-Lopez[1], P. Leone[2], M. Garcia-Canadas[1].* 1) Human DNA Variability, GENYO(Pfizer - University of Granada & Andalusian), Granada, Spain; 2) Andalusian Stem Cell Bank, Center for Biomedical Research, University of Granada, Spain.

Long Interspersed Element-1 (LINE-1 or L1) is the most abundant autonomous non-LTR retrotransposon that shapes the human genome, representing approximately 17% of its genomic mass. Despite their abundance, only 80 to 100 copies per human remain retrotransposition competent (RC-L1s). RC-L1s are 6-kb in length elements that encode two Open Reading Frames (ORF1 and ORF2) required for their mobilization. ORF1 encodes an RNA binding protein while ORF2 encodes a protein with both ENdonuclease (EN) and Reverse Transcriptase (RT) activities. RC-L1s encoded proteins are also used by Short Interspersed Elements (SINEs) (Alu, SVA and others selected RNAs) to mediate their mobility in trans. L1 and Alu insertional mutagenesis has resulted in a diverse range of diseases, and their retrotransposition provides a constant source of human genetic diversity. L1 and Alu might ensure their evolutionary transmission to new generations through the accumulation of new insertions during early embryonic development or in germ cells. In fact, endogenous L1s are expressed in Human Embryonic Stem Cells (hESCs) and embryonic carcinoma cell lines (hECs). In addition, engineered human L1s can retrotranspose into both hESCs and hECs. Recent reports have revealed that pluripotent cells silence de novo L1 insertions by epigenetic mechanisms. Additionally, recent findings have revealed that selected somatic tissues (like Neuronal Progenitor Cells) can accommodate the mobilization of engineered L1s. However, it remains to be determined if all cultured human cells are equally receptive to accommodate L1 retrotransposition. To obtain an overview of the retrotransposition load that human pluripotent cells can accommodate, we have compared the retrotransposition rate of engineered L1 elements in a panel of human cells (hESCs, hECs, hESC-derived NPCs, a Neuroblastoma cell line, and differentiated cell lines as control). Remarkably, we found that the rate of L1 mobilization can vary up to 200-fold among cultured pluripotent cells. Interestingly, those cell types characterized for their neuronal nature support the highest rate of engineered L1 retrotransposition. Thus, it seems that neuronal cells have the intrinsic property to accommodate higher levels of L1 mobilization, which may have a direct impact in the degree of mosaicism generated during human embryonic development.

851W

Using the repeat architecture to discover new hotspots of copy number variation associated with autism spectrum disorder. *S. Girirajan, M.Y. Dennis, B.P. Coe, C. Baker, T.H. Vu, M. Malig, C. Alkan, E.E Eichler.* Department of Genome Sciences, Howard Hughes Medical Institute, University of Washington School of Medicine, Seattle, WA.

Repeat architecture of the human genome predisposes certain regions to non-allelic homologous recombination (NAHR) resulting in copy number variants (CNVs). Rare (<0.1% frequency) CNVs created by NAHR between large (>10 kbp) segmental duplications (SDs), termed genomic hotspots, are already implicated in >6% of pediatric cases with intellectual disability as well as more complex neurological phenotypes such as epilepsy, autism spectrum disorder, and schizophrenia. Despite advances in sequence technology, the discovery and genotyping of smaller (>5 kbp) genetic variants associated with disease have been challenging especially in repeat-rich regions. Based on the original model of NAHR, we systematically identified 1,340 gene-rich regions for recurrent CNVs ranging in size from >5 kbp to 5 Mbp. These novel hotspots (termed mini hotspots and micro hotspots) are flanked by high identity (>95%) repeat sequences including *Alu*s, LINEs, and smaller (1-10 kbp) SD blocks, or identical pairs of >100 bp sequences, respectively. We designed a custom targeted, high density (a probe every 50 bp-1 kbp) microarray to identify CNVs in a total of 1,460 hotspot regions including 120 SD-mediated hotspots, 733 mini hotspots and 607 micro hotspots with lower density (a probe every 14 kbp) in the genomic backbone. We tested 1,833 individuals with sporadic autism and compared to CNV data from 8,635 control individuals. As expected, we identified SD-mediated CNVs in 16p11.2 (8/1,833), 15q13.3 (3/1,833), 17q12 (2/1,833), 7q11.23 (3/1,833), and 1q21.1 (5/1,833). We also identified 220/1,340 mini and micro hotspot regions to be variant in one or more individuals with 35 events observed exclusively in individuals with autism spectrum disorder. Notable examples include smaller (>5 kbp) events disrupting neurologically relevant genes such as *FOXP1*, *NRXN1*, *A2BP1*, *DPP10*, *CNTN4*, *GRID1*, and *CNTNAP2* previously associated with autism spectrum disorder as well as *DISC1*, *YWHAE*, *EHMT1*, *CHRNA7*, *ATXN1*, *CDH8*, *PRDM9*, *PTCHD3*,*CTNNA3*, and *GTF2I*—genes not previously associated with autism spectrum disorder. We have identified a previously undetermined set of regions of recurrent rearrangement contributing to rare CNVs and potentially conferring risk to neurodevelopmental disorders. While the biological significance is yet to be determined, these novel hotspot events are excellent candidates for testing genomic imbalance contributing to autism spectrum disorder.

852W

Sequencing of the 22q11.2 deletion in VeloCardioFacial syndrome to identify genetic variants predisposing to Schizophrenia. *M. Guipponi[1,2], X. Bonilla Bustillo[1], C. Gehrig[2], S. Dahoun[2], S. Eliez[3], S. Antonarakis[1,2].* 1) Genetic Medecine, University of Geneva Medical School, Geneva, Geneva 4, Switzerland; 2) Genetic Medecine, University Hospitals of Geneva, Geneva 4, Switzerland; 3) Office Médico-Pédagogique Research Unit, Department of Psychiatry, University of Geneva Medical School, Switzerland.

Hemizygous deletions of the chromosome 22q11.2 region result in the 22q11.2 deletion syndrome also known as DiGeorge or Velocardiofacial syndrome (VCFS). The phenotype is variable and includes dysmorphic features and cardiac defects. Patients with VCFS also frequently have cognitive and behavioral symptoms and about one third of them develop schizophrenia during early adulthood. The incidence of schizophrenia in patients with VCFS is 30x higher than in the general population; thus del22q11.2 is an important risk factor for schizophrenia. The 22q11.2 deletion effectively reduces the normal diploid to an haploid state and therefore can result in the unmasking of otherwise recessive alleles that may remain on the intact homolog. By sequencing the remaining copy of the chromosome 22q11.2 in patients with VCFS and comparing the detected genetic variants between schizophrenic and non-schizophrenic patients, we expect to identify variations in one or more genes or functional elements that could increase the risk for the schizophrenic phenotype. We have selected 38 VCFS patients carrying the typical deleted region of 3Mb on chromosome 22q11.2; half of them showed no psychiatric symptoms, and the rest showed delirium, hallucinations, psychosis and/or schizophrenia. Age, sex, prevalence of cardiac defects and palatal abnormalities do not statistically differ between the two groups. DNA libraries for Illumina paired-end sequencing were prepared for these 38 samples and organized in seven pools of 5 or 6 libraries, each sample being tagged with a specific barcode. The 22q11.2 3Mb deletion from the non-deleted chromosome plus 200kb of upstream and downstream genomic sequences were captured using an Agilent SureSelect custom made enrichment system. Sanger sequencing of enriched, barcoded samples revealed an average enrichment of 400x. The seven pools are currently being sequenced on an Illumina GAIIx machine. Identification of variant predisposing to schizophrenia in this discovery set will be presented and discussed. Detection of genetic variants in the remaining allele of haploinsufficiency syndromes may reveal risk alleles for specific phenotypes.

853W

The Allen Human Brain Atlas: Multi-Scale Transcriptional Architecture of the Human Brain. *E. Shen, D. Bertagnolli, C. Dang, A. Ebbert, D. Feng, A. Guillozet-Bongaarts, M. Hawrylycz, J. Hohmann, C. Lau, C. Lee, E. Lein, J. Miller, L. Ng, J. Phillips, Z. Riley, K. Smith, S. Sunkin, P. Wohnoutka, A. Jones.* Allen Institute for Brain Science, Seattle, WA.

Neuroanatomically precise, genome-wide maps of transcript distributions are critical resources to complement genomic sequence data, yet are currently limited to rodent model systems. We report here a transcriptional atlas of the normal adult human brain, the Allen Human Brain Atlas, an online multimodal atlas of human brain that integrates anatomic and genomic information with a suite of visualization and data mining tools. A key component of the resource is genome wide microarray-based gene expression profiling across ~1,000 structures and nuclei from 2 normal non-diseased postmortem brains. The atlas contains T1- and T2-weighted magnetic resonance (MR) and diffusion tensor (DT) data as well as high-resolution histological data. An anatomic ontology was developed that enables repositioning and visualization of the 3D samples. Search functionality includes the ability to examine differences in gene expression between anatomic structures and genes. Normalized gene expression data are viewable in heatmap format across multiple structures and gene probes. Preliminary analysis reveals a dramatic multi-scale differential expression structure of the human brain with conserved architectural patterns between major structures. By using conventional and network analysis based methods, major modules of gene expression contributing to neuronal, glial, primary sensory, and certain structural patterns can be identified. Localized profiling in specific structures, such as the hippocampus or myencephelon, reveals modules for transcription machinery, metabolism, and other key functions. By combining gene expression, imaging, and supporting metadata the atlas can help elucidate the relationships between genomics and neuroarchitecture in novel ways. For example, when examining highly differentially expressed genes in the cortex, expression relationships of these genes can be shown to recapitulate the basic architectural plan of the major lobes and respective gyri. The Allen Human Brain Atlas integrates imaging, anatomy and genomics data modalities. Expression profiles from ~1,500 distinct anatomic samples from 2 individuals are currently available, with additional data and more sophisticated application features expected to be added throughout the remainder of this 5-year project. The Atlas can be accessed via the Allen Brain Atlas data portal at www.brain-map.org.

854W

BrainSpan Atlas of the Developing Human Brain. *S. Sunkin[1], A. Bernard[1], T. Chen[2], G. Coppola[3], C. Dang[1], S.-L. Ding[1], A. Ebbert[1], O. Evgrafov[4], B. Fischl[5], M. Gerstein[6], D. Geschwind[3], A.L. Guillozet-Bongaarts[1], J. Hohmann[1], S. Horvath[3], T. Hyde[7], A. Jones[1], Y. Kawasawa[6], J. Kleinman[7], P. Levitt[4], M. Li[6], S. Mane[6], E. Shen[1], K. Smith[1], A. Stevens[5], D. Weinberger[7], P. Wohnoutka[1], M. Hawrylycz[1], J.A. Knowles[4], N. Sestan[6], E. Lein[1].* 1) Allen Institute for Brain Science, Seattle, WA; 2) USC, Los Angeles, CA; 3) UCLA, Los Angeles, CA; 4) Zilkha Neurogenetic Institute of the Keck School of Medicine of the University of Southern California; 5) Massachusetts General Hospital; Harvard Medical School, Boston, MA; 6) Yale University, New Haven, CT; 7) NIMH, Bethesda, MD.

Though the complete sequence of the human genome has been available for over a decade, similar systematic efforts to map detailed gene expression patterns in the human brain have been lacking, particularly relating to development. To fill this void, we describe here a consortium project aimed at creating a unique multimodal transcriptional atlas of the pre- and postnatal developing human brain as a publicly accessible online resource for neuroscience, genome and medical research communities. This resource integrates transcriptomic, cellular resolution histology and imaging data in the context of human brain development through a portal for viewing, searching and mining of spatiotemporal gene expression patterns. Specifically, Illumina RNA-seq was used to generate quantitative transcriptome profiles of hippocampus, amygdala, thalamus, striatum, cerebellum and 11 neocortical regions, spanning early fetal neocortical development through adulthood. This broad spatiotemporal data set is supplemented with a high anatomical resolution microarray analysis of >300 laser microdissected structures from mid-gestational fetal human brain. To complement the transcriptome data, a large scale in situ hybridization (ISH) data set was generated to analyze cellular distributions using an industrial scale histology platform. ISH gene selection was biased towards genes associated with human neurological and neuropsychiatric disorders, as well as a portion matching the NIH Blueprint NHP Atlas (www.blueprintnhpatlas.org) to allow a direct comparison of gene expression patterns between human and non-human primate model systems across postnatal brain development. Finally, de novo fetal histological reference atlases and MRI and DWI data sets spanning human brain development are being created to provide a neuroanatomical and neurodevelopmental context for understanding spatiotemporally regulated transcriptional programs. Preliminary analysis of the transcriptome profiles from the adult timepoint will be presented. The product, integrating these large-scale data sets with tools for data visualization and analysis, aims to create a lasting resource for relating specific transcriptional programs to processes of human brain development and a normative data set for understanding the genetic basis of neuropsychiatric disease. BrainSpan (http://www.developinghumanbrain.org/) is publicly accessible through the Allen Institute for Brain Science portal (www.brain-map.org).

855W

Gene-based association analysis in pathways relevant to brain arteriovenous malformation. *S. Weinsheimer[1], L. Pawlikowska[1], N. Bendjilali[1], P.Y. Kwok[2,3], J.G. Zaroff[4], S. Sidney[4], C.E. McCulloch[5], W.L. Young[1,6], H. Kim[1,5].* 1) Dept Anesthesia, Univ California, San Francisco, San Francisco, CA; 2) Cardiovascular Research Institute, UCSF; 3) Institute for Human Genetics, UCSF; 4) Division of Research, Kaiser Permanente Northern California, Oakland, Calif., USA; 5) Departments of Epidemiology and Biostatistics, UCSF; 6) Departments of Neurology and Neurological Surgery, UCSF.

Background: The genetic basis of brain arteriovenous malformation (BAVM), a tangle of abnormal vessels directly shunting blood from the arterial to venous circulation, is unknown. However, tissue and candidate gene studies have suggested several pathways associated with BAVM biology. We performed a gene-based analysis of a case-control genome-wide association study (GWAS) dataset, focusing on 7 biological pathways implicated *a priori* in BAVM, to identify candidate genes that may not reach significance in GWAS analysis. **Methods:** Affymetrix SNP 6.0 data from a case-control study of BAVM in Caucasians (334 cases; 504 controls) was used. Gene-based analysis in PLINK was conducted as follows: 1) single SNP association was tested using multivariate logistic regression adjusting for age, gender, and top 3 principal components of population stratification; 2) gene sets were defined by subsets of unlinked, nominally associated SNPs ($p<0.05$, $r^2<0.5$, maximum of 5 SNPs per gene) mapping within 20kb of genes in 7 pathways: MAPK signaling (n=261 genes), TGF-beta signaling (n=85), VEGF signaling (n=74), NOTCH signaling (n=46), vascular development (n=57), angiogenesis (n=51), and inflammatory response (n=124); and 3) p-values controlling the error rate at the gene level were generated using 10,000 permutations of case-control status, keeping r^2 between SNPs constant. Pathway-wide level of significance was set using Bonferroni adjustment for the total gene count per pathway. **Results:** A total of 37 genes, including *THBS3*, *BMP7*, *IL18*, *NFKB2*, and *BDNF* were associated with BAVM (gene level $p<0.05$) that function in TGF-beta signaling (n=5 genes), VEGF signaling (n=1), angiogenesis (n=2), vascular development (n=2), inflammatory response (n=12), and MAPK signaling (n=15). However, none of these genes met the pathway-wide level of significance. Our top associated gene was thrombospondin 3 (*THBS3*) (Bonferroni-adjusted $p=0.06$), which is involved in TGF-beta signaling but not previously known to be a candidate for BAVM. **Conclusions:** We used a gene-based approach to study the association of genes in 7 candidate biological pathways for BAVM. Several genes were associated with BAVM at nominal $p<0.05$, including *THBS3*; however, no genes were associated at the pathway-wide level. These novel candidate genes and variants can be tested for replication in larger BAVM cohorts.

856W

Gene-set analysis of a genome-wide association study for colorectal cancer. *S. Castellvi-Bel[1], J.J. Lozano[2], C. Ruiz-Ponte[3], C. Fernández-Rozadilla[3], A. Abulí[1], A. Castells[1], A. Carracedo[3], Gastrointestinal Oncology Group of the Spanish Gastroenterological Association.* 1) Gastroenterology Department, Hospital Clinic-IDIBAPS-CIBERehd-CEK, Catalonia, Barcelona, Spain; 2) Bioinformatics Unit, Hospital Clinic-IDIBAPS-CIBERehd-CEK, Catalonia, Barcelona, Spain; 3) FPGMX, Genomics Medicine Group, Hospital Clinico-CIBERER-USC, Santiago de Compostela, Galicia, Spain.

Colorectal cancer (CRC) is the second leading cause of cancer related death in Western countries, representing in Spain the most frequently detected neoplasm in men and women. As other complex diseases, CRC is caused by both genetic and environmental factors. Twin studies showed that genetic susceptibility plays a major role and 30 percent of CRC present familial aggregation. Familial adenomatous polyposis and Lynch syndrome are the more frequent hereditary CRC syndromes and they are caused by germline mutations in APC, MUTYH and DNA repair genes. Recently, new common low-penetrance single nucleotide polymorphisms (SNP) for CRC have been identified by case-control genome-wide association studies (GWAS) permitting to point out so far 16 loci for CRC genetic susceptibility. GWAS have become a popular strategy to find associations of genes to traits of interest. However, success of its application in real studies has been limited by the testing strategy that considers SNPs independently. Gene-set analysis (GSEA), a different approach based on testing the association of modules of functionally related SNPs/genes, takes into account the combined effect of markers and it could be considered as an alternative method. The i-GSEA4GWAS (improved GSEA for GWAS) tool was recently developed as an open platform (NAR 2010;38:W90-5). A GWAS for CRC was recently concluded in the EPICOLON consortium, a Spanish epidemiologic, prospective, multicentre and population-based study. Genotyping was performed with the Affymetrix Genome-Wide Human SNP Array 6.0 in 881 CRC cases and 667 controls. After quality control, data was available from 848 cases and 629 controls for 674.578 SNPs. The i-GSEA4GWAS tool was used to perform a GSEA analysis on our data. Pathways/gene sets with a false discovery rate (FDR)<0.05 and a p value<0.001 were considered as overrepresented in our GWAS results. They included categories such as positive regulation of cell proliferation, glycerolipid metabolism, cell death, tumor necrosis family, ERK1/ERK2/MAPK family, growth, T-cell receptor signaling, ectoderm development, cell-cell signaling, apoptosis, cell activation, NFKB, or focal adhesion. Genes with significant SNPs in our GWAS from the above category sets comprised POU3F2, IL9, CDH13, LAMA1, ALDH1A3, NFKB1, MAP2K4, JUN, IL2, BMPR1B, SMAD3 and SPINK5. GWAS GSEA could be used as a complementary strategy to help selecting SNPs in phase 1 to be genotyped in consecutive replicas.

857W

An integrated high-throughput automated workflow for RNA and DNA extraction from FFPE samples for Second Generation Sequencing. *T. Guettouche[1,2], D. Hedges[1], J. Rantus[1], K. Slosek[2], I. Konidari[1], B. Hulme[1], A. Andersen[1], A.L. Diaz[1], R. Gentry[1], Y. Pasco[1], M. Pericak-Vance[1], J. Gilbert[1].* 1) Hussman Institute for Human Genomics, University of Miami, School of Medicine, Miami, Florida; 2) Oncogenomics Core Facility, Sylvester Comprehensive Cancer Center, University of Miami, School of Medicine, Miami, Florida.

Millions of formalin-fixed, paraffin embedded (FFPE) samples are stored in Biorepositories around the world. These samples contain a vast compendium of phenotypic, histological and pathological data. For genomics studies, such as biomarker discovery, targeted resequencing or gene expression profiling of this resource has been largely unutilized because sample extraction can be challenging and the quality of the extracted nucleic acids is often poor. Currently, RNA and DNA extractions from FFPE samples are commonly performed manually and involve laborious protocols that are not amenable to high throughput processing. In addition there are no standard quality control methods for downstream applications such as second generation sequencing. Here we present a fully automated RNA and DNA extraction method (Tissue Preparation System, Siemens Healthcare Diagnostics; Tarrytown,NY) for IVD and research use that allows simultaneous or separate extraction of DNA and RNA from up to 48 samples in 4h with minimum operator interaction. In contrast to most other FFPE extraction protocols this method uses an innovative de-paraffinization step on the instrument that does not require incubation with Xylene or other solvents. We have extracted DNA and RNA from over 100 Melanoma, Breast and Prostate cancer FFPE samples and are currently processing 350 additional samples from 7 different tumor types including lung, pancreas, bladder and cervix. From adjacent 10uM paraffin sections the automated system, on average, extracts longer nucleic acid fragments as judged by Bioanalyzer traces and similar amounts of RNA and DNA measured by the Qubit method compared to a standard commercially available manual protocol. Matched fresh frozen and FFPE samples are being analyzed by second generation sequencing on a HiSeq2000. We present an integrated FFPE analysis workflow that includes standardized and fully automated nucleic acid extraction and quality controls for high-quality, high-throughput preparation of FFPE samples (96 samples/8 hr workday) for downstream analysis such as resequencing or transcriptome analysis by second generation sequencing. Our integrated workflow should significantly simplify utilization of FFPE samples for downstream genomic analyses such as second generation sequencing and as a consequence unlock a largely untapped source of information.

858W

From “gene desert” to cancer stem cell biomarker: Using public genomic data to take 8q24 from GWAS to the clinic. *A. Hsu, J. Madhusoodanan, J. Su, M. Shekar, I. Kupershmidt.* NextBio, Cupertino, CA.

The SNP rs6983267 and others in the 8q24.21 region have been identified in numerous genome-wide association studies of several different types of cancer. At the time of the original publications, the region was thought to be a “gene desert”, containing only the pseudogene POU5F1B. POU5F1B has since been found to encode a functional isoform of the transcription factor POU5F1, though POU5F1B's roles in cancer and normal development are still not understood. Using the NextBio platform, we performed meta-analyses of curated public genomic data. Strong evidence from multiple independent studies show that 1) POU5F1B is overexpressed in embryonic stem cells (ESCs) compared to differentiated tissues and cells; 2) POU5F1B is up-regulated and/or amplified in several types of cancer; 3) POU5F1B is highly up-regulated when reprogramming differentiated cells into induced pluripotent stem cells via overexpression of a 4-gene cocktail comprising SOX2, KLF4, POU5F1/OCT4, and MYC; and 4) POU5F1B is down-regulated when differentiating ESCs by overexpression of the tumor suppressor INHBA. These multiple lines of evidence—**not discoverable in the biomedical literature**—suggest that POU5F1B may be a useful biomarker for cancer stem cells, as well as a lead in understanding the progression of certain types of cancer.

859W

RNA-sequencing and allele-specific expression analysis identify strong allelic expression imbalance (AEI) for a bladder cancer associated variant rs2294008 within the prostate stem cell antigen (PSCA). *I. Kohaar, A. Mumy, W. Tang, Y-P. Fu, P. Porter-Gill, L. Prokunina-Olsson.* Laboratory of Translational Genomics, DCEG/NCI/NIH.

A recent GWAS identified a SNP rs2294008 within the prostate stem cell antigen (PSCA) at 8q24.3 as a risk factor for bladder cancer, [OR=1.13 (1.09-1.17), p=4E-11, Rothman, 2010]. PSCA encodes a GPI-anchored cell membrane glycoprotein expressed in the prostate, bladder, stomach and pancreas. A transcript with the non-risk C allele of rs2294008 encodes a protein of 114 amino acids, while the risk T allele creates a novel upstream translation start site (acg->aTg) that extends the N-terminal leader peptide by 9 amino acids, to generate a protein of 123 amino acids. A humanized monoclonal anti-PSCA antibody is already used in clinical trials for prostate and pancreatic cancer, but the functional roles of PSCA and its genetic variants in normal and cancer conditions remain unknown. We aimed to explore mRNA expression of PSCA in relation to rs2294008 and bladder cancer risk. RNA-sequencing of 6 bladder tissues (3 tumors and 3 normal adjacent) and one normal prostate tissue detected PSCA expression in all samples. Analysis of three samples heterozygous for rs2294008 (bladder tumor, bladder normal and prostate normal) showed evidence of strong allelic imbalance (AEI) - 90%; of all PSCA transcripts carried the risk T allele, while only 10%; showed the non-risk C allele. A similar pattern was observed for 11 additional transcribed SNPs within the PSCA gene, but not for SNPs located in neighboring genes, Ly6K and JRK, suggesting that the AEI is specific for PSCA and not a result of AEI of the whole region. Analysis of total PSCA expression in 42 tumor and 39 normal bladder tissue samples showed that total PSCA expression was strongly increased in carriers of risk TT and TC genotypes, up to 10-fold compared to carriers of non-risk CC genotypes (p trend=0.00064), even after adjustment for sample status (normal/tumor). By using an allele-specific expression assay that measured allelic ratio of rs2294008 in PSCA transcripts, we also confirmed AEI detected by RNA-seq. We are currently evaluating regulatory potential of SNPs in strong LD with rs2294008 and located upstream of PSCA ansd exploring functional significance of the alternative leader peptide of PSCA created by rs2294008 risk allele. In conclusion, we suggest that either rs2294008 or other variants in LD with it regulate mRNA expression of PSCA and cause strong AEI that leads to almost monoallelic expression of PSCA transcript that carries the bladder cancer associated T allele of rs2294008.

860W

Integrative molecular profiling in serous epithelial ovarian cancer for identification of biomarkers of chemoresistance. *M. Koti[1], R. Vidal[1], P. Nuin[1,2], A. Haslesurst[1], J. Weberpals[3], T. Childs[4], P. Bryson[4], H. Feilotter[1], J. Squire[1], P. Park[1].* 1) Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada; 2) Ontario Cancer Biomarker Network, Toronto, Canada; 3) Division of Gynecologic Oncology, Ottawa Health Research Institute, Ottawa, ON, Canada; 4) Department of Obstetrics and Gynecology, Queen's University, Kingston, ON, Canada.

Development of primary resistance to carboplatin and paclitaxel poses a major challenge in the management of ovarian cancer. To identify the molecular mechanisms underlying this process, we used integrative microarray analyses to profile the 1) copy number alteration and SNP, 2) mRNA, 3) miRNA and 4) methylation signatures in a cohort comprising 11 chemoresistant and 19 sensitive tumour samples, as defined by the RECIST criteria. The profiles were analyzed by Bayes statistics, based on R/Bioconductor packages, and the relevant pathways determined using Ingenuity Pathway Analysis. The data from each array platforms were integrated using bioinformatics tools developed in house to decipher the most critical biological pathways, and to identify the molecular mechanisms driving the pathways. Copy number analysis shows significant alterations in the chemoresistant group (gains on chromosomal regions, 4q, 6q, 8p, 8q, 19q, 7q and 22q; losses on 8p and 10q) compared to the sensitive group. Preliminary mRNA expression analysis identified an enrichment of upregulated genes involved in cellular growth and proliferation, development as well as differential gene expression changes in the, TGFB1, TNF, , ERBB2, CTNNB1, IFNG, LH and FSH networks, in the resistant compared to the sensitive group. The major molecular and cellular functions associated were cell-to-cell signaling, molecular transport and cellular movement. Similarly, the major gene networks by IPA analysis of most significantly downregulated genes were the IFNG, IRF3, IFN alpha, IL-1B. Differences were also seen in the PI3K, TP53, and the TNF networks. Interestingly, these changes were consistent with PTEN loss which has been frequently observed in serous low grade tumours. Additionally, genes involved in activation of NF2B pathway and the TP53 network showed differential expression in the two groups.Analysis is being performed on correlating the gene expression data with miRNA, methylation and copy number alterations. Ongoing comparative analyses and integration of miRNA profiles within this cohort have also identified several differentially expressed transcripts including mir-34b, mir-155, mir-214, mir-200c and mir-143. Some of these miRNAs have been earlier reported to be associated with tumour progression. Further integrated analysis will elucidate the synergistic roles that the genetic and epigenetic alterations in the deregulation of these and other pathways involved in primary chemoresistance.

861W

HUMAN GENETICS. *b. nasasira.* Mbarara university of science and technology, Kampala, Mbarara, Uganda.

Human genetics describes the study of inheritance as it occurs in human beings. This encompasses a variety of overlapping fields including molecular genetics, biochemical genetics, Population genetics, Developmental and clinical genetics among others. The study of human genetics is vital in that it helps us understand the diseases and develop effective disease treatment, as well as helping us understand human life. Genes are the common factor of the qualities of most human-inherited traits. These are carried on the DNA of the individual, each DNA carrying several genes that determine the organism's characteristics. The sum of the entire DNA contained within an organism is its genome. The structural and functional study of which is called genomics. Population genetics deals with investigating processes that cause changes in allele and genotype frequencies in populations based on inheritance. The human karyotype, that is the picture of homologous chromosomes;44 autosomes and 2 sex chromosomes during metaphase 1 arranged according to their length provides a way of diagnosing chromosomal abnormalities in clinical genetics. These anomalies, normally caused by non-disjunction during cell division include, Trisomy21, Down's syndrome and many more. However, allelic disorders also occur; such as albinism, sickle cell anemia among others. Here particular genes are affected. Some traits are sex linked in that the genes determining such are carried on sex chromosomes. These include Hemophilia, muscular dystrophy. Genetic anomalies are often inherited from parent to daughter organisms. This is by autosomal recessive inheritance, autosomal dorminant inheritance or sex linked inheritance. Genetic knowledge of such anomalies provides a basis for their diagnosis and treatment. Genes also have a strong influence on human behavior. IQ is largely heritable. We must not forget that the sex of an organism is also genetically determined so that an individual with XX sex chromosomes is a female, one with XY is a male.

862W

Integrative analysis of whole-genome and transcriptome sequence data for metastatic triple negative breast cancer. *A. Siddiqui[1], O. Sakarya[1], C. Barbacioru[1], J. Aldrich[2], S. Sinari[2], A. Christoforides[2], T. Izatt[2], J. Keifer[2], L. Hoang[1], S. Mousses[2], J.D. Carpten[2], D. Von Hoff[2], D.W. Craig[2].* 1) Life Technologies, Foster City, CA; 2) The Translational Genomics Research Institute (TGen) Phoenix, AZ.

Triple negative breast cancer (TNBC) is characterized by the absence of expression of estrogen receptor, progesterone receptor and Her2-neu, accounting for 15% of all breast cancer diagnosis. Targeted therapies have a reasonable likelihood of improving the cure rate of early stage TNBC, and promising therapies discovered in the metastatic setting are rapidly advancing through clinical trials. We present integrated analysis of matched normal and tumor whole genome data, along with tumor transcriptome sequencing data of multiple individuals with metastatic chemo-resistant TNBC. In each case, two independent 1.5kb Mate-pair libraries were generated for both tumor and germ-line derived genomic DNA and sequenced using SOLiD® version 4.0 paired 50mers to a target of 30x depth. The tumor transcriptome was sequenced on four replicates and compared to transcriptome sequencing from ethnicity-matched population-based control hyperplastic breast tissue. Genome analysis was performed using multiple aligners and variant callers. Transcriptome alignment was performed using Life Technologies Bioscope pipeline, and differential expression analysis was performed using EdgeR and DESeq. We prioritized annotated germline and somatic variants by integrative analysis with differential expression results. Several striking examples of intronic events correlating with either altered splicing or differential expression were observed in genes relevant to cancer treatment, suggesting that transcriptomic data may have high value in interpreting somatic events that fall outside of coding regions. Final integration of data was validated through knowledge mining and convergence of somatic events and expression. For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

863W

Detecting Neoplasms of Hematopoetic or Lymphatic Tissue as an Incidental Finding of GWAS within the electronic Medical Records and Genomics (eMERGE) Network. *U. Schick[1], A. McDavid[1], N. Weston[2], K. Ehrlich[2], K.N. Newton[2,3], P.K. Crane[4], C. Laurie[5], C. Laurie[5], A.P. Reiner[1,6], R.D. Jackson[7], U. Peters[1], G.P. Jarvik[4,8], E.B. Larson[2,4], C.S. Carlson[1,6].* 1) Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Group Health Research Institute, Seattle, WA; 3) School of Public Health, University of Washington, Seattle, WA; 4) Department of Medicine, University of Washington, Seattle, WA; 5) Department of Biostatistics, University of Washington, Seattle, WA; 6) Department of Epidemiology, University of Washington, Seattle, WA; 7) Department of Internal Medicine, Ohio State University, Columbus, OH; 8) Department of Genome Sciences, University of Washington, Seattle, WA.

Collaborators at the Gene, Environment Association Studies Consortium (GENEVA) have developed an algorithm that detects large copy number variants (>50 kb) and mosaic copy-neutral loss of heterozygosity with high sensitivity and specificity from Illumina high-density SNP genotyping data. In this study, the GENEVA detection method was applied to population-based cohort of 2,771 elderly participants enrolled in a study of aging and dementia, where GWAS genotypes were available from the eMERGE consortium. Genotyping was performed on the Illumina Human 660W-Quad1 platform using DNA derived from peripheral blood samples. We hypothesized that karyotypic anomalies detected in DNAs collected at enrollment would be associated with a subsequent occurrence of neoplasms of the lymphatic or hematopoietic tissue and tested this hypothesis using electronic data derived during ongoing care in an integrated delivery system during a median 6 years of follow-up after enrollment samples were collected. Through the application of the GENEVA method, 109 individuals were observed to have at least one chromosomal anomaly greater than 1.5 megabase in length. An association was observed between these large chromosomal anomalies and a subsequent diagnosis of leukemia, lymphoma or multiple myeloma in this cohort (odds ratio, 3.69; 95% Confidence Interval: 2.08-6.27). Odds ratios for each type of neoplasm were consistent, and several of the anomalies observed in participants later diagnosed with neoplasms have previously been reported as recurrent findings in leukemia (e.g. trisomy 12). These findings suggest that this method may have utility in the early detection of neoplasms of hematopoetic or lymphatic origin. Replication efforts are underway to confirm the association of apparently acquired copy number variants with hematopoetic or lymphatic neoplasms in an independent sample of 10,497 individuals from the Women's Health Initiative.

864W

Genetic Architecture of Adiponectin in Hispanic Americans from the IRAS Family Study. *S.S. An[1], N.D. Palmer[1], A. Hanley[2], J.T. Ziegler[1], W.M. Brown[1], S.M. Haffner[3], J.M. Norris[4], J.I. Rotter[5], X. Guo[5], Y.D.I. Chen[5], L.E. Wagenknecht[1], C.D. Langefeld[1], D.W. Bowden[1].* 1) Biochemistry, Wake Forest University School of Medicine, Winston-Salem, NC; 2) University of Toronto, Toronto, Ontario; 3) University of Texas Health Sciences, San Antonio, TX; 4) University of Colorado, Denver, CO; 5) Cedars-Sinai Medical Center, Los Angeles, CA.

Adiponectin is a widely studied adipocytokine that has been implicated in glucose, lipid and energy homeostasis. Adiponectin has been found to be inversely correlated with obesity, insulin resistance and diabetes. Plasma adiponectin levels are highly heritable, with heritabilities ranging between 40-70%. Previous studies have suggested the the ADIPOQ locus itself is the major genetic determinant of plasma adiponectin levels. Common variations in the ADIPOQ gene, however, have accounted for less than 10% of the observed variance in adiponectin levels. Previously, we identified a low frequency (1.1%) coding variant, G45R, in Hispanic Americans from the Insulin Resistance Atherosclerosis Family Study (IRASFS) which accounted for an estimated 16.7% of the proportion of variance in plasma adiponectin levels in the Hispanic population. The current study was designed to quantify the variation in adiponectin attributable to clinical traits, low frequency variants, and common variants in ADIPOQ, in a Hispanic sample. Through resequencing and bioinformatic analysis, we identified 45 low frequency (MAF<5%) and common (MAF>5%) SNPs in ADIPOQ which were genotyped in 822 Hispanics in IRASFS in 90 pedigrees. To test for association and estimate contributions to adiponectin variation, a variance components model was computed as implemented in SOLAR. Eight of the 12 low frequency SNPs (MAF 0.01-0.04) were associated with adiponectin levels (p-value 0.013-5.03E-40), with G45R having the strongest association. We postulate that low frequency variants contribute a major component of variance in plasma adiponectin. In addition to genetic contributions to adiponectin variation, we estimated the variation attributable to an extensive list of 14 clinical variables (age, gender, recruitment center, BMI, glucose homeostasis, adiposity, PAI-1, fibrinogen, CRP, and blood pressure). In 822 individuals with complete clinical data, the clinical measures accounted for 31% of the variance in adiponectin. Low frequency genetic variants accounted for 24% of the variance and common variants accounted for only 4% of the variance. Cumulatively, the clinical data, low frequency and common SNPs accounted for an estimated 58% of variance in plasma adiponectin. In conclusion, low frequency variants and clinical characteristics account for the majority of the variance observed and in combination with common SNPs account for a substantial proportion of the variation in adiponectin levels.

865W

In Search of Regulatory Variants in Exfoliation Glaucoma. *M.A. Hauser[1,2], Y. Liu[1], S.E. Williams[3,4], B.T. Whigham[1], J. Wheeler[1], T.R. Carmichael[4], X. Qin[1], R.R. Allingham[2].* 1) Ctr Human Gen, Duke Univ Med Ctr, Durham, NC; 2) Department of Opthalmology, Duke Medical Ctr, Durham, NC; 3) St John Eye Hospital, University of Witwatersrand, Johannesburg, South Africa; 4) University of Witwatersrand, Johannesburg, South Africa.

Exfoliation (pseudoexfoliation) glaucoma (XFG) is the most common identifiable form of open-angle glaucoma in the world. XFG is defined by the inappropriate production and extrusion of fibrillar extracellular protein by multiple tissues within the eye and systemically. Abnormal protein aggregates damage the aqueous humor outflow pathways of the trabecular meshwork, causing elevated intraocular pressure. A recent genome wide association study and several followup studies have found that the non-synonymous coding variant rs3825942 in the lysyl oxidase-like 1 gene (LOXL1) is highly associated with increased risk of XFG. Our analysis of a native South African population of 113 cases and 107 controls has demonstrated that this variant is not causal. It is highly associated ($p=6x10^{-21}$; odds ratio 6.3 (95%CI 3.7-10.8)); however, the risk allele is reversed. The A allele of this SNP is associated with risk rather than the G allele seen in other populations, indicating that the actual functional variants are nearby but remain undiscovered. We hypothesize that causal variants in LOXL1 will contribute to disease risk by regulating the level of expression of LOXL1. In order to identify these risk variants, we have sequenced the full genomic LOXL1 locus in 50 XFG cases and 50 controls from South Africa. The sequence region includes all exons and introns as well as 10kb upstream of the gene (a total of 40kb). Our sequence analysis and followup association studies in this subset of our study population show that the original rs3825942 SNP is highly associated with XFG ($P=10^{-10}$). Eight additional eight sequence variants in the first 5000 bases of intron 1 were also significantly associated with XFG ($P= 10^{-4}$ - 10^{-10}). Interestingly, the risk alleles for these variants were also reversed in comparison to those in our Caucasian XFG dataset. These data suggest possible DNA recombination events around the exon1/intron 1 in the LOXL1 gene. We are currently sequencing additional upstream regions of LOXL1 in both Caucasian and African samples.

866W

Analysis of targeted exome-plus sequencing in a kidney transplantation outcome cohort. *B.M. Herrera[1], S.M. Kurian[2], P. Langfelder[3], S. Horvath[3], T. Mondala[2], P.L.F Tang[1], E.T. Lam[1], D. Solomon[2], P. Kwok[1].* 1) CVRI, UCSF, San Francisco, CA. University of California, San Francisco 555 Mission Bay Blvd South, MC-3118 San Francisco, CA 94158-9001; 2) Molecular and Experimental Medicine (MEM-241) The Scripps Research Institute, La Jolla, CA, 92037; 3) Department of Biostatistics UCLA School of Public Health Los Angeles, CA 90095-1772.

Despite vast efforts in matching kidney donors with recipients and the use of immunosuppressive therapy, only 30% of recipients have a well functioning graft (TX) 10 years after transplantation, while 70% will develop Chronic Allograft Nephropathy (CAN). Furthermore, diagnosis of the degree of graft rejection requires invasive procedures which further contribute to graft injury. Therefore, the identification of genetic markers indicative of risk for developing CAN, is of paramount importance and may eventually assist in donor-recipient matching. To test the hypothesis that rare coding mutations affect the outcome of renal transplantation. We have sequenced the exome (38MB) and regulatory regions of over 2000 genes which were previously implicated in outcome studies through differential expression and as part of a weighted correlation network analysis. Whole exome-plus capture was carried out in a cohort of 100 unrelated renal transplant recipients (CAN= 50 and TX=50) collected as part of the Transplant Genomic Collaborative Group. Sequence capture was performed using the SureSelect kit v1.0 (38Mb+6Mb) and parallel DNA resequencing was carried out on the HiSeq-2000. 2x100bp paired-end sequence reads were aligned to the hg19 reference genome using the Burrows-Wheeler alignment tool (BWA 0.5.7). Duplicate reads, resulting from PCR clonality or optical duplicates and reads mapping to multiple locations were excluded from downstream analysis. The unified genotyper utility from the Genome Analysis Toolkit was used for variant calling and then filtered using rigorous quality controls. In the first 55 exomes sequenced (CAN=28, TX=25), on average, 80.02% of reads were aligned to the genome, of these, 87.13% are on or near target intervals with a mean target coverage for bases is ~ 70.7x. We identified an average of 21,866 variants per individual, of which ~5% are novel. Filtering of variants was done by comparing to dbSNP131 and 1000 Genomes pilot SNP calls and annotated using SeattleSeq. Thus far we have identified up to 18 novel coding variants with different frequencies between the CAN and TX samples suggesting genetic involvement, among these, several variants clustered around the Protocadherin alpha-8 isoform-1 precursor gene (PCDHA8, chr-5q32) and Double-stranded RNA-binding protein Staufen (STAU1, chr-20q13). In addition we will investigate IN/DELS and CNVs and explore the role of these observations in kidney transplant outcome.

867W

Tumor exome sequencing in patients with isolated bilateral Micronodular Adrenocortical Disease identifies pathogenic somatic and germline mutations. *A. Horvath[1], R.B. Alexandre[1], E. Saloustros[1], C. Wassif[1], A. Manning[1], P. Paschou[1], P. Briasoulis[1], S. Sigh[1], J. Epstein[1], I. Levi[1], J. Neimela[2], J.B. Oliveira[2], J.A. Carney[3], F.D. Porter[1], C.A. Stratakis[1].* 1) NICHD, NIH, Bethesda, MD; 2) CC, DLM, Bethesda, MD; 3) Mayo Clinic, Rochester, Minnesota 55905, USA.

Micronodular Adrenocortical Disease (MAD) is a form of bilateral adrenocortical hyperplasia associated with corticotrophin (ACTH)-independent Cushing's syndrome. It has been seen isolated or as a part of multi-neoplasia syndromes, such as Carney complex and McCune-Albright Syndrome. Both isolated MAD (iMAD) and MAD in the context of Carney complex or McCune-Albright Syndrome have been related to molecular defects in cAMP- signaling pathway. Carney complex is caused by germline mutations in regulatory subunit type 1-alpha (*PRKAR1A*) of Protein Kinase A (PKA), while McCune-Albright Syndrome results from early postzygotic mutations of the alpha subunit of the stimulatory G protein gene (*GNAS*). Despite the recent identification of several new genes implicated in the adrenocortical tumors predisposition, including at least two members from the family of the phosphodiesterases (P*DE11A* and *PDE8B*), a subset of iMAD tumors remain with undiscovered genetic cause. The genetic etiology of iMAD is clearly supported by very early onset and invariable bilateral appearance. We applied targeted exome enrichment (Agilent technologies) and subsequent massive parallel sequencing on SOLiD4 platform (Applied Biosystems) on adrenocortical tumors from 5 unrelated patients with bilateral iMAD. An average coverage of 50-fold per targeted base was achieved, and filtering criteria to reduce false positive calls were applied. After filtering against existing mutation databases, and our own clinically unrelated next-generation sequencing datasets, between 298 and 452 novel sequence-altering variants per tumor were identified, these including missense, nonsense, and splice base substitutions (up to 3 bp from the junction), small (up to 9bp) and large indels. The variants were sorted according to their presence in maximum number of patients, expression in the adrenal cortex, functional relevance and pathogenic potential, and a short list of candidate genes was generated. Among the most favored candidates are genes involved in the cAMP-signaling and Wnt-signaling pathways, cell growth and differentiation control, and genes known to have altered expression in related tumor conditions; experiments further confirming selected genetic variants are ongoing.

868W

In search of a new vascular dementia: An exome sequencing approach to a Swedish multi-infarct family. *M. Junna[1], A. Börjesson Hanson[2], C. Sundal[3], O. Andersen[3], M. Baumann[4], H. Kalimo[5], M. Pöyhönen[6,7], M. Viitanen[8,9].* 1) Dept. of Medical Biochemistry and Genetics, University of Turku, Turku, Finland; 2) Dept. of Psychiatry and Neurochemistry, Neuropsychiatry Epidemiology Unit, Institute of Neuroscience and Physiology, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden; 3) Dept. of Neurology, Institute of Neuroscience and Physiology, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden; 4) Protein Chemistry Unit, Institute of Biomedicine/Anatomy, University of Helsinki, Helsinki, Finland; 5) Dept. of Pathology, Haartman Institute, University of Helsinki, Helsinki, Finland; 6) Dept. of Clinical Genetics, Helsinki University Central Hospital, Helsinki, Finland; 7) Dept. of Medical Genetics, University of Helsinki, Helsinki, Finland; 8) Dept. of Neurobiology, Care Sciences and Society, Karolinska Institutet, Stockholm, Sweden; 9) Dept. of Geriatrics, University of Turku, Turku, Finland.

In 1977, Sourander and Wålinder described a case of hereditary multi-infarct dementia (MID) in a Swedish family. Later their disease was suggested to be the cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). The clinical picture of recurrent strokes, (but without the frequently seen characteristic migraine) with MRI that shows subcortical white matter degeneration and vascular changes resembles CADASIL, but in our studies we did not detect any pathogenic mutations in the entire 8091 bp reading frame of *NOTCH3* nor found clear evidence for the *NOTCH*3 gene linkage. Neither did we find any evidence of the CADASIL-typical granular osmiophilic material (GOM) in skin biopsies. These multiple approaches suggest that in this Swedish family the hereditary MID suspected to be CADASIL is a different disorder with dissimilar pathological features, and belongs to the growing group of genetically uncharacterized familial small vessel diseases (SVDs).

In order to search for the pathogenic mutation behind the disease, we did a whole-exome sequencing for one healthy and three affected family members. Their genomic DNA was extracted from peripheral blood lymphocytes, the exon targeting was done with NimbleGen's sequence capture (Roche Inc. USA) and the sequencing with Illumina's sequencing platform (Illumina Inc. USA).

After sequencing, the results were filtered against public SNP databases and the data from the Thousand Genomes Project in order to identify the sequence variants that have not been published previously as polymorphisms. Subsequently, we concentrated on the variants that are shared by all the three patients and excluded the variants that are also shared by the healthy control. Since we are searching for a dominant disease we narrowed our search by excluding the variants that are homozygous in all patients. Detailed results of these analyses and the possible pathogenic variation or variations will be presented at the congress.

869W

Genetic contribution to neuronal cell death in hippocampus induced by prolonged seizures in rats. *C. Martin[1], P. Lema[2], L. Carmant[2], P. Cossette[1].* 1) Notre-Dame Hospital, University of Montreal, Montreal, PQ, Canada; 2) Pediatric department, Neurology, Ste Justine Hospital, Montreal, PQ, Canada.

Temporal lobe epilepsy (TLE) is the most common form of epilepsy and is often associated with hippocampal sclerosis. The disease usually evolves in three different stages: 1) the acute phase, associated with an initial insult (brain trauma, status epilepticus, infection); 2) the latent period where the person shows no sign of epilepsy; and 3) the chronic phase, characterised by the apparition of recurrent spontaneous seizures. Rats reproduce the pattern of evolution of TLE after the induction of a prolonged seizure (status epilepticus), making them a good model for the disease. Hippocampal sclerosis (HS) is associated with intractable TLE and has been reproduced in mice models of epilepsy. However, the molecular mechanisms underlying this neuronal death and gliosis in the hippocampus remain to be determined. Our hypothesis is that HS found in TLE occurs in individuals that are genetically predisposed. In a preliminary study, we measured the severity of the HS in 4 consanguineous strains of rats (SHR, Brown-Norway, Dahl, Lewis) after the induction of a status epilepticus (SE) using kainate acid (n=10/strain). Dahl and Lewis showed a similar sensitivity to KA and underwent a comparable SE. Interestingly, only the Dahl rats suffered from severe HS (n=9; 90%). The level of expression of all the glutamatergic receptors was measured, and no significant difference was observed. Galanin is known to have neuroprotective effect in the mice model of TLE. We therefore measured the basal level of expression of Galanin, Galanin receptor 1 (Galr1) and Galanin receptor 2 (Galr2) in the hippocampus of our control rats. The results showed that the level of expression of Galr1 was 4 times higher in the Lewis compared to the Dahl (p=0.02). We sequenced the coding region of Galr1 as well as its promoter in both strains, but no genetic variation was found. Our results suggest that decreased expression of Galr1 is associated with susceptibility to HS induced by SE. The exact genetic variation leading to decreased Galr1 expression remains to be identified.

870W

Genetic variations in matrix metalloproteinases are associated with increased risk of Ulcerative Colitis. *A.R. Morgan[1,4], D.Y. Han[1,4], W.J. Lam[1,4], C.M. Triggs[2,4], A.G. Fraser[3,4], M. Barclay[5,6], R.B. Gearry[5,6], L.R. Ferguson[1,4].* 1) Discipline of Nutrition, FMHS, The University of Auckland, Auckland, New Zealand; 2) Department of Statistics, FoS, The University of Auckland, Auckland, New Zealand; 3) Department of Medicine, FMHS, The University of Auckland, Auckland, New Zealand; 4) Nutrigenomics New Zealand; 5) Departmentof Medicine, University of Otago, Christchurch; 6) Departments of Gastroenterology & Clinical Pharmacology, Christchurch.

Matrix metalloproteinases (MMPs) are believed to play an important role in the pathogenesis of inflammatory bowel disease (IBD), functioning as pro-inflammatory cytokines, chemokines and other immune and inflammation regulators, as well as playing a role in extracellular matrix turnover.

We undertook an extensive candidate gene SNP study of the MMP family and their inhibitors and investigated MMP's -1, -2, -3, -7, -8, -9, -10, -12, -13, -14 and tissue inhibitor of metalloproteinases (TIMPs) -1, -3 and -4 for association with Ulcerative Colitis (UC). We identified tagging SNPs across these genes, and genotyped these SNPs in a Caucasian New Zealand dataset consisting of 419 UC patients and 907 controls.

Analyses showed SNPs in a number of MMP genes to be associated with UC. After correcting for multiple testing using false discovery rate analyses, SNPs in MMP-3, MMP-8, MMP-10 and MMP-14 remained significant in their associations with UC. Data from an international meta-analysis provide confirmatory results.

The data from this study provides preliminary evidence to suggest that genetic variation in the MMPs may explain a significant part of inter-individual differences in IBD susceptibility and clinical outcome, consistent with data from in vitro studies and from animal experiments.

871W

Search for candidate genes that contributes to or protect from diabetic nephropathy in type 1 diabetes through exome sequencing. *M.G. Pezzolesi[1,2], H.A. Keenan[1,2], J.C. Mychaleckyj[3], J.S. Dunn[1], S.S. Rich[3], J.H. Warram[1], G.L. King[1,2], A.S. Krolewski[1,2].* 1) Section on Genetics and Epidemiology, Joslin Diabetes Center, Boston, MA; 2) Department of Medicine, Harvard Medical School, Boston, MA; 3) Center for Public Health Genomics, University of Virginia School of Medicine, Charlottesville, VA.

Diabetic nephropathy (DN) is a late complication that affects 30% of all patients with type 1 diabetes (T1D). Only a minority of patients with T1D, approximately 5%, survive without this complication for 50 or more years following the onset of disease. Despite its known familial aggregation and intense effort to determine the genetic components that underlie its risk, no major gene that either contributes to or protects from its susceptibility has been identified. To identify genetic variants that contribute to either risk of or protection from nephropathy in T1D, we initiated a project to resequence approximately 180,000 exons for 18,673 protein-coding genes in 3 individuals who developed DN after a short duration of T1D and 5 individuals who remained free of kidney complications despite 50 or more years of disease. Following in-solution capture using Nimblegen's SeqCap EZ Exome SR, whole exome resequencing was performed on an Illumina GAIIx Genome Analyzer. The resulting data were aligned to the human genome using BWA and processed using Samtools. These data provided a mean on-target read depth of 56-80X and 20X coverage for greater than 80% of the targeted region. Multi-sample variant calling was implemented using GATK. In total, we identified 32,682 non-reference SNPs, including 2,459 (7.5%) novel variants not annotated in dbSNP or present in data available from the 1000 Genomes Project and 14,084 (43%) non-synonymous SNPs that map to 6,939 distinct genes. We identified an average of 6,574 non-synonymous SNPs, including 6,534 and 40 nonsense variants, per exome. Among these non-synonymous SNPs, 1,581 and 4,163 were unique to individuals with and without DN, respectively. Nine-hundred thirteen genes contained non-synonymous SNPs that were exclusively observed in patients with early onset DN, while 2,565 genes contained non-synonymous SNPs that were exclusively observed in patients without kidney complications. Together, these data implicate several potential candidate genes in the pathogenesis of diabetic nephropathy in type 1 diabetes. Efforts to expand this project to included ~20 individuals who developed DN after a short duration of T1D and ~20 individuals who remained free of kidney complications despite 50 or more years of disease are currently underway. We anticipate that these studies will aid in identifying genes that contain functional changes associated with either the risk of or protection against this complication.

872W

Candidate Gene Identification in a Pediatric Patient with Immune Mediated Enteropathy by Whole Genome Sequencing and VAAST Analysis. *P.G. Ridge Jr[1,2,6], R.L. Margraf[1,6], S.L. Guthery[3,6], M. Singleton[4], J.D. Durtschi[1], B. Moore[4], L.B. Jorde[4], M. Yandell[4], A. Kumanovics[1,5], K.V. Voelkerding[1,5].* 1) ARUP Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, UT; 2) Department of Biology, Brigham Young University, Provo, UT; 3) Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, UT; 4) Department of Human Genetics, Eccles Institute of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT; 5) Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT; 6) Authors contributed equally.

A 4-year-old male presented with persistent severe watery diarrhea and small intestinal total villous atrophy with dense lymphocytic infiltrate. Celiac disease antibody studies were negative and the patient failed to respond to a gluten-free diet. Coexisting hypothyroidism, hyperglycemia, and reduced numbers of FOXP3 positive CD4+ T cells suggested IPEX syndrome, but sequencing of the FOXP3 gene revealed no mutations. Under a clinical research protocol, the genomes of the patient, and asymptomatic parents and male sibling were sequenced.

Whole genome sequencing was performed using the Illumina Hiseq 2000 achieving ~30x coverage for each genome. Reads were aligned to human genome reference hg19 using BWA, with variant and indel detection performed with SAMtools. A recently developed probabilistic disease-causing variant discovery algorithm termed VAAST was used to identify candidate disease genes. VAAST performs a likelihood ratio test on variants within a given feature (gene) to calculate the probability that the target disease genotype differs significantly from the background healthy genotype. VAAST employs the use of mode of inheritance (dominant/recessive) and family pedigree information when calculating the maximum likelihood ratios.

The proband, mother, father, and unaffected sibling each had expected numbers of SNVs and indels (3.5 Million (M)/506,000, 3.6 M /500,000, 3.5 M/506,000, and 3.6 M/536,000, respectively). Each had between 10,000 and 10,500 missense variants. VAAST analysis of missense variants, assuming recessive inheritance, identified 24 candidate genes, two of which, LY9 and MICB, are of particular interest due to their known roles in immunity and previous reports suggesting roles in autoimmune diseases.

The current study, evaluating the VAAST algorithm for candidate gene identification in whole genome analyses has generated several candidate genes, including two with known roles in immunity and autoimmunity. These data provide the foundation for additional genetic and functional studies in this patient with enteropathy. Further studies will investigate other inheritance patterns.

873W

ASSOCIATION ANALYSIS OF POLYMORPHISMS LOCATED IN INFLAMMATION-RELATED GENES IN OBESITY MEXICAN PATIENTS. *Y. Saldaña-Alvarez[1], MG. Salas-Martínez[1,2], S. Jiménez-Morales[1], A. Luckie-Duque[3], G. García-Cárdenas[4], AN. Pale- Hernández[1], H. Vicenteño-Ayala[5], A. Carnevale-Cantoni[1], L. Orozco[1,2].* 1) Instituto Nacional de Medicina Genómica. Periférico Sur 4809, Col. Arenal Tepepan, Del. Tlalpan, México, D.F. C.P. 14610; 2) Posgrado en Ciencias Genómicas-UACM. San Lorenzo 290, Col. del Valle, Del. Benito Juárez, México, D.F. C.P. 03100; 3) H. R. "1o. de Octubre: ISSSTE". Av. Instituto Politécnico Nacional 1669, Col. Magdalena de las Salinas, México, D.F. C.P. 07360; 4) Clinica de Diagnostico Autorizado, ISSSTE. AV. Universidad 1321 Col. Florida, Del. Alvaro Obregon. Mexico, D. F. C.P. 01030; 5) H.R. "Lic. Adolfo Lopez Mateos". ISSSTE. AV. Universidad 1321 Col. Florida, Del. Alvaro Obregón, Mexico, D. F. C.P. 01030.

Obesity alters adipose tissue metabolic and endocrine function and leads to an increased release of proinflammatory molecules and adipokines, which have been implicated in the pathogenesis of the chronic inflammation and insulin resistance. In this way, some studies has reported a strong relationship between polymorphisms located in inflammation-related genes, such as Interleukin 6 (IL-6), Tumor Necrosis Factor (TNF( ) and C-Reactive Protein (CRP), and increasing body mass index (BMI). To investigate the possible role of inflammation-related genes, six common SNPs within IL-6, TNF(and CRP were screened in 541 obese cases (BMI>30) and 366 non-obese Mexican controls (BMI<25). Cases and controls were recruited from two tertiary Hospitals in Mexico City. We analyzed the variants rs2069827G/T and rs1554606G/T from IL-6; TNF SNPs rs1800629G/A and rs361525G/A and the variants r1205C/T, rs1130864C/T and rs1417938T/A localized on CRP gene. Genotyping was carried out by 5' exonuclease assay (TaqMan). The association test, Hardy-Weinberg Equilibrium (HWE) and haplotypes were evaluated using EPIDAT, FINETTI and Haploview software, respectively. Genotype distributions of all SNPs were in HWE in both patients and controls. When genotypes and alleles frequencies were compared between cases and controls, no significant differences were observed in any of the variants analyzed. However, when sample was stratified by severity, the CRP rs1130864T and rs1417938A alleles were found to be associated with protection against morbid obesity (allele frequency: p=0.0052, OR=0.635, 95% CI 0.461-0.874, and p= 0.0123, OR=0.665 95% CI 0.483-0.916; and genotype frequency: p=0.0221, OR=0.440 95% CI 0.215-0.900 and p= 0.0221, OR= 0.440 95% CI 0.215-0.900, respectively). Consistently, haplotype analysis reveled a severity obesity protection haplotype (CTA), p= 0.0048, OR=0.70 IC 95% 0.54-0.90 when we campared patients with BMI>35 and controls. In conclusion, polymorphisms within the CRP gene could be severity obesity modifiers in Mexican population, however, in contrast with data previously reported, our results suggest than variants in the IL6R and TNF-(genes are not associated with BMI in Mexican population. These findings reveal a genetic heterogeneity among populations and support the need to determine the behavior of polymorphisms in each population.

874W

A polygenic risk profile for Achilles tendinopathy incorporating components of the extracellular matrix degradation pathway. *A.V. September[1], E. Nell[1], J. Cook[2], C.J. Handley[3], M. Collins[1,4].* 1) MRC/UCT Research Unit for Exercise Science and Sports Medicine, Department of Human Biology, University of Cape Town, South Africa; 2) School of Primary Health Care, Faculty of Medicine, Nursing and Health Sciences, Monash University, Victoria, Australia; 3) School of Human Biosciences and the Musculoskeletal Research Centre, La Trobe University, Melbourne, Australia; 4) MRC/UCT Research Unit for Exercise Science and Sports Medicine, South African Medical Research Council, Cape Town, South Africa.

INTRODUCTION Achilles tendinopathy (AT) is a degenerative condition for which several risk factors have been implicated including components of the extracellular matrix (ECM) degradation pathway. OBJECTIVE The aim was to identify a polygenic risk profile using 6 variants within 5 genes involved in the ECM degradation pathway for AT. METHODS 191 asymptomatic controls (CON) and 110 participants diagnosed with Achilles tendinopathy (TEN) from South Africa and Australia were genotyped for 6 variants within 5 genes (COL5A1, MMP3, GDF5, IL-1beta, PTGER4). Total genotype scores (TGS) were calculated (2=risk, 1=neurtral, 0=protective). A Mann-Mitney test was used to determine differences in TGS distribution between CON and TEN. Median and inter-quartile ranges, sensitivity and specificity were calculated and a receiver operator characteristic (ROC) curve plotted to determine the accuracy of TGS to predict risk of AT. Statistical significance was accepted at $p<0.05$. RESULTS The TGS in CON (56.4±16.2) was significantly lower ($p<0.001$) than in TEN (62.8±13.9). ROC analysis showed a significant discriminating accuracy of risk of AT (AUC=0.62; 95%CI 0.55-0.767; $p<0.001$). CONCLUSION This study demonstrates that a polygenic profile incorporating components of the ECM degradation pathway discriminating between individuals with and without AT is feasible.

875W

A Genome-wide analysis of Copy Number Variations in Dyslexic families reveal Duplications in Language gene *PCDH11X*. *A.M. Veerappa[1], J.S. Marita Priya[2], P. Padakannaya[2], N.B. Ramachandra[1].* 1) Genomics Lab, DOS in Zoology, University of Mysore, Mysore, India; 2) DOS in Psychology, University of Mysore, Mysore, India.

Purpose: Developmental Dyslexia (DD) is a specific learning disability with unexpected difficulty in learning to read and spell despite adequate intelligence, education, environment and normal senses affecting 5-12% of school age children. Recent evidences suggest that structural variation, including copy number variations (CNVs), across the genome is common and likely contributes to human disease. **Methods:** Fifteen certified DD subjects from eight families were subjected to power analysis using standard parameters. Genomic DNA from each member of the family was extracted using Promega Wizard® Genomic DNA purification Kit. Genome-Wide genotyping was performed using an Affymetrix Genome-Wide Human SNP Array 6.0 chip. All SNPs that were called using Birdseed v2 algorithm had a Quality Control (QC) call rate of >97%. Contrast QC across all samples was > 2.5. Eigenstrat method and Bonferroni correction were used to avoid possibility of spurious associations and for multiple testing. **Results:** Testing for dyslexia showed impairment of mapping sound to symbol than mapping symbol to sound in the neurocognitive process. Association testing and CNV analysis revealed a microduplication at Xq21.3 in 5 dyslexic subjects from two families. This duplication varied in size from 113 kb to 212 kb and also the position of duplication site varied from upstream region through coding region to downstream regions. Further analysis revealed the location of the language gene, *PCDH11X* in the Xq21.3 region. Duplication sites in the subjects had a direct impact on the exon structure of the PCDH11X, which is known as protocadherin 11 X-linked. **Discussion:** *PCDH11X* codes for a cell adhesion molecule belonging to the cadherin superfamily. The protein products are expected to play a role in intercellular communication acting as axonal guidance factors and influencing the connectivity of the cerebral cortex. *PCDH11X* phenotypically is associated with verbal ability, language processing and cerebral asymmetry suggesting that this is a major new promising candidate gene involved in reading-spelling deficits in developmental dyslexia. Thus, partial duplications of *PCDH11X* affects the production and function of PCDH11X proteins causing dyslexia.

876W

15q11.2-13.3 chromatin analysis reveals epigenetic regulation of CHRNA7 with deficiencies in Rett and autism brain. *DH. Yasui[1], HA. Scoles[1], S. Horike[2], M. Meguro-Horike[2], KW. Dunaway[1], DI. Schroeder[1], JM. LaSalle[1].* 1) Dept Medical Micro and Immuno, Univ California Davis, Med Sch, Davis, CA. 95616 011-530-754-7906, dhyasui@ucdavis.edu; 2) Kanazawa University, Frontier Science Org. Kanazawa Japan,.

Genomic copy number variants (CNVs) within human 15q11.2-13.3 show reduced penetrance and variable expressivity in a range of neurologic disorders. Therefore, characterizing the epigenetic chromatin organization of 15q11.2-13.3 is important for understanding the structural basis of this locus in normal neuronal development. Deletion of the Prader-Willi imprinting center (PWS-IC) disrupts long range imprinted gene expression resulting in Prader-Willi syndrome. Previous results establish that MeCP2 binds to the PWS-IC and is required for optimal expression of the 15q11.2-13.3 genes GABRB3 and UBE3A. To examine the hypothesis that MeCP2 facilitates 15q11.2-13.3 transcription by linking the PWS-IC to distant loci, chromosome capture conformation on chip (4C) analysis for a 13 Mb region of 15q11.2-13.3 was performed on human SH-SY5Y neuroblastoma cells. Differentiated SH-SY5Y neurons displayed 2.84 fold fewer 15q11.2-13.3 chromatin loops formed by PWS-IC interactions than SH-SY5Y undifferentiated neuroblasts, revealing a developmental increase in chromatin decondensation. Of the total 15q11.2-13.3 PWS-IC interactions identified by 4C and MeCP2 binding sites previously identified, five sites were found to significantly overlap. Remarkably, overlapping PWS-IC and MeCP2 bound loci mapped to 15q13.3 containing CHRNA7 encoding the cholinergic receptor, nicotinic, alpha 7. Consistent with these results, siRNA knockdown of MeCP2 levels abolished PWS-IC interaction with CHRNA7. Subsequent quantitative transcriptional analyses of frontal cortex from Rett syndrome and autism patients with reduced MeCP2 protein revealed significantly reduced CHRNA7 expression compared to control cortices. PWS-IC interaction with CHRNA7 was independently confirmed by FISH analysis of SH-SY5Y neuroblasts and neurons. Together, these results suggest that transcription of CHRNA7 is modulated by chromatin interactions with the PWS-IC. Loss of long-range chromatin interactions within 15q11.2-13.3 may contribute to multiple human neurodevelopmental disorders.

877W

Functional characterization of DcR3 in EBV transformed cell lines from IBD patients of different allelic background and role in disease pathogenesis. *R. Pandey, C. Cardinale, S. Panossian, F. Wang, E. Frackelton, C. Kim, M. Frank, R. Chiavacci, K. Kachelries, S. Grant, R. Baldassano, H. Hakonarson.* Centre for Applied Genomics, Children's Hospital Of Philadelphia, Philadelphia, PA., Select a Country.

Aim: Decoy receptor3 (DcR3), a soluble receptor for FasL, plays significant role in immune suppression and tumor progression by neutralizing the FasL mediated apoptosis signal. Here we investigate the possible immuno-modulation mediated by DcR3 in EBV transformed control and patient derived cell lines with and without risk variants in the TNF Receptor Superfamily 6B gene, TNFRSF6B captured by the tagging SNP, rs2315008). Methods: Expression of DcR3 and its kinetics were examined by immunoblot analysis in whole cell lysates from EBV transformed control and patient derived cell lines of different genotype background for rs2315008 (AA, AT, TT). DcR3 induced rapid activation of nuclear factor 2B (NF-2B) monitored by immnunoblot analysis of IkB(. DcR3 knockdown was performed using specific DcR3 siRNA. Cell proliferation and cell death was measured by MMT assay. Caspase8, caspase9, caspase3, and Bcl2 expression were analyzed by western blots. Results: EBV transformed cell lines derived from IBD patients harboring risk variants in TNFRSF6B (A allele) exhibit differential pattern of DcR3 expression and NF-2B kinetics in comparison with wild type. siRNA mediated knockdown post 24hrs of nucleofection results in decreased DcR3 expression, increased cell death and decreased cell proliferation, effects that were also genotype-dependent. Conclusion: EBV cell lines from IBD patients harboring risk variants in the TNFRSF6B gene exhibit differential pattern of DcR3 expression and NF-2B activation and promote inflammation in Crohn's disease by inhibiting FasL-induced apoptosis.

878W

Detection of the 16p11.2 chromosomal rearrangements in two patients with global developmental delay and dysmorphism. *E. Shin[1], c. Jung[1], k. lee[1], h. yeun[1], h. jung[2].* 1) Genome Res Ctr, NeoDin Med Inst, Seoul, seoul, Korea; 2) Dept. of Pediatrics, NHIC Ilsan Hospital.

Recurrent microdeletion and microduplication of approximately 550 kb at 16p11.2 was reported to occur in developmental delay, cognitive impairment, autism spectrum disorder, seizures, minor dysmorphic features without a consistent pattern and abnormal head size. Recently, many new diagnoses of 16p11.2 microdeletions and microduplications have been made by array CGH (comparative genomic hybridization) and congenital anomalies and abnormal brain findings were frequently observed in patients with these rearrangements. We report two patients identified by microarray analysis with copy number variation (CNV) of 16p11.2 (one deletion and one duplication) using Roche NimbleGen 135K (Roche NimbleGen Inc., USA). Case 1 is a 1 year old boy with dysmorphic features and mild Rt/Lt ventriculomegaly with brain MRI and has deletion in 16p11.2. And, case 2 is a 3 year old boy with developmental delay, hypotonia, failure to thrive and low set ear and has duplication in 16p11.2. Two all patients have a heterozygous loss or gain corresponding to chromosomal coordinates (chr16:26,594,890-30,100,123) with a minimal size of 535.23 kb. Recurrent reciprocal 16p11.2 deletion and duplication are charaterised by a spectrum of primarily neurocognitive phenotype that are subject to incomplete penetrance and variable expressivity. Two of our cases indicates that recurrent 16p11.2 deletions and duplications are associated with variable clinical outcome, most likely arising from haploinsufficiency of one or more genes.

879W

Strategies for analyzing allele specificity in ChIP-seq data. *V. Vacic[1], N. Dewal[1], T. LaFramboise[2], M.L. Freedman[3,4], I. Pe'er[1].* 1) Department of Computer Science, Columbia University, New York, NY; 2) Department of Genetics, School of Medicine, Case Western Reserve University, Cleveland, OH; 3) Department of Medicine, Harvard Medical School, Boston, MA; 4) Medical Oncology Service, Dana-Farber Cancer Institute, Boston, MA.

Allele specificity can be used as a powerful tool for interrogating the relationship between genetic and functional variation. It opens the possibility for novel discoveries, such as identifying distal promoters or associating regulatory elements to genes, by probing functional variation at heterozygous sites in a single sample. In addition, analysis of allele specific effects is appealing because it eliminates the influence of environment and minimizes potential noise from otherwise variable factors across multiple experiments. We examine single nucleotide variants and how they influence distributions of histone modification marks and binding sites of DNA binding proteins. Unfortunately, it has been demonstrated that naïve analysis of allele specificity performed via aligning ChIP-seq reads against a default human reference introduces subtle biases which hamper discovery. We devise a pipeline to facilitate unbiased analysis of allele specificity in functional sequencing. When genetic variation is known upfront, biases can be mitigated by aligning reads against a “personalized” set of two haploid reference copies, modified with appropriate variants. We demonstrate that this removes bias. Under the same quality measurements we align about 0.75 to 1% more reads which span het SNPs. The unbiased ascertainment of such reads is essential for identifying sites of allelic imbalance and estimating their significance. In addition we evaluate the power to call het SNPs within deeply-sequenced transcription factor-binding peaks or histone modification domains -- which are the sites of interest in functional sequencing -- using ChIP-seq reads only. We tested this approach on HapMap sample NA12878, for which a complete catalogue of variation is known due to the 1000 Genomes Project. Using the publicly available single-end ChIP-seq data from ENCODE project the power was in the 16-26% range while on our previously unpublished paired-end ChIP-seq data the power was 84-90%. These results underscore the benefits of deep paired-end sequencing in allele specificity studies. Our results open a possibility of using allelic imbalance in ChIP-seq disease studies of samples which did not have their whole DNA sequenced and are directly applicable to studies of regulatory perturbations in cancer.

880W

MitoDx and MitoNucleomeDx: Next Generation (NextGen) Sequencing of the Mitochondrial Genome and Nuclear Genes Synergistically Enhances the Diagnosis of Mitochondrial Disease. *N. Neckelmann[1], C. Buzin[1], W. Scaringe[1], A. Zare[1], C. Boysen[1], M. Pold[1], R. Boles[2], S. Sommer[1].* 1) MEDomics, LLC, Azusa, CA., Select a Country; 2) Los Angeles Childrens Hospital.

90+ percent of the cellular energy is derived from oxidative phosphorylation (OXPHOS) within mitochondria. The human mitochondrial DNA (mtDNA) contains only 37 genes (13 proteins required for OXPHOS, 2 ribosomal RNA genes, and 22 tRNA genes), but these genes mutate rapidly because of (i) high exposure to free radicals and other mutagenic agents, and (ii) only rudimentary mechanism are known to exist for the repair of DNA damage. However, the vast majority of genes required for oxidative phosphorylation are found within the nuclear genome. Evidence from more limited previous technology suggests that most mitochondrial disease mutations in children result from nuclear gene mutations, while most mitochondrial disease in adults arises from mitochondrial genome mutations. The revolutionary power of NextGen sequencing enables the comprehensive analysis of mtDNA mutations at ultra-low heteroplasmy levels, via ultra-deep sequencing, and the analysis of the many mitochondrial nuclear genes listed in OMIM as OXPHOS-associated diseases, as well as 120+ additional genes involved in OXPHOS and related biochemical pathways. Synergistic NextGen Sequencing of both the mitochondrial genome and OXPHOS nuclear genes allows analysis of patients suspected of having mitochondrial diseases with unprecedented rigor. In addition, sequencing of both the mitochondrial genome and the mitonucleome facilitates the analysis of interactions between mtDNA and nuclear gene variants which may affect the penetrance and/or severity of the disease. Three illustrative cases will be presented. It is hoped that coordinated NextGen sequence-based testing of DNA derived from blood samples will send most muscle biopsies to hell, where they belong.

881W

A Comprehensive Next-Generation Sequencing Panel for Molecular Testing of Primary Ciliary Dyskinesia (PCD) and PCD-like Ciliopathies. *S. Lee, S. Mexal, W. Guo, A. Kammesheidt.* Ambry Genetics, Aliso Viejo, CA.

Primary Ciliary Dyskinesia (PCD) is a genetically heterogeneous, usually autosomal recessive disorder with impaired ciliary function leading to progressive sinopulmonary disease. Upper and lower respiratory tract manifestations are key features of PCD and are often present at birth. Upper respiratory tract features include chronic nasal drainage, sinusitis, and otitis media. Lower respiratory features include neonatal respiratory distress, chronic productive cough, chronic bronchitis, recurrent pneumonia, and bronchiectasis. Approximately half of the PCD patients have situs inversus (also known as Kartagener's syndrome) and at least ~6% have situs ambiguous (heterotaxy). Fetal cerebral ventriculomegaly and hydrocephalus can also occur due to impaired circulation of the cerebrospinal fluid. In adults with PCD, male infertility and female sub-fertility are also common features. Prompt diagnosis of PCD is critical for the prevention of secondary respiratory complications, such as bronchiectasis, pneumonia and/or progressive loss of lung function. Diagnosis of PCD has relied on identification of ciliary dysmotility and specific ciliary ultrastructural defects. Most PCD patients (80-90%) have defective outer dynein arms (ODA), and/or inner dynein arms (IDA). A few patients have defective radial spokes (RS) or central apparatus (CA). Because ultrastructural studies are challenging and not readily available, the diagnosis is often delayed or missed or made incorrectly. In some patients, PCD is confirmed by genetic studies despite normal ciliary ultrastructure. Thus far, disease-causing mutations have been identified in eleven genes coding for ODA proteins (DNAI1, DNAI2, DNAH5, DNAH11, TXNDC3), RS proteins (RSPH4A, RSPH9), cytoplasmic proteins involved in DA assembly (C14orf104/KTU, LRRC50) and in RPGR and OFD1. Full gene sequence analysis in molecular testing of PCD is costly and time-consuming due to the relatively large size of many of the disease genes and the extensive locus heterogeneity. Targeted resequencing offers an alternative, more cost-effective approach for enrichment and next-generation sequencing (NGS) of all exons of candidate genes in parallel. RainDance targeted amplification of exons and exon/intron boundaries of eleven genes associated with PCD was carried out followed by Illumina's NGS. This study presents performance data of the Ambry panel for Primary Ciliary Dyskinesia (PCD) and PCD-like ciliopathies.

882W

Detection of copy-number changes in Bardet-Biedl syndrome by targeted array CGH. *A. Lindstrand[1], C. Carvalho[2], J.R. Lupski[2,3], N. Katsanis[1].* 1) Center for Human Disease Modeling, Duke University Medical Center, Durham NC, USA; 2) Department of Molecular and Human Genetics and Department of Pediatrics, Baylor College of Medicine, Houston TX, USA; 3) Texas Childrens Hospital, Houston TX, USA.

Bardet-Biedl syndrome (BBS: MIM209900) is a multisystemic developmental disorder characterized by retinal degeneration, obesity, polydactyly, mental retardation, renal dysfunction, and hypogonadism. Extensive in vitro and in vivo studies have highlighted BBS as a model ciliopathy, with at least 16 causal genes. In addition, cis and trans modifier alleles contribute to variable expressivity and reduced penetrance both in BBS and across the ciliopathy spectrum, supporting the hypothesis that the mutational load in the ciliary proteome plays a critical role in defining disease presentation. As part of our systematic analysis of the ciliary proteome in BBS families irrespective of their primary genetic lesion, we have initiated a systematic examination of CNV in disease burden. To establish the genetic contribution of CNVs in the known BBS loci and to identify new BBS genes, we tested 102 BBS patients by a targeted custom array CGH with 180,000-60mer oligos/array and 4 arrays/slide focusing on 772 `ciliary proteome'-genes. On average, coding sequences had a probe density on 1/100bp and non-coding sequences 1/500bp. Target genes were selected based on known association with ciliopathies, known association with in vivo ciliary dysfunctions and genes from the ciliary proteome. The results showed the presence of multiple CNVs in known BBS genes including either single or multiple exons (for example ARL6, BBS4, BBS5 and BBS12), as well as in other ciliopathy genes (such as NPHP1); indicating that this mutational mechanism contributes significantly to the disease burden. In addition, we found new candidate BBS loci that are presently undergoing in vivo and in vitro validations. Functional studies will also demonstrate whether more common copy number changes might be causal and/or represent modifiers in BBS.

883W

ChIP-Enrich: An application for improved gene set enrichment testing of ChIP-Seq results. *C. Lee[1], R.A Smith[2], R. Welch[1], L. Scott[3], M.A. Sartor[1,3].* 1) Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI; 2) Human Genetics, University of Michigan, Ann Arbor, MI; 3) Biostatistics, University of Michigan, Ann Arbor, MI.

Gene set enrichment testing is used to identify predefined biologically related sets of genes that are over- or under-represented in data from a biological experiment. ChIP-Seq experiments allow assessment of genome-wide protein-DNA interactions. Microarray based gene set enrichment testing methods have been used to analyze ChIP-Seq peaks, but direct use of these methods can create false positives and negatives. Unlike microarray data each ChIP-Seq peak has to be assigned to a gene. Genes with greater distance to their nearest neighbors are more likely to contain a peak and biological categories such as nervous system, development, cell adhesion, and transcription are enriched for such genes. Furthermore, some gene loci are more mappable than others, and peaks are more likely to be identified in highly mappable loci. Existing methods do not take mapability into account; this can affect the results, because as we show, mapability is also associated with functional gene categories. We developed an empirical approach to correct for potential biases introduced by differing locus lengths and mapabilities. We estimated weights for each gene based on the relationship between the mappable locus length and presence of a peak in the overall data using a binomial smoothing spline. We perform a modified version of the random sets method, weighing genes to correct for peak bias due to locus length and mapability. Our method is available in the application, ChIP-Enrich. We tested our method on simulated and experimental ChIP-Seq data. For example, we simulated ChIP-Seq peaks such that a specified percent were false positives (randomly assigned to the genome), and a randomly chosen Gene Ontology (GO) term, e.g. reproduction, was purposely enriched. We used Fisher's exact test and ChIP-Enrich to test for enrichment. Fisher's exact test identified nervous system development and other GO terms with longer loci lengths as most significantly enriched, whereas ChIP-Enrich correctly identified reproduction and related GO terms. Simulating data under the null hypothesis, ChIP-Enrich showed the expected uniform distribution of p-values. With experimental data, we show that ChIP-Enrich provides expected results, as opposed to Fisher's Exact test, which resulted in nervous system development as the top enriched GO term. Our results suggest that our method corrects for bias due to gene locus length and mapability, thus allowing for discovery of biologically enriched gene sets.

884W

Computational Genomics for genome sequencing: development of a pipeline graphical workflow environment. *F. Macciardi[2,3], F. Torri[2,3], P. Petrosyan[1], Z. Liu[1], A. Zamanyan[1], P. Eggert[1,4], J. Pierce[1], A. Genco[1], J.A. Knowles[5], A.P. Clark[5], J.D. Van Horn[1], J. Ames[2], C. Kesselman[2], A.W. Toga[1,2], S.G. Potkin[2,3], I. Dinov[1,2].* 1) Laboratory of Neuro Imaging (LONI), UCLA, Los Angeles, CA; 2) Biomedical Informatics Research Network (BIRN), Information Sciences Institute (ISI-USC), Los Angeles, CA; 3) Department of Psychiatry and Human Behavior, UCI School of Medicine, Irvine, CA; 4) Department of Computer Science,UCLA, Los Angeles, CA; 5) Zilkha Neurogenetic Institute, USC Keck School of Medicine, Los Angeles, CA.

Background and rationale Whole-genome and exome sequencing in simple traits have already proven to be powerful methods to identify genes responsible for several disorders. These methods are also beginning to be applied to complex traits as well, other than being adopted as the current mainstream approach in population genetics. These achievements have been possible mostly thanks to next generation sequencing technologies; however, a substantial bioinformatics work is needed to analyze sequence data. Within the framework of a joint collaboration between LONI (Laboratory of Neuro Imaging) at UCLA and BIRN (Biomedical Informatics Research Network) at UCI, ISI and USC, we have developed a pipeline environment for genomics computations that may enable genetic investigators to simplify and speed-up their approaches to real genome sequencing projects. **Methods: Implementation of Genomics and Informatics Pipeline Protocols** To develop a complete Pipeline biomedical solution to a well-defined genomic computational challenge as analyzing sequencing data, there are essential steps to follow: protocol design, tool installation, module definition, workflow implementation, and workflow validation. Implementing these steps for specific sequencing issues and using the framework of the already existing LONI pipeline environment originally created for neuroimaging analyses[1,2], we have developed modules for basic sequence alignment and search, sequence data quality control, SNPs and CNVs identification, molecular annotation and visualization of results, These represent the essential basic steps of any genome sequencing task. **Results** We developed the Graphical Pipeline for Computational Genomics and Visual Informatics (GPCG) with a flexible graphical infrastructure for efficient biomedical computing and distributed informatics research. The GPCG is intended to satisfy needs of geneticists and computational scientists who are interested in whole genome sequence projects. Our aim is to allow advanced sequence data processing, integrate diverse arrays of computational tools and services, promote community-based protocol validation, and openly share and disseminate knowledge, tools and resources. The current version of the pipeline is available for interested researchers at both http://www.birncommunity.org/ and http://pipeline.loni.ucla.edu [1] Rex DE et al 2003-Neuroimage doi:10.1016/S1053-8119(03)00185-X [2] Dinov I et al 2010-PLoS ONE doi:10.1371/journal.pone.0013070.

885W

Molecular characterization of mutations in CYP21A2 gene from indian congenital adrenal hyperplasia patients. *N. Rehal[1], A. Bhansali[2], R. Walia[2], D. Dayal[3], G. Kaur[4], R. Prasad[1].* 1) Biochemistry, PGIMER, Chandigarh, India; 2) Endocrinology, PGIMER, Chandigarh; 3) Pediatrics , PGIMER, Chandigarh; 4) Physiology, GMCH, Chandigarh.

The congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder that occurs due to presence of mutations in CYP21A2 gene, which expresses 21-hydroxylase enzyme. It catalyzes the conversion of progesterone to deoxycortisone. More than 90% of cases are caused by 21-hydroxylase deficiency, and the severity of the resulting clinical symptoms varies according to the level of 21-hydroxylase activity. The CAH is associated with different phenotypes, viz simple virilizing, salt wasting and nonclassical CAH. It is noteworthy that there are only few reports available on spectrum of mutations in CYP21A2 gene from Indian CAH population. In view of this fact, the present study was conducted to establish spectrum of mutation in CYP21A2 gene from Indian patients. In this study 35 CAH patients and their families were included. Out of the 35 patients, 43% were males and 57% were females. The serum 17OHP levels were found to be several folds higher than the normal in CAH patients. Additionally, hyponatremia and hyperkalemia were also observed in the patients. In salt wasting cases, minimum and maximum ages of onset were found to be at birth and 26 days of life respectively. In simple virilizing cases, the minimum and maximum ages of onset were found to be at birth and 16yrs respectively. In nonclassical CAH cases, the minimum and maximum ages of onset were found to be at 13 yrs and 19 yrs respectively. Out of 35 patients 50% were of simple virilizing, 29% were of salt wasting and 21% were of nonclassical CAH. Further, the studies were carried out to establish the spectrum of mutations in CYP21A2 gene from CAH patients. At first instance, most common mutations present in other populations were analysed using RFLP, further these mutations were molecularly characterized by subsequent DNA sequencing. The genetic analysis study revealed that maximum patients were heterozygous for R356W mutation, followed by gene deletion. It was ascertained that P30L and V281L, I172N and i2g , R356W, E6 cluster, Q318X, and gene deletion are associated with non classical, simple virilizing and salt wasting forms of CAH respectively. In conclusion the most common mutation (R356W) was found with 54% frequency. Our results have shown a good genotype/phenotype correlation in the case of most common mutations.

886W

***De novo* transcriptome assembly by pooling samples in case-control studies identifies novel events missed by alignment-based approaches.** *M.W. Snyder[1], R.P. Nair[2], L.C. Tsoi[1], T. Tejasvi[2], P.E. Stuart[2], B. Li[1], H.M. Kang[1], J.T. Elder[2,3], G.R. Abecasis[1].* 1) Department of Biostatistics, University of Michigan, Ann Arbor, MI; 2) Department of Dermatology, University of Michigan, Ann Arbor, MI; 3) Ann Arbor Veterans Affairs Hospital, Ann Arbor, MI.

Next-generation shotgun sequencing technologies allow the characterization of the transcriptomes of individual tissues. Typically, many short sequence reads are generated from a cDNA library, which provides a snapshot of gene expression in a particular sample. These short sequence reads can either be mapped to known transcript sequences (or the reference genome) or can be assembled *de novo*. While technically more challenging, *de novo* transcriptome assembly has enhanced ability to detect completely novel transcripts, including variation not present in the reference genome that might be missed by alignment-based analyses.

Here, we used next generation sequencing technologies to characterize gene expression in punch biopsy samples of lesional skin from 92 psoriasis patients and site-matched samples of normal skin from 82 controls. We generated an average of 38 million 80-bp reads per sample. We first pooled reads across individuals and assembled potential transcripts using the software ABySS. After assembly, contigs were filtered by length and number of supporting reads, and compared against known transcript and EST collections to identify candidate novel events. At the time of abstract submission, we identified 18,919 known transcripts representing 14,335 genes present in both cases and controls, along with smaller numbers of transcripts unique to each set (1,084 in cases 1,864 in controls). We further assembled 8,585 and 13,076 contigs 100bp or longer in cases and controls, respectively, which were unmapped to reference collections and potentially represent previously unannotated transcripts or portions of transcripts. We then searched for evidence of differential gene expression between cases and controls and identified 2,049 differentially expressed genes. The set of highly differentially expressed genes included IL17D, IL20RA and IL20RB, and IFNGR2, consistent with previous reference-based analyses, but also identified differential expression of previously unidentified transcripts such as an extended 3' UTR in DSC1.

Our results show that *de novo* transcriptome assembly in case-control samples can yield insights into the genetic architecture of complex diseases.

887W

Complex structural polymorphisms predispose to 15q13.3 deletion syndrome. *F. Antonacci[1], PH. Sudmant[1], JA. Rosenfeld[2], L. Vives[1], A. Stuart[3], TA. Graves[4], RK. Wilson[4], LG. Shaffer[2], CT. Amemiya[3], EE. Eichler[1].* 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) Signature Genomic Laboratories, LLC, Spokane, WA 99207, USA; 3) Benaroya Research Institute at Virginia Mason, 1201 9th Avenue, Seattle, WA 98101, USA; 4) The Genome Institute at Washington University, Washington University School of Medicine, St Louis, MO 63108, USA.

The human genome is a highly dynamic structure that shows a wide range of genetic variation ranging from SNPs to a variable number of tandem repeats and structural alterations such as deletions, duplications and inversions. Complex structural polymorphisms are significantly enriched in regions of segmental duplications but most of this diversity has yet to be characterized at the sequence level. Different duplication architectures have been shown to predispose and protect specific haplotypes as well as different population groups to microdeletions associated with genomic disorders. The 1.5 Mbp microdeletion at chromosome 15q13.3 is one of the most common recurrent mutations associated with intellectual disability, schizophrenia and epilepsy. We developed next-generation sequencing coupled with cytogenetic-based assays to characterize the organization, orientation and segmental duplication architecture of this region in multiple individuals from different ethnicities. Using fluorescence in situ cohybridization experiments, we identified a total of four alternate structural configurations of the 15q13.3 region ranging in size from 2.2 to 2.8 Mbp. In particular, the inverted haplotype shows a significantly smaller and less complex organization than the directly oriented haplotypes. Our preliminary analysis of 809 human genomes suggests an inversion allele frequency of 6% in individuals from different populations. Analyzing multiple individuals from two outgroup species of great apes (chimpanzee and orangutan), we show that the large inversion polymorphism is specific to the human lineage. To resolve the complex segmental duplication organization of the 15q13.3 region, we are creating a BAC library resource from 16 human genomes. Complete sequencing of a contiguous set of 17 BAC clones from one haplotype corresponding to this 2.8 Mbp region on 15q13.3 has discovered a smaller inversion of a 150 kbp region not detectable by cytogenetic assays. This inversion places pairs of highly identical segmental duplications, flanking the disease-critical region, in direct orientation creating a configuration predisposing to 15q13.3 microdeletion. Using high density and targeted array-based comparative genomic hybridization (CGH) experiments, we examined 13 microdeletion samples and mapped the breakpoints of the disease-critical region to these large blocks of segmental duplications, which we posited might mediate the recurrent rearrangement associated with disease.

888W

Hydroxyurea induces de novo copy number variants in human cells. *M.F. Arlt[1], A.C. Ozdemir[1], S.R. Birkeland[2], T.E. Wilson[2], T.W. Glover[1].* 1) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 2) Department of Pathology, University of Michigan, Ann Arbor, MI.

Copy number variants (CNVs), defined as deletions or duplications of a few kilobases to over a megabase, are widely distributed throughout the human genome, where they contribute to genetic variation and phenotypic diversity. Spontaneous CNVs are also a major cause of genetic and developmental disorders, including intellectual disability, neuropsychiatric disorders and structural birth defects, and arise frequently in cancer cells. As with all mutation classes, genetic and environmental factors almost certainly increase the risk for new and deleterious CNVs. However, despite the importance of CNVs, there is limited understanding of these precipitating risk factors and the mechanisms responsible for a large percentage of CNVs. Here we report that low doses of hydroxyurea, an inhibitor of ribonucleotide reductase and an important drug in the treatment of sickle cell and other diseases induces a high frequency of de novo CNVs in cultured human cells that resemble pathogenic and aphidicolin-induced CNVs in size and breakpoint structure. We exposed normal human fibroblasts to HU-mediated replication stress and generated clonal cell populations, which were analyzed for CNVs using high-resolution SNP arrays. We found that treatment with low doses of HU, equivalent to patient serum levels, resulted in de novo CNVs at a frequency of 1.8-2.4 CNVs/clone, a significant increase over the 0.7 CNVs/clone seen in untreated cells ($p<0.001$). These CNVs are distributed throughout the genome, and several hotspots of de novo CNV formation were found, including 3q13.31 at the LSAMP gene and 7q11.22 at AUTS2. Sequencing revealed that CNV breakpoint junctions are characterized by short microhomologies, blunt ends, and short insertions, consistent with the hypothesis that these rearrangements are the result of nonhomologous repair processes and not homologous recombination repair. These data provide direct experimental support for models of replication-error origins of CNVs and further suggest that any agent or condition that leads to replication stress has the potential to induce deleterious CNVs. In addition, they point specifically to a need for further study of the genomic consequences of the therapeutic use of hydroxyurea.

889W

A Genome-Wide Investigation of Copy Number Variation in Patients with Sporadic Brain Arteriovenous Malformation. *N. Bendjilali[1], H. Kim[1], S. Weinsheimer[1], P.Y. Kwok[1], J.G. Zaroff[2], S. Sidney[2], C.E. McCulloch[1], W.L. Young[1], L. Pawlikowska[1].* 1) University of California , San Francisco, CA; 2) Kaiser Permanente Division of Research, Oakland, CA.

Background: Brain arteriovenous malformations (BAVM) are a tangle of poorly formed blood vessels with abnormal connections between arteries and veins. AVMs often occur in patients with hereditary hemorrhagic telangiectasia, an autosomal dominant disease with mutations in TGF-beta pathway genes, but most are sporadic. Copy number variation (CNV) has been reported in TGF-beta pathway genes and in other brain vascular malformations. We hypothesized that CNVs (defined as deletions or duplications of a DNA segment >1kb in size) may be associated with sporadic BAVM. **Methods:** 338 BAVM cases and 510 healthy controls of Caucasian ethnicity were genotyped on the Affymetrix 6.0 SNP array. We used PennCNV to identify deletions and duplications on the 22 autosomes, adjusting for genomic waves. We used standard quality control (QC) filters, excluded outliers, and further restricted analysis to CNVs called based on >10 markers. CNVs passing QC were merged into CNV regions (CNVR) using a reciprocal overlap threshold of >70%. We performed both a CNVR-based analysis and a gene-based analysis to assess for association with BAVM compared to controls using one-sided Fisher's exact tests. A Bonferroni correction was used to adjust for multiple testing. To validate our findings, we repeated the above analyses using CNVs called by the Birdsuite algorithm. **Results:** After QC, we observed a total of 34,253 CNV calls in 270 cases and 457 controls using PennCNV. 5119 CNVRs were constructed and tested for association with BAVM; 2 CNVRs (duplications) on 1p36.13 and 4p16.3 were significantly enriched in cases compared to controls using PennCNV and Birdsuite. From the gene-based analysis, 28 genes showed statistically significant enrichment of duplications in cases versus controls. Of those, 13 genes were replicated using Birdsuite. 4 of these 13 genes map to the associated CNVRs on 1p36.13 (NBPF1 and NBPF10) and 4p16.3 (ZNF595 and ZNF718). **Conclusions:** We conducted the first genome-wide study of CNVs in sporadic brain AVM, and have identified two CNVR loci that significantly differ between cases and controls. These CNV loci overlap NBPF genes known as candidate tumor suppressor genes linked to neuroblastoma, where ZNF595 and ZNF718 genes may be involved in transcriptional regulation. These results need to be validated experimentally and replicated in an independent brain AVM cohort.

890W

CNV load in recessive carrier status - preliminary findings from exon-targeted array comparative genomic hybridization (aCGH). *P.M. Boone[1], C. Shaw[1,2], S.W. Cheung[1,2], A.L. Beaudet[1,2,3,4,5], P. Stankiewicz[1,2,6], A. Patel[1,2], J.R. Lupski[1,2,4,5].* 1) Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Medical Genetics Laboratories, Baylor College of Medicine, Houston, TX; 3) Dept. of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX; 4) Dept. of Pediatrics, Baylor College of Medicine, Houston, TX; 5) Texas Children's Hospital, Houston, TX; 6) Dept. of Medical Genetics, Institute of Mother and Child, Warsaw, Poland.

We previously reported that exon-focused clinical aCGH, in which array probes are concentrated in exons of disease genes, enables the detection of small copy-number variations (CNVs) affecting single genes or even single exons. We now show that, in addition to detecting intragenic CNVs associated with specific dominant clinical phenotypes, exon-targeted aCGH unveils heterozygous single-gene mutations in recessive disease genes (i.e. establishing carrier status) and in disease genes for late onset dominant disorders (i.e. potentially predicting disease susceptibility). DNA from 9,005 anonymized clinical patients was analyzed by exon-targeted aCGH. Computational analyses revealed CNVs in 3,946 individuals, 1,295 of whom exhibited a single CNV affecting a single gene. Eighty-six of these CNVs encompassed or disrupted one of 242 genes implicated in severe, pediatric, recessive disease [Bell et al. Sci Transl Med 3:65ra4 (2011)] and for which at least one exon had enhanced probe coverage on our array. In total, 39 unique genes were affected, including *AHI1* (Joubert syndrome, type 3), *ATR* (Seckel syndrome, type 1), *BCKDHB* (classic maple syrup urine disease, type 1B), and *CRTAP* (osteogenesis imperfecta, type IIB). For 77/86 individuals, a brief (1-10 words) clinical indication was available; 6 of these descriptions suggested the possibility of recessive disease corresponding to the affected gene [e.g. “dysmorphic features” and *VPS13B* (Cohen syndrome)], though no indications were pathognomonic for any disorder. Our cohort also includes examples of potentially damaging single-gene CNVs in genes associated with susceptibility to dominant late onset disease, for example *SPAST* (autosomal dominant hereditary spastic paraplegia, type 4) and *EXT2* (multiple exostoses, type 2). Previous investigation of carrier status and genetic load, whether population-based or grounded in recently acquired personal genome sequences, has focused on simple nucleotide variation (SNV). Our findings demonstrate that assessing CNV using clinical exon-targeted aCGH is an effective means not only for diagnosing genetic illnesses, but also for identifying CNV resulting in recessive carrier states and potential predisposition to late onset dominant disease. Which of these many variants are likely medically actionable and reportable to clinicians is an evolving question, made particularly challenging when the mutation is novel and has not been functionally or epidemiologically assessed.

891W

Copy Number and Allelic Variation of Immunoglobulin Variable Gene IGHV1-69 and HIV Progression. *F. Breden[1], C. Watson[1], J. Willsey[1], M. Brockman[2], F. Pererya[3], B. Walker[3].* 1) Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada; 2) Molecular Biology and Biochemistry and Faculty of Health Sciences, Simon Fraser University, Burnaby, British Columbia, Canada; 3) Ragon Institute of MGH, MIT and Harvard, Charlestown, Massachusetts, USA.

The immunoglobulin Variable (V) gene IGHV1-69 is used in at least one neutralizing antibody against HIV, and is greatly enriched in the antibody responses to HIV and influenza. This gene is also elevated in several autoimmune diseases and is characteristic of CLL (chronic lymphocytic leukemia). Human IGH haplotypes exhibit extensive copy number (CNV) and allelic variation for IGHV1-69, although how this variation is distributed among geographical populations is unknown. Also, it is not clear how well human SNP assays can determine these IGHV1-69 haplotypes, and therefore possible associations between germline variation and human diseases remains untested by conventional genome-wide association studies (GWAS). To test for such associations, we have developed a TaqMan assay to measure copy number and allelic variation in order to rigorously genotype individuals at IGHV1-69. We validated this approach by comparing CNV and allelic state of several human HapMap samples by cloning and sequencing and by our new TaqMan assay. The results agreed in all cases. We then compared spontaneous controllers of HIV infection to normal HIV+ progressors, and observed an increase in copy number for alleles with a distinctive hydrophobic amino acid in CDR-H2 in the spontaneous controllers. This allele is known to be associated with anti-HIV and anti-flu antibodies.

892W

Hutterite genome sequencing identifies potential asthma genetic susceptibility variants. *C.D. Campbell[1], M. Malig[1], A. Ko[1], L. Vives[1], B.J. O'Roak[1], C. Alkan[1], P.H. Sudmant[1], L. Han[2], M. Abney[2], M.J. Rieder[1], C. Ober[2], E.E. Eichler[1,3].* 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) Department of Human Genetics, University of Chicago, Chicago, IL; 3) Howard Hughes Medical Institute.

Copy number variants (CNVs) may be important in the development of asthma: many associations between common CNVs and disease were with immune-related phenotypes, and immune genes are overrepresented in CNV regions. We hypothesized that CNVs, including recurrent CNVs, are a source of genetic risk for asthma. To test this hypothesis, we studied individuals from an extended 13 generation Hutterite pedigree who have reduced genetic heterogeneity due to a small founding gene pool and reduced environmental heterogeneity as a result of a communal lifestyle. We selected six individuals for whole-genome sequencing: three had asthma and were from asthma-enriched families and three did not have asthma and were from asthma-poor families. All six individuals are related to each other within four to ten generations. We generated 10-15X effective sequence coverage of the genome for each of these six individuals using the Illumina HiSeq2000 platform. Using sequencing read-depth, we have genotyped 7,550 known CNVs. Of these CNVs, 3,456 (46%) were copy number variable in these six individuals; 97 CNVs were enriched in the asthmatics or had an asthmatic individual as an outlier compared to the controls and to 45 CEU individuals (1000 Genomes Project). We tested 31 of these CNVs using microarray comparative genomic hybridization in an additional 74 Hutterites with asthma and 201 Hutterite controls and found three (9.7%) that showed a nominally significant difference between asthmatics and controls. Of particular interest was the *beta-defensin* cluster of genes on chromosome 8, where we found that the median copy number of asthmatic individuals was significantly lower than controls ($p = 0.019$). Defensins are antimicrobial peptides, and copy number of this locus has been implicated in other immune-related diseases. In addition, we have identified previously unreported genetic variants, including 750 deletions from pair-end mapping (including 44 that affect coding sequence), 56 frame-shifting indels, 25 nonsense mutations, 34 splice-site mutations, and 1,302 missense mutations. We are integrating both CNV and other disruptive mutations into the haplotype structure of these six individuals to identify regions of identity-by-descent that may help to explain the enrichment of asthma in some families and paucity of asthma in other families of this large pedigree.

893W

dbVar: A database of genomic structural variation. *D. Church, T. Hefferon, J. Lopez, J. Garner, N. Husain, M. DiCuccio, J. Paschall, M. Feolo, S. Sherry, D. Maglott.* Natl Ctr Biotech Infor/NIH, Bldg 45 Rm 5AS43, Bethesda, MD.

As part of NCBI's comprehensive approach to facilitate research into the discovery and understanding of human variation, we have developed an archive of large-scale variation called dbVar. In collaboration with our sister archive DGVa, we currently hold over 50 human studies, including those assessing individuals with no determined phenotype, such as 1000 Genomes, as well as studies looking at complex structural variation in phenotyped individuals, such as data from the ISCA consortium and data from a tumor and matched normal set. This collection consists of a diverse set of variants ranging from deletions, insertions, inversions and more complex events. One of the challenges of capturing such data is that discovery methods often do not define the precise breakpoints of the events being assayed. Additionally, different analytic methods can produce multiple interpretations of the same underlying data. Thus, we have developed a data model that attempts to capture the evidence for a particular variant call, as well as validation information when available. Additionally, we store and display ambiguity when breakpoint resolution has not been obtained. Each variant region is assigned a unique identifier of the type SV (nsv|esv) and each variant instance (or example) is given a unique identifier of the type SSV (nssv|essv). NCBI has taken an integrated approach to managing human variation data. Information from individuals that have not consented to have their DNA sequence made publicly available can submit controlled access data to dbGaP. Genotype information can be deconstructed, after which summary data can be submitted to dbVar. This approach has been used to make summary data from several studies, including data from the ISCA consortium, widely available. While dbVar serves a primary role in defining molecular events, integration with the ClinVar database allows for clinically important information to be layered on the variants in dbVar. We also make use of other NCBI resources such as Gene, PubMed, dbSNP and our assembly alignment and analysis tools to provide a comprehensive resource that allows for both the molecular definition of large-scale variation and integration with other biological information to aid in the interpretation of this data.

894W

Genome-wide copy number detection and analysis in a large diabetes cohort implicates rare yet recurrent imbalances in *INS*, *ABCC8* and *KCNJ11*. *L. Davis[1], J. Below[1], A. Konkashbaev[1], K. Aquino-Michaels[1], V. Paz[1], E. Cook[2], G. Bell[1], N. Cox[1].* 1) Section Genetic Medicine, The University of Chicago, Chicago, IL; 2) Department of Psychiatry, The University of Illinois, Chicago, IL.

Type 1 diabetes mellitus (T1DM) is a chronic disease resulting from autoimmunological destruction of the insulin-secreting cells in the pancreas. Complications of T1DM can be serious and include nephropathy retinopathy, neuropathy and peripheral vascular disease. The Genetics of Kidneys in Diabetes (GoKinD) cohort was collected to study these complications. Our study sought to investigate the role of copy number variation (CNV) in T1DM and diabetic complications in the GoKinD data set. DNA samples from patients and unaffected parents (2,726 individuals with T1DM with and without diabetic complications) were tested for CNV using the Affymetrix Genome-Wide Human SNP Array 5.0. Signal intensity values generated from the SNPs and copy number probes present on the array were then analyzed with PennCNV which utilizes a Hidden Markov Model to detect deviations in signal intensity indicative of CNVs. After sample-based and CNV-based quality control measures were implemented, a complete set of 66,719 CNVs was generated consisting of CNVs detected in patients (N= 1930) and unaffected parents (N=956). We identified a number of rare yet recurrent CNVs in patients including known candidate genes for diabetes and nephropathy. Mutations in these genes are associated with hypo- and hyperglycemia. These CNVs have are currently undergoing validation with real time PCR methods and are exceedingly rare as evidenced by their absence from the comprehensive Database of Genomic Variants (DGV). Additionally, we have identified a small number of deletions and duplications indicating monogenic forms of diabetes (MODY) masquerading as T1DM in this large cohort (e.g. *HNF1B* and *HNF4A* deletions). The results are consistent with the hypothesis that copy number variation plays a role in the development of T1DM and its complications.

895W

Comparative Analysis of High Resolution Array Platforms Reveals Diverse Genome-wide Copy Number Variation Detection in Humans. *R.R. Haraksingh[1,2], A. Abyzov[3,4], M. Gerstein[4,5], A.E. Urban[1,6], M. Snyder[1].* 1) Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520; 2) Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305; 3) Program in Computational Biology & Bioinformatics, Yale University, New Haven, CT 06520; 4) Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520; 5) Department of Computer Science, Yale University, New Haven CT 06520; 6) Department of Psychiatry and Behavioral Sciences, School of Medicine, Stanford University, Stanford, California 94305.

Accurate and efficient genome-wide detection of copy number variants (CNVs) is essential for understanding human genomic variation, genome-wide CNV association type studies, cytogenetics research and diagnostics, and independent validation of CNVs identified from sequencing based technologies. Numerous, array-based platforms for CNV detection exist utilizing array Comparative Genome Hybridization (aCGH), Single Nucleotide Polymorphism (SNP) genotyping or both. We have quantitatively assessed the abilities of eight leading genome-wide CNV detection platforms to accurately detect Gold Standard sets of CNVs in the genome of HapMap CEU sample NA12878, and found significant differences in performance. The technologies analyzed were the NimbleGen 2.1M and 3x720K Whole Genome and CNV focused arrays, the Agilent 2x400K CNV and SNP+CGH arrays, the Illumina Human Omni1Quad array and the Affymetrix SNP 6.0 array. The Gold Standards used were a 1000 Genomes Project sequencing-based set of 3997 validated CNVs and an ultra high-resolution aCGH-based set of 756 validated CNVs. We found that sensitivity, total number, size range and breakpoint resolution of CNV calls were highest for CNV focused arrays. Our results are important for cost effective CNV detection and validation for both basic and clinical applications.

896W

Copy Number Variations in Gene Networks Involving Alternative Splicing Impact Lifespan. *C.E. Kim [1], J.T. Glessner [1], A.V. Smith [2,3], D. Hadley [1], K. Wang [1,4], S. Panossian [1], N. Takahashi [5], K. Thomas [1], F. Wang [1], T. Harris [6], L. Launer [6], B. Keating [1], G. Lyon [1], J. Connolly [1], P.M.A. Sleiman [1], J.D. Buxbaum [5], S.F.A. Grant [1,7,8], V. Gudnason [2,3], H. Hakonarson [1,7,8].* 1) Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA, 19104, USA; 2) Icelandic Heart Association, Heart Preventive Clinic and Research Institute, Kopavogur, Iceland; 3) University of Iceland, Reykjavik, Iceland; 4) University of Southern California, Los Angeles, CA, 90007, USA;; 5) Laboratory of Molecular Neuropsychiatry, Department of Psychiatry, Mount Sinai School of Medicine, New York, NY 10029 USA; 6) Laboratory of Epidemiology, Demography, and Biometry, National Institute on Aging, National Institutes of Health, Bethesda, Maryland; 7) Division of Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA, 19104, USA; 8) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA, 19104, USA.

Longevity has a strong genetic component evidenced by family-based studies. Lipoprotein metabolism, FOXO proteins, and insulin/IGF-1 signaling pathways in model systems have shown polygenic variations predisposing to shorter lifespan. To test the hypothesis that rare variants could predispose individuals to shorter lifespan, we compared the rates of CNVs in healthy children (0-18 years of age) with individuals 67 years or older. CNVs at a significantl higher frequency in the young cohort were considered risk variants impacting lifespan, while those in the old cohort were considered longevity protective variants. We performed a whole-genome CNV analysis on 7,313 children and 2,701 adults of European ancestry genotyped with 302,108 SNP probes. Positive finding were evaluated in an independent cohort of 2,079 young and 4,692 old subjects. We detected 8 deletions and 10 duplications that were enriched in the young group (P=3.33x10-8 1.6x10-2 unadjusted), while only one duplication was enriched in the old cohort (P=6.3x10-4) These CNVs replicated in the independent cohort (combined P=2.33x10-16-1.8x10-4) and were experimentally validated, using qPCR. Evaluation of these genes for pathway enrichment demonstrated one-half involved in alternative splicing (P=0.0077 Benjamini and Hochberg corrected). We conclude that genetic variations disrupting RNA splicing could have long-term biological effect impacting lifespan.

897W

Copy number variation and transcription levels of the PXE-gene ABCC6 and its pseudogenes. *M.K. Kringen [1], C. Stormo [2], R.M. Grimholt [2], J.P. Berg [2], A.P. Piehler [2].* 1) Department of Pharmacology, Oslo University Hospital, Ullevål, Oslo, Norway; 2) Department of Medical Biochemistry, Oslo University Hospital, Ullevål, Oslo, Norway.

Background: The ATP-binding cassette transporter ABCC6 is located on chromosome 16 along with its two pseudogenes (ABCC6P1 and ABCC6P2). Mutations in ABCC6 cause pseudoxanthoma elasticum. Previously, we have shown that ABCC6P1 is transcribed and affect ABCC6 at the transcriptional level. The aim of this study was to determine copy number variations in ABCC6, ABCC6P1 and ABCC6P2. Moreover, we sought to study the transcription pattern of ABCC6P2 in 39 differen human tissues. Methods: Genomic DNA from fiv populations (Chinese (n=24), Middle East (n=20), Mexicans (n=24), Caucasians (n=50) and Africans (n=24), were examined for copy number variation of ABCC6 and its pseudogenes. Due to the high sequence similarities of the three genes, relative copy numbers were determined by pyrosequencing. Previously, we determined the expression pattern of ABCC6 and ABCC6P1 in 39 human tissues by RT-qPCR. By pyrosequencing, we were now able to quantify the relative transcription of ABCC6P2 to ABCC6 and ABCC6P1 in the same tissues, and thereby to deduce the relative expression of ABCC6P2 from the previous expressional data of ABCC6 and ABCC6P1. Results: Copy number variation of ABCC6 was very rare (2/142). However, deviation in copy number was more frequent for ABCC6 pseudogenes. In Africans, no deviation from the normal copy number of two was found for ABCC6 pseudogenes. In Chinese, deletions or duplications of ABCC6P1 were more frequent than in any other population (9/24). Furthermore, in the total population (n=142), 1 or 3 copies of ABCC6P1 was relatively common (3% and 8%, respectively). Only one person had 1 copy of ABCC6P2 while none had 3 copies. The transcription pattern of ABCC6P2 was highly similar to ABCC6 and ABCC6P1, with highest transcription in liver and kidney. Interestingly, the total transcription of pseudogenes, ABCCP1 + ABCC6P2 was higher than ABCC6 in most tissues, including liver and kidney. Conclusions: By pyrosequencing, we were able to quantify copy number variation of ABCC6 pseudogenes. Having less or more copies of ABCC6 pseudogenes is quite common, especially in populations of Chinese ancestry. The expression pattern of ABCC6P2 in 39 human tissues was highly similar to that of ABCC6 and ABCC6P1 suggesting similar regulatory mechanisms for ABCC6 and its pseudogenes. Furthermore, the findin of a higher expression of ABCC6 pseudogenes than ABCC6 in many tissues, strongly support that they may have an important biological function.

898W

Genome-wide analysis of rare CNVs in control populations. *L.-P. Lemieux Perreault [1, 2], E. Giannoulatou [1], M.-P. Dubé [2], C. Spencer [1], P. Donnelly [1,3], The Wellcome Trust Case Control Consortium 2.* 1) The Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 2) Montreal Heart Institute, Université de Montréal, Montréal, Québec, Canada; 3) Department of Statistics, University of Oxford, Oxford, United Kingdom.

Recently, a number of genome-wide associations studies have been conducted using rare copy number variations (CNVs) typed on SNP arrays. The power to detect associations relies on the accuracy of CNV detection (SNP density, algorithm, etc.) and on appropriate quality control filters. To minimize the false positive rate, researchers tend to filte calls based on fixe criteria such as size, number of probes, confidenc measure and presence of rearrangement-prone regions. There are also differen ways to specify CNV regions (such as reciprocal overlap) in the presence of imperfect CNV detection. Differen criteria, thresholds, or definition will result in substantially differen calls, due to a change in both false positive and false negative rates. For associations with rare CNVs false negative calls (failure to detect the CNV in individuals) can lead to spurious associations. To study the impact of differen detection methods and quality control procedures in association studies, control samples from three sources genotyped on both Affymetri 6.0 and Illumina custom array Human 1.2M-Duo have been used. The control samples consisted of approximately 9,000 healthy individuals from the National Blood Service, the 1958 Birth Cohort and the Wellcome Trust funded People of the British Isles study. Technical replicates of the HapMap sample NA12878 were also genotyped on Affymetri 6.0 (n=345) and Illumina Human660W (n=68). The firs phase was to perform an evaluation of concordance of CNV calls in between NA12878 replicates, as well as in between platforms and calling algorithms. A comparison of these calls with a well-characterized data set (1000 Genomes Project) using differen overlap definitio and thresholds has been performed, showing a high variability. The second phase was to compare the CNV burden between control groups, as previous studies have found an increase in genome-wide CNV burden in cases compared to controls. Fold change between control groups for the mean number of calls, the mean size of calls, the mean total size of calls and the mean number of overlapped genes have been compared between control groups. The third and last phase was to test for association, treating one control group as cases and another as controls, using a gene-centric approach consisting offi nding how many calls overlaps a given gene for each group. This study highlights difficulti underlying rare CNV calling, filterin and association analyses.

900W

Identification of a novel locus associated with the common birth defect spina bifida. *L. Muthuswamy[1], AG. Bassuk[2], R. Boland[3], M. Hakeman[3], J. Dierdorf[3], A. Hulstrand[3], G. Bonde[4], R. Cornell[4], DW. Houston[3], JR. Manak[2,3,5].* 1) Ontario Institute for cancer Research, Toronto, Canada; 2) Department of Pediatrics, University of Iowa, College of Medicine; 3) Department of Biology, University of Iowa, Iowa City, IA; 4) Department of Anat and Cell Biology; 5) Carver Center for Genomics, University of Iowa, Iowa City, IA.

Neural Tube Defects (NTDs) such as spina bifida are common birth defects of complex etiology. Family and population studies have confirmed a genetic component underlying NTDs. However, despite more than three decades of research, the genes involved in human NTDs remain largely unknown. We used array-based comparative genomic hybridization (aCGH) to identify copy number variants (CNVs) in 122 spina bifida cases, including 20 trios. We performed two types of analyses: one looking for rare spontaneous copy number variations (SCNVs), and one looking for rare genes enriched in the disease population compared to unaffected parents and the publically available database of genomic variants. We identified 28 de novo copy number variants (SCNVs) comprising 105 genes and approximately 480 genes within rare regions of duplications and deletions in the disease population. One de novo heterozygous deletion removed Pax3, a previously identified candidate gene for spina bifida, among several other genes. Another smaller de novo heterozygous deletion removed glypican 5 (GPC5) and part of glypican 6 (GPC6). Glypicans act by modulating the activity of morphogens such as Wnts, which themselves are candidate genes for spina bifida. However, up to now, glypicans have not been considered to be candidate genes for this disorder. To determine whether GPC5 or 6 has a role in neural tube closure, we used antisense morpholino oligonucleotides to inhibit their function in both the frog, Xenopus tropicalis, and the fish, Danio rerio. We show that knockdown of GPC5/6 results in neural tube closure defects in both vertebrate model systems, providing strong evidence that loss of glypican function is etiologic for human spina bifida.

901W

Copy number variations are associated with osteoporotic fracture: The Framingham Osteoporosis Study. *K. Nandakumar[1], C.L. Cheung[1], H. Zhou[1], Y. Zhou[2], C.T. Liu[2], S. Demissie[2], D. Karasik[1], A. Cupples[2], D.P. Kiel[1], Y.H. Hsu[1].* 1) Institute for Aging Research, Boston, MA; 2) Boston University, Boston, MA.

Osteoporosis is a skeletal disorder characterized by low bone mineral density (BMD) and the deterioration of bone tissue, predisposing to an increased risk of fracture. Though vertebral fractures are the most common type of osteoporotic fractures, non-vertebral fractures, are associated with high levels of morbidity and mortality. Although, GWAS have successfully discovered novel SNPs associated with osteoporosis related skeletal phenotypes, these common variants explain only 10% of BMD variance in Caucasians. Furthermore, there have been no GWAS for fractures. To discover additional genetic determinants of bone phenotypes, we conducted a genome-wide CNV association analysis of osteoporotic fractures in 8,700 adult men and women from the Framingham Osteoporosis Study. The CNVs were called from the Affymetrix 500K chip using the PennCNV package, and then confirmed by the Golden Helix SVS CNV module. The PennCNV software uses Hidden Markov Models to predict CNV events, whereas Golden Helix uses the optimal segmenting algorithm. A total of 112,745 CNV events were estimated in the Framingham population. Non-vertebral osteoporotic fractures (Non-vt O-Frx) (excluding fractures resulting from high-impact trauma, skull, spine, finger and toe fractures) were ascertained by medical records/X-ray images in most cases or were self-reported. There were 1,671 men and 2,160 women with at least one Non-vt O-Frx. Association analysis was performed by using a burden test for rare/low frequency CNV regions. The PLINK software was used to perform the burden test by chromosomes (http://pngu.mgh.harvard.edu/~purcell/plink/). From the burden test we found chromosomes 8, 9 and 15 to be significantly associated with non vertebral fracture based on the number of segments (or CNV events) and proportion of samples with one or more segments. To further identify rare events, regional tests were performed for CNV regions on each chromosome. One of the regions on 15q11.2 was shown to be significantly (adjusted P=0.02) associated with Non-vt O-Frx This is the first study showing the contribution of CNVs to the susceptibility to osteoporotic fracture for which replication will be sought in other cohorts.

902W

High resolution map of canine copy number variation. *E. Nevalainen[1,2,3], J. Berglund[6], C. Hitte[4,5], A. Nyström[6], C. André[4,5], M. Webster[6], H. Lohi[1,2,3], K. Lindblad-Toh[6,7], LUPA Consortium.* 1) Veterinary Biosciences, Univ Helsinki, Helsinki, Finland; 2) Department of Medical genetics, University of Helsinki, Finland; 3) Department of Molecular Genetics, Folkhälsan Institute of Genetics, Finland; 4) CNRS, UMR6061, Institut de Génétique et Développement de Rennes, F-35000 Rennes, France; 5) Université de Rennes 1, IFR 140, F-35000 Rennes, France; 6) Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden; 7) Broad Institute of Harvard and Massachusetts Institute of Technology.

Copy number variations (CNVs) are an important form of structural variation in mammalian genomes. Substantial amount of phenotypic differences between healthy individuals have been attributed to CNVs and they also contribute to disease risk in several human disorders including autism spectrum disorders, schizophrenia and epilepsy. Dog breeds as genetic isolates have recently emerged as a new model for human inherited diseases. Hundreds of genetic diseases have been identified in dogs, many of which are similar to human diseases and are likely to have a similar genetic background. CNVs are expected to play a role in several disease conditions in dogs and contribute to breed-specific characters. This study aims to map and catalogue CNVs in 50 dogs from 17 breeds (2-10 dogs/breed) and 3 wolves by comparative genome hybridization (aCGH) using Nimblegen's arrays with 2.1 million probes giving a high resolution of approximately 1 kb median probe spacing across the canine genome. This density gives considerably higher resolution than previously published studies. A Finnish Boxer DNA was used as a reference for other breeds. The results of this high resolution analysis of the CNVs in a large number of breeds provide important discoveries likely to contribute to several phenotypes in dogs and are being followed up in many cohorts available in the European canine genomics effort, LUPA consortium. Furthermore, findings associated with genetic diseases can be tested on human cohorts and eventually this will improve the health of both species. The new CNV data will be discussed in the meeting.

903W

Detection of copy number variations (CNVs) in PARK2 and SNCA in Parkinson disease patients using NanoString® technology. *K. Nuytemans[1,2], G. Bademci[1,2], L. Wang[1,2], S. Zuchner[1,2], F. Nahab[3], C. Singer[3], T. Guettouche[1,2], J.M. Vance[1,2].* 1) John P. Hussman institute for Human Genomics, University of Miami, Miami, FL; 2) Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami, Miami, FL; 3) Department of Neurology, Miller School of Medicine, University of Miami, Miami, FL.

CNVs are recognized as a prevalent form of structural variation in the genome, contributing to genetic variability and susceptibility for complex diseases such as Parkinson disease (PD). Disease causing CNVs have been reported in four out of five major PD genes. Therefore, detection of CNVs is of major research and clinical interest. Interestingly, whole gene multiplication of *SNCA* is more common than missense mutations, while up to 50 percent of mutations identified in *PARK2* represent CNVs of one or more exons of the gene. CNVs in other PD genes appear to be far less common. Current CNV detection technologies can lead to high false-positive rates, can be expensive and labor intensive when screening in a large set of samples for many genomic regions. We employed a novel direct digital detection method developed by NanoString to screen for exonic CNVs in *SNCA* and *PARK2*. Multiplexed, fluorescent barcoded probes bind directly to genomic DNA without any amplification step, avoiding biased overrepresentation of specific genomic regions. This digital method allows high-throughput detection of multiple CNVs in a single reaction. In a total of 125 individuals, we included both positive and negative controls, previously screened with SNP and CGH arrays. Family members of the positive controls were also included to investigate segregation. Presence or absence of CNVs was confirmed in 13/13 positive and 64/64 negative controls, indicating that the NanoString technology provides accurate results. Additionally, segregation of *PARK2* CNVs with disease was shown in six out of nine families. Interestingly, the three instances of non-segregation to other affected relatives involved isolated deletions or duplications of *PARK2* exon 2. In summary, we are able to utilize this high-throughput technique that circumvents many of the previous technology-based issues to detect CNVs in PD related genes. Our results indicate that the NanoString technology is very reliable and user-friendly. We expect this analysis system will be a promising alternative for current CNV detection methods. The reason for non-segregation of the *PARK2* exon 2 CNVs is not clear. These CNVs might not be the causal PD mutations in these families. Currently, we are sequencing these families for other potential *PARK2* mutations. However, these results emphasize the importance of determining CNV segregation when evaluating pathogenicity.

904W

Detection of novel copy number variation in individuals with autism spectrum disorder using a comparative genomic hybridization array. *A. Prasad[1], C.R. Marshall[1,2], B. Thiruv[1], J. Wei[1], J.L. Howe[1], D. Pinto[1], J. Rickaby[1], Q. Tran[1], S.W. Scherer[1,2].* 1) The Centre for Applied Genomics and Program in Genetics and Genome Biology, The Hospital for Sick Children, Toronto, ON., Canada; 2) McLaughlin Centre, Faculty of Medicine, University of Toronto, ON., Canada.

Autism spectrum disorder (ASD) is a neurodevelopmental condition with a strong genetic component. Previous studies have reported rare copy number variations (CNVs) to be associated with autism and implicated several contributing risk loci/genes. There are a variety of platforms available to detect CNVs including comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) arrays. Each platform has its own advantages and disadvantages and can be complementary. In the present study, we used the Agilent 1M CGH array to detect novel rare CNVs in more than 700 unrelated ASD probands which have been previously run on other SNP microarrays. The high-resolution Agilent 1M CGH array has a total of 974,016 probes providing uniform whole genome coverage. The ASD cases were competitively hybridized to a pool of fifty sex-matched Caucasian controls as a reference. The CNV calling was performed using Aberration Detection Module 2 (ADM2) algorithm of DNA Analytics 4.0.85 and a circular binary segmentation (CBS) algorithm implemented in the DNAcopy package. The CNVs detected by both algorithms were defined as stringent and were utilized for further analyses. The calls were compared with the CNV data from Illumina 1M single and duo, Affymetrix 500K, Affymetrix 6.0 & Illumina 2.5M arrays. For example, 350 samples in our dataset were run previously on Illumina 1M single array which was analyzed using two algorithms-iPattern and QuantiSNP. The average number of stringent calls generated for Agilent 1M and Illumina 1M is 36 and 18, respectively. We found that only 27% of the total Agilent 1M call set was detected using the Illumina 1M platform. Conversely, 46% of the Illumina 1M calls were not detected using the Agilent 1M platform. The difference in the CNVs detected using multiple platforms is mainly due to the differences in probe distribution and sensitivity of the detection algorithms used. In addition, we have detected several novel rare CNVs using Agilent 1M array which were missed by other SNP genotyping platforms. Some of the novel CNVs encompasses genes that are known to play a potential role in neurodevelopment e.g. *NF1*, *CTNNA2*, *DPYD*, *YWHAE*, *NRXN1*, *DMD* and have been experimentally validated. Our data suggests that use of multiple platforms is advantageous for both maximizing CNV discovery and validation rates in case-cohorts.

905W

Discovery of Pseudoautosomal region 3 redefines the structure and function of Human X and Y chromosomes. *N.B. Ramachandra[1], A.M. Veerappa[1], J.S. Marita Priya[2], P. Padakannaya[2].* 1) Genomics Lab, DOS in Zoology, University of Mysore, Mysore, India; 2) DOS in Psychology, University of Mysore, Mysore, India.

Purpose: Recombination is restricted to the tips of the short and long arms of the X and Y chromosomes, which contain pseudoautosomal region 1 (PAR1) and pseudoautosomal region 2 (PAR2). Beside the PARs, homology also exists in the X-transposed region (XTR), which is at Xq21.3 and Yp11.2.When the PAR2 block and XTR block were both created by duplication and have a high level of sequence homology, then the question arises as to why recombination would be limited only to the PARs? **Methods:** DNA of fifteen dyslexic subjects, their parents and unaffected siblings, from eight families were subjected to whole genome genotyping using an Affymetrix Genome-Wide Human SNP Array 6.0 chip. All SNPs and CNVs had a Quality Control (QC) call rate of >97% across all subjects and controls. Contrast QC across all samples was > 2.5. Genomic DNA of these subjects was amplified by PCR using X and Y specific primers for validation. **Results:** A mother of a dyslexic subject from one of the families revealed two transposed blocks of 144 kb and 102 kb of Yp11.2 in the Xq21.3 region of one of her X chromosomes. The presence of these transposed blocks was validated by using Y- and X-specific primers for PCR. A further analysis of the blocks revealed the presence of *PCDH11Y* exons of the Yp11.2 region. These transposition sites had a direct impact on the exon structure of *PCDH11X*. The subject had a total of 6 exon transpositions, from the 1st to the 6th exon, which truncated the gene arrangement from the sixth exon. **Discussion:** Xq21.3 region has a history of duplication and transposition activity by means of a duplicated and later transposed block of 3.5 Mb from the X to Y chromosome approximately 5-6 MYA. This process is very similar to the origination of the PAR2. Like the PARs, XTR has 98.78% identity, and *PCDH11X* in the XTR has a 99.1% identity with its PCDH11Y allelic homolog. A few genes in PAR1 and PAR2 are known to escape inactivation in the inactivated chromosome. Likewise, a few genes in Xq21.3/*PCDH11X* escape inactivation. PAR2 and XTR (PAR3) share similar type of origin and creation. PAR2 is found to exhibit a much lower frequency of pairing and recombination than PAR1. Thus, one can expect varied frequency of recombination in each of these PAR regions, which could be due to the differential structural dynamics of the X and Y chromosomes. Therefore, this recombination event in the XTR can be regarded as a PAR3 region.

906W

CNV detection Using Uncertainty of Read Mapping. *z. wang[1], f. hormozdiari[1], w. yang[2], e. eskin[1].* 1) Computer Science Department, University of California, Los Angeles, Los Angeles, CA; 2) Bioinformatics Program, University of California, Los Angeles, Los Angeles, CA.

Copy number variation (CNV) has widely been known as a genetic risk factor for disease. The development of high-throughput sequencing (HTS) technologies has provided great opportunities to detect CNV regions in mammalian genome. Millions of short reads generated from donor genome using HTS are mapped back to the reference genome. Then depth-of-coverage information can be used for identification of copy variation regions. However, a large fraction of the reads can be mapped to more than one position. Most existing methods only consider reads that can be uniquely mapped to the reference genome, and therefore have low power in detecting CNV, especially those located within repeat sequences. We proposed a probabilistic model that utilizes the uncertainty of read mapping. We use maximum likelihood to estimate locations and copy number of copied regions, and implemented an expectation-maximization (E-M) algorithm to update the mapping posterior probabilities of each read. One important contribution of our model is that we can distinguish between regions in the reference genome that slightly differ from each other. We can also predict the CNV with high resolution. Previous methods either divide the genome into small windows or use paired-end reads to find a rough boundary. These strategies make the resolution of predicted copy regions low. Our method takes each single nucleotide as the basic unit, and thus can predict the breakpoints precisely. We applied our method to simulation datasets and achieved higher accuracy.

907W

Characterizing copy number variation at the human immunoglobulin heavy chain locus. *C.T. Watson[1], K.M. Steinberg[2], R.L. Warren[3], F. Hach[4], J.B. Joy[1], T. Graves[5], R.K. Wilson[5], C. Sahinalp[4], R.A. Holt[3], E.E. Eichler[2], F. Breden[1].* 1) Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada; 2) Department of Genome Sciences, University of Washington, Seattle, Washington, USA; 3) Genome Sciences Centre, BC Cancer Agency, Vancouver, British Columbia, Canada; 4) School of Computing Science, Simon Fraser University, Burnaby, British Columbia, Canada; 5) The Genome Institute, Washington University, St. Louis, Missouri, USA.

The human immunoglobulin heavy chain (IgH) locus spans approximately 1 MB of chromosome 14, and consists of 120-130 functional and nonfunctional variable (V) gene segments that reside adjacent to ~27 diversity (D), 9 joining (J), and 11 constant (C) functional and non-functional gene segments. Each of these groups of gene segments is the result of duplication and divergence, making the IgH locus one of the most segmentally duplicated regions of the human genome. As in many parts of the genome, segmental duplication in IgH has lead to extensive copy number variation (CNV), the extent and distribution of which are not fully known. For example, upwards of 15 expressed V genes remain uncharacterized at the genomic level, and are not included in the current human reference sequence. Large insert clones, such as bacterial artificial chromosomes (BACs) and fosmids provide a useful resource for characterizing CNV-containing haplotypes in human populations. As part of a larger project seeking to describe polymorphisms across immunoglobulin loci that are relevant to human disease, we analyzed complete sequences of 30 fosmid and BAC clones mapping to the IgH region from multiple human populations, including the first complete haploid sequence for IgH from a single human chromosome constructed using a hydatidiform mole BAC library. BAC and fosmid sequences allowed for the characterization and mapping of many previously undescribed insertions and deletions within IgH, including multiple V genes with known roles in infectious and autoimmune diseases. These data are now being used to (1) assess the mutational mechanisms directing CNV in Ig loci, (2) design genotyping assays to assess the frequency of these polymorphisms across human populations as well as test for associations with infectious and autoimmune disease susceptibility, and (3) assess the efficacy of next-generation short-read sequence mapping approaches and tag SNPs to interrogate these polymorphisms.

908W

dbSNP: Database of Short Genetic Variations. *R. Maiti, H. Zhang, M. Kholodov, D. Shao, E. Shekhtman, D. Rudnev, K. Sirotkin, M. Ward, D. Maglott, M. Feolo, S. Sherry, L. Phan.* National Center for Biotechnology Information (NCBI), National Library of Medicine (NLM), National Institutes of Health (NIH), Bethesda, MD.

The National Center for Biotechnology Information (NCBI) develops and maintains dbSNP as an open source centralized database of short genetic variations. There are three major functionalities in this resource: 1) archiving submitted variants, 2) integrating the variant information with relevant NCBI resources, and 3) disseminating variant information in a biological context to the scientific community worldwide. dbSNP not only catalogs millions of variations from large projects such as The SNP project, The International HapMap Consortium, and 1000 Genomes, but also catalogs thousands of variations with clinical properties from Locus Specific Databases (LSDB) and clinical sources. Both neutral and clinically significant variations are accessioned in a standard process for publication, and are accessible for search and download. dbSNP archives submissions, and assigns a unique identifier to each variant for citing in manuscripts and providing the appropriate acknowledgement to the submitter. Data submitted as changes relative to a reference sequence, e.g., an HGVS expression, are validated against that reference and returned to the submitter if any discrepancy is identified. The original data provider is given access to a specific link to maintain their own submissions. Data are integrated with other NCBI resources such as Sequence Viewer, RefSeqGene, Gene, PubMed, ClinVar, and OMIM®, for interpreting the variants in a variety of biological contexts as such as positions on the genome, genes or proteins, functional implications, clinical relevance, and scholarly publications. Variant records can be accessed through NCBI's web search system using different parameters and filters, such as type of variation, clinical significance, somatic allele origin, minor allele frequency (MAF), location on the genome, or suspected artifact. For example, users wanting to retrieve only single nucleotide polymorphisms can ask for variations of type `single nucleotide variation' with MAF > 0.01. Complete variation datasets can be downloaded from the dbSNP FTP site in such formats as FASTA, XML, and ASN.1, and VCF. Data from clinical sources are displayed in the gene-centric Variation Viewer, where clinical significance and testing status are reported. LSDB and other clinical researchers may continue to submit via the specialized data submission system (http://www.ncbi.nlm.nih.gov/SNP/tranSNP/VarBatchSub.cgi); data will be integrated into ClinVar.

909W

Determination of *RET* sequence variation in an MEN2 unaffected cohort using multiple-sample pooling and massively parallel sequencing. *R. Margraf[1], J. Durtschi[1], J. Stephens[1], M. Perez[1], K. Voelkerding[1,2].* 1) Research & Development, ARUP Institute for Clinical & Experimental Pathology®, Salt Lake City, UT; 2) Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT.

Determination of sequence variation within a genetic locus is essential for developing clinically relevant databases, designing molecular assays and clinical test interpretation. In this study, multi-sample, non-indexed pooling combined with Illumina Genome Analyzer (GA) sequencing was used to discover sequence variation within the *RET* proto-oncogene for a cohort known to be unaffected by Multiple Endocrine Neoplasia type 2 (MEN2). Known unaffected DNA samples (113 Caucasians and 23 of other ethnicities) were amplified for the *RET* gene (from intron 9 to intron 16, ~9Kb amplicon). Amplicon concentrations were normalized using Sequalprep and quantified by Picogreen. Between 27 and 29 Caucasian samples' amplicons were pooled before Illumina library prep and GA sequencing (4 pools total). The ethnic cohort was sequenced in a separate pool. Two controls were also included in this study (previously GA sequenced for the 9Kb *RET* amplicon and Sanger sequenced for *RET* exons): a single sample and also a pool of 50 samples. The single sample control was used for the subtraction correction method on the pools. All 59 variants previously detected in the 50 pool were present. Of the 57 variants detected in the unaffected cohort, 6 and 10 variants of low variant frequency were unique to the 113 Caucasian sample set and ethnic pool, respectively. A total of 20 variants were novel changes, not seen in the 50 pool control or in dbSNP132. Several variants within the unaffected cohort were validated by high-resolution melting analysis and Sanger sequencing, and the expected allele frequencies correlated with the GA sequencing frequencies determined for the pools. The variant results from the unaffected cohort will be added to the *RET* MEN2 database and also used for comparison in case reports. These methods can be applied to other pooled samples (such as genetic locations for GWAS follow up) and natural pools (such as mitochondrial heteroplasmy or mixed tumor populations).

910W

ALFRED: A resource for research and teaching. *H. Rajeevan[1,2], U. Soundararajan[1], A.J. Pakstis[1], J.R. Kidd[1], K.K. Kidd[1].* 1) Genetics, Yale University, New Haven, CT; 2) Center for Medical Informatics, School of Medicine, Yale University School of Medicine, New Haven, CT.

ALFRED (http://alfred.med.yale.edu) is a unique resource of allele frequency data on human population samples available for use by the scientific and educational communities. A five-fold increase in allele frequency tables over the past year (663,410 polymorphisms, 710 populations and 35,227,249 frequency tables (one population typed for one site)) has necessitated creation of efficient search and summary functions. Though not all polymorphisms have been studied on all populations, there are over 150 different polymorphisms (including SNPs, InDels, and STRPs) with data on over 100 different populations and over 6,000 on more than 52 populations. The Illumina ~650K marker set have data on 50 populations (HGDP-CEPH panel and the Kidd Lab Korean population). We highlight developments to facilitate effective use of ALFRED: one to use the available search options to search and identify a gene, polymorphism or population and another to use the various summary tables to identify polymorphisms that can be explored further within ALFRED and the databases to which active links exist. In response to user feedback we have revised the `homepage' to make it easier for first-time users (especially students) to learn how to search and navigate within ALFRED. Other new additions include highlighting interesting examples in ALFRED as part of increased effort to make ALFRED a better didactic tool through empiric human population genetics. Some such highlighted SNPs include those for earwax type (rs17822931 at ABC11), hair thickness (rs3827760 at EDAR), skin color (rs16891982 at SLC45A2), and bitter taste (haplotypes of rs713598, rs1726866, and rs1024639 at TAS2R38). The textual materials in the `Educational Material' section under the `Documentation tab' also provide suggestions and starting points to help with possible classroom projects. ALFRED is supported by US NSF BCS0938633.

911W

Tracing experimental process flow to the original biologics in genomic relational databases. *S. Saccone, J. Quan.* Department of Psychiatry, Washington University, St Louis, MO.

There is growing evidence that the results of genetic association studies are enriched for certain kinds of genomic data such as eQTLs and biochemical pathways. The integration of genetic association studies with genomic databases may therefore provide new insights into these studies and further their impact on public health. In order to viably integrate genome-wide genetic association results with today's exciting, diverse and ever-expanding genomic databases, we need new methods and tools for assessing credibility by systematically tracing genomic data through the experiments that generated it back to the original biologics. Our solution to this challenge is the introduction of the new biologic-experiment-result (BERT) relational database model. The BERT model viably captures the sequence of experiments that produced the data in a genomic database. Its implementation will enable investigators to assess credibility by tracing key information back through these experiments to, whenever possible, the original biologics and samples. We have implemented the BERT model in an interactive web application for graphically visualizing experimental process flow. The web application allows investigators to explore the data from all parts of the experimental process in high detail using methods based on our dbSNP-Q application (http://cgsmd.isi.edu/dbsnpq). One example that we have incorporated with particularly high detail is the HapMap relational database. Because of its ubiquity in designing commercial genotyping arrays and guiding disease association studies, there is a substantial need to assess the credibility and reliability of HapMap data. For all 11 populations in the HapMap Phase III Release 3 and Public Release 28 databases, the web application enables investigators to trace experimental results, such as allele frequency and linkage disequilibrium estimates, back through processes such as statistical analysis, genotyping and quality control assessment to detailed information on DNA extraction for the original subjects. Public genomic databases rarely provide a systematic means of tracing the data back to its experimental origins. The BERT model and its implementation in a web application represents a key step in overcoming this major obstacle to the viable integration of diverse genomic databases for studying the genetics of human disease.

912W

dbGaP: genotype data processing and QC. *N. Sharopova, J. Paschall, Z. Wang, L. Hao, Y. Jin, M. Kimura, S. Stefanov, S. Pretel, A. Sturcke, M. Feolo, S. Sherry*. NCBI/NLM/NIH, Bethesda, MD.

Data sharing in human genetics benefits the community by reducing research costs, time and by providing opportunities for data verification. However the necessity of combining genomic datasets generated by more than one independent research team and/or across various technologies raises concerns about standards of data sharing, accessibility of proper data annotation/quality metrics, as well as availability of standard methods and tools for routine data merging and evaluation. In addressing these concerns, NCBI's dbGaP (http://www.ncbi.nlm.nih.gov/gap) has implemented submission and quality control processing steps necessary for the deployment of community accepted quality standards. Today, dbGap stores and provides access to genotype data for more than 350,000 subjects. The dbGaP's genotype curators are actively involved with investigators in the curation of submitted genotype datasets. Based on recommendations from NIH and UW investigators, genotype processing pipelines were developed to ensure internal and cross-dataset consistency in annotation, quality control, and representation of genotype data. Considering the size of genomic datasets, the dbGap team constantly searches for more flexible ways of serving genotype data to the community. Data flow for genotype data processing, quality control, and accepted quality standards will be presented.

913W

A relational database for next-generation sequencing data. *C. Xu, Q. Tian, J. Li, H.W. DENG*. Center for Bioinformatics and Genomics, Department of Biostatistics and Bioinformatics, School of Public Health & Tropical Medicine, Tulane University, New Orleans, LA 70112.

Rapid advances in high-throughput DNA sequencing technologies, along with dramatically reduced cost, have allowed increased scales in whole-genome sequencing of multiple individuals. Management and analyses of the huge amount of data generated from whole-genome sequencing studies demand dedicated and efficient software. To satisfy such increasing needs, we have designed and implemented a relational database, with a focus on the sequencing data generated on the Complete Genomics, Inc. platform. Our software is easy to use and provides multiple functionalities such as integrated management of sequencing data from multiple individuals, variant extraction based on types and/or from pre-selected genomic regions, and annotation information extraction and comparison across multiple sequences. Incorporated comparison and analyses of different forms of genomic data, such as SNP, DNA sequence, RNA sequence, from the same individual can also be easily performed. In addition, similar operation on sequencing data from other platforms is also under development. Our database is a sophisticated tool and can provide excellent help for next-generation sequencing based research.

914W

An automated workflow for library preparation, target enrichment, and bioinformatics processing for large-scale 2nd Generation resequencing studies. *D. Hedges[1], T. Guettouche[1, 2], C. Siebert[1], A. Griswold[1], C. Kroner[1], S. Slifer[1], M. Schmidt[1], P. Whitehead[1], I. Konidari[1], W. Hulme[1], A. Andersen[1], A. Lekas-Diaz[1], R. Gentry[1], Y. Pasco[1], A. Aviram[1], G. Beecham[1], M.A. Pericak-Vance[1], J.R. Gilbert[1].* 1) Center for Genome Technology, Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, Florida; 2) Oncogenomics Core Facility, Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, Florida.

Second Generation sequencing in the context of large population-based genetic studies has necessitated the development of automated workflows that minimize human intervention. Manual execution of protocols in high-throughput scenarios is laborious and presents numerous opportunities for the introduction of human error. To improve throughput for a large-scale 2000 sample autism targeted resequencing study covering 17Mb (corresponding to the exons of 689 genes), we have developed an automated library and target enrichment workflow. The laboratory automation operates in tandem with a downstream informatics processing pipeline that navigates samples through alignment, quality control, genotype calling, and genomic annotation. The laboratory component of our workflow automates the Illumina library preparation and Agilent SureSelect capture protocol using the Sciclone G3 and Zephyr Liquid Handling Workstations (Caliper). We divided our workflow into pre-PCR (Sciclone G3) and post-PCR (Zephyr) components. Library preparation and target enrichment included optimization of purification to maximize sample recovery. Our approach includes methods for quality control (QC), post-shearing quantitation and normalization, and post-hybridization amplification. The hybridization set up is carried out within an open deck layout and includes an innovative liquid handling step to account for temperature differences and evaporation. In contrast to manual protocols, our workflow currently has a maximum throughput of 384 samples per five day work week, and the resulting sequencing metrics are comparable to manual protocol performance. Following Illumina HiSeq2000 sequencing, an informatics pipeline was developed to automate the processing of samples through BWA alignment, quality control, genotype calling (via GATK Unified Genotyper), and genomic annotation. Results are then archived in VCF format for downstream statistical analysis. Our bioinformatics pipeline targets the LSF load distribution system for parallelization on a 5000 core Linux cluster (PEGASUS). The pipeline allows for complete informatics processing of a HiSeq2000 flow cell (56 multiplexed samples) in approximately 5 hours, enabling downstream data process to provide rapid feedback to the laboratory. Our performance data indicate that the workflow is an efficient and robust solution for large-scale genetic studies requiring targeted resequencing.

915W

Flexible Desktop Software for Genome Assembly and Variation Analysis. *M. Keyser[1], T. Durfee[1], T. Schwei[1], S. Baldwin[1], R. Nelson[1], D. Nash[1], D. Jurzcak[1], C. Stern[1], J. Stieren[1], A. Pollack-Berti[1], K. Maxfield[1], E. Ma[1], A. Niffenegger[1], K. Dullea[1], P. Pinkas[1], R. Solberg[1], G. Plunkett III[1,3], F. Blattner[1,2,3].* 1) DNASTAR, Inc., Madison, WI; 2) Scarab Genomics LLC; 3) University of Wisconsin.

The new biotechnology and biomedical frontiers opened up by next generation sequencing (NGS) also pose significant hurdles in terms of data management, assembly and analysis. Most available software solutions require cobbling together disparate programs that typically require substantial computing resources and bioinformatics expertise. In addition, these systems may be fine tuned to a particular sequencing platform. At DNASTAR, we have developed an integrated suite of software programs for assembling and analyzing NGS data from all of the major platforms and supporting the key workflows on a desktop computer. At the core of the suite is the SeqMan NGen assembler, which runs on Windows, Macintosh and Linux operating systems, and assembles single and paired end data from all the major NGS technologies: Illumina, Roche 454, Ion Torrent, Helicos and SOLiD. The assembler shows excellent performance on modestly priced desktop computers. For example, whole human genome assemblies of deep Illumina data (35x) take less than 24 hours on a $2500 desktop computer with 16GB of RAM while human exome assemblies (~150x coverage) take about three hours on the same computer. Probabilistic SNPs/small indels and genotype calls are included in the output with known variants identified by their dbSNP IDs. The new assembler version expands on this foundation in two crucial areas. First, a structural variation report is included for identification of large indels, inversions and translocations. Second, multiplexed (e.g. MID-tagged) samples can be processed and assembled either as individual data sets or as one or more groups. These capabilities make SeqMan NGen a truly fully functional, high performance desktop assembler for discovering genetic variation from NGS data sets of any size or configuration. Interactive alignment and strategy views within SeqMan Pro, together with tables for SNPs, structural variation, coverage, and features, allow for efficient navigation through even large projects such as human genome assemblies. New multi-sample views and tables provide for analysis of MID-tagged or indexed samples in a single project. For large multi-sample projects composed of hundreds of individual data sets, SeqMan NGen generated variation tables can now be exported to ArrayStar, which has tools for filtering, set comparison and clustering that support a number of different workflows, such as identifying candidate polymorphisms in a NGS-based association study.

916W

Droplet Digital PCR™ as a Powerful Microscope to Explore and Optimize Genomic Assay Designs. *R.T. Koehler, S. Saxonov, N. Heredia, J. Regan.* QuantaLife , Pleasanton, CA.

Droplet Digital PCR (ddPCR) is a new molecular detection system whereby samples are first partitioned into thousands of nanoliter droplets, a PCR reaction is run to completion, then each droplet is read as either positive or negative. With these counts, statistics are used to estimate the concentration of the target DNA/RNA present in the original sample. Compared with real-time PCR, ddPCR takes PCR from a measure of relative quantification to one of digital absolute quantification. This high precision enables DNA detection applications well beyond real-time PCR. For example, ddPCR can readily differentiate copy number variants (CNV) of five from six copies, discern gene expression differences as small as 10% among samples, and detect rare mutant alleles in a high background of normal sequences.

We find that most published and commercial PCR assays work well in our ddPCR format without any modifications to either sequence or experimental conditions. This holds true across the board for applications including CNV and SNP genotyping, RNA quantification, and rare event detection, as well as for target characteristics like genomic complexity and GC content. However, given the unprecedented precision afforded by our system, it is clear that some assays perform better than others. With the goal of optimizing assay design, we ask: Why do some assays perform better than others and can we learn to predict this?

Here we show how ddPCR can illuminate subtle aspects of assay performance that would be impossible to detect with other systems. Using well-characterized TaqMan assays as reference points, we quantitatively explore the affects of perturbing sequence and experimental conditions in several assay-relevant dimensions. Sequence changes include shifting primer and probe position, switching target strand, altering oligo length and predicted melting temperature, and introduction of mismatches at different positions. Experimental variations include PCR conditions such as annealing temperature, choice of polymerase and restriction enzymes, and effects of buffer additives. Our results quantitatively illustrate how different aspects of assay design influence performance and robustness, helping to optimize genomic assay design rules not only for ddPCR but for PCR systems in general.

917W

The CIDR Infinium WebLIMS: A Modular, Multi-Product Laboratory Information Management System for Illumina Infinium Genotyping Platforms. *D. Leary, M. Barnhart, J. Goldstein, B. Craig, J. Romm, K. Doheny, L. Watkins, Jr.* Center for Inherited Disease Research, Johns Hopkins University, Baltimore, MD.

The Center for Inherited Disease Research provides high quality next-gen sequencing, genotyping, and statistical genetics consultation to investigators working to discover genes that contribute to common disease. CIDR began using Illumina® Infinium™ genotyping arrays in 2005, with the Infinium 100K (Human-1) product. A LIMS was unavailable from Illumina at the time, so we developed our own using a Java Swing front end and a MySQL relational database back end. This required approximately 500 person-hours over 2 1/2 months by a three-person software development team. The HumanHap300 product was released in 2006; leveraging the existing codebase, we developed a similar LIMS for the HumanHap300 product in about 30 person-hours over a one-month period. This was followed by a LIMS for the HumanHap550 product. As new Infinium arrays followed every few months, it became obvious that we needed an "all-inclusive" Infinium LIMS that would be flexible enough to allow us to add new products while preserving the workflows for existing products. For this new LIMS, we abandoned Swing in favor of a Web-based front end coded in HTML, CSS, and JavaScript, using Java Servlet technology in the application layer. Building the second-generation LIMS took about 1225 person-hours over 9 months. External to the LIMS, but integrated with the back end, are: a utility for uploading plated sample information from a designated robot, an automated QC pipeline, and an extensive collection of reports. The LIMS can also generate worklists for "cherry-picking" individual samples from plates. This is especially helpful for re-dos of failed assays. Adding a LIMS module for a new genotyping array involves duplicating the codebase and database lookup tables for a product with a similar workflow, then modifying source files as necessary. This results in some redundancy and code bloat but preserves the integrity of existing modules. Portions of the module creation process have been automated, reducing the time required to add a module for a product whose workflow is similar to that of an existing product to as little as eight hours, including testing. The advent of next-generation sequencing has brought additional challenges; sample pre-testing is performed with Infinium genotyping arrays, but the need to accommodate differences between sequencing projects and genotyping projects has led to a proliferation of LIMS modules. To date, the LIMS comprises 30 modules (10 of them now obsolete).

918W

AnnTools -Genome annotation toolkit for SNP and CNV data from whole exome and whole genome sequencing data and microarrays. *V. Makarov[1,2], T. O'Grady[6], G. Cai[1,2], J. Lihm[1,2,7], J. Buxbaum[1,2,3,4,5], S. Yoon[1,2].* 1) Seaver Autism Center, Mount Sinai School of Medicine; 2) Department of Psychiatry, Mount Sinai School of Medicine; 3) Neuroscience, Mount Sinai School of Medicine; 4) Genetics and Genomic Sciences, Mount Sinai School of Medicine; 5) Friedman Brain Institute, Mount Sinai School of Medicine; 6) Levy Library, Mount Sinai School of Medicine; 7) Department of Applied Mathematics and Statistics, Stony Brook University.

As massively parallel sequencing is becoming technically and economically feasible, the amount of sequence data grows exponentially. Dramatic increase in the detection of novel sequence variants requires reliable and comprehensive annotation. To meet this need, we developed a fast and robust bioinformatics tool set for annotation of the genomic variants generated from the sequencing and microarray data. For each Single Nucleotide Variant (SNV) and Copy Number Variant (CNV) we specify gene name and chromosomal location. The tool allows to indicate whether SNV is in a promoter region, exon, intron, UTR, or intergenic. Using pre-calculated data tables, we specify coding changes and type of mutation for both known and novel SNV. For the exonic variants we provide the exon number and the total number of exons in the gene. Optionally, the tool can indicate an overlap with the conserved transcription factor binding sites, known regions of segmental duplications, artifact prone regions such as parts of antibodies (abParts), immunoglobulin (IG), T-cell receptor regions, and disease/trait associated CNV regions. If the gene has several isoforms, annotation is provided for each isoform. Finally, we generate a short summary report to indicate the number of variants in each location. AnnTools can accept custom tracks in a BED format downloaded from UCSC or generated by the user to meet the specific user's needs. Our genome annotator mines data from several reliable and constantly updated sources, such as dbSNP (NCBI) and UCSC Genome Browser tables. AnnTools accepts input files in the VCF or pileup formats, but user specified formats are also possible. Output is in the VCF 4.0 format used by the 1000 Genomes project, which will facilitate data exchange between working groups. High annotation speed (over 1000 lines per min) makes our application suitable for high throughput genomic facilities. The Python source code can easily be parallelized, making it suitable for high-performance computer clusters. On the other hand, a low memory footprint and CPU requirements allow the application to run even on a personal computer. We provide installation scripts, demos and a useful set of extra tools, such as pileup-to-VCF, Tab-to-VCF and VCF-to-BED conversion tools. Availability: AnnTools is freely available for academic and non-commercial use from http://anntools.sourceforge.net/.

919W

Generating Large Scale pedigree drawings for Genetic Studies. *R. Mayani[1], G. Mehta[1], E. Deelman[1], K. Seth[1], J. Vöckler[1], F. Wang[2].* 1) Information Sciences Institute, University Of Southern California, Marina Del Rey, CA; 2) Department of Psychiatry, Washington University, St Louis, MO.

Pedigree diagrams are common and useful tool in documenting the genetic history of a family over several generations. Most large-scale studies done by NIH and NIMH to determine disease linkages to genes include these charts including the full genotypic and phenotypic data. There are several software solutions for drawing these charts but most of them tend to have drag-and-drop or click-based user interface to draw pedigree charts for one family at a time.Curating and drawing pedigrees for large-scale data across several different studies of the same disease consisting of 1000's of families can be a cumbersome, long, tiring, and error prone job. Also currently most pedigree software have very basic error checking and reporting functionality to check mistakes in the pedigree drawing in terms of relationships, age, or other parameters.

We have created a Pedigree plotting service for the Center For Collaborative Genetic Studies on Mental Disorders funded by NIMH at https://www.nimhgenetics.org/pedigree that allows users to quickly generate large-scale pedigrees in a matter of minutes instead of months of manual work. The pedigree service allows users to upload a CSV file consisting of hundreds of families, totaling thousands of individuals. Several quality control steps check age, gender, and relationships as well as other aspects. It also provides the user options to select different shading patterns for each affected status. In matters of seconds to minutes the service generates large, high-quality pedigree graphs in PDF format, and makes it available for download. The PDF contains a well-documented legend page and an index page organized by family identifiers. The table of contents is hyper-linked for an improved online viewing experience. This service is free for use and is also freely available as a source to download.

The CGSMD pedigree plotting service is based on an open source software Madeline 2.0 Pedigree Drawing Engine developed at University of Michigan Kellogg Eye Center [1].

[1] Madeline 2.0 PDE: A new program for local and web-based pedigree drawing, E. Trager; R. Khanna; A. Marrs; L. Siden; K. Branham; A. Swaroop; J. Richards. Bioinformatics 2007; doi: 10.1093/bioinformatics/btm242.

920W

Automated high-throughput analysis of personal genome sequences: towards clinical interpretation. *A. Russell[1], S. Chervitz[1], M. Yandell[2], E. Kiruluta[1], M. Reese[1], F.M. De La Vega[1,3].* 1) Omicia Inc, Emeryville, CA, USA; 2) Department of Human Genetics, Eccles Institute of Human Genetics, University of Utah and School of Medicine, Salt Lake City, Utah, USA; 3) Department of Genetics, Stanford School of Medicine, Stanford, CA, USA.

Recent advances in high-throughput genome sequencing and the ensuing outpouring of genomic information have accelerated the convergence of discovery science and clinical medicine. Cheap genome sequencing is now producing analysis bottlenecks, especially as regards the clinical interpretation of genetic variants. The ability to automatically annotate variants, to combine data from multiple projects, and to prioritize subsets of variants for downstream analyses is becoming a critical step in the adoption of genomics in personalized medicine. Here we present an new automated and integrated analysis system, the Omicia Genome Analysis System (GAS), a comprehensive pipeline for the clinical interpretation of personal genomes data integrating medical relevant annotations from such diverse data sources and algorithms such as OMIM, HGMD, LSDB, PharmGKB, dbSNP, 1000Genomes, Harrison's anchored disease ontologies, drug sets and clinically actionable gene set and algorithms such as PolyPhen, SIFT and VAAST. GAS includes a web-based application for genome-enabled clinical decision support that allows users to drill down from genome-wide views of variant data to individual genes and their variants; allows users to generate customized subsets of variants germane to particular clinical analyses, and provides a unique disease-gene ontology that ties variants and genes to clinically relevant disease literature. This functionality speeds the clinical decision-making process - from weeks to hours - and enables innovative meta-analyses of personal genomic data from gene panels, exomes and whole-genomes. GAS also integrates a newly developed tool called VAAST, the Variant Annotation, Analysis and Selection Tool. VAAST provides simple means to identify disease genes and causative variants within sets of genomes, making possible case-control style studies of personal genome sequences. We present here several genome analyses and show how GAS can be used to identify variation hotspots both along the chromosome, and within gene ontologies, disease classes and metabolic pathways. Special emphasis is placed upon the impact of data quality and ethnicity, and their consequences for further downstream analyses. We also show how variant calling procedures, pseudogenes and gene families all combine to complicate clinically-oriented analyses of personal genome sequences in ways that only become apparent when cohorts of genomes are analyzed.

921W

SNP genotyping using the Affymetrix® Axiom® Genome-Wide Yoruba (YRI) Array Set. *M. Shapero, Y. Lu, M. Purdy, M. Shen, J. Gollub, Y. Zhan, T. Webster.* Affymetrix, 3420 Central Expressway, Santa Clara, CA 95051.

Genome-wide association studies (GWAS) in African populations pose unique challenges, given the extensive genetic heterogeneity that has been observed. The landscape of human genetic variation is rapidly changing as next-generation sequencing is used to survey an increasing number of populations, enabling the continued identification of common and rare SNPs across diverse sample sets. Thus, sufficiently powered GWAS can be designed using array-based SNP panels that provide high genetic coverage of populations of interest. To this end, the Axiom® Genome-Wide YRI Array Set has been designed to maximize coverage of common and rare variants in populations of Yoruba (YRI) ancestry and admixed populations. This array set contains ~2.2 million markers (SNPs and insertions/deletions) obtained from HapMap, dbSNP, the 1000 Genomes Project, and the Southern African Genomes Project. In addition to high genome-wide coverage, these markers were also selected for coverage of the following categories: chromosomes X and Y, mitochondria, coding regions, recombination hotspots, ADME, miRNA, and disease-associated regions. The array set has been tested using manual and automated workflows with multiple sample types such as cell line, saliva, and blood-derived DNA. Assay performance was evaluated and shown to have an average sample call rate greater than 99.0%; trio concordance and average sample concordance to independent DNA genotype information (HapMap) were greater than 99.5%, and intra- and inter-run reproducibility were greater than 99.8%. Genetic coverage estimates for Yoruba, Asian (Chinese and Japanese), European, African-American, Luhya, and Maasai populations will be presented, as well as a discussion of how the array set can be applied to genome-wide association, disease association, and replication studies.

922W

Statistical approach to predicting functional effects of allelic variants from comparative sequence analysis. *S. Sunyaev[1], I. Adzhubey[1], D. Jordan[1,2].* 1) Div Gen, Dept Med, Brigham & Women's Hosp, Boston, MA; 2) Biophysics, Harvard University, Cambridge, MA.

Rapid advances in DNA sequencing technology enabled massive identification and cataloging of human allelic variation in research and clinical setting. A key challenge for human genetics today is to identify, among the myriad of alleles, those variants that have an effect on molecular function and phenotypes. Discovery of allelic variants of functional significance can be facilitated by computational predictions based on comparative sequence analysis. Specifically, massive exome sequencing projects aiming at identifying genes that harbor rare coding variants involved in human phenotypes require highly accurate, easy to use and fast methods for annotating large numbers of sequence variants. Although a number of computational methods for predicting the functional effect of human missense mutations and SNPs exist, limited prediction accuracy remains the major bottleneck in applications of these methods. We propose a new statistical approach that benefits from the newly available comparative genomics dataset of 46 vertebrates and the large size of training set based on over 60,000 missense disease mutations reported at HGMD. For positions with zero or one substitution per site in vertebrates, we directly estimate the probability that a given amino acid change at a site with a given phylogenetic pattern would be pathogenic. For variable positions we represent the observed phylogenetic pattern by a set of features and use a machine learning approach. Using a set of cross-validation experiments, we demonstrate that this approach benefiting from rich phylogenetic and training data substantially improves prediction accuracy over existing methods.

923W

Building an infrastructure for 21st century research: VIVO. *M.R. Tennant[1,2], K.L. Holmes[3,4], M. Conlon[5], VIVO Collaboration.* 1) Health Science Center Libraries, University of Florida, Gainesville, FL; 2) UF Genetics Institute, University of Florida, Gainesville, FL; 3) Bernard Becker Medical Library, Washington University School of Medicine in St. Louis, St. Louis, MO; 4) Department of Genetics, Washington University School of Medicine in St. Louis, St. Louis, MO; 5) Clinical and Translational Science Institute, University of Florida, Gainesville, FL.

VIVO is an open source semantic web discovery tool that enables exploration of research and scholarship across and among institutions. Discovery is facilitated across disciplinary and administrative boundaries through interlinked profiles of people and other research-related information. VIVO allows scholars to visualize academic and social networks and display information related to their activities and expertise, such as research interests, publications, grants, teaching, service, awards and more. Researcher information can be automatically ingested from authoritative data sources such as institutional human resources, bibliographic and grant databases. VIVO allows students and others to showcase their research - essential when applying for fellowships and postdoctoral and faculty positions. VIVO does more than provide current profiles. Through VIVO, researchers can keep abreast of the literature in their areas of expertise as well as in peripheral areas of interest. VIVO can help identify potential collaborators, events, seminars, programs, facilities and resources on the local campus and beyond. VIVO's linked set of structured data provides a foundation for search, navigation and additional tools for research discovery. One can begin with a search for a term, locating grants, papers, and people associated with the term. Click on a grant, then click on its principal investigator or other key personnel, click on the investigator's department, program or college, click on additional grants from those units, traversing the works. In each case, details are discovered, revealing the connections between each concept. VIVO supports learning and searching across domains. For example, linking researchers and clinicians to the genes they work on provides a capability to search for efforts based on the human diseases linked to those genes. These types of powerful linkages enable discovery of interdisciplinary activity as well as potential gaps and opportunities. Adoption, ontology usage, data provision, application development, and data consumption are all ways in which institutions and individuals can become involved with VIVO. The VIVO community enjoys a robust open source space on SourceForge, and the software and ontology are publicly available at http://vivo.sourceforge.net along with content that supports implementation, adoption, and development efforts.

924W

High-throughput annotation of genomic variants using a relational database system. *L. Wiley, W. Bush.* Center for Human Genetics Research, Vanderbilt University School of Medicine, Nashville, TN.

With waves of new data arriving from next-generation sequencing studies, there is a growing need for computational tools to annotate and characterize both common and rare genetic variants. Ideally, genomic annotations could be stored in a centralized relational database system established specifically for this purpose. Relational databases have many advantages over standalone software, most notably that information is stored and processed on disk rather than in memory. However, even after appropriate optimizations, retrieval time for interval-based queries (i.e. Does this position fall within any known genomic intervals/annotations?) is not trivial. In 2007, Alekseyenko and Lee presented a nested containment list (NCList) data structure which queried ranges 5-500 fold faster than current database indexing methods. This algorithm has been implemented successfully in many tools including JBrowse, a popular genome browser, and PeakAnalyzer, a utility that processes ChIP-seq and ChIP-chip data, however the authors note that for optimal incorporation into existing workflows and pipelines, the algorithm should be implemented within a relational database system. Here we present an implementation of the NCList algorithm in the popular database software MySQL, with the goal of providing an easily extendible collection of genomic annotations for rapid querying of next-generation sequence data. Our implementation consists of a pre-processing Perl script that establishes the initial database structure, and stored procedures for single position query, batch query, and batch update of the data structure. This collection currently contains over 500,000 annotations and can query 150 positions in approximately ten seconds. All tools accept genomic annotations from the widely used bed-file format.

925W

Biases in coverage and mutation detection of human coding exome by next generation sequencing. *G. Wu[1], M. Edmonson[2], L. Wei[1], M. Rusch[1], J. Easton[1], X. Chen[1], C. Mullighan[1], M. Dyer[1], T. Le[1], D. Alford[1], D. Zhao[1], A. Ulyanov[1], J. Downing[1], J. Zhang[1].* 1) Department of Computational Biology, St Jude Children's Research Hospital, Memphis, TN; 2) National Cancer Institute, Bethesda, MD.

Advances in next-generation sequencing (NGS) technology has made it possible to sequence a human genome with an average of 20-30x haploid coverage, enabling discovery of somatic alterations and genetic variations for biomedical research. Although poorly-covered exons in known human cancer genes have been noted, the extent and the impact of the coverage bias of the entire human coding exome has not been comprehensively analyzed. In this study, we evaluated coding exon coverage of two major cancer genome sequencing projects: The Cancer Genome Atlas (TCGA) and the St Jude Children's Research Hospital - Washington University Pediatric Cancer Genome Project (PCGP). Our analysis included matching normal samples of 58 patients of 5 different cancers sequenced by three NGS platforms of either whole-genome or exome. Approximately 2.1%-14% coding exons are poorly covered with an average coverage below 10x. They are highly enriched in GC-rich exons (e.g. exons with >60% GC content) as 26-90% of the GC-rich exons are poorly covered. 12-58% of the genes in Cancer Gene Census have at least one poorly-covered coding exon. This observation is universal regardless of platforms, data types (exomes or WGS), sequencing centers and diseases. To evaluate the impact of NGS coverage bias on mutation detection, we compared the distribution of somatic mutations deposited in the COSMIC database (http://www.sanger.ac.uk/genetics/CGP/cosmic/) in 12 large-scale candidate gene sequencing projects with those detected by NGS. 22% of the somatic mutations detected by Sanger sequencing are in GC-rich exons compared with 15% by NGS, indicating that poor coverage in GC-rich exons by NGS may cause the reduction of somatic mutations identified in these regions. For PCGP project, we explored possible solutions for improving coverage in GC-rich exons and found both RNA-seq and custom exon capture targeting poorly-covered exons can “re-cover” approximately 80% of the poorly-covered exons.

926W

A novel algorithm for detecting low frequency variants and somatic mutations at 1% frequency in hundreds of genes from heterogeneous research samples using the 5500 Genetic Analyzer. *X. Xu, M. Storm, S. Mangul, Y. Sun, J. Ichikawa, R. Padilla, D. Hom, M. Rhodes, F. Hyland.* Biological Information System, Applied Biosystems, part of Life Technologies, Foster City, CA.

The identification of low frequency variants resulting from sub-populations is important to elucidate the underlying biology of cancer. Here we describe the reliable detection of low frequency variants in hundreds of genes from heterogeneous research samples by utilizing the accuracy of the 5500 Genetic Analyzer. We developed a new algorithm to detect low frequency variants in whole exome or other targeted resequencing research applications. We leveraged the error correcting capability of the 5500 Genetic Analyzer to generate highly accurate data and detect extremely rare variations, down to an allele ratio of 1%. To test the performance of the algorithm, we diluted a known PCR enriched HapMap sample (NA12878) with a background of HuRef DNA of the same enriched 2 MB regions to create a series of concentration ratios, and applied our algorithms to the targeted resequencing data mapped with LifeScope Genomic Analysis Software, with an average coverage of 1400x. We measured the true positive, false positive and false negative rate for rare variants based on the known genotypes of these two samples. We demonstrated the ability to identify somatic mutations to a frequency of 5% with high sensitivity and high specificity and to a frequency of 1% and below with some loss of sensitivity. *For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.*.

927W

Accurate and Sensitive Somatic Mutation Detection in Heterogeneous Cancer Research Specimens by castPCR Technology. *I. Casuga[1], D. Merrill[1], B. Ching[1], Y. Bao[1], D. Deng[1], D. Le Corre[2], S. Desai[1], R. P. Petraroli[1], T. Hartshorne[1], H. Blons[2], P. Laurent-Puig[2], C. Chen[1].* 1) Life Technologies, 850 Lincoln Centre Drive, Foster City, CA; 2) UMR-S775, INSERM, Paris, France.

The discovery of pivotal genetic alterations and the understanding of their role in cancer is leading to remarkable successes in therapeutics and patient care. Molecular diagnosis methods such as DNA sequencing and conventional genotyping of tumor biopsies have advanced research in this field, but are limited in sensitivity due to stromal contamination and by genetic heterogeneity in cancer. We have recently developed competitive allele specific TaqMan® PCR (castPCR) assays for detecting cancer-associated sequence variations. The technology has demonstrated detection of as little as 1 mutant allele molecule in 10,000,000 wild type molecules. We report here sensitive and accurate detection of cancer-associated KRAS mutations within formalin-fixed paraffin-embedded (FFPE) heterogeneous cancer specimens. Eight FFPE model cell lines were initially used to investigate the assay capabilities (NCI-H2009:p.G12A; SW1463:p.G12C; PANC-1:p.G12D; PSN-1: p.G12R; A549:p.G12S; SW480:p.G12V; DLD-1:p.G13D; Jurkat:-Wild Type). Mutant FFPE cell line DNAs were titrated into the FFPE wild type cell line DNAs from 100% to 0.1%. Mutations were unambiguously identified and clearly distinguished from background signal at the level of 0.1% with high reproducibility. In a follow up set of experiments, 21 anonymous tumor tissues and 12 non-tumor tissues from FFPE specimens were evaluated for somatic mutations. Seven different KRAS gene mutations in each of the tumor derived samples were examined by castPCR assays. No mutations were detected in non-tumor tissues. The results obtained by castPCR for the 21 tumor tissues were concordant to those previously reported by three different methods (Taqman® PCR, Taqman® PCR + PNA and Sequencing). Our results demonstrate that castPCR, as a new rare mutation detection technology, has greater sensitivity, specificity and can thereby facilitate accurate mutation detection of heterogeneous cancer specimens.

928W

A New Sequencing Workflow Increases Accuracy and 5' Resolution for Capillary Electrophoresis Resequencing Applications. *C. Davidson[1], P. Ma[1], S.-C. Hung[1], S. Berosik[1], S. Schneider[1], V. Bourdon[2], T. Noguchi[2], H. Sobol[2], M. Wenz[1].* 1) Life Technologies, Foster City, CA; 2) Institute Paoli Calmette, Marseille, France.

The MLH1 and MSH2 are mismatch repair genes that play an essential role in DNA repair. Variants in MLH1 and MSH2 are highly heterogeneous and contribute to genome instability, and for this reason are associated with increased risks of cancer including Lynch syndrome or hereditary nonpolyposis colorectal cancer (HNPCC). MLH1 and MSH2 are large with more than 200 unique variants characterized for each gene. The size of the MLH1 and MSH2 genes and the sheer number of sequence variations requiring confirmation across a large genetic region makes interrogation by Sanger sequencing the preferred technique. To enable the identification of MSH2 variants, we have employed an improved capillary electrophoresis Sanger sequencing workflow that offers substantial time savings compared to previous workflows. The sequencing-based mutation detection methodology described here improved the resolution of fragments at the 5' end of all amplicons investigated in the MSH2 gene. 40% of the new method reads have⁄ 25 bases with QV20+ scores when compared to the same amplicons sequenced using a standard cycle sequencing workflow. Electrophoresis migration time was improved from 145 minutes to 75 minutes while maintaining read length and preserving excellent resolution starting at the first base after the gene-specific primer sequence. Further, the modified chemistry employed in the workflow resolved the peak compression problems that can occur when sequencing with conventional chemistries. All steps in the workflow, from the PCR reaction to loading on the capillary, were performed in the same reaction tube without the need to transfer material. As a consequence of workflow enhancements, the amount of hands-on time was reduced by 40% with the new method requiring 4 steps taking approximately 5 hours compared to 5 processing steps and up to 8 hours for a conventional cycle sequencing workflow. By minimizing the number of manual handling steps and eliminating the possibility of transfer errors, the likelihood of experimental variability is reduced compared to standard sequencing workflows. A unique one-step PCR purification-cycle sequencing reaction was also employed, further enabling a rapid sample to sequence turn-around time and improving the rate of variant detection in the MSH2 gene. Taken together, the workflow described here is a simple, streamlined procedure that increases throughput, and improves the accuracy of MSH2 genetic variant identification.

929W

Development of SureSelect Target Capture Methods for Sequencing on the Ion Torrent PGM System. *A.C. Giuffre[1], J. Ong[1], M. Guadalupe[1], S. Joshi[1], H. Ravi[1], M. Visitacion[2], C. Pábon-Peña[2], E. Lin[2], B. Novak[2], M. Hamady[2], F. Useche[2], D. Roberts[2], E. LeProust[2], S. Happe[1].* 1) Agilent Technologies, Cedar Creek, TX; 2) Agilent Technolgies, Santa Clara, CA.

New powerful methods for massively-parallel sequencing have emerged, allowing for enhanced ease-of-use, faster turnaround times, and focused analyses. However, limited read output still requires that samples be enriched for targets of interest prior to sequencing. To determine the efficacy of the Agilent SureSelect Target Enrichment System as a method for targeted resequencing on the Ion Torrent Personal Genome Machine (PGM), we developed methods to prepare genomic DNA libraries and perform hybrid capture, followed by analysis of sequencing performance. For PGM library construction, we utilized the Ion Fragment Library kit to append specific adapters. Hybrid capture was performed with specially-designed blocking reagents to prevent cross-hybridization of adapters. Following sequencing on the PGM, we demonstrated high specificity for capturing targets of interest, excellent uniformity, and even coverage across the targeted regions. The method was ideally suited for targeting regions < 1Mb, but higher coverage of larger regions could be achieved by sequencing across multiple chips. High performance was achieved across different sample types captured with SureSelect libraries of varying complexity and content. Data obtained was used to efficiently identify non-reference variants, and showed high correlation with previously-determined HapMap genotypes. These findings illustrate the utility of the SureSelect method for target enrichment on the Ion Torrent PGM, and provide a path forward for variation discovery in small targeted regions , validation of hits from other platforms, and profiling variation within large sample sets in minimal time.

930W

Genomic Structural Rearrangement Detection in Highly Aneuploid cancers using Long Fragment Read Technology. *B.A. Peters[1], B.G. Kermani[1], O. Alferov[1], J. Haas[1], R. Drmanac[1], T.D. Barber[2].* 1) Research Department, Complete Genomics, Inc., 2071 Stierlin Court, Mountain View, CA 94043; 2) Translational Science, Eli Lilly and Company, Lilly Corporate Center/DC 0428, Indianapolis, IN 46285.

Detection of genomic structural rearrangements in cancer is currently limited by the read length and mate pair gap length of next generation sequencing (NGS) technologies. The typical NGS read length of 35-200 base pairs with a mate pair gap of 200-2000 base pairs can make translocations difficult to detect. Using a library preprocessing method called Long Fragment Read (LFR) technology, which generates data similar to single molecule sequencing of 50kb fragments, we generated approximately 2 TB of data on 2 matched tumor and normal hepatocellular carcinoma samples. We show that complicated genomic rearrangements can be easily mapped using standard NGS library construction and sequencing when LFR is employed. Further, by using the long fragments to phase variants in tumor samples we demonstrate that potential false positive somatic mutations, which are inconsistent with assembled haplotypes, can be effectively identified and removed from further analysis. Importantly this work was performed using ~130 pg of DNA an amount equivalent to what is contained within 20 human cells. This technological breakthrough will enable similar analyses to be performed on circulating tumor cells, cancer stem cells, fine needle aspirates, and other DNA samples extracted from extremely low abundance tissue sources.

931W

5500 GATM System in Combination with Enhanced Call Chemistry (ECC) Enables the Detection of Low Frequency Genetic Variation in Heterogeneous Samples. *C. Storm, J. Ichikawa, R. Padilla, D. Hom, G. Meridith, C. Adams, X. Xu, YM. Sun, F. Hyland, M. Rhodes.* Applications, Life Technologies, San Francisco, CA.

The propagation of next generation sequencing has opened up many new and exciting avenues for cancer research. One such area is to actively search for and determine the clinical relevance of genetic sub populations in circulating tumor samples. There are two main considerations that have to be accounted for when undertaking this type study. First the sequencing throughput has to be adequate to detect the variation at low frequencies; for example, theoretical calculations show that low frequency variants occurring at 1% may require as much as 1000 fold coverage to maximize the ratio between true positives and false negatives. Secondly, the accuracy of the sequencing will determine the ability to ascertain with any level of confidence that novel variation is actually true and not an artifact of the sequencing process. Accuracy of the sequencing chemistry is paramount to address both these issues. The difference between an accuracy of 99.95 and 99.99 for a minor allele that occurs at 1% translates in to an additional 100 fold coverage. And at a certain point there is no benefit to additional sequencing, since the number of false positive variants will soon vastly outnumber the number of true variants. Further higher accuracy and a lower number of false positive variation calls will significantly reduce the amount and complexity of the downstream validation work that has to be done. In this poster we describe how the addition of Enhanced Call Chemistry (ECC) to the already highly accurate two base encoding of the SOLiDTM platform in combination with a new set of computational algorithms designed specifically to take advantage of this chemistry shows a significant improvement to current abilities to detect variation occurring at an exceedingly low frequency in a background of “normal” material. For this proof of concept we have looked at two different enrichment methods, one PCR based and one based on hybridization of complimentary probes. For both methods we targeted a number of genes commonly associated with cancer. Further, we chose to simulate the frequency of circulating tumor cells in a normal background by pooling samples at predetermined levels, down to 0.1%. In this poster we demonstrate feasibility to detect these variants down to the lowest level as well as describing the pros and cons of each type of enrichment for this type of study.

932W

Microsatellite Instability & Immunohistochemistry Testing of Colorectal Tumors among U.S. Cancer Programs & Follow-up of Abnormal Results. *L.C. Beamer[1], D.J. MacDonald[1], C. Huizenga[1], H. Hampel[2], K.R. Blazer[1], M. Grant[3], J.N. Weitzel[1].* 1) Clinical Cancer Genetics, City of Hope, Duarte, CA; 2) Division of Human Genetics, The Ohio State University, Columbus, OH; 3) Nursing Research & Education, City of Hope, Duarte, CA.

PURPOSE: Reflex microsatellite instability (MSI) and immunohistochemistry (IHC) testing of CRC tumors to screen for Lynch syndrome, a hereditary cancer predisposition syndrome, is an emerging practice in the U.S. To date, no formal studies described this practice. We sought to explore the practice of MSI/IHC testing by type of cancer program and number of CRC cases, and patient follow-up of abnormal results. DESIGN: A 12 item survey was developed following input from expert panels and pilot testing in 22 cancer programs (99.5% return rate). METHODS: The study sample included 39 NCI-designated Comprehensive Cancer Centers (NCI-CCCs), 50 randomly-selected Community Hospital Comprehensive Cancer Programs (CHCCPs) and 50 randomly-selected Community Hospital Cancer Programs (CHCPs). An invitation letter, survey, and online-survey option was emailed to a contact at each cancer program. Data were merged into one SPSS file. Program characteristics and MSI/IHC data were summarized using frequencies and percentages. The association between cancer program type and current reflex testing practice was measured using a chi-square test of independence. Multinomial logistic regression was implemented to create a model to predict reflex testing. RESULTS: The overall response rate was 50% (63% for NCI-CCCs, 50% for CHCCPs, and 40% for CHCPs). Seventy-one percent of the NCI-CCCs, 36% of the CHCCPs, and 15% of the CHCPs were using a reflex MSI and/or IHC test. Only 30% of the programs used a pre-surgical information packet, consent, or opt-out option. Nearly one-half of the programs had patients who did not engage in a recommended genetics clinic visit. A significant association was found between the type of cancer program and current reflex testing practice ($X^2(2, n=69),=14.543, p=.001$). NCI-CCCs were significantly more likely to currently engage in reflex testing than the CHCCPs and CHCPs. Number of CRC cases predicted current practice of using reflex MSI/IHC testing (p= .049). Similarly, NCI-CCC status predicted current reflex MSI/IHC testing (p=.013); CHCCP status did not predict current reflex MSI/IHC testing. CONCLUSIONS: Most NCI-CCCs use MSI/IHC whereas most CHCPs do not; nearly two-thirds of CHCCPs currently or plan to use this testing. Clinic follow-up is suboptimal. CLINICAL RELEVANCE: Findings will guide the creation a toolkit to enhance MSI/IHC testing and follow-up of abnormal results.

933W

A Single SNP Surrogate for HLA-Cw6 Genotyping in Diverse Populations. *R.P. Nair[1], P.E. Stuart[1], T. Tejasvi[1], P.A. Shaiq[4,5], R. Qamar[4], G.K. Raja[5], P. Kullavanijaya[3], J. Ding[2], Y. Li[2], J.J. Voorhees[1], G.R. Abecasis[2], J.T. Elder[1].* 1) Dept Dermatology, Univ Michigan, Ann Arbor, MI; 2) Dept of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, MI; 3) Institute of Dermatology, Bangkok, Thailand; 4) COMSATS Institute of Information Technology, Islamabad, Pakistan; 5) PMASAA University, Rawalpindi, Pakistan.

Psoriasis is an autoimmune disease of the skin affecting ~2% of the Caucasian population. Several studies have conclusively established the Cw6 allele of HLA-C, a highly polymorphic MHC Class I gene, as the most strongly associated among 24 known psoriasis susceptibility loci. Because of the high degree of polymorphism, direct assay of HLA-Cw6 requires sequencing, serological assays or highly temperamental allele-specific PCR, and a reliable surrogate marker for Cw6 has not been available. We have previously established a 7-SNP typing system to identify the Cw6 allele with a high degree of accuracy [Nair et al., AJHG 78:827, 2006]. Here we report a single SNP that can predict HLA-Cw6 status with 99% accuracy. In attempting to further delineate the MHC psoriasis locus, which appears to harbor multiple susceptibility genes, we genotyped a dense set of 1332 markers spanning the MHC. The most significantly psoriasis-associated markers that emerged from this analysis are rs12191877 ($p = 1.2 \times 10^{-35}$, OR = 2.67) and rs4406273 (1.2×10^{-34}, OR = 2.94). Comparison with 2332 samples typed for HLA-Cw6 showed that the A allele of rs4406273 tagged HLA-Cw6 with high specificity (genotype concordance 0.989, LD $r^2 = 0.983$, Pearson correlation = 0.991). We developed a single base extension assay (Snapshot, Applied Biosystems) for rs4406273 and genotyped 4995 North American Caucasian samples, 308 from Thailand and 837 from Pakistan, and compared the results with HLA-Cw6 types assayed using our previously reported method. The concordance rates were 99.1% for Caucasians, 98.7% for Thai and 99.5% for the Pakistani samples. Our results show that the A allele of rs4406273, located 26.5 kb centromeric of HLA-C and 55.3 kb telomeric of HLA-B can serve as a highly accurate surrogate for HLA-Cw6.

934W

A novel proteomics approach for identification of protein-RNA complex associated with specific gene loci using a human artificial chromosome (HAC). *T. Seko[1], S. Yamaguchi[1,2], Y. Yoshimura[1,2], Y. Nakayama[3], M. Kato[4], T. Obayashi[2], M. Oshimura[1,5], H. Kugoh[1,5].* 1) Dept. of Biomed. Sci., Inst. of Regenerative Med., Tottori Univ.,Tottori,Japan; 2) Div. of Laboratory Animal Sci., Res. Ctr. for Biosci. and Technology, Tottori Univ. Tottori,Japan; 3) Div. of Functional Genomics, Ctr. for Biosci. and Technology., Tottori Univ.,Tottori,Japan; 4) Div. of Human Genomic Sci., Dept. of Mol. and Cellular Biology, Sch. of Life Sci., Faculty of Med., Tottori Univ., Tottori,Japan; 5) Chromo. Eng. Res. Ctr., Tottori Univ.Tottori,Japan.

Functional analysis of DNA, RNA and protein-protein interaction has progressed rapidly in recent years. Numerous protein-complex on chromatin are involved in the gene regulation. Therefore, identification of molecular complex of specific genome loci is critical to understanding genomic network. Here, we propose a novel method using HAC, termed chromosome immunoprecipitation (ChrIP), which allows for efficient purification of protein complex on targeted gene loci. ChrIP assay consist of the following three steps to purify proteins on specific genome loci. 1. The introduction of targeted genomic region and tag marker to HAC. 2. Recover of the HAC by chromatin immunoprecipitation. 3. Identification of proteins that specifically bind to targeted genomic region. In this study, we introduced a direct repeats of the lac operator (lacO) into chinese hamster ovary (CHO) chromosome to confirm whether it is able to recover genomic DNA equivalent to the DNA size of HAC. The lacO was produced by gene amplification using a dihydrofolate reductase (DHFR) expression vector with methorexarte selection. To identify the CHO cells that carry the lacO in the CHO chromosome, we performed fluorescence in situ hybridization (FISH). As a result, one of 23 clones was introduced lacO sequence to the CHO chromosome. Next, we introduced the LacI-GFP vector that produce LacI-GFP protein complex into one of the lacO positive clones. We report here that a large size of CHO chromosome was recovered by anti-GFP antibody. These results suggest the possibility of recovery of the HAC by chromatin immunoprecipitation. Furthermore, our colleague has developed a new HAC vector with multi-integrase recombination sites.[1] MI-HAC enables to introduce various large genes or genomic loci. Further studies to introduce MI-HAC that contains the lacO repeat sequences and targeted gene loci are still in progress. [1] A method for Producing Transgenic Cells Using a Multi-Integrase System on a Human Artificial Chromosome Vector. PLoS one February 2011 vol.6.

935W

Efficient computing of genotype calling for GWAS. *S. Leo[1], I. Zara[1], M. Valentini[1], S. Sanna[2], G. Zanetti[1].* 1) CRS4, Pula, CA, Italy; 2) IRGB, CNR, Monserrato, CA, Italy.

Genotype Calling (GC) represents an important challenge for Genome-Wide Association Studies (GWAS), given the noise associated with high-throughput genotyping and batch effects when performing plate-by-plate processing. Confounding can be drastically serious for certain genotyping platforms, and in order to reduce batch effects and other error sources, it is mandatory that all available samples are processed in one run. However, as the number of samples grows, job running time and computational resource usage increase dramatically, making it difficult to scale efficiently to thousands of samples. Even partitioning data into probeset subsets, thus allowing a certain level of parallelization, does not solve the problem, since the core of currently available algorithms requires accessing all data samples at once. For instance, a GC run performed at our site using the Affymetrix Power Tools (APT), took two weeks for processing 6863 samples by using 18 nodes with 8 CPUs and 16 GB of RAM for each job (one node for each probeset subset). Moreover, these computational costs make it cumbersome to perform a systematic analysis of the robustness of results with respect to changes in the dataset used. We developed a new distributed GC application that scales efficiently with the number of samples, harnessing the full power of a large computational cluster. For example, we were able to perform GC on the aforementioned 6863 samples in about 16 hours using 30 nodes with 8 CPUs each. Results obtained with our tool are comparable with those of the Birdseed v2 algorithm included in the APT. Specifically, the average call rate, 94.983%, was slightly higher than the one obtained with the APT Birdseed v2 implementation (94.794%), which resulted in calling about 12 million additional genotypes over all 6863 samples. The average allelic discordance rate with respect to the original run was 3.6×10^{-4}, with a standard deviation of 0.1×10^{-4}; 6838 out of 6863 samples (99.6%) had an allelic mismatch rate $\leq 7 \times 10^{-4}$. The new tool is implemented as a Hadoop MapReduce application that uses the Pydoop Python API (http://pydoop.sourceforge.net) to interact with Hadoop, and Numpy plus Boost.Python extension modules for APT for core computation and Affymetrix-specific data I/O. The software will be available as open source in the near future.

936W

Pegasus WMS: Enabling Bioinformatics using Workflow Technologies. *G. Mehta[1], E. Deelman[1], K. Vahi[1], Y. Wang[2,4], A. Clark[3], R. Mayani[1], T. Chen[4], J. Knowles[3].* 1) Information Sciences Institute, University of Southern California, Marina Del Rey, CA; 2) Department of Automation, Xiamen University, China; 3) Department of Psychiatry, USC Keck School of Medicine, Los Angeles, CA; 4) Department of Biological Sciences, University of Southern California, Los Angeles, CA.

Recent advances in bioinformatics from genome sequencing techniques, to protein analysis to bacterial RNA studies have resulted in large amounts of raw data being generated. This data needs to be analyzed, mapped to genomes as well as handled in a robust, efficient and secure manner. Generally most laboratories lack the tools or the manpower to create complex pipelines to analyze these datasets and run these pipelines on the computing infrastructure present either in the laboratories or on campus or the commercial cloud environments.

To enable scientists to run their bioinformatics analyses on large-scale computational resources we use Pegasus WMS, a Workflow Management System that can manage large-scale scientific workflows across local, Grid, and Cloud resources simultaneously. Pegasus WMS provides a means for representing the application as a workflow in an abstract XML form, agnostic of the resources available to run it on and the location of the input data and executables. It then, compiles these workflows into executable workflows by querying catalogs and farming computations across local and distributed computing resources, as well as emerging commercial and community cloud environments in an easy and reliable manner. Pegasus WMS optimizes the execution as well as data movement by leveraging existing Grid and cloud technologies via flexible pluggable interfaces and provides advanced features like data reuse, automatic cleanup of generated data, and hierarchal workflows with deferred compilation. It also captures all the provenance of the workflow from the compilation stage to the execution of the generated data, helping scientists accurately measure performance metrics of their workflow as well as tackle data reproducibility issues.

Pegasus WMS has also been packaged in an easy to use Virtual Machine that can be used on local machines or clusters to run RNA Sequencing Workflows using different Genome mapping tools and expression calculators for the NIMH Transcriptional Atlas of Human Brain Development project. It was recently used to compute more then 200 samples of human RNA samples on a campus cluster. Pegasus WMS was initially developed to support large-scale high-energy physics and astrophysics experiments and supports a wide variety of applications from earthquake simulation, bacterial RNA studies, helioseismology to bioinformatics.

937W

Handling the data management needs of high-throughput sequencing data and GWAS: SpeedGene, a C++ library for the fast and efficient storage of genetic data. *D. Qiao, W. Yip, C. Lange.* Department of Biostatistics, Harvard School of Public Health, Boston, MA.

The pedigree file-format is one of the most commonly used input formats for genetic data analysis software, e.g. FBAT, PBAT, PLINK. For high-throughput sequencing data and data from genome-wide association studies, the sizes of these pedigree files can reach several tetra-bytes of disk space. Unnecessarily large files result in waste of disk space and loading time during analysis. We introduce here the SpeedGene-library that includes an optimized "storage-and-load" algorithm for genetic data. Depending on the minor allele frequency, the SpeedGene algorithm chooses among three different compression-methods to minimize the disk and memory space for storage. The SpeedGene-format does not require any CPU-time for decompression. The library provides functions for loading of the compressed files and retrieving any part of the original data. Our new algorithm performs better than currently available compression formats for pedigree files by several magnitudes. The compression factor of the algorithm depends on the genotype frequency distribution of the markers and the number of subjects in the dataset. The compression factor ranges from 16 to several hundreds. Furthermore, it takes only few seconds/minutes to load an entire file with the library, and our implementation supports parallel processing of the dataset, i.e. loading of subsets of markers. This greatly decreases the loading time when parallel jobs are dispatched in clusters. The library provides direct-data-retrieving functions, which allows the compressed dataset to be accessed by any other C++ programs. In conclusion, the SpeedGene library enables the storage and the analysis of next generation sequencing data in existing hardware environment, making system upgrades unnecessary.

938W

Software library and tools for efficient and flexible processing of sequence reads and variant calls. *M. Trost, G.R. Abecasis, H.M. Kang.* Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI.

As the volume of large scale next-generation sequence data increases from terabytes to petabytes, the need for high quality, high performance analytic tools is becoming crucial. The Sequence Alignment/Map (SAM) format and its binary counterpart (BAM) are the most widely used formats for storing alignment information. The Variant Call Format (VCF) is commonly used for storing genetic variations discovered from sequence reads. We developed an object-oriented C++ library that allows us to efficiently and flexibly process these files and built a set of useful tools to automate common tasks for the manipulation and analysis of these files.

Our generic C++ library for robustly and interchangeably handling SAM or BAM files provides a rapid, validated, and modularized interface to access aligned sequence read information without the detailed knowledge of the data format. The library is designed to provide a clear and convenient interface to SAM/BAM files in order to facilitate the development of sequence data processing software. In addition, by handling VCF files, our C++ library allows us to flexibly perform various types of data manipulation and association analysis from variant calls identified from the sequence data.

A number of tools for handling SAM/BAM/VCF files have been built using our library. The bam diff tool compares a pair of SAM/BAM files produced by different analytic pipelines outputting discordant records. The bam revert tool allows us to undo realignment or recalibration operations by recovering original alignment information from ancillary information stored in info tags. To reduce the size of a BAM file, the squeeze bam tool drops unessential fields and duplicate reads and edits the representation of base sequences to reduce file size. This saves on storage space and facilitates storage and long-term archiving. The VcfCooker tool allows data manipulation and the filtering of variants. The verifyBamID tool detects contamination in the sequence data using known genotypes or allele frequency information.

Our software tools and library are publicly available at http://genome.sph.umich.edu/wiki/Software.

939W

Improving mate-paired sequencing for submicrogram human samples. *Z. Chen, M. Wei, T. Biorac, B. Li, X. Xu, G. Marnellos, R. Bennett.* Life Technologies, 5791 Van Allen Way, Carlsbad, CA 92008.

Mate paired sequencing is critical for shotgun based whole genome sequencing and structural variation study using next generation massively parallel sequencing technologies. Recent published studies showed that mate paired sequencing detected 69% more structural variation by average in the primary breast cancer samples than paired end sequencing method and over 2-fold more in the breast cancer cell lines. To construct long mate-paired (LMP) library with high complexity it usually requires tens of micrograms of input DNA, which is rarely the case for primary tumor samples. The least efficient step in the LMP protocol is a circularization step, which joins two ends of a molecule together to an internal adaptor to form a mate pair. We have developed a new intramolecular circularization (NIC) method, which improves efficiency of this circularization step dramatically. We used the nick translation method in SOLiD™ 4 LMP protocol to generate even-sized mate-paired tags with defined average length, and further optimized the protocol to generate longer mate tags and tighter tag size distribution for 2x60 mate-paired sequencing. Using the NIC method and improved procedure to construct LMP libraries with HuRef genomic DNA, we demonstrate 3 to 10 fold improvement on yield of LMP library over SOLiD™ 4 LMP method for 1-3kb insert LMP libraries, and a reduced number of false positive mate reads. Furthermore, we develop a unique mate paired barcoding strategy so that submicrogram starting material can be used and 8 to 12 samples can be pooled after barcoded mate paired adaptor ligation in the early library construction workflow to simplify the library preparation for processing 8 to 12 LMP libraries parallel. This approach enables us to increase LMP library throughput and improve the library complexity for sequencing. This low input mate-paired library construction method will further broaden the application of mate paired sequencing in human genome research. For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

940W

Methods for systematically reducing the uncertainty in calling copy number variations by combining information from multiple sources and technologies. *D.S. Greer, D. Malhotra, W. Wu, J. Sebat, Psychiatric GWAS Consortium.* Psychiatry, University of California, San Diego, La Jolla, CA.

We address the general computational problem of more accurately determining copy number variations (CNVs) by consolidating data gathered from multiple experiments and diverse technologies. While the underlying duplication or deletion of a genetic region has theoretically well-defined breakpoints, at present, common methods for ascertaining these locations produce results that are only rough approximations. Many algorithms, which operate on high-throughput sequencing or microarray data, implicitly overstate their actual accuracy by reporting only the estimated breakpoints. Consequently, CNV calls made on the same subject by various analysis software vary significantly, and the differences are difficult to evaluate.

We are developing algorithms that can accept information from heterogeneous technologies and from multiple experiments to progressively refine the estimates of the CNV regions with increasing certainty and accuracy. Rather than producing a final set of calls, a data structure is retained which is continually updated by incorporating additional results from additional tests. This facilitates not only the detection of CNVs, but also the evaluation of different calling methods.

In order to systematically represent and analyze the uncertainty, CNV calls from a single individual are used to divide the genome into disjoint segments. The segments can vary in length from a single base-pair to very long regions which do not contain structural variations. As calls are added to an existing segment table, segments are split where they span one of the endpoints. For each segment, a feature vector is extracted from the multiple data sources. The features may be derived from sequence matching, average microarray intensity values, or estimated probability functions. Since the segment data structure contains at most a few thousand regions (compared to one that explicitly represents all three billion base-pairs) it can be used in complex computations that would otherwise be intractable. These include signal processing and machine learning algorithms that attempt to make the optimal use of all the available information. By extracting the abstract mathematical properties of CNVs, the segment table structure is extensible, flexible, independent of specific measurement techniques, and capable of representing the results of many repeated and diverse experiments.

941W

Chromosome Copy Number Variation by Digital PCR. *J.T. Wang, J.T. Kuykendall, J.F. Stevens.* qPCR R&D, Life Technologies, Foster City, CA.

Chromosomal rearrangement is a common feature in transformed tumor derived cell lines. The number of chromosomes (karyotype) of different tumor cell lines can be unique and may be used for identification of the cell line lineage. The number of duplicate chromosomes in transformed cells can be quite high. At these high copy numbers small differences can be difficult to detect using traditional qPCR (quantitative real-time Polymerase Chain Reaction) methods as the measuring method is base on logarithmic scale. Digital PCR is an advanced utilization of PCR technology where the number of nucleic acid template is accurately counted. This technology relies on diluting the nucleic acid template to the Poisson distribution limit where each PCR reaction contains at least a single copy of template or none. The 1 and 0 scoring of the reactions gives the digital nature of this PCR application. The average number of copies per reaction can then be calculated using Poisson statistics. Digital PCR technology's linear scale measurement offers the precision and accuracy required to discriminate variations in higher copy number. Here we describe an approach using Digital PCR to detect chromosomal aberration in tumor cell lines. We use 48 TaqMan® Copy Number Assays (2 assays for 24 chromosomes - 22 autosomes and 2 sex chromosomes) to count the copy number of chromosomes. We characterized genomic DNA extracted from 6 different human derived transformed cancer cell lines as well as 3 normal cell lines. We found the digital method provides advantage in accuracy of copy number is that the linear scale of the quantification method allows discrimination between two high copy numbers. The digital PCR technique allows precise copy determination of chromosome locus assayed.

942W

Evaluation of Recruitment Strategies for Web-based Genetic Studies. *E.M. Drabant, K.E. Barnholt, C. Dijamco, J.Y. Tung, A.K. Kiefer, J.L. Mountain, A. Wojcicki.* 23andMe, Mountain View, CA.

Many of the hundreds of web-based genetic studies conducted by 23andMe's research arm rely solely on organic recruitment through 23andMe's personal genome service. For a few studies, however, 23andMe actively recruits individuals who meet particular inclusion criteria. Two examples are 23andMe's Parkinson's disease and sarcoma studies. Recruitment of participants for these studies is conducted via a number of strategies. We evaluated our recruitment strategies by reviewing the changes in enrollment rate in response to each type of effort. The recruitment strategies that have been most successful include paper-based mailings from advocacy organizations such as the Michael J. Fox Foundation and the Parkinson's Institute, and email and website campaigns conducted by advocacy organizations. Grass-roots efforts led by 23andMe members affected with the disease in question have been a significant source of recruitment through online support groups and blog posts. Both studies have garnered attention in the press, which has enhanced enrollment to some extent. The strategies that have been least successful include attending and sponsoring disease-focused events, listing the study on www.clinicaltrials.gov, and speaking to disease-focused support groups. Together these efforts have resulted in rapid recruitment for both studies. In the two months after the Parkinson's study was launched, more than 2,000 patients enrolled. Two years later, the study includes 5,100 patients, the largest single cohort of Parkinson's patients to date. Since the sarcoma study was launched in April 2010, 23andMe has enrolled 519 patients with sarcoma, again, the largest cohort of its kind. Web-based genetic studies allow for global recruitment and require no participant travel. However, such studies do require participants to have Internet access and to navigate the Internet. These requirements are likely correlated with the relative success rates of the recruiting strategies in that individuals who learn of the study via the Internet or email are likely to successfully navigate the recruitment and participation process. Understanding which strategies are most effective for recruitment of web-based genetic research will inform future efforts to engage patients and develop large cohorts for research in a variety of diseases and conditions.

943W

Scalable data management and computable framework for large scale longitudinal studies. *L. Lianas[1], G. Cuccuru[1], S. Leo[1], I. Zara[1], M. Pitzalis[2], M. Zoledziewska[2], F. Deidda[2], S. Sanna[3], G. Zanetti[1].* 1) CRS4, Pula, Italy; 2) Dipartimento di Scienze Biomediche, Università di Sassari, 07100 SS, Italy; 3) CNR-IRGB, Monserrato, CA, Italy.

The increasing rate of data production and decrease of experimental cost of the latest generation of high throughput technologies facilitates large scale high resolution studies even in medium size laboratories. However, once the pure experimental data acquisition hurdle has been overcome, success will depend on the ability to properly manage and process the high-dimensional data sets that are generated, which in turn requires to adopt advances in informatics. Apart from the computational problems connected to the data analysis proper, one key issue is the increasing number of items that need to be tracked, for instance the network of connections and dependencies from the same biological sample to different experiments. Moreover, for clinically motivated studies, the amount of related information, from diagnosis to laboratory results, starts to be comparable to what is handled by a Clinical Information System of a small hospital. We have implemented a computable biobank (CB) in support of two large scale longitudinal studies (~10,000 individuals) on autoimmune diseases and longevity conducted in Sardinia. The CB is designed to represent, using a uniform computational formalism, the information on all the relevant objects (e.g., physical samples, experimental and derived data, clinical data) and the network of actions, performed during the experiments and the computational analysis, that relate one to the other. The system supports type introspection on all its objects and reflects OpenEHR archetypes, an open standard specification in health informatics that describes the management storage, retrieval and exchange of health data in electronic health records (EHRs). The latter choice guarantees a robust, computable, uniform and implementation independent description of the clinical data. The results of computation, e.g., genotype calling data, is held in specialized data structures that directly support further parallel processing and analysis. The current implementation of CB is built upon the core services of OME Remote Objects (OMERO), an open source software platform that includes a number of storage mechanisms, remoting middleware, an API, and client applications for biological data management developed by the Open Microscopy Environment. Currently available CB aware external programs include suites of map-reduce programs for GWAS and sequencing applications, as well as basic chain-of-custody inspection tools and tools for clinical data import.

944W

The clinical and molecular genetic characteristics of Korean patients with Argininosuccinate Synthetase (ASS1) deficiency. *B.W. Jeon[1], B.H. Lee[1,2,3], G.H. Kim[1,2], J.M. Kim[1,2], J.H. Choi[1,3], H.W. Yoo[1,2,3].* 1) Genome Research Center for Birth Defects and Genetic Disorders, Asan Medical Center, Seoul, Korea; 2) Medical Genetics Center, Asan Medical Center, Seoul, Korea; 3) Department of Pediatrics, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine.

The urea cycle, consisting of a series of six enzymatic reactions, plays key roles to prevent the accumulation of toxic nitrogenous compound and synthesize arginine. Argininosuccinate synthetase (ASS1) deficiency is a urea-cycle disorder characterized by a defect in ammonium elimination in liver, leading to hyperammonemia.Citrullinemia type I (CTLN1). This study was undertaken to investigate the clinical and molecular genetic characteristics of Korean patients with CTLN1. A total of 21 patients from 20 unrelated Korean families have been diagnosed with CTLN1. Presenting manifestations were hyperammonemic encephalopathy (14pts, 73.7%) and vomiting and irritability (3 pts, 15.8%). The remaining two patients (2 pts, 10.6%) were identified in asymptomatic period, one by neonatal screening test and another patient by family member screening test. Fifteen patients (71.4%) were identified in neonatal period, 2 pts (9.5%) in infantile period, and 4 pts (19%) in adulthood period. The *ASS1* gene analysis revealed 11 different mutations in 39 of 40 alleles (97.5%).p.G230A, p.E298K, and p.I394N were novel mutations. These mutations were located on highly-conserved residues of ASS1, and In silico analysis predicted these variants as pathogenic. Notably, three variants.c.421-2A>G (12/40, 30.0%), G324S (10/40, 25.0%), and c.1127-9_1185dup67 (8/40, 20.0%), were common mutations in Korean CTLN1 patients. The mutation spectrum of the ASS1 gene in Korean population was different from those in other populations, even from those in other East Asian countries. Our report supports wide spectrum of genetic heterogeneity in CTLN1. Further study for the functional characterizations of novel variant is needed.

945W

Investigation of ECM1 mutation in lipoid proteinosis (LP). *F. mahjoubi[1], s. samanian[1], f. izadi[2], mm. tavakoli[2], m. farhadi[2].* 1) National Institute of Genetic Engineering and Biot, tehran, Iran; 2) HAZRAT Rasool Hospital, Tehran University of Medical Sciences and Health Care Services,.

Purpose: Lipoid proteinosis is a rare autosomal recessive disorder (OMIM 247100) that results from mutations in extracellular matrix protein 1 (ECM1), a glycoprotein that is expressed in several tissues. Lipoid proteinosis is a clinically heterogeneous disease. Affected individuals show differing degrees of skin scarring, variable signs of hoarseness and respiratory distress, neurological abnormalities such as temporal lobe epilepsy, hyperkeratosis in knee and elbow and shortening of tongue. Methods used: In this study DNA was extracted from peripheral blood samples taken from affected individuals and their parents, exons were amplified using PCR and then sequenced the PCR product. The project was approved by the local ethical committee of National Institute for Genetic Engineering and Biotechnology (NIGEB) and written informed consent was obtained from all cases. Specimens (peripheral blood) were collected from Hazrat Rasool hospital. Results: One of the patients was a 21-year-old Iranian female who had hoarseness, skin scarring on the face and keratosis in knee and elbow. To best of our knowledge a new homozygous missense mutation C269Y in exon 7 was observed, parents were both heterozygous for this mutation. Conclusion: Our results suggest that this mutation is responsible for LP disease in this patient.

946W

Proteinuria in Infantile-Onset Spinocerebellar Ataxia, Type 2. *R.K. Brar[1], C. Moore[2], L. Eviatar[3], R. Smith[3], C. Sethna[4], M.G. Bialer[2].* 1) Dept. of Pediatrics, Nassau University Medical Center, East Meadow, NY; 2) Div. of Medical Genetics, North Shore LIJ Health System, New Hyde Park, NY; 3) Div. of Pediatric Neurology, North Shore LIJ Health System, New Hyde Park, NY; 4) Div. of Pediatric Nephrology, North Shore LIJ Health system, New Hyde Park, NY.

Spinocerebellar ataxia, type2 (SCA2) is an autosomal dominant progressive neurodegenerative disorder. It typically presents during adulthood in the 4th decade as gait ataxia and incoordination. Other symptoms include hyporeflexia, dysarthria, dysphagia and oculomotor dysfunction. Paternal transmission instability of the causative CAG repeat can, in some cases, lead to extreme repeat expansion, resulting in earlier onset of disease. Infantile-onset of SCA2 is characterized by hypotonia, dysphagia, infantile spasms, myoclonus, dystonia, myokymia, pigmentary retinopathy and abnormal eye movements. There is often rapid progression of disease with early death. We saw a 7 month old girl with axial hypotonia, appendicular hypertonia, microcephaly and global developmental delay. Delays had initially been noted at 5 months with poor head control and lack of tracking. Ophthalmology evaluation revealed cortical visual impairment. EEG showed hypsarrhythmia. CT scan of the head showed parenchymal volume loss. A strong family history of SCA2 exists across 3 generations. The father had onset in his 20's and had a CAG expansion of 44 in the ATXN2 gene. The paternal grandfather had onset in his 30's. Our patient had a heterogeneous expanded repeat allele of 300-350 repeats. At about 12 months she developed nephrotic range proteinuria (protein:creatinine ratio 4-7, normal <0.2). DNA sequencing of known nephrotic syndrome genes (NPHS2, WT1, LAMB2, PLCE1 and NPHS1) did not reveal any definitive pathogenic mutations. Of note, the patient's brother presented similarly as an infant with blindness, developmental delay, seizures, and respiratory and feeding problems. He also developed nephrotic syndrome. A brain CT scan showed cerebral atrophy with a small cerebellum. He died at 2.5 years without a definitive diagnosis. SCA2 gene analysis was never performed. Our case demonstrates considerable molecular instability and, surprisingly, it appears to have occurred twice in the same family. A number of infantile-onset SCA2 cases have been reported in the medical literature, but to the best of our knowledge, this is the first clinically reported case with an expansion significantly more than 200 CAG repeats. To our knowledge, renal involvement has not previously been reported in SCA2. SCA2 should be seriously considered in the differential diagnosis of infants with non-specific neurodevelopmental delay and a family history of cerebellar ataxia.

947W

Investigation of triplet repeat nucleotide in ATXN8OS Gene in one SCA8 suspected patient. *Z. Ghaderi Ardekani[1, 2], M. Houshmand[1, 3], O. Aryani[1], N. Chaparzadeh[2].* 1) Genetic Lab.,Special Medical Center, Tehran, Iran; 2) Depatment of Biology, Azarbaijan University of Tarbiat Moallem; 3) National Institute of Genetic Engineering and Biotechnology Depatment of Medical Biotechnology.

ABSTRACT Introduction: SCAs are progressive neurodegenerative disease that produced from three nucleotide repeat expansion in special region in specific Genes. In SCA8, CTG triplet repeats in untranslated region in ATXN8OS Gene on 13q21 choromosome, are increased above 80 units. Normal range has 15-50 repeats. Intermediate range are Alleles of questionable significance. Materials and Methods : Sample was one patient with clinical features similar to SCA symptoms that had refered to Special Medical Center. DNA was extracted from blood and ATXN8OS Gene was amplified, PCR product ran in polyacrylamide gel. by this formula, Number of repeats measured (N - 231)/3) that N is the PCR product length. Results: The patient was similar to his mother that had 55 repeats in ATXN8OS Gene in one allele and both of them had another allele in normal range, his father had two alleles in normal range. Discussion: By considaration of dominant inheritance manner in this disease, exist of one allele in intermediate range, can be justifier of seen clinical features. By attention to intermediate alleles in correlation to penetrance percentage, personal qualifications and some undefined parameters can or can't illustrate disease, therefore it can clarify that his mother despite of one allele in intermediate range due to uncomplete penetrance didn't show clinical features but the child by inheritance of this allele was patient.

948W

CHARGE SYNDROME; REPORT A NEW CASE. *E. Hernandez Gomez[1], L. Hernández Gómez[2], S.G. Juárez García[3], D.O. Gómez Torres[4].* 1) Biology, Facultad de Estudios Superiores Iztacala, Mexico, Mexico; 2) Instituto Nacional de Rehabilitación, Audiología. Universidad del Valle de México, Campus Coyoacan; 3) Instituto Nacional de Rehabilitación. Patología de Lenguaje; 4) Instituto Nacional de Rehabilitación.

CHARGE syndrome is a recognizable (genetic) pattern of birth defects which occurs in about one in every 9-10,000 births worldwide. It is an extremely complex syndrome, involving extensive medical and physical difficulties that differ from child to child. The vast majority of the time, there is no history of CHARGE syndrome or any other similar conditions in the family. Caused by a change (mutation) in a single gene, most often CHD7. In August, 2004, the first major gene for CHARGE syndrome was reported by a group of researchers in the Netherlands. Newborn with CHARGE syndrome are often born with life-threatening birth defects, including complex heart defects and breathing problems. They spend many months in the hospital and undergo many surgeries and other treatments. Most have hearing loss, vision loss, and balance problems which delay their development and communication. : coloboma of the eye, heart defects, atresia of the choanae, retardation of growth and/or development, genital and/or urinary abnormalities, and ear abnormalities and deafness. The clinical diagnosis is made using a combination of Major and Minor features. Major features are characteristics that are quite common in CHARGE syndrome but relatively rare in other conditions, and are, for the most part, diagnosable in the newborn period We present a new case: female Mexican child at 4 years old: product of the first gestation no consanguineous young parents; present circular umbilical cord in utero, with growth arrest and malnutrition. she. born at 42 weeks by cesarean section, low weight, course with cyanosis, the diagnosis of congenital hypothyroidism, membranous choanal atresia, and ventricular septal defect. Hearing loss was detected at one year of age, language delay. Used hearing aids. Physical examination: Female to conduct hearing, normal, language impairment. hypertelorism, facial asymmetry, ear hypoplasia helix bilaterally, tortuous ear canals, tympanic membranes opaque. Rhinoscopy was normal. High palate, uvula bifida. Audiometric test showed sensorineural severe hearing loss. Speech audiometric test showed sensorineural deficit. Timpanogram: curves As of Jerger bilateral Stapedial reflex absent bilaterally. ABR wave V to 100 dB bilateral, elongated latency, low amplitude waves. Psychological assessment: normal IQ. . Computed tomography of the temporal bones normal.

949W

OTOFACIOCERVICAL SYNDROME, IN A MEXICAN FAMILY. *L. Hernandez-Gomez[1], E. Hernández Gómez[3], S.G. Juárez García[2], D.O. Gómez Torres[4].* 1) Instituto Nacional de Rehabilitación Audiología. Universidad del Valle de México, Campus Coyoacan; 2) Instituto Nacional de Rehabilitación. Patología de Lenguaje; 3) Facultad de Estudios Superiores Iztacala. Biología; 4) Instituto Nacional de Rehabilitación.

Otofaciocervical syndrome may be caused by a contiguous gene deletion involving the EYA1 gene or by mutation in the EYA1 gene (Gene map locus: 8q13.3). The otologic features were conductive hearing loss and prominent auricles with large conchae and preauricular fistulas just in front of the helix. Distinguishing features were a long face with narrow nose, high arched palate, prominent and dysmorphic ears, long neck, sloping shoulders and clavicles, winged, low and laterally set scapulae, tetralogy of Fallot, and deafness secondary to cochlea malformation. The absence of preauricular tags, lacrimal duct stenosis, and renal malformations, and the presence of distinct facial and radiographic findings and markedly downward sloping shoulders in their patient led Dallapiccola and Mingarelli (1995) to suggest that the syndrome is a unique, clinically recognizable entity. We report a Mexican family with 3 affected members with hearing loss in 2 generations, In which father, his daughter and his son are affected. CASE 1: male patient 47-year-old, propositus father,hearing loss onset at 8-year-old, tinnitus at 44 year old. Physical exploration: male with auditory conduct hearing loss, voice and language with alterations. Ears: malformations of helix and antihelix, ducts, external auditory permeable, left with hypoplasia, tympanic membrane opaque with myringosclerosis. Cleft palate, micrognathia. CASE 2: female 17 year old, hearing loss onset at 15-year-old. Physical exploration: female with auditory conduct hearing loss, voice and language with alterations. Ears: Prominent auricles, malformations of helix, preauricular fistulas, ducts, external auditory permeable. Tympanic membrane opaque, long face, narrow nose, cleft palate, micrognathia, long neck, sloping shoulders, low-set clavicles. CASE 3 male 9 year old, left microtia, language impairment l hearing loss onset at 7-year-old Physical exploration: male with auditory conduct hearing loss, voice and language with alterations. Ears: Prominent auricles, malformations of helix and antihelix, preauricular and lateral cervical fistulas, ducts, external auditory permeable. Tympanic membrane normal, long face, eyelid left coloboma, narrow nose, cleft palate, micrognathia, long neck. At 3 patients were performed audiometry, speech audiometry, tympanometry and stapedial reflex, at 2 pediatric patients were also carried out nasal endoscopy, urinalysis, renal ultrasound, computed tomography of temporal bone.

950W

Velocardiofacial syndrome: report of case. *S. Juarez-Garcia[1], L. Hernández Gómez[2], E. Hernández Gómez[3], F. Castillo Lorca[4], D.O. Gómez Torres[5].* 1) INSTITUTO NACIONAL DE REHABILITACION. NEUROPSICOLOGIA; 2) INSTITUTO NACIONAL DE REHABILITACION. AUDIOLOGIA. UVM; 3) FACULTAD DE ESTUDIOS SUPERIORES IZTACALA; 4) S.S.A. TABASCO; 5) INSTITUTO NACIONAL DE REHABILITACION.

Velocardiofacial syndrome are caused by a 1.5 to 3.0-Mb hemizygous deletion of chromosome 22q11.2. The del22q11 syndrome is associated with a highly variable phenotype despite the un-iformity of the chromosomal deletion, characterized by the following frequent features: cleft palate, cardiac anomalies most commonly tetralogy of Fallot, and learning disabilities. Ophthalmologic abnormalities, including tortuous retinal vessels, small optic discs, or bilateral cataracts. In some case presence of progressive sensorineural deafness, whereas in del22q11 the hearing loss was mostly conductive due to palate deformities and velopharyngeal insufficiency. The subjects showed higher verbal than nonverbal IQ scores, assets in verbal memory, and deficits in the areas of attention, story memory, visuospatial memory, arithmetic performance relative to other areas of achievement, and psychosocial functioning. We present a caso: female mexican, child 3 year old, is the child of non consanguineous parents, pregnancy without adversities prenatal. She present tetralogy of Fallot, submucosal cleft palate, bilateral cataracts, hearing loss sensorineural and language disabilities. Normal IQ. ABR with V wave at 100dB. FISH with 22q deletion.

951W

Novel Locus Identified for Branchio-Oto-Renal Syndrome. *A. Sun, K. Taylor, N. Kramer, JM. Graham.* Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA.

Branchio-Oto-Renal (BOR) syndrome is an autosomal dominant disorder characterized by branchial arch anomalies, ear malformations, hearing loss, and renal abnormalities. It has a prevalence of 1 in 40,000 and accounts for 2% of children with profound hearing loss. To date, 3 genes have been identified to cause BOR syndrome. *EYA1* is implicated in 40% of cases, and *SIX1* and *SIX5* together account for approximately 5% of cases. The remaining 55% of individuals with BOR syndrome have an unknown etiology. The EYA1 protein is expressed in renal mesenchyme, cochlear and vestibular neuroepithelia, and other parts of the ear. EYA1 directly interacts with members of the SIX gene family as part of the Six-Eya-Dach regulatory network. Once EYA1 binds SIX1 or SIX5, the complex translocates into the nucleus where the phosphatase activity of EYA1 de-represses target genes. Mutations in *EYA1* can either result in haploinsufficiency of the protein or reduced phosphatase activity. Mutations in *SIX1* and *SIX5* decrease the ability of the proteins to bind EYA1 or DNA. Aberrant gene activation ultimately results in abnormal morphogenesis of the branchial arches, ears, and kidneys. We present a 5 generation family with BOR syndrome. Sequence analysis of *EYA1* did not identify any mutations. A SNP chromosomal microarray showed no significant genomic deletions or duplications. Linkage analysis demonstrated an area on the short arm of chromosome 8 with a LOD score of 3.02. This region does not overlap with any of the existing genes known to cause BOR syndrome and likely represents a novel locus for this disorder. Further association analysis will be done to narrow down the area of interest followed by high throughput sequencing to screen candidate genes. The causative gene may be another member of the SIX or EYA gene families or encode a cofactor that participates in this regulatory network. Once the gene has been identified, we plan to screen other families with BOR syndrome in whom a genetic change has yet to be found.

952W

Inherited systemic hyalinosis: a novel mutation and association with marked alpha-ketoglutaric aciduria. *F. Al Murshedi[1], S. Al Sinani[2], R. Abdwani[2].* 1) Dept of Genetics; 2) Dept of Child Health, Sultan Qaboos University Hospital, Muscat, Oman.

Inherited Systemic Hyalinosis (ISH) is an autosomal recessive condition characterized by progressive hyaline deposition in the papillary dermis and other tissues, namely gastrointestinal mucosa. The infantile form typically presents at birth with reduced spontaneous movements secondary to severe pain and progressive joint contractures. Progressive thickening of skin and appearance of hyperpigmented patches over bony prominences of the joints is a hallmark. Protein-losing enteropathy is the main life threatening complication. ANXR2 gene is the only gene known to be associated with ISH. We hereby report a classic case of infantile ISH. Sequencing of ANTXR2 gene revealed a novel homozygous 79 bp deletion of the entire exon 11 (c.867_945del). Work-up also revealed persistent and marked ketoglutaric aciduria. Association of ketoglutaric aciduria with ISH is not previously documented. A female baby was born at full term with birth weight of 3.3 kg. She was noticed at day one of life to have reduced spontaneous movements and pain with handling. Clinical findings at age of 8 months included tense shining skin marked over bony prominences, joint contractures affecting small joints of hands and knees with overlying skin thickening, hyperpigmentation and nodules. Development, other than gross-motor, was age-appropriate. Other features included gum hypertrophy and perianal tags. Parents are first-cousins and three siblings (2 males, one female), undiagnosed, died with a similar presentation in infancy. Work-up revealed osteopenia and bilateral femur green-stick fractures on skeletal X rays. Biochemically, alkaline phosphatase and parathyroid hormone were elevated with low phosphate consistent with rickets. Urine organic acids done at age of four, six and eight months respectively revealed large increase of alpha-ketoglutaric acid (>500 umol/mmol creatinine). Baby had normal cognitive development and normal plasma and urine lactate excluding the possibility of concurrent alpha-ketoglutarate dehydrogenase deficiency. Diarrhea started at around the age of 6 months, progressively worsened despite dietary modifications and a trial of Octreotide. Patient passed away at age of 15 months with intractable diarrhea. Although mild ketoglutaric aciduria is a common non-specific finding, this consistently profound elevation of the Krebs cycle intermediate may reflect disturbance in mitochondrial function as a contributing factor into the pathogenesis of ISH.

953W

A Report of Three Patients with MMP2 Associated Hereditary Osteolysis. *M.S. Aglan[1, 2], S.A Temtamy[1, 3], S. Ismail[1], A.M. Ashour[1], L.A. Hosni[1], T.H. El-Badry[4], E.H.A. Aboul-Ezz[4, 2], K. Amr[5], E. Fateen[6, 2], T. Maguire[7], K. Ungerer[7], A. Zankl[8].* 1) Clinical Genetics Dept, National Research Centre(NRC), Cairo, Egypt; 2) Board member of the National Society of Human Genetics (NSHG), NRC, Egypt; 3) Head of NSHG, NRC, Egypt; 4) Orodental Genetics Dept, NRC, Egypt; 5) Medical Molecular Genetics Dept, NRC, Egypt; 6) Biochemical Genetics Dept, NRC, Egypt; 7) Queensland Health Pathology Services, Brisbane, Australia; 8) UQ Centre for Clinical Research, The University of Queensland, Brisbane, Australia.

Osteolysis disorders are a group of inherited diseases characterized by rheumatoid like destruction and resorption of affected small joints with associated swelling and pain. Historically, the existence of three autosomal recessive osteolysis syndromes was suspected: Torg syndrome, Winchester syndrome and Nodulosis-Arthropathy-Osteolysis syndrome or Multicentric Osteolysis, Nodulosis and arthropathy. As they are all caused by MMP2 mutations, they were grouped under the nomenclature of Torg-Winchester syndrome (TWS). The current study reports three new patients from two unrelated Egyptian families with TWS. Clinical examination, radiological, biochemical and molecular studies were conducted. Clinical and radiological findings suggested the diagnosis of TWS. The three affected patients were homozygous for novel missense MMP2 gene mutations which confirmed the diagnosis. In both our families, based on the amino acid position, the mutation seems to be far from the catalytic domain with subcutaneous nodules as a prominent clinical feature. The patients reported here further define the clinical spectrum of TWS. They are the first to be reported from Egypt thus, supporting the pan ethnic nature of the disease.

954W

Chronic Tibial Nonunion in a Rothmund-Thomson Syndrome Patient. *A.M. Carlson[1], S. Kirmani[2], K.B. Thomas[3], N.M. Lindor[2].* 1) Mayo Medical School, Mayo Clinic College of Medicine, Rochester, MN; 2) Department of Medical Genetics, Mayo Clinic, Rochester, MN; 3) Department of Radiology, Mayo Clinic, Rochester, MN.

Background: Rothmund-Thomson Syndrome (RTS) is a rare disorder caused by biallelic mutations in RECQL4, a DNA helicase involved in chromosomal stability and DNA excision repair. Patients with RTS present with a characteristic phenotype of diffuse poikilodermatous rash, sparse hair, skeletal abnormalities, short stature, photosensitivity, and a predilection to developing osteosarcoma and cutaneous malignancies. Known skeletal abnormalities include osteopenia, radial ray defects, and congenital dislocations. **Purpose:** To present an adult with RTS who had chronic nonunion of the proximal tibias bilaterally due to minor trauma and later developed generalized lower extremity osteopenia with normal calcium homeostasis and calcitriol levels. **Methods:** Medical records from July 7, 1998 to January 27, 2011 were reviewed and abstracted; radiographs were correlated with clinical course. A comprehensive literature search was performed to identify previously reported skeletal abnormalities and rates of occurrence. **Results:** Despite 400 U of vitamin D daily and calcium supplementation, the patient's osteopenia progressed and nonunion of the proximal tibias bilaterally continued without resolution over nine years of observation. Additional fractures such as an ununited intra-articular fracture of the proximal right ulna and stress fractures in multiple metatarsals were also noted. Bony abnormalities are quite common in RTS patients, as Mehollin-Ray et al. found in 2008 that 75% of their cohort had at least one bony abnormality. However, this literature review showed chronic bony nonunion was a novel and unexplained finding in RTS. **Conclusions:** As treatments for osteosarcoma improve and RTS patients survive into later decades of adulthood, osteopenia and congenital malformations present a source of morbidity. Treatment options for osteopenia are limited by RTS patient's predilection for osteosarcoma. Further work is needed to determine the mechanism for osteopenia and nonunion of fractures in this patient and whether or not this is a common event in individuals with RTS.

955W

De-novo microdeletion of chromosome region 1p36.12: unmasking a rare autosomal recessive form of spondyloepimetaphyseal dysplasia. *L. Dupuis[1], A. Guerin[1], E. Goh[1], A. Chaudhry[1], M. Shago[2], R. Zeller[3], D.J. Stavropoulos[2], R. Mendoza-Londono[1].* 1) Division of Clinical & Metabolic Genetics, Hospital for Sick Children, Toronto, Canada; 2) Department of Paediatric Laboratory Medicine, Hospital for Sick Children, Toronto, Canada; 3) Department of Orthopedic Surgery, Hospital for Sick Children, Toronto, Canada.

Classically, microdeletions are not associated with autosomal recessive phenotypes. However, here we report clinical, radiologic and molecular cytogenetic findings in a female of Portuguese descent with a de-novo microdeletion of chromosome region 1p36.12 involving HSPG2. The patient was born at 38 weeks gestation with a birth weight of 2.6 kg (5-10th centile) and length of 48 cm (25th centile). She was diagnosed postnatally with a form of spondyloepimetaphyseal dysplasia (SEMD). She developed progressive limitation of the range of motion of multiple joints and became wheelchair-bound by 4 years of age. At age 14, she was significantly short at 122 cm (50th centile for a 7 year old). Her bone mineral density was low (Z score -3.8). Her intellectual development has been normal. Skeletal survey at 12 years of age showed irregularities of the endplates of the cervical spine with fusion of the bodies of C2 and C3 and kyphoscoliosis of the thoracolumbar spine. She had severe deformities of the pelvis with small iliac wings and shortening of the pelvic bones bilaterally. The femoral heads were irregular and showed sclerosis. In addition, there was broadening of the femoral neck. The epiphyses of the distal femur and tibia were enlarged and there was generalized shortening of the long bones. Her elbow joints were dislocated bilaterally and there was radio-ulnar dislocation. Array CGH identified a deletion of chromosome region 1p36.12 with an estimated size of 1.357 Mb. This deletion results in the loss of 17 UCSC RefSeq genes, which include OMIM gene HSPG2. Mutation analysis of HSPG2 revealed a base pair deletion in the other allele, c. 9326delA. This mutation has been previously described as a pathogenic mutation. The mutation was identified in a family of Brazilian origin making it likely that it arose in Portugal. Mutations in HSPG2 have been implicated in a rare autosomal recessive form of SEMD: Schwartz-Jampel syndrome type 1 (SJ) and Silverman-Handmaker type of dyssegmental dysplasia (SHDD). Although this patient was deficient in both copies of HSPG2, her skeletal findings were different from those typically seen in SJ or SHDD, thus expanding the phenotype of HSPG-related disorders. This case highlights the importance of considering the unmasking of a recessive phenotype when a microdeletion is identified.

956W

Vitamin D Status & Decreased Muscle Function in Children with Neurofibromatosis Type 1. *S.M. Huson[1], C.W. Hockett[1,2,6], J. Eelloo[1], S. Roberts[3], J. Berry[4], C. Chaloner[5], Z. Mughal[6].* 1) Neurofibromatosis Centre, Genetic Medicine, Manchester Biomedical Research Centre, Manchester Academic Health Sciences Centre, University of Manchester and Central Manchester University Hospitals Foundation Trust, M13 9WL UK; 2) Department of Biology, South Dakota State University, Brookings, SD; 3) Biostatistics Group, School of Epidemiology & Health Sciences, University of Manchester; 4) Vitamin D Research Group, Medicine, Manchester Royal Infirmary; 5) Department of Clinical Biochemistry,, Royal Manchester Children's Hospital, Manchester, UK; 6) Department of Paediatric Endocrinology, Royal Manchester Children's Hospital, Manchester, UK.

Background - Recent studies have reported significantly lower mean serum 25-hydroxyvitamin D (25OHD) concentration in adults with NF1. Vitamin D deficiency not only has a negative effect on bone, but is also important in muscle function. One of the main factors influencing bone formation and size is the mechanical load that is placed on bone. If there is decreased muscle function, loading forces on bone may be reduced leading to increased bone resorption and low BMD. Therefore, muscles play a key role in the sculpting of bone size and maintenance of bone integrity. Previous studies have shown that NF1 patients have slender long bones, which may be due to decreased muscle strength. Objective - The aim of this cross-sectional pilot study was to assess the vitamin D status and muscle function in children with NF1 compared with unaffected siblings. Methods - We identified NF1 children between 5 to 18 years of age and who had at least one unaffected sibling from the NF1 clinic records. Along with 25OHD serum concentration, we also measured serum concentrations of calcium, inorganic phosphate alkaline phosphatase activity, parathyroid hormone and 1,25-dihydroxyvitamin D concentrations in the study subjects. The Leonardo Mechanography Ground Reaction Force Platform was used to measure peak jumping power, peak jumping force and peak jump height. Results - The mean serum 25OHD concentration between NF1 children and their non-affected sibling was not different. There was no significant relationship between vitamin D status and muscle function. After controlling for weight and height, the least squares means for non-NF1 and NF1 children for peak jumping power was 1.64 (1.53, 1.75), 1.40 (1.29, 1.51) respectively, force 1.05 (0.99,1.10), 0.91 (0.85,0.96) respectively, and height 0.35 (0.27, 0.44), 0.31 (0.22, 0.39). From the least squares means, we found that NF1 children had significantly lower maximum jump power (p=0.004) and force (p=0.001) than non-NF1 children. Conclusions - We did not find low levels of vitamin D in children with NF1. Lammert et al (2006) showed a correlation between vitamin D and neurofibroma number, as significant numbers of these lesions are only present in adults with NF1 this is one possible explanation.NF1 children have impaired jumping power and jumping force, when compared to a child not affected by NF1.Decreased muscle function may contribute to the osseous abnormalities observed in NF1 children.

957W

Association study of common polymorphisms in human lysyl oxidase genes with adolescent idiopathic scoliosis. *T.L. McGregor[1,8], C.A. Gurnett[2,3], M.B. Dobbs[3,4], C.A. Wise[5,6], J.A. Morcuende[7], T.M. Morgan[1,8], R. Menon[9,10], L.J. Muglia[1,11].* 1) Dept of Pediatrics, Vanderbilt University Medical Center, Nashville, TN; 2) Depts of Neurology and Pediatrics, Washington University School of Medicine, St. Louis, MO; 3) Dept of Orthopedic Surg, Washington University School of Medicine, St. Louis, MO; 4) Saint Louis Shriners Hospital for Children, Saint Louis, MO; 5) Sarah M. and Charles E. Seay Center for Musculoskeletal Research, Texas Scottish Rite Hospital for Children, Dallas, TX; 6) Dept of Orthopaedic Surgery and McDermott Center for Human Growth and Development, University of Texas Southwestern Medical Center, Dallas, TX; 7) Dept of Orthopedic Surgery and Rehabilitation, University of Iowa, Iowa City, IA; 8) Center for Human Genetics Research, Vanderbilt University School of Medicine, Nashville, TN; 9) Perinatal Research Center, Nashville, TN; 10) Dept of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA; 11) Dept of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, and Vanderbilt Kennedy Center for Human Development, Vanderbilt University, Nashville, TN.

Background: Although adolescent idiopathic scoliosis affects approximately 3% of adolescents, the genetic contributions have proven difficult to identify. Work in model organisms, including zebrafish, chickens, and mice, has implicated the lysyl oxidase family of enzymes in the development of scoliosis. We hypothesized that common polymorphisms in the five human lysyl oxidase genes (*LOX, LOXL1, LOXL2, LOXL3,* and *LOXL4*) may be associated with the phenotype of adolescent idiopathic scoliosis. Methods: This was a case-control genetic association study. A total of 112 coding and tag SNPs in *LOX, LOXL1, LOXL2, LOXL3*, and *LOXL4* were genotyped in a discovery cohort of 138 cases and 411 population controls. Genotypes were tested for association with adolescent idiopathic scoliosis by logistic regression with a two degree of freedom genotypic model and gender as a covariate. Fourteen SNPs with $p < 0.1$ in the discovery phase were genotyped in an independent replication cohort of 400 cases and 506 controls. Results: No significant associations were found between coding or tag SNPs in *LOX, LOXL1, LOXL2, LOXL3,* and *LOXL4* and the phenotype of adolescent idiopathic scoliosis. Conclusions: Despite suggestive evidence in model organisms, common variants and known coding SNPs in the five human lysyl oxidase genes do not confer increased genotypic risk for adolescent idiopathic scoliosis. The above methodology does not address rare variants or individually private mutations in these genes, and future research may focus on this area.

958W

Expanding the Phenotype of the Novel Ehlers-Danlos caused by mutations in the *CHST14* gene. *R. Mendoza-Londono[1,2], D. Chitayat[1], W. Kahr[1], A. Hinek[2], S. Blaser[1], L. Dupuis[1,2], E. Goh[1], A. Howard[2], L. Mittaz[3], A. Superti-Furga[3], S. Unger[3], G. Nishimura[4], L. Bonafe[3].* 1) Div Clinical & Metabolic Gen, Hosp Sick Children and University of Toronto, Toronto, ON, Canada; 2) The Bone Health Centre, The Hospital for Sick Children, Toronto, ON, Canada; 3) Division of Molecular Pediatrics, CHUV, Laussanne, Switzerland; 4) Department of Radiology, Tokya Metropolitan Kiyose Children's Hospital, Kiyose, Japan.

Ehlers-Danlos Syndrome (EDS) is a heterogenous group of disorders characterized by varying degrees of connective tissue hyperextensibility. In 2010 a new type of EDS was described in patients of Japanese and of Turkish descent presenting with craniofacial characteristics, contractures and joint and skin laxity. All patients had mutations in the *CHST14* gene coding for the enzyme dermatan 4-O-sulfotransferase. We present the detailed clinical findings in 2 sisters of Afghani descent born to consanguineous parents and 1 male patient of Somali descent. All patients presented with multiple contractures, progressive joint and skin laxity and hemorrhagic diathesis following minor trauma. Prenatal evaluation of patient 2 identified possible Dandy-Walker variant, prominent amnion-chorionic separation, bilateral club feet and clenched hands.In addition to the findings above patient 3 has significant ocular defects and microphthalmia. Platelet aggregometry and electron microscopy revealed normal function and ultrastructure. Cultured fibroblasts produced normal amounts of fibronectin and elastin but demonstrated lack of collagen III production, lack of extracellular deposition of collagen I fibers and a peculiar intracellular retention of Collagen I. All patients were found to be homozygous for novel missense mutations in the *CHST14* gene. The identification of three additional patients with this disorder raises awareness of the pan-ethnicity of the condition and expands our knowledge regarding the clinical manifestations. Our studies show that the condition has to be considered in cases presenting prenatally with clenched fists and amnion-chorion separation, that the facial features are characteristic regardless of ethnicity and the bleeding diathesis in these patients is not the result of thrombocytopathy. We also expand the knowledge regarding the collagen abnormalities in this condition.

959W

Cherubism: clinical, radiological and familial aspects in a large French family. *G. Morin[1], S. Demachy[2], E. Cadet[3], B. Demeer[1], S. Testelin[2], S. De Broca[2], J. Rochette[3], B. Devauchelle[2], M. Mathieu[1].* 1) Clinical Genetic Unit, Amiens University Hospital, Amiens, France; 2) Maxillofacial Surgery and Stomatology Service, Amiens University Hospital, France; 3) Molecular Biology Laboratory, Amiens University Hospital, France.

Cherubism is a rare genetic osseous dysplasia responsible of oligodontia, giant cysts of maxillaries, and deformations of the lower part of the face. The mode of inheritance is autosomal dominant and the expressivity very variable. The maxillary involvement is generally asymmetric and the teeth disorganization often spectacular, associated with malocclusion, gum hypertrophy, and mastication problems. Some extra-maxillary involvements are possible: exophthalmia, ptosis, retraction of the lower eyelids, cervical lymphadenopathies, skin's café-au-lait spots, other skeletal lesions. The clinical features generally appear during the first five years of life and progress until the late teens. However, a regression of the cysts is often observed during the third decade. The genetic nature of the disease was quickly suggested, and a linkage to the chromosome 4p16.3 region was discovered in 1999. Mutations of the SH3BP2 gene were reported in 2001. They all concern the exon 9 and modify only 3 amino acids located 31 to 36 amino acids upstream of the SH2 domain and 205 to 210 amino acids downstream of the SH3-binding domain. This gene plays a critical role in regulation of myeloid cell responses and mutations are probably responsible of a gain of function. We report a large family affected by cherubism on at least 3 generations with at least 8 affected persons including 2 monozygotic twins. A mutation was found in the exon 9 of SH3BP2 gene (c.1253C>A / p.418H). This mutation was already reported in affected patients (Ueki et al 2001). We describe the clinical, radiological and familial aspects of the disease in this large French family. The outcome of the affected family members is also considered.

960W

Ischiospinal dysostosis results from mutations in BMPER and is allelic with diaphanospondylodysostosis. *L. Nevarez[1], S. Nampoothirii[2], O. Kim[3], A. Superti-Furga[4], D.H. Cohn[1].* 1) MCDB, UCLA, Los Angeles, CA; 2) Amrita Institute of Medical Sciences and Research Centre, Cochin, Kerala, India; 3) Department of Radiology, Ajou University Hospital, Suwon, Korea; 4) Department of Pediatrics, Univ. of Lausanne, Switzerland.

Ischiospinal dysostosis (ISD) is a non-lethal skeletal dysplasia named for the severe hypoplasia of the vertebral bodies and the hypoplastic ischia that characterize the disorder. Delayed ossification and hypoplasia of the vertebral bodies can lead to a short trunk phenotype and to significant kyphoscoliosis. In some patients, ossification defects of the ribs, termed rib gaps, have also been observed. In addition to the dysostosis, defects in the kidneys, ranging from a single cystic kidney to enlarged kidneys with nephroblastomatosis, have been seen in several cases. The combination of defects in vertebral segmentation and nephroblastomatosis is also seen in diaphanospondylodysostosis (DSD), a perinatal lethal skeletal dysplasia recently found to result from recessively-inherited, inactivating mutations in BMPER. BMPER encodes the BMP-binding endothelial cell precursor-derived regulator, a pro-BMP signaling molecule, implying that defective BMP signaling underlies the phenotype. To determine whether DSD and ISD are allelic, mutation analysis of BMPER was carried out in two ISD cases. One of the affected individuals was a compound heterozygote for two stop codon mutations, similar to the types of mutations observed in DSD. The newborn phenotype of this individual resembled DSD but over time evolved into the characteristic ISD phenotype, indicating that factors other than the BMPER genotype may determine clinical outcome. The second affected individual studied, who was the offspring of consanguineous parents, was homozygous for a F375S missense mutation in the gene. The phenotype was milder, with ossification defects in the lumbar vertebrae and mildly hypoplastic ischia, but there was still significant shortening of the trunk. The mutation altered a highly evolutionarily conserved residue within the von Willebrand D domain of BMPER, which appears to tether the molecule to the cell surface to facilitate its signaling role. These findings thus demonstrate that DSD and ISD are allelic recessive disorders.

961W

PARRY ROMBER SYNDROME. MULTIDISCIPLINARY EVALUATION OF SIX PATIENTS BY ORTHODONTICS AND GENETICS. HOSPITAL PARA EL NINO POBLANO, MEXICO. *S. Ochoa[1], J. Aparicio[2,3], E. Huitzil[3].* 1) Orthodontics; 2) Genetics, Hospital para el Nino Poblano, Puebla, Puebla, Mexico; 3) Estomatology, Benemerita Universidad Autonoma de Puebla.

Hemifacial atrphy or Romberg disease, Parry Romberg syndrome and facial trophoneurosis are a rare clinical entity. It is characterized due to facial lipid tissue, skin, bones and cartilaginosous tissue atrophy. The early diagnosis in pediatrics is sometime difficult due to be a rare event. Actual orthodontics is a medical discipline that must be treated by a multidisciplinary medical team in estomatology and pediatrics. Some of the dentofacial malocclusions and deformities have a familiar distribution that makes necessary that ortodonthics and genetics work together including lift and palate deformities. Pediatric cases are analyzed in this study, where hemifacial hemiatrophy are observed more frequently in female than males, and on the left facial side according to the literature. Six pediatrics cases are studied where four (Males) and two (females) were observed. 3 males and a one female patient had a left facial side affected and two male and one female patient on the right side. All of the patients had a multidisciplinary attention both at the departments of orthodontics and genetics.

962W

Skeletal anomalies and severe language disorder with submicroscopic deletion in 12q13 including HOXC cluster. *N. Okamoto[1], K. Shimojima[2], T. Yamamoto[2].* 1) Dept Medical Genetics, Osaka Med Ctr/Res Inst, Osaka, Japan; 2) Tokyo Women's Medical University Institute for Integrated Medical Sciences, Tokyo, Japan.

HOX genes have an important role in the initial formation of the body. We report a patient with severe kyphoscoliosis and digital abnormalities with a submicroscopic deletion of 12q13. He also exhibited cardiac anomalies and a severe delay in language development. The deletion was detected by array-CGH and confirmed by FISH. The patient was haploinsufficient for the HOXC gene cluster. No human genetic disorders due to HOXC abnormalities are yet known. A haploinsufficiency of the HOXC gene cluster may result in severe kyphoscoliosis and digital abnormalities. Radiologic features of the fingers had some similarities with those for multiple synostosis syndrome. Deformation of the spine seen may be partially explained by a haploinsufficiency HOXC-4. The flexion contracture and other digital abnormalities in our patient may have some association with a HOXC-8 haploinsufficiency. A haploinsufficiency of other genes may contribute to the cause of language disorder.

963W

Hereditary Disorders of Connective Tissue in Patients with Intracranial Hypotension Syndrome. *E. Reinstein[1], W. Schievink[2], M. Pariani[1], D. Rimoin[1].* 1) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Dept. of Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, CA.

Introduction: Intracranial hypotension syndrome (ICH) is a rare and understudied condition with an estimated prevalence of 1:50,000. It is an under diagnosed and frequently misdiagnosed condition, even by experienced clinicians. Consequently, the diagnosis and treatment are often significantly delayed. This has major consequences on the quality of life of the patients and their families, as well as on the medical system in terms of excessive financial and time expense, unnecessary investigations, incorrect therapies, and preventable deterioration of the disease state. The clinical presentation of patients with ICH is a postural headache that worsens in the upright position. The most common cause of ICH is spontaneous cerebrospinal fluid leak and an underlying weakness of the spinal dura is generally suspected. Hereditary disorders of connective tissue comprise a clinically and genetically heterogeneous group of disorders, characterized by generalized fragility of connective tissues with an incidence of 1:5000. Several case reports and small studies suggested a link between hereditary disorders of connective tissue and spontaneous cerebrospinal fluid leaks but it is not clear what the exact prevalence is. Objective: To determine the prevalence of hereditary disorders of connective tissue among patients with ICH in a prospective study using a large cohort of patients. Methods: We enrolled a consecutive group of 45 patients presenting with ICH. All patients have been carefully examined for clinical features of hereditary disorders of connective tissue, and based on findings patients underwent genetic testing. Ancillary diagnostic testing included echocardiography and histopathological examinations of skin and dura biopsies in selected patients. Results: Preliminary results based on 45 patients indicate that hereditary disorders of connective tissue, including Marfan syndrome, Ehlers-Danlos syndrome and other unclassified hereditary disorders of connective tissue are significantly more prevalent in patients with ICH compare to the general population (20% as opposed to .0002%; P<.001). Conclusion: Hereditary disorders of connective tissue are common among patients with ICH syndrome, and cerebrospinal fluid leak may be the first clinical presentation of the disorder.

964W

The Singleton-Merten Syndrome Calcification Paradox is Potentially Associated with Decreased *TGFBRII* Expression. *F. Rutsch[1], Y. Nitschke[1], I. Buers[1], O. Mamaeva[2], J. Dong[2], C. Müller[1], HG. Kehl[3], J. Kleinheinz[4], P. Barth[5], M. Daudon[6], D. Bazin[7], R. Hennekam[8], M. MacDougall[2].* 1) Dept. of General Pediatrics, University Children's Hospital, Muenster, Germany; 2) Institute of Oral Health Research, School of Dentistry, University of Alabama, Birmingham, AL, USA; 3) Dept. of Pediatric Cardiology, Muenster University Children's Hospital, Muenster, Germany; 4) Dept. of Cranio-Maxillofacial Surgery, Muenster University Children's Hospital, Muenster, Germany; 5) Institute of Pathology, Muenster University Hospital, Muenster, Germany; 6) Service de Biochimie A, AP-HP, Hopital Necker, Paris, France; 7) Laboratoire de Physique des Solides, Universite Paris Sud, Orsay, France; 8) Dept. of Pediatrics, Academic Medical Center, University of Amsterdam, The Netherlands.

Ectopic artery calcification is frequently associated with decreased bone mineral density or disturbed bone-turnover. This contradictory association, referred to as the calcification paradox might be due to opposite regulation of the OPG-RANK-RANKL system in bone and vasculature by TGF-beta. Extensive calcification of the ascending aorta and cardiac valves and early loss of rootless secondary teeth is characteristic of Singleton-Merten syndrome (SMS), a rare inborn disorder of autosomal dominant inheritance. We report on the first three European patients with SMS (two siblings of both sexes and one unrelated girl), who presented with delayed eruption of their deciduous teeth, loss of secondary dentition during adolescence, widened medullary cavities of the phalanges and calcifications of the ascending aorta and heart valves, requiring cardiac valve replacement in two of the patients before 25 years of age. Explanted calcified cardiac valves from one patient were subjected to HE staining, FTIR spectroscopy and scanning electron microscopy. Primary dental cells (Pulp and enamel organ epithelial, EOE) from a Singleton-Merten tooth were established and analyzed for mineralization potential by in situ alkaline phosphatase histochemistry and XO staining for calcium deposition. Quantitative RT-PCR and gene array analysis was performed to compare gene expression profiles to normal age- and sex-matched controls. Histologically the cardiac valves showed an effacement of their normal structure by extensive fibrosis of all layers and amorphous calcifications without significant inflammation. The calcifications contained mainly hydroxyapatite crystals. Dental pulp cells showed decreased mineralization potential under osteogenic media conditions. Of 72 genes of an osteogenesis array panel, 40 genes (47.6%) were down-regulated in the EOE cells while 39 (46.2%) were down regulated in pulp cells. TGFBRII receptor was dramatically down-regulated in both cell types (-4,627 and -785, respectively). Singleton-Merten syndrome is a model disease for the calcification paradox. Decreased TGF-beta signaling is potentially a good candidate for the driving force of decreased bone and tooth mineralization and increased arterial calcification in this syndrome.

965W

Crommelin-type symmetrical tetramelic reduction deformity; Report of a case with review of prenatal and postnatal findings, differential diagnosis and etiology. *S. Sawyer[1], O. Caluseriu[1], J. Kohlhase[2], A.M. Innes[1].* 1) Department of Medical Genetics, Alberta Children's Hospital, Calgary, Alberta, Canada; 2) Institute for Human Genetics and Anthropology, University of Freiburg, Freiburg, Germany.

We report on a 3 year old girl with upper limb amelia and lower limb phocomelia. Her features are in keeping with the Crommelin-type symmetrical tetramelic reduction syndrome[1]. This case report contributes to our knowledge of the long term prognosis for this rare condition. The limb deformities were first noted on a 22 week fetal ultrasound. Post-natal X-rays confirmed complete absence of the arms with normal scapulae and clavicles. In the lower limbs the acetabulae were poorly formed and femurs were absent bilaterally. There was a single bone bilaterally in the legs and 4 rays in the feet with soft tissue syndactyly of the lateral two rays. X-rays a year later suggested a femoral ossification centre on the right lower limb, and an unusual appearance of the calcaneus and talus bilaterally. There were no other abnormalities on fetal ultrasound or fetal echocardiogram. There was no exposure to Thalidomide during pregnancy. Amniocentesis confirmed a normal karyotype without evidence of centromeric `puffing'. At age 3 she has normal cognitive development and despite her striking limb anomalies is currently learning to walk. Genetic investigations, including karyotype and array CGH, were normal. As WNT3 and WNT7A have been implicated in syndromes with limb malformations [2] they were both sequenced in our patient. No point mutations or deletions were found. The differential diagnosis for this condition includes Fibula-Femur-Ulna complex, however the striking symmetry in Crommelin-type tetramelic reduction appears to set this condition apart. To date there has been no underlying genetic etiology described that causes Crommelin-type symmetical tetramelic reduction. Further cases are being identified in preparation for possible next generation sequencing studies. References 1. Harewood, L., et al., `Crommelin-type' symmetrical tetramelic reduction deformity: a new case and breakpoint mapping of a reported case with de-novo t(2;12)(p25.1;q23.3). Clinical dysmorphology, 2010. 19(1): p. 5-13. 2. Woods, C.G., et al., Mutations in WNT7A cause a range of limb malformations, including Fuhrmann syndrome and Al-Awadi/Raas-Rothschild/Schinzel phocomelia syndrome. American journal of human genetics, 2006. 79(2): p. 402-8.

966W

Head and Neck Pain in EDS: Significant Co-Morbidity. *B. Tinkle.* Div Human Gen, Cincinnati Children's Hosp, Cincinnati, OH.

Ehlers Danlos Syndrome (EDS) is a group of heritable connective tissue disorders characterized largely by joint laxity and skin/soft tissue fragility. While chronic joint pain or even widespread pain are features of EDS (Sacheti, 1997), it is underappreciated that the craniocervical junction is one of the most mobile joints and also susceptible to joint instability and pain. A significant portion of EDS patients complain of chronic or recurrent head pain. Head pain could have several sources from headache, sinus pain, temporomandibular dysfunction (TMD; including myofascial pain), and neck pain. Chronic headaches are estimated to affect about half of EDS patients (Sacheti 1997; Jacome, 1999; Castori, 2010) with majority suffering from migraine (Bendik, 2011). Estimates for the prevalence of neck pain in EDS range from 37% to 67% (Hudson, 1995; Sacheti, 1997). The Brief Pain Inventory results show that 67% of 118 EDS patients experience head and neck pain of some kind (our data). Fifty percent of the patients complained of neck pain, 19.5% complained of posterior cranial pain, and 33.1% complained of facial pain. TMD can also be associated with face pain and headache. It was seen in 67% (20/30; our data) of EDS patients which was comparable to previous reports (Castori, 2010). Further, the mean TMD Disability Index of 48 EDS patients demonstrated moderate disability (our data). Of 52 EDS patients, the mean Northwick Park Neck Pain score was 36.9% revealing a moderate disability; however, several patients had no pain but 17 had severe and 10 had crippling disability. Similarly, the Neck Pain Disability Index of another group of 30 EDS patients had a group mean of 18.8% consistent with moderate disability with the clear majority of patients (16/30) also scoring in this category. Common symptoms that these patients complained of in clinic included: numbness and tingling in the upper extremities; frequent spells of dizziness; nausea; fatigue; muscle spasms; tinnitus; jaw “popping”, “clicking”, or locking; sleep difficulty; history of dropping things; palpitations; unusual visual impairment (e.g. blurriness, double vision, or tunnel vision); and difficulty swallowing. These symptoms, along with the head and neck pain, significantly decreased the quality of life of these patients. Recognition and acknowledgment of the patient's symptoms will focus management and have a better chance of improving the quality of life of those suffering with EDS.

967W

Candidate gene analysis of nonsyndromic sagittal craniosynostosis.

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Craniosynostosis, the premature fusion of one or more cranial sutures, occurs in approximately 1 in 2,500 live births. Among various forms, midline sagittal nonsyndromic craniosynostosis represents the most prevalent type. In syndromic craniosynostosis, causative mutations are known on fibroblast growth factor receptors (FGFRs), TWIST1, RAB23, and other genes. However, the etiology of nonsyndromic craniosynostosis is largely unknown. We used data from an ongoing, population-based case-control study in Iowa and New York State to identify novel candidate genes for nonsyndromic sagittal craniosynostosis. Diagnosis was confirmed by radiographic images. Family history data are being collected from mothers, and saliva samples are being collected from the index child and birth parents in case and unaffected control families. In our initial analyses, we evaluated 96 nonsyndromic sagittal synostosis cases and performed extensive candidate gene analysis by direct sequencing. Two isoforms of FGFR1, FGFR2, TWIST1 and RAB23 were selected based on their expression patterns, animal models, and/or roles in human craniosynostosis syndromes such as Carpenter syndrome, which is associated with midline craniosynostosis and RAB23 mutations. We identified three novel variants among different patients. In case 1, we found a frameshift variant in exon 6 of FGFR1 isoform 6 (c.732_733insG) which was predicted to abolish the entire immunoglobin III domain, including the ligand binding region. In case 2, a variant in TWIST1 exon 1 (c.439C>G) was observed at the highly conserved loop domain and may affect the DNA binding ability; the mother of the case reported to have had jaw surgery and was also confirmed to harbor the same variant. In case 3, we identified a variant in exon 6 in RAB23 (c.546A>C) that led to the amino acid substitution from glutamic acid to aspartic acid and was inherited from the mother. These variants detected in our study were unique and did not occur in 116 alleles from healthy control children from the same population or in more than 200 alleles previously screened in controls or in the NCBI dbSNP and CHIP Bioinformatics databases. Our data suggest that mutations in these candidate genes may contribute to nonsyndromic sagittal craniosynostosis, although they would account for a small proportion of total cases, and add to the perception of craniosynostosis as a complex developmental anomaly under potential polygenic control.

968W

Rasmussen encephalitis associated with other autoimmune disorders.

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Background: Rasmussen's encephalitis (RE) is a rare chronic inflammatory disease characterized by intractable focal epilepsy, often epilepsia partialis continua, progressing to hemiparesis, cognitive impairment, and unilateral cerebral atrophy. The etiology of RE remains unknown. We have previously reported the occurrence of two rare autoimmune disorders, Behcet's disease and RE, in a father and son respectively, and suggested that they might be genetically susceptible to developing autoimmune conditions which have been precipitated by separate environmental triggers. Purpose: To report four individuals with a dual diagnosis of RE and other autoimmune disorders (AID), and to discuss the possible mechanisms of this association. Results: The diagnosis of RE in all four patients was based on previously reported criteria, and was pathologically confirmed in patients 2, 3 and 4. In family 1, a patient with RE onset at 2y 8mo developed Hashimoto's thyroiditis at 15 years; he also had a brother with Type 1 (autoimmune) diabetes mellitus. In family 2, the patient had RE onset at 3y 9mo, and developed ulcerative colitis at 10y 9mo. In family 3, the patient had RE at the age of 6 y, and developed Crohn's disease at age 14y. In family 4, the patient had RE onset at 14y and onset of systemic lupus erythematosus at 31y. In these four families, the second AID appeared a number of years after the onset of RE, with a mean interval of 11 years (median 10 years). Discussion: We suggest that the occurrence of a second autoimmune disease in RE patients may be higher than by chance; however, the true frequency and relative risk are unknown. The delay between the appearance of RE and the second autoimmune disease might seem long, but in fact the mean age of onset of these other AID is younger than average. Although the role of the immune system in RE, causing or merely reacting to the disease, is still unclear, our observations suggest that there may be a common predisposing factor for the development of RE and other AID. One hypothesis could be a common underlying genetic susceptibility to developing autoimmune conditions; these may then be precipitated by exposure to two different hits, e.g. separate environmental triggers or other susceptibility genes. Further study of the long term evolution of RE patients, as well as detailed family histories, and use of newer molecular techniques such as CGH microarray and whole exome sequencing, are warranted to clarify this.

969W

Action Myoclonus-Renal Failure Syndrome (AMRF): Founder Effect in French Canadians. *E. Andermann[1], D. Amrom[2], J. Mulley[3], M. Bayly[4], M. Jomphe[5], S. Berkovic[6], F. Andermann[7], L. Dibbens[4].* 1) Neurogenetics Unit, Montreal Neurological Hospital and Institute; Depts of Neurology & Neurosurgery and Human Genetics, McGill University, Montreal, Canada; 2) Neurogenetics Unit, Montreal Neurological Hospital and Institute; Dept of Neurology & Neurosurgery, McGill University, Montreal, Canada; 3) Department of Genetic Medicine, Women's and Children's Hospital, North Adelaide, Australia; 4) School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia; 5) BALSAC Project, Université de Chicoutimi, Quebec, Canada; 6) Epilepsy Research Center, Department of Medicine (Neurology), University of Melbourne; Austin Health, Heidelberg West, Melbourne, Australia; 7) Epilepsy Service and Seizure Clinic, Montreal Neurological Hospital and Institute; Depts of Neurology & Neurosugery and Pediatrics, McGill University, Montreal, Canada.

BACKGROUND: AMRF, an autosomal recessive form of progressive myoclonus epilepsy with renal failure, was initially described in three French Canadian families in 1986. Additional families were reported in the USA, Cuba, Germany, Portugal and Australia. The gene for this disease was recently found to be SCARB2/Limp2, encoding a lysosomal-membrane protein. A nonsense mutation c.862C>T (Q288X) in exon 7 of SCARB2 was identified in one of the original French-Canadian families. PURPOSE: To characterize the SCARB2 mutations in French Canadian patients with AMRF, and to determine whether these mutations can be explained by a founder effect. DESIGN/METHODS: DNA was obtained on 105 family members in 7 families, and exon 7 of SCARB2 was sequenced for the Q288X mutation. Genealogies of the AMRF families sharing this mutation were analyzed employing the BALSAC population register. Haplotype analysis using highly heterozygous markers was also performed. RESULTS: Four new patients in three families were homozygous for the Q288X mutation. Thirty-three unaffected individuals in five families who were not obligate carriers were found to be heterozygous for the Q288X mutation. In a sixth family, the mother was found to carry another SCARB2 mutation, suggesting allelic heterogeneity. One patient of French-Canadian extraction living in New England was found to be a compound heterozygote for both mutations. Genealogies of the 5 families sharing the Q288X mutation demonstrated three links 6 generations back, and one 9 generations back. The mean kinship coefficient was significantly higher than in control French Canadian genealogies. Haplotype analysis resulted in a shared haplotype of over 7.2 Mb involving 6 heterozygous markers, strongly suggesting identity by descent. CONCLUSIONS: By combining genealogical and molecular data, we have established that six French Canadian families share the same SCARB2 mutation that can be explained by a founder effect. Carrier screening for the founder mutation in this population can be carried out without sequencing the entire SCARB2 gene. This has important implications for genetic counseling, prenatal and preclinical diagnosis, and prevention of this debilitating disease.

970W

Brain Abnormalities in Individuals with Beckwith-Wiedemann Syndrome. *D. Chitayat[1, 3, 8], K. Gardiner[2], S. Choufani[3], C. Shuman[4, 8], S. Blaser[5, 8], D. Terespolsky[6], P. Ray[7, 8], B. Baskin[7, 8], R. Weksberg[3, 4, 8].* 1) Prenatal Diag & Med Gen, Mount Sinai Hosp, Toronto, ON, Canada; 2) Sarah Lawrence College, Bronxville, NY; 3) Genetics and Genome Biology, Research Institute, The Hospital for Sick Childrenm Toronto, On; 4) Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, On; 5) Diagnostic Imaging, The Hospital for Sick Children, Toronto, On; 6) Genetics Department, Credit Valley Hospital, Mississauga, On; 7) Molecular Genetics Division, Paediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, On; 8) Faculty of Medicine, University of Toronto, Toronto, On.

Beckwith-Wiedemann syndrome (BWS) is an overgrowth disorder with variability in clinical manifestations and molecular etiology. Developmental issues identified in patients with BWS can, in most cases, be ascribed to neonatal hypoglycaemia or chromosome abnormalities involving copy number variation for genes beyond the critical BWS region on chromosome 11p15.5. Brain malformations are not known to be part of the BWS phenotypic spectrum. A retrospective review of 450 BWS patients included in our registry detected 7 cases with brain abnormalities including 2 with Blake's pouch cyst, 2 with Dandy-Walker variant (hypoplasia of the inferior part of the vermis), 1 with Dandy-Walker malformation and 1 with DWM, dysgenesis of the corpus callosum and brain stem abnormality. Molecular analysis showed loss of methylation at imprinting center (IC2) in 3, CDKN1C mutations in 2, and 11p14-15 microdeletion in 1. One had no detectable mutation/methylation abnormalities. These data suggest that genes in imprinted domain 2 are involved in normal midline and posterior fossa brain development. Brain ultrasound/MRI should be considered for BWS patients with developmental delay, especially if they have molecular alterations in imprinted domain 2 of chromosome 11p15.5.

971W

Glut1 deficiency syndrome with a SLC2A1 splice site mutation and normal erythrocyte glucose uptake. *N. Ishihara[1], Y. Azuma[1,2,3], K. Yanagihara[4], S. Yokoi[1], T. Nakata[1,2], K. Aso[3], K. Ohno[2], J. Natsume[1].* 1) Dept Pediatrics, Nagoya Univ Graduate Sch Medicine, Nagoya, Japan; 2) Div Neurogenetics and Bioinformatics, Nagoya Univ Graduate Sch Medicine, Nagoya, Japan; 3) Dept Pediatrics, Aichiken Aoitori Medical and Welfare Center, Nagoya, Japan; 4) Dept Developmental Medicine, Osaka Medical Center Res Inst for Maternal and Child Health, Osaka, Japan.

Glucose transporter type 1 deficiency syndrome (Glut1-DS) is characterized by infantile seizures, acquired microcephaly, developmental delay, hypoglycorrhachia, and decreased erythrocyte glucose uptake. These are caused by a mutation of the Glut1 gene (SLC2A1) leading to brain energy failure. Since Glut1 protein is highly expressed on the erythrocyte membranes, the state of Glut1-deficiency usually can be revealed by erythrocytes glucose transport assays. Here we report a 5-year-old boy, who had typical symptom of Glut1-DS and hypoglycorrhachia, but normal erythrocytes glucose uptake caused by new mutation of SLC2A1. He was born to non-consanguineous parents with moderate mental retardation. His motor development was delayed; head was controlled at 8 months of age, and he walked with support at 18 months. He had loss of consciousness during febrile illnesses several times since 16 months of age, and his first afebrile seizure occurred at 28 months of age. Treatment with phenobarbital caused unsteady standing and diziness. Head MRI was normal. EEG revealed diffuse irregular spike-waves during fasting period. The CSF glucose level was 27 mg/dl and blood-glucose level was 75 mg/dl, thus a CSF/blood glucose ratio was decreased to 0.36. He was suspected to have Glut1-DS, but his erythrocyte glucose uptake was normal. Mutational analysis of SLC2A1 was performed with genomic DNA extracted from white blood cells, and we found heterozygous mutation at intron 9, c1279-1G>A. Next we performed RT-PCR with mRNA extracted from white blood cells, and found 2 different splice variants which were not seen in healthy controls. Glut1 protein is expressed in vascular endothelial cells comprising blood-brain barrier. As SLC2A1 is also expressed in erythrocytes, the diagnosis of Glut1-DS is confirmed by a decreased uptake of 3-O-methylglucose into erythrocytes. In our case, in spite of the clinical features which strongly suggested Glut1-DS, erythrocyte glucose uptake revealed normal. Wang et al. reported two patients with a Glut1-DS phenotype associated with a heterozygous Glut1 T295M mutation and normal erythrocyte glucose uptake. The T295 places intracellular loop 6-7 and it co-operated with intracellular C-terminal of Glut1. As both of our splice variants make truncation of C-terminal of Glut1, we speculated that our mutation could also lead to decreased uptake of glucose into blood-brain barrier without decreasing erythrocyte uptake.

972W

Distinct de novo deletions in a brother-sister pair with RTT: A case report. *K. Mittal[1], N. Gupta[2], M. Kabra[2], R. Juyal[3], B.K. Thelma[4].* 1) Molecular Neuropsychiatry and Development Laboratory, Centre for Addiction and Mental Health, Toronto, Ontario, Canada; 2) All India Institute of Medical Sciences, New Delhi, India; 3) National Institute of Immunology, New Delhi, India; 4) University of Delhi South campus, University of Delhi South campus.

Rett syndrome (RTT), a neurodevelopmental disorder caused by mutations in the X-linked gene encoding methyl-CpG-binding protein2 (MeCP2), is a leading cause of mental retardation in females. Majority of cases are sporadic (99%) but some familial cases have also been observed. We describe a familial study with a brother-sister pair with symptoms of RTT and exhibiting distinct deletions in the MECP2; with the sister exhibiting deletion in exon3 (MBD domain)and the brother with deletion in exon4 (TRD and DPR domains). The non-shared de novo deletion in the two sibs provides important insights into the disease etiology, especially for male sibs showing varied phenotypes as compared to the classical ones seen in the females.

973W

Identification of a novel recurrent microdeletion at 2q11.2 associated with speech delay and ADHD. *A. Patel[1], P. Hixson[1], P. Stankiewicz[1], S.W. Cheung[1], P.I. Bader[2].* 1) Dept Molecular and Human Gen, Baylor Col Medicine, Houston, TX; 2) Parkview Health Systems,Fort Wayne, IN.

A rare recurrent microdeletion involving chromosome 2q11.2 of approximately 900 kb in size was detected by a custom-designed exon-targeted clinical microarray in four individuals from approximately 15,000 cases evaluated by our laboratory. The deleted segment is flanked by low copy repeats suggesting nonallelic homologous recombination is the mechanism of origin. The common clinical features observed include speech delay and attention-deficit/hyperactivity disorder (ADHD). The deletion is de novo in one individual and was inherited from an affected mother of a sibling pair. Copy number variation in this region has not been reported in any normal individuals (Database of Genomic Variants). There are at least 21 genes mapped in this interval, including 13 OMIM genes. Of the genes whose function is known, the most intriguing gene in this region with respect to the phenotype observed in these individuals is the ADRA2B (adrenergic, alpha-2B receptor) gene. This gene belongs to a family of receptors which play a critical role in regulating neurotransmitter release from sympathetic nerves and from adrenergic neurons in the central nervous system. A functional deletion variant of this gene that consists of an inframe deletion of 3 acidic residues in the third intracellular loop of the receptor, acts as a loss of function polymorphism in regulation of emotional memory. Carriers of this deletion exhibit increased activation of the amygdala and inferior frontal gyrus in response to acute stress. Interestingly, the prefrontal cortex is one of the brain structures implicated in mood disorders and ADHD. In summary, we describe the molecular and clinical features of a novel recurrent microdeletion at 2q11.2 associated with speech delay and ADHD further supporting that deletion of the ADRA2B gene is likely responsible for the observed ADHD phenotype.

974W

Neonatal Epilepsy as a Presenting symptom of Infantile Cerebral and Cerebellar Atrophy in a Caucasus Jewish Child. *A. Singer[1], A. Shaag[2], D. Lev[3,4], C. Vinkler[3,4].* 1) Dept Clinical Gen Unit, Barzilai Med Ctr, Ashkelon, Israel; 2) The Department of Genetic and Metabolic Diseases, Hadassah, Hebrew University Medical Center, Jerusalem, Israel; 3) Institute of Medical Genetics, Wolfson Medical Center, Holon, Israel; 4) Metbolic Neurogenetic Clinic, Wolfson Medical Center, Holon, Israel.

Infantile cerebral and cerebellar atrophy is a new syndrome recently described. Five patients have been described so far. They all presented with swallowing difficulties at the age of 1-2 month leading to failure to thrive, poor visual fixation and lack of tracking, jitteriness and truncal arching. At a few months of age they started with overt seizures. Progressive microcephaly is observed with a head circumference of -6 SD at about 9 months of age. They did not show any acquisition of developmental milestones and any dysmorphic features. Brain MRI revealed severe diffuse cerebral and cerebellar atrophy already evident at few months of age with poor myelination. All five patients were of Caucasus Jewish origin. Using homozygosity mapping, a common mutation- L371P, in the MED 17 gene was found. We describe a 4y old patient with a severe clinical presentation of intractable epilepsy right after birth. He is the first child of young unrelated parents of Caucasus Jewish origin born at term following an uneventful pregnancy. Initially, extensive evaluation, including brain ultrasound and CT, were normal. EEG was abnormal with epileptogenic foci especially remarkable in the right temporal area. At first, he responded well to phenobarbital treatment. His head circumference at birth was 35 cm. At 4 months he was reevaluated because his seizures recurred and could not be controlled by drugs, he had roving eye movement and developmental delay . At the age of one year, his head circumference was -4 SD and brain MRI showed sever diffuse cerebral and cerebellar atrophy with poor myelination. At 3.5 y, he had profound mental retardation, overt epilepsy and dysmorphic features. Molecular analysis of the MED17 gene revealed the common mutation L371P. The underlying genetic etiologies of neonatal seizures are diverse. Some of the known mutated genes cause channelopathies. Others are related to metabolic diseases. Another group of genes relate to syndromes involved with brain structural anomalies. The mechanism by which a mutation in the MED17 gene causes neonatal onset epilepsy is not clear. Our patient presented with neonatal epilepsy. Microcephally, abnormal MRI of the brain and dysmorphic features were apparent only at a later stage. We would like to suggest that mutation in the MED17 gene should be included in the differential diagnosis of neonatal seizures in patients of Caucasus Jewish origin.

975W

46, XY Pure Gonadal Dysgenesis and Pontocerebellar Hypoplasia. *K. Siriwardena[1], A. Guerin[1], S. Blaser[2], D. Chitayat[1].* 1) Division of Clinical & Metabolic Genetics, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Radiology, Hospital for Sick Children, Toronto, Ontario, Canada.

We present an infant with XY pure gondal dysgenesis, developmental delay, spasticity, and ponto-cerebellar hypoplasia. This is only the second patient with this unique combination as described by Huq and Nigro, Pediatr Neurol 23(4):357-60. The phenotypically female infant was seen at 10 months of age for severe global developmental delay and cerebellar hypoplasia. She was born to non-consanguineous Caucasian parents at term after an uneventful pregnancy and vaginal delivery with a birth weight of 3.75kg. Family history was unremarkable. She required nasogastric tube feeds until 2 weeks of age. Developmental milestones were delayed with patient smiling at 4 months, holding objects at 8 months and rolling but not sitting at 10 months. Episodes of back arching were noted at 4 months but an EEG showed no evidence of epileptiform activity. Audiology, eye examination and VEP testing were normal. On examination she had minor craniofacial dysmorphic features with a flat occiput, prominent glabellar with nevus flammeus, broad nasal root and tip. External genitalia were unambiguously female with a clitoris and labia minora with posterior labial agglutination. At 10 months, she had significant head lag and truncal hypotonia associated with increased distal tone and brisk deep tendon reflexes but no clonus. She was able to fix and follow well but bilateral intermittent esotropia was noted. A thorough metabolic screen including isoelectric focusing was normal. A microarray analysis showed a 46 XY complementation with maternally inherited 2q12.3 duplication. While this region contains known genes- GCC2, SULT1C3, SULT1C4, SULT1C2 and SLC5A7- it is considered to be a variant. FISH for SRY was positive. Molecular testing for ATRX and DHCR7 was negative. Repeat MRI at 17 months showed reduced periventricular white matter and size of corpus callosum, thalami and pons in addition to cerebellar hypoplasia. MRI imaging of the pelvis followed by examination under anesthesia showed the presence of a blind ended vagina and bilateral “gonadal” structures noted to be Fallopian tubes on pathological examination. FSH was elevated at 85 IU/L with low androgens levels. Our patient continues to gain milestone: was crawling by 17 months. Our patient and the patient described by Huq and Nigro appear to have the same rare disorder of sexual differentiation with as yet unknown genetic etiology.

976W

Mutation in *MKI-67* in a family with intellectual disability and microphthalmia. *M. Srour, M.E. Samuels, J.L. Michaud.* Human Genetics, University of Montreal, Montreal, Quebec, Canada.

Microphthalmia is a heterogeneous condition, whose genetic basis remains poorly characterized. We recently observed a new form of syndromic microphthalmia in a consanguineous Lebanese family. The proband was a girl with severe intellectual disability, central hypotonia, peripheral spasticity and severe anterior chamber eye defects consisting of aphakia and sclerocornea. Brain MRI and array genomic hybridization were normal. To our knowledge, this association of severe intellectual disability and microphthalmia has never been reported. The mother of the proband subsequently became pregnant of a fetus with evidence of bilateral microphthalmia on ultrasound and MRI. The pregnancy was terminated. Autopsy demonstrated similar ocular defects.

To study the molecular basis of this new phenotype, we performed whole genome SNP genotyping of the proband and the fetus using the Illumina 610 Quad panel. We identified 7 regions of homozygosity (RH) larger than 1 Mb shared by the proband and the fetus. The largest RHs (>3 Mb) were: 1) chr1:43,071,708-53,738,134 (10.7 Mb); 2) chr10:77,586,965-131,072,928 (53.5Mb). With the exception of *FOXE3*, which maps to the chromosome 1 interval, none of the known microphthalmia genes (*PAX6, RAX, SIX3, SIX6, OTX2, SOX2, CHX10,GDF6, MITF*) lie in the candidate RHs and we did not find any rare variants in *FOXE3*. Whole-exome sequencing was performed in the affected child using an Agilent SureSelect biotinylated RNA library and the Illumina Genome Analyzer II (GAII) system. After filtering out common variants, we identified a homozygous nonsense mutation in *MKI-67* (Antigen identified by monoclonal antibody Ki-67) (c.7402C>T, p.2468Q>X) in the largest interval of homozygosity. The mutation is predicted to abolish the distal third of the protein. Sanger sequencing confirmed that both affecteds are homozygous and their parents heterozygous for this mutation. This mutation was not found in 152 Middle-Eastern control chromosomes, nor was it identified in the 1000 genome project. *MKI-67* encodes a ubiquitous nuclear protein whose exact function is unknown, though it is widely used as a marker of cellular proliferation. Knock-down experiments in the zebrafish are currently underway. The implication of this gene in neural and ophthalmologic development is novel and suggests new avenues for research.

977W

Description of a patient with characteristic dysmorphic features and mental retardation associated with FGF12 gene deletion. *L. Telvi[1], A. Rouen[1], A. Coussement[1], J.M. Dupont[1], I. Desguerre[2], M.C. Leroux[2].* 1) Cytogenetics Department, Hospital Cochin-St Vincent de Paul, Paris, France; 2) Department of Genetics, Hospital Necker, Paris, France.

We describe a 21 years-old patient with characteristic dysmorphic traits as large forehead, hypothelorism, long nose, broad nasal base, short philtrum and macrognathia associated to a mild mental retardation. The patient showed a normal caryotype after RHG banding. The oligonucleotide array analysis using Nimblegen whole genome tiling CGX 135K HG18 demonstrate that the patient carry an 112 kb deletion localized at 3q28, which include FGF12 gene. The RP11-466I16 probe was used for FISH analysis and confirms this deletion. The phenotypically normal father was found carrying the same deletion after using oligonucleotide array and FISH analysis. The phenotypical differences between the patient and his father could be explained by a haploinsufficency. The patient had phenotypically normal two sisters. The patient's mother and one of the sisters showed no deletion at this chromosomal site after FISH analysis with the same probe. The fibroblast growth factors are referred as fibroblast homologous factors (FHFs) and comprise a family of related polypeptides with broad mitogenic and cell survival activities. Smallwood et al. (1996) showed that FHFs are expressed principally in the nervous system and are therefore likely to play a role in nervous system development and/or function. Nie X (2006) was described the FGF12 gene as having function in the developing mouse cranial base, and showed that the FGF12 was transitorily expressed at early chondrocranium. We discuss the possible relationship between the deletion of the FGF12 gene and phenotypical abnormalities of the patient.

978W

Ramon Syndrome in a 38 years old male. *S.B. Turyk, M. Sakurai, C. Galliuss, E. Maloberti.* Servicio de Genetica, Hospital Britanico de Buenos Aires, Buenos Aires, Buenos Aires, Argentina.

Ramon Síndrome (RS) was initially described by Ramon et al. (Oral Surg. 24:436-48,1967). Since that time there have been few patients reported in the literature with this rare, presumed autosomal recessive syndrome. Described patients were mostly children. We report a new case of RS, a rare disorder, characterized primarily by gingival overgrowth, congenital generalized hypertrichosis, mental retardation and epilepsy. Our proband was a caucasian 38 year old male. Third son of non consanguineous parents and his older brothers of whom were unaffected. He was born full-term by normal vaginal delivery and his birth weight was 2 kg. Developmental milestones were delayed. Reportendly, he attended regular school up to the 9th grade. Our patient had mental deficiency, epilepsy, gingival fibromatosis, cherubism due to the fibrous dysplasia of the maxillae, hypertrichosis and pigmentary changes in the retina. Chromosomal study of peripheral blood lymphocytes confirmed the 46,XY karyotype. Ocular features were not described in the original report on this syndrome. Recent reports of other families with R.S. describe pigmentary retinopathy and optic disc pallor. It may be concluded that ocular abnormalities are another feature of Ramon syndrome and are developing later.

979W

Cutaneous manifestations in 9 patients with biallelic germline MUTYH mutations. *F. Caux[1], A. Lévy[2], P.O. Schischmanoff[3], F. Coulet[4], F. Soubrier[4], Y. Parc[5], L. Laroche[1], C. Colas[4].* 1) Reference Center for Genetic Skin Diseases & Dpt of Dermatology, Avicenne Hospital, Bobigny, France; 2) Dpt of Pathology, Avicenne Hospital, Bobigny, France; 3) Laboratory of Biochemistry, Avicenne Hospital, Bobigny, France; 4) Laboratory of Oncogenetics, Pitié Salpétrière Hospital, Paris, France; 5) Dpt of Digestive Surgery, Saint Antoine Hospital, Paris, France.

Biallelic germline MUTYH mutations (M-/-) are associated with autosomal recessive adenomatous polyposis and colorectal cancer. Skin lesions such as sebaceous adenomas, hyperplasia or carcinomas may occur in association. Prevalence of these lesions is unknown since only case reports have been described. In order to assess prevalence and describe histologically these lesions, we performed a systematic clinical examination of 9 individuals M-/-. A dermatological examination has been proposed to all patients M-/- seen in oncogenetic counseling (n=36). Nine patients were examined including 8 by the same dermatologist. Six skin biopsies were performed in 4 patients. Colonic manifestations were gathered in order to search for phenotype-genotype correlation. There were 5 males and 4 females with a mean age of 49.3 years (range 26-65). Six out of 9 patients had skin lesions with a mean age of beginning of 43 years (range 33-54). These lesions were yellowish papules in a variable number occurring on the face (forehead, cheeks). Analysis of 6 lesions always demonstrated sebaceous hyperplasia, none showing microsatellite instability (n=3). Colonic polyposis was present in 8 patients with a mean beginning age of 40.6 years (range 30-49) and a variable number of polyps (range 5-150). A colorectal cancer was associated in 6 patients. MUTYH analysis demonstrated homozygous mutations in 7 patients (p.Gln337Ter, p.Gly382Asp, p.Tyr165Cys, p.Glu396GlyfsX43) and compound heterozygous mutations in 2 patients (p.Tyr165Cys and p.Glu466del; p.Gly382Asp and p.Pro391Leu). No phenotype-genotype correlation was detected. Treatment with isotretinoin completely cleared skin lesions (n=3). Prevalence of sebaceous lesions is high (66%) in our patients M-/-. These lesions constantly were hyperplasia while 14 sebaceous tumors (8 adenomas, 4 hyperplasia, 2 carcinomas) have been described in the literature. Dermatological appearance of these patients may evoke Muir-Torre syndrome. However Muir-Torre syndrome is autosomal dominant and characterized by sebaceous adenomas. Skin findings also share similarities with multiple sebaceous hyperplasia seen in transplant recipients under immunosuppressive therapies. Finally we observed a high prevalence of sebaceous hyperplasia in patients M-/-. Dermatologists have to be aware that multiple facial yellowish papules in a young individual is not specific of Muir-Torre syndrome but may also lead to search for MUTYH mutations and to perform colonoscopy.

980W

A new case of Gorlin Syndrome in Mexican female. *N. DAVALOS, A. RINCON, C. ISLAS, R. LOPEZ, J. ONTIVEROS, D. GARCIA, S. RAMIREZ, J. OLMOS, S. ALONSO, Genetic variation group.* Inst Human Genetics, Univ Guadalajara, Guadalajara, Mexico.

Gorlin Syndrome also know as Nevoid Basal Cell Carcinoma (NBCCS) present a pattern hereditary autosomical dominant. Cases is caused by a novo mutation, was reported clinical variability in populations and ethnic groups.The prevalence is 1/57000 to 1/256000 among.The pourpouse of the new case in Mexican famele, with age 39-year-old,product of the fourth pregnancy ,of non consanguineous parents,family history revealed by the maternal breast and thyroid, was operated on 13 times for removal of keratocysts and nevus mainly from the frontal and thorax. Grandmother and two maternal aunts showed breast cancer and sister thyroid cancer.Psychomotor development was normal,dermatologist reported more than twenty nevi with different body distribution which have removed many times,beginning with dental anomalies ten years keratocysts detected, and the date has since been operated on 13 times this reason, also present history abortions reported two three wings near and ten weeks of gestation, asocited with bicornuate uterus. Proband present attention deficit within the average IQ bordering. Other findings in proband is magraine, a quiet cooperative patient with face feature characteristics for macrocephaly,hypertelorism,frontal bossing, any more palmoplantar pits,more than twenty nevi on the face and neck,high-class,dermatology has surgically resected nevi in at least thirteen occasions where pathology reports type basal cell carcinoma, pigmented and superficial multifocal micronodular variant, micro surgical side edges and free of neoplasia, hands and feet has many pits. TAC of nervious system showed calcification falx cerebri and microcalcifications in the frontal zone,through grooved maxillofacial,reported poor dental occlusion sclerotic radiolucent edge of the branch at the left maxillary and images supported by multiple scar lesion was excised by keratocysts, gynecology. We conclude than this is the first case which shows the association between abortion-Gorlin syndrome repetitions for bicornuate uterus and multiple microcalcifications in the central nervous system.

981W

Pulmonary anomalies in a novel syndrome of macrocephaly, nephromegaly, and Wilms tumor reveals mechanistic etiologies for overgrowth syndromes. *J.A. Martinez-Agosto.* Department of Human Genetics, University of California, Los Angeles, Los Angeles, CA.

The molecular basis for several overgrowth syndromes, including Beckwith-Wiedemann (BWS) and Sotos, has been identified. However, the etiology for many others remains unknown. Here we describe a novel overgrowth syndrome characterized by macrocephaly, congenital pulmonary cysts, nephromegaly, and Wilms tumor predisposition. The presence of multicytsic lung disease in these cases prompted a search of the literature and patient database records for the presence of pulmonary malformations in other overgrowth conditions. An exhaustive search did not identify any overgrowth syndrome cases with pulmonary anomalies. The unique association of these findings with cystic lung disease suggests that they represent a novel overgrowth syndrome. In contrast, bullous pulmonary lesions have been described in a subset of patients with Proteus syndrome. We compare the Proteus lung phenotype to our patients and have identified unique similarities that support a distinct common molecular etiology. Birt-Hogg-Dube syndrome includes multiple lung cysts as well as renal tumors and is due to mutations in FLCN, which regulates PTEN/Akt/mTor signaling. As mutations in PTEN have been identified in hamartoma syndromes that share many findings with Proteus, dysregulation of PTEN/Akt/mTor signaling likely underlies the somatic overgrowth and lung defects in these syndromes. This is supported by the pulmonary phenotype in tuberous sclerosis, which presents with lung cysts prior to development of lymphangiolyomyomatosis. In addition, mouse knockouts for the TAZ gene, a transcriptional coactivator and downstream target of the Hippo pathway, develop pulmonary cysts. TAZ is required for lung alveolarization, where it regulates expression of connective tissue growth factor. We propose that dysregulation of the Hippo and PTEN/Akt/mTor pathways cause anomalies that lead to somatic overgrowth and cystic lung changes in this subset of overgrowth syndromes. The advent of massively parallel/whole exome sequencing technologies has allowed the genetic characterization of these sporadic disorders and we are currently using this approach to identify the causative gene for this syndrome.

982W

Gorlin Syndrome: A familial case. *I.M. Salazar-Dávalos[1], M.A. Aceves-Aceves[1], D. García-Cruz[2], N.O. Dávalos[2], M.G. González-Mercado[2,3], J.A. Cruz-Ramos[2,3], A. González-Mercado[2,3], S.A. Alonso-Barragán[1,2], C. Roa[4], I.P. Dávalos[2,3].* 1) Facultad de Medicina, CUCS, Universidad de Guadalajara; 2) Instituto de Genética Humana, Doctorado en Genética Humana, CUCS, Universidad de Guadalajara; 3) División de Genética, CIBO, Instituto Mexicano del Seguro Social; 4) UMAE, Hospital de Pediatría, CMNO, Instituto Mexicano del Seguro Social. Guadalajara, Jalisco, México.

Introduction: The Gorlin syndrome, also known as Gorlin-Goltz syndrome, Nevoid Basal Cell Carcinoma Syndrome (NBCCS) and Basal Cell Nevus Syndrome (BCNS), is an infrequent multisystemic disease, inherited as an autosomal dominant disorder, with complete penetrance and variable expressivity. It is characterized by odontogenic keratocysts of jaws, multiple basal cell nevi and skeletal abnormalities, other features include palmar and plantar epidermal pits, facial dysmorphism including broad facies, frontal and biparietal bossing, ocular anomalies, cleft lip/palate, medulloblastoma as well as intracranial ectopic calcifications. BCNS is caused by mutations in the PTCH1 gene (601309.0001) on chromosome 9q22, the PTCH2 gene (603673.0003) on chromosome 1p32, or the SUFU gene (607035) on chromosome10q24-q25. Case report: We studied a 16 year-old male, product of the 1st pregnancy, mother of 36 and father 38 years old, a brother of 10 years and a sister of 5 years old. His father had a previous history of an odontocyst. In the present case at 13 years old were detected multiple odontogenic cysts in mandible and maxillary regions by orthopantomography, the cysts were surgically removed and histopathological analysis reported keratocystic odontogenic tumors (KCOTs). Physical examination showed height of 163 cm, weight of 53 and head circumference of 56 cm, frontal bossing, moderate hypertelorism, pits of the palms and soles. Radiological studies showed bridging in sella turcica. At 15 year-old presented a mass in the left lumbar region and in inframaleolar dorsal region, both masses were surgically removed and histopatologically diagnosed as benign lipomatous tumor of skin and subcutaneous tissue. The father with a previous odontogenic keratocyst was clinically examinated showing palmar/plantar pits and multiple nevi. These datae led us to consider a familial BCNS. Conclusion: Several studies include KCOTs, basal cell nevi and skeletal anomalies as major clinical features of BCNS. The diagnosis criteria of BCNS require the presence of two major criteria or one major and two minor criteria (Kimonis et al, 1997). The aim of this work is to present a familial case of BCNS, reaffirming early diagnosis and clinical evaluation are necessary in Gorlin syndrome patients and relatives, as well as to reduce exposure to UV radiation to prevent BCC (Basal Cell Carcinoma).

983W

Association between rs642961 and cleft lip and palate in Patagonia (ECLAMC) population. *F.M. de Carvalho[1], A.R. Vieira[2,3,4], F. Poletta[5,6], J.C. Mereb[7], R.F. Fonseca[1], J.L. Camelo[5,6,8], E.E. Castilla[5,6,9], I.M. Orioli[1].* 1) ECLAMC and INAGEMP at Genetics Department, Federal University of Rio de Janeiro, RJ, Brazill; 2) Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, USA; 3) Pediatric Dentistry Department, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, USA; 4) Center for Craniofacial and Dental Genetics, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, USA; 5) ECLAMC and INAGEMP at CEMIC (Center for Medical Education and Clinical Research), Buenos Aires, Argentina; 6) CONICET (National Research Council of Argentina), Buenos Aires, Argentina; 7) ECLAMC and INAGEMP at Hospital Zonal El Bolson, El Bolson, Rio Negro, Argentina; 8) ECLAMC at IMBICE /CONICET, La Plata, Argentina; 9) ECLAMC and INAGEMP at Epidemiology Laboratory of Congenital Malformations, Oswaldo Cruz Institute, Rio de Janeiro, Brazil.

Cleft lip with or without cleft palate (CL/P) birth prevalence is about 1/1,000 live births. In South America four CL/P high prevalence regions was detected, one of them being the Patagonia (Argentina). Significant association between the ancestral allele of the single nucleotide polymorphisms (SNPs) rs2235371(C>T) in the IRF6 gene and CL/P was inconsistently shown in multiple populations. Recently it was proposed that the ancestral allele of rs2235371(C>T) does not have an association with clefting independently of the mutated allele of the SNP rs642961(G>A) near the IRF6 region. We tested for association between these SNPs in IRF6 region (rs2235371C>T and rs642961G>A) and the risk of CL/P, using case-parent trio design in a high frequency CL/P population from Patagonia (Argentina). Five-hundred and twenty-six individuals from 129 families were selected through the Latin-American Collaborative Study of Congenital Malformations (ECLAMC). Genotyping was carried out by using TaqMan®SNP genotyping assays on the ABI Prism 7900HT. Haplotype and transmission distortion analyzes were performed with the Family Based Association Test (FBAT). We found an association between rs642961(G>A) around the IRF6 gene and CL/P with over transmission of the ancestral allele (f (G)=0.70, Z=2.042, p=0.04) in this population. This association remained when we analyzed a subgroup of 52 families (217 individuals) from El Bolsón and neighborhood villages (Lago Puelo, Ñorquincó, El Maitén, El Mallín Ahogado, Los Menucos, Maquinchao, Ingeniero Jacobacci) (f(G)=0.69, Z=2.616, p=0.009) and disappeared when we excluded this subgroup from the analysis (f(G)=0.71, Z=0.533, p=0.60), suggesting some kind of heterogeneity in this population. The variant rs2235371 is not associated with CL/P in this population. The association between the rs642961 (G>A) and CL/P, through the ancestral allele in some populations and through the mutated allele in other suggests that this SNP is not causal to CL/P.

984W

IRF6, SNAP91, PRSS35 and CRISPLD2 contribute to the etiology of oral clefts in a Latin American population (ECLAMC). *R.F. Fonseca[1], F. Poletta[2,3], F.M. Carvalho[1], E.E. Castilla[2,3,4], A.R. Vieira[5,6,7], I.M. Orioli[1].* 1) ECLAMC and INAGEMP at Department of Genetics, Federal University of Rio de Janeiro, RJ, Brazil; 2) ECLAMC and INAGEMP at CEMIC, Buenos Aires, Argentina; 3) CONICET (National Research Council of Argentina), Buenos Aires, Argentina; 4) ECLAMC and INAGEMP at Epidemiology Laboratory of Congenital Malformations, Oswaldo Cruz Institute, Rio de Janeiro, Brazil; 5) Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, USA; 6) Pediatric Dentistry Department, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, USA; 7) Center for Craniofacial and Dental Genetics, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, USA.

The aetiology of oral clefts is complex and very heterogeneous, with multiple genetic and environmental factors playing a role. The most recent estimative suggest that from 3 to 14 genes contribute to oral clefts. The aim of this study was to test if 26 single nucleotide polymorphisms (SNPs) distributed in in seven loci previously associated with oral clefts [MTHFR (2 SNPs), IRF6 (7 SNPs), TGFA (5 SNPs), 6q (six SNPs), FOXE1 (2 SNPs), CRISPLD2 (2 SNPs), and PVR (2 SNPs)] were associated with oral clefts. Genotypes were generated using TaqMan chemistry and real time PCR equipment. From years 2003-2006, DNA samples were collected from 304 triads through the Latin-American Collaborative Study of Congenital Malformations (ECLAMC) for this case-parent trio association study. These triads were from hospitals in: Argentina (103), Bolivia (14), Brazil (16), Chile (87), Ecuador (50), Colombia (13) and Venezuela (19). Analyses of association were performed by FBAT (Family Based Association Test) and TRIMM (Triad Multi-Marker) in total sample and in groups according to type of cleft diagnosed: 76 associated cases, 43 isolated cleft lip, 50 isolated cleft palate, 133 isolated cleft lip with cleft palate and 176 cleft lip with or without cleft palate. We found a statistically significant association between IRF6 (rs2235371:G>A, P=0.0001), and genes located in 6q [PRSS35 (rs7753918:A>G, P=0.004) and SNAP91 (rs217325:C>G, P = 0.04)] and oral clefts. For cleft palate only we found association with IRF6 (rs2013162:A>C, P=0.003; rs861019:A>G, P=0.01 and rs2073487:C>T, P= 0.048) and CRISPLD2 (rs1546124:C>G, P=0.02). The TRIMM analysis confirmed the FBAT results and did not shown any association with the maternal genotype. The results of this study support the hypothesis that IRF6 contributes to the aetiology of oral clefts and suggest SNAP91, PRSS35 and CRISPLD2 as candidates for these defects in a Latin American population.

985W

Phenotype characterization of a sibship with *SDCCAG8*-related disease. *E. Héon[1, 2], C. Deveault[2], G. Billingsley[2].* 1) Ophthalmology, Vision Science; 2) Genetics and Genome Biology Program, The Hosp For Sick Children, Toronto, ON, Canada.

Mutations in *SDCCAG8* were recently identified to cause a retinal-renal ciliopathy which included patients with the clinical diagnosis of Senior-Loken syndrome (SLSN) as well as Bardet-Biedl syndrome (BBS). The full clinical phenotype has yet to be defined. We assessed a subgroup of patients with the clinical diagnosis of BBS, for which no mutation was identified, and identified a *SDCCAG8*-mutation positive sibship that we thoroughly phenotyped. A sibship of East Indian origin carried the putative clinical diagnosis of BBS and previously published *SDCCAG8*(NM_006642.3) compound heterozygous mutations in exon12 (c.1444delA, p.Thr482LysfsX12) and exon14 (c.1627-1630delATAG, p.Asp543AlafsX24) which segregated with the disease phenotype. Revision of comprehensive systemic and ocular assessments was performed. No digit anomaly was observed. The kidney disease was early onset and required kidney transplant at the ages of 9 and 11 years old. Other features such as increased insulin, transaminases, parathyroid hormone and cholesterol levels were observed in both sisters. Both sibs had abnormal echogenicity of the kidneys, liver and pancreas by age 7y. BMI at the age of 10 was 25.5 and 27.1, respectively. Both sibs also have high blood pressure, asthma and are growth hormone deficient. The patients have been followed for 10 years. Unlike what is most commonly seen in BBS, the central visual acuity was preserved in the teenage years with a recordable electroretinogram despite a constricted visual field. Optical coherence tomography showed a preservation of the retinal lamination as seen in other forms of BBS. However, the rate of vision loss was less than that usually seen in BBS. Brain MRI, hearing, smell and cardiac function assessment were normal. No neurological anomalies were documented. The only variability observed in this sibship was at the level of weight gain and cognitive ability. Distinctive features of this *SDCCAG8* genetic variant appear to be severe renal disease combined with lack of digit anomaly and slower rate of vision loss when compared to patients with *BBS* gene mutations. This is the first comprehensive phenotyping of this genetic variant.

986W

Mutation screening of the *LPP* gene in patients with esophageal atresia, tracheoesophageal fistula, and VACTERL association. *A. Hernandez-Garcia[1], E. Brosens[2,3], H. Zaveri[1], Z. Yu[1], C. Fernandes[4], A. Johnson[1,5,6], S. Lalani[1], D. Tibboel[3], A. de Klein[2], D.A. Scott[1].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, the Netherlands; 3) Department of Paediatric Surgery, Erasmus Medical Center, Rotterdam, the Netherlands; 4) Department of Pediatrics, Baylor College of Medicine, Houston, TX; 5) Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX; 6) Department of Pediatric Surgery, Baylor College of Medicine, Houston, TX.

Esophageal atresia (EA) and tracheoesophageal fistula (TEF) are life threatening birth defects that affect ~1 in 3,500 newborns. Approximately 50% of cases occur in association with additional anomalies while VACTERL (Vertebral, Anal, Cardiac, TracheoEsophageal fistula, Renal, Limb) association is found in ~10% of infants with EA/TEF. The genes responsible for most cases of EA/TEF and VACTERL have yet to be identified. Recently, Arrington et al. described a 451 kb interstitial deletion involving only the LIM domain containing preferred translocation partner in lipoma (*LPP*) gene in an individual diagnosed with VACTERL association. This diagnosis was based on cardiac defects—tetralogy of Fallot (TOF) with a right aortic arch and left superior vena cava draining to the coronary sinus—EA/TEF, and small kidneys. They also described an intronic deletion in *LPP* that segregated with TOF in a small nuclear family and was associated with reduced *LPP* expression in a qPCR assay. To determine if alterations in *LPP* are a common cause of EA/TEF or VACTERL association, we screened 170 patients with these diagnoses for *LPP* deletions by microarray and a smaller cohort of 42 patients for sequence changes. No deletions involving *LPP* were identified. However, we found a missense change (c.1162C>T) in an African American male diagnosed with VACTERL association. He presented with a persistent left superior vena cava draining to a mildly dilated coronary sinus, a mildly dilated ascending aorta, vertebral anomalies, unilateral renal agenesis, hypoplastic ribs, polydactyly, and a bladder diverticulum. This sequence change results in an R388C substitution that affects a highly conserved region upstream of the first LIM domain and was predicted to be probably damaging by Polyphen2. Although this change was not reported in the SNP database, subsequent testing revealed the same change in the patient's apparently unaffected mother and in 1 out of 78 African American control samples. This suggests that this change is unlikely to be the sole cause of our patient's phenotype. No other novel sequence changes were identified in our cohort. We conclude that alterations in the *LPP* gene are not a common cause of EA/TEF or VACTERL association. If changes in *LPP* contribute to the development of VACTERL-related phenotypes they likely do so in combination with other genetic and/or environmental factors.

987W

Case-parent trio genome-wide association study identifies several candidate loci for nonsyndromic sagittal craniosynostosis. *C.M. Justice[1], Y. Kim[1], G. Yagnik[2], I.A. McMullen[3], H. Ling[3], P.A. Sanchez-Lara[4], V. Kimonis[5], J. Stoler[6], M. Cunningham[7], A.F. Wilson[2], S.A. Boyadjiev[1].* 1) Genometrics Section, Inherited Disease Research Branch, NHGRI, NIH, Baltimore, MD 21224; 2) Section of Genetics, Department of Pediatrics, University of California Davis, Sacramento, CA 95817; 3) Center for Inherited Disease Research, Johns Hopkins University, Baltimore, MD 21224; 4) Division of Genetics, Department of Pediatrics, University of South California, Los Angeles, CA 90027; 5) Division of Genetics, Department of Pediatrics, University of California Irvine, Irvine, CA 92697; 6) Division of Genetics, Children Hospital Boston, Harvard University, Boston, MA 02115; 7) University of Washington Department of Pediatrics, Division of Craniofacial Medicine and Seattle Children's Craniofacial Center, Seattle, WA 98101.

Craniosynostosis is a common malformation in which one or more of the cranial sutures in an infant skull (metopic suture, coronal sutures, sagittal suture and lambdoid sutures) fuse prematurely. Sagittal craniosynostosis is the most common type of craniosynostosis, accounting for 40 to 58% of all cases, with an estimated prevalence of 1.9-2.3 per 10,000 live births. The International Craniosynostosis Consortium (genetics.ucdmc.ucdavis.edu/icc.cfm) has been established with the aim of identifying the genetic causes of nonsyndromic craniosynostosis and more than 720 families with at least one affected individual have been recruited. In an attempt to identify genetic variants associated with nonsyndromic sagittal craniosynostosis, we performed a genome-wide association analysis of 201 case-parent trios and 13 nuclear families with two affected siblings. A total of 662 individuals in 214 families (70% Caucasian families, 30% mixed ethnicity families) were genotyped on the Illumina 1M Human Omni1-Quad array. Quality control measures reduced the number of SNPs available for analysis from 1,140,419 to 914,402 markers. Association between sagittal craniosynostosis and each SNP was measured using the transmission disequilibrium test (TDT) as implemented by PLINK v1.0.7. The strongest association was with rs1884302 ($p = 3.79 \times 10^{-14}$) in the flanking 3' UTR of BMP2 on chromosome 20. BMP2 belongs to the transforming growth factor-beta (TGFB) gene family and is involved in bone and cartilage formation. Genome-wide significant ($p \leq 5 \times 10^{-8}$) associations were also detected for SNPs intronic to BBS9 (rs1420154, $p=3.51 \times 10^{-13}$) on chromosome 7, which is thought to be involved in parathyroid hormone action in bones. Additionally, we have strong but not genome-wide significant signals on DLG1 (rs12152266, $p= 1.44 \times 10^{-7}$) on chromosome 3, RPS12 (rs9493468, $p=2.8 \times 10^{-7}$) on chromosome 6 and LOC643631 (rs1948330, $p=4.78 \times 10^{-7}$) on chromosome 5. Loss of heterozygosity mapping identified several chromosomal regions which are in process of further analysis.

988W

Statistical and biological evidence for AXIN2/IRF6 interaction in oral facial clefting. *A. Letra[1], B. Bjork[2], M. Copper[1], H. Szabo-Rogers[3], C. Brandon[1], J. Resick[1], K. Bardi[1], F.W.B. Deleyiannis[4], L.L. Field[5], A.E. Czeizel[6], L. Ma[7], G.P. Garlet[8], F.A. Poletta[9,10], J.C. Mereb[11], J.S. Lopez-Camelo[9,12], E.C. castilla[9,13], I.M. Orioli[14], S.K. Wendell[1], K.L. Liu[3], M.L. Marazita[1], A.R. Vieira[1,15], R. Menezes[1].* 1) Dept Oral Biology and Center for Craniofacial and Dental Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Division of Genetics, Harvard University, Boston, MA; 3) Department of Craniofacial Development, King's College London, London, United Kingdom,; 4) Departments of Surgery and Otolaryngology, University of Colorado School of Medicine, Denver, CO; 5) Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 6) Foundation for the Community Control of Hereditary Diseases, Budapest, Hungary; 7) School of Stomatology, Beijing University, Beijing, China; 8) Department of Biological Sciences, University of São Paulo, Bauru, Brazil; 9) ECLAMC (Latin American Collaborative Study of Congenital Malformations) at CEMIC (Center for Medical Education and Clinical Research), Buenos Aires, Argentina,; 10) ECLAMC at INAGEMP-CNPq (National Institute of Population Medical Genetics) at CEMIC, Buenos Aires, Argentina; 11) ECLAMC at Hospital de Area El Bolson, Rio Negro, Argentina; 12) ECLAMC at Imbice (Multidisciplinary Institute of Cellular Biology), La Plata, Argentina,; 13) ECLAMC at INAGEMP-CNPq (National Institute of Population Medical Genetics) in Department of Genetics, Osvaldo Cruz Foundation, Rio de Janeiro, Brazil,; 14) ECLAMC (Latin American Collaborative Study of Congenital Malformations) at INAGEMP (National Institute of Population Medical Genetics) at Department of Genetics, Institute of Biology, Center of Health Sciences, Federal University of Rio de Janeiro, Rio de; 15) Department of Pediatric Dentistry, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, United States.

Oral-facial clefts (OFC) have a multigenic etiology that involves many different signaling pathways. We recently reported that families segregating OFCs present an increased history of cancer when compared to control families, and that AXIN2 is associated with OFCs in a US population. Since Axin2 is an important negative regulator of the Wnt pathway, and the Wnt pathway is integral to craniofacial development, we expanded our initial study to explore the association of AXIN2 markers with OFCs in 724 cleft families from North America, Europe, Asia, and Latin America. Thirteen AXIN2 SNPs were genotyped using TaqMan chemistry and tested for association with OFC using the Family-Based Association Test (FBAT). An association between SNP rs7224837 and OFC was identified in the pooled populations ($P=0.001$), while SNP rs3923086 showed association with Chinese families from Beijing and Shanghai presenting cleft lip and palate individuals only ($P=0.004$). We found statistical evidence of interaction between these AXIN2 SNPs and IRF6 SNPs rs2235371 and rs642961 ($P<0.05$). We assessed mouse Axin2 expression during mouse palatogenesis, and also detected co-localization of Axin2 and IRF6 proteins in the palate, oral and molar tooth epithelia. Taken together, our results support an important role for AXIN2 during craniofacial and palatal development. Further, these results provide insights into potentially etiologic AXIN2-IRF6 interactions that may contribute to the etiology of OFCs in humans.

989W

Refining the 2p15-p16 Microdeletion Syndrome. *R. Lozano, W. Wilcox.* Lozano, Reymundo. Cedars Sinai, Los Angeles, CA.

In 2007 Rajcan et al., reported the clinical and molecular cytogenetic findings of two individuals with a similar microdeletion at 2p15p16. Additional 4 more patients have been reported. We report here the youngest case that represents the earliest development phenotype, with one of the smallest 2p15-16.1 microdeletions, that helps to narrow the critical region previously suggested by Felix et al. to 0.08KB (Ch:2:61.24-61.32) containing 4 genes. CLINICAL REPORT: The patient is 2 year-old Mexican-boy born to non-consanguineou parents. He is developmentally delayed sitting at 1.2 years, does not walk unsupported, and has only a few spoken words. At 25 months old, his height is 85cm (25th percentile), weight of 10.3kg (below 5th percentile), head circumference of 45cm (5 standard deviations below the mean for age). He is microcephalic and has bitemporal narrowing, low anterior hairline, a wide and prominent nasal bridge, thick-bushy eyebrows, long eyelashes, epicanthal folds and ptosis. CYTOGENETIC AND FISH ANALYSIS: Array CGH was performed and showed a 1.11 MB deletion includes four OMIN genes (BCL11A, REL, PUS10, and PEX13) and 7 other genes (PAPOLG, FLJ16341, KIAA1841, LOC339803, C2orf74, AHSA2 and USP34). DISCUSSION: This is the sixth report of a patient with 2p15-16.1 microdeletion syndrome. The child described here has a clinical history, facial features and associated malformations similar to the cases reported (table 1). The 1.1 Mb loss detected in our patient falls within the critical region previously suggested by Felix et al. (2010) and overlaps only a small region of the case with the smallest deletion reported by E. Chabchoub et al; The deletion of the present report is the first that excludes XPO1 and SNORA70B, refining the critical region and leading us to hypothesized that the genes contain in this small region are causative of the typical dysmorphic features and cognitive impartment. The genes contain in the critical region are KIAA1841, C2orf74, AHSA2 and USP34. Only deletions In the C2orf74 gene have been describe as a normal variants of unknown clinical significance. Little information is known about these genes and their functions. The KIAA1841 gene has significant higher expression in brain. We suggest KIAA1841gene as a good candidate gene not only for the neurodevelopment condition, but also the dysmorphic features, since it is expressed in human fetal brain, adult whole brain, the hippocampus and amygdala.

990W

Microduplication on chromosome 17q23.1q23.2 involving TBX4 is not a common cause of nonsyndromic clubfoot. *W. Lu[1], C.A. Bacino[2], S. Richards[3], J.T. Hecht[1].* 1) University of Texas Medical School, Houston, TX; 2) Baylor College of Medicine, Houston, TX; 3) Texas Scottish Rite, Dallas, TX.

Clubfoot is a common birth defect characterized by inward posturing and rigid downward displacement of one or both feet. It occurs as an isolated birth defect in 1/700-1000 newborns in the US each year or as an associated anomaly in more than 200 syndromes. The etiology of syndromic forms of clubfoot is varied and the causes of isolated clubfoot are not well understood. A microduplication of 2.2 Mb on chromosome 17q23.1q23.2 and including TBX4, a hind limb-specific gene and 16 other genes was recently identified in 3 of 66 families with reported nonsyndromic clubfoot. While other malformations were identified in some of the family members who also had this microduplication, other family members with the microduplication reportedly had no malformations suggesting that there was nonpenetrance. To determine whether this microduplication was a common cause of nonsyndromic clubfoot, 609 probands (from multiplex and simplex families) with nonsyndromic clubfoot where evaluated by copy number and oligonucleotide array CGH testing. Only one multiplex family with 12 affected individuals had a 350kb microduplication, which included the complete duplication of TBX4 and NACA2 and partial duplication of BRIP1. The microduplication was transmitted in an autosomal dominant pattern of inheritance and all those with the microduplication had either severe clubfoot or short wide feet and short toes. Hands and stature were normal. These results suggest that there is variable expressivity associated with this microduplication and that modifier genes may be affecting whether there is a clubfoot or milder foot phenotype. The 17q23.1q23.2 microduplication occurred in only 0.16% of our isolated nonsyndromic clubfoot probands indicating that this microduplication is not a frequent cause of this common orthopedic birth defect.

991W

Association Analysis of *MSX1*, *AXIN2*, and *PAX9* with Palatally Displaced Canines in Subjects Exhibiting No Lateral Incisor Agenesis . *L.A. Morford[1, 2], D.L. Kujak[3], G. Falcão-Alencar[2], D.W. Fardo[4], J.V. Macri[5], J.K. Hartsfield, Jr.[2, 3].* 1) Deptartment of Oral Health Practice, Univ of Kentucky College of Dentistry, Lexington, KY USA; 2) Center for Oral Health Research, Univ of Kentucky College of Dentistry, Hereditary Genomics Laboratory, Lexington, KY USA; 3) Division of Orthodontics, Univ of Kentucky College of Dentistry,Lexington, KY USA; 4) Department of Biostatistics, Univ of Kentucky, College of Public Health, Lexington, KY USA; 5) Department of Orthodontics and Oral Facial Genetics, Indiana University School of Dentistry, Indianapolis, IN USA.

Objective: Palatal displaced canines (PDCs) are maxillary canines that are developmentally displaced to the palate, frequently resulting in impaction. PDCs can occur concurrently with several other tooth anomalies including: small or peg-shaped lateral incisor(s) and lateral incisor agenesis. This simultaneous occurrence of PDCs with multiple incisor anomalies suggests that incisor development may influence canine eruption patterns. This study investigated the hypothesis that genetic variation in tooth development genes such as *MSX1*, *AXIN2,* and *PAX9* could influence the formation of PDCs when lateral incisors were present. **Methods:** DNA was isolated from the buccal swabs of 136 Caucasian subjects undergoing orthodontic treatment (29 subjects with unilateral PDCs, 10 with bilateral PDCs and 97 controls). TaqMan-based® SNP genotyping of *MSX1* (rs12532), *AXIN2* (rs7591, rs2240308, rs3923086), and *PAX9* (rs10141087, rs17176643) was completed on the Roche LightCycler480®. PDC phenotypes were determined by treatment records and radiographs. Hardy-Weinberg Equilibrium (HWE) and Chi-Square analysis for association were performed assuming a co-dominant mode of inheritance with significance at ($p<0.05$). **Results:** PDCs were identified more often in women than men (1.8-to-1). All genotypes tested maintained HWE within the control population. *MSX1* rs12532 was analyzed in 38 PDC subjects (18AA/17GA/3GG) and 96 controls (43AA/44GA/9GG) with no association identified ($p=0.95$). *AXIN2* rs7591 was analyzed in 38 PDC subjects (10TT/19TA/9AA) and 96 controls (37TT/47TA/12AA) with no significant association ($p=0.19$). Association analyses for the *AXIN2* SNPs, rs3929086 and rs2240308, were also not significant ($p=0.55$ and 0.72, respectively). *PAX9* rs17176643 was analyzed in 36 PDC subjects (18CC/17CA/1AA) and 89 controls (37CC/39CA/13AA) with no association ($p=0.16$). Similarly, no association was observed for *PAX9* rs10141087 ($p=0.98$). **Conclusions:** This study presents no evidence that *MSX1*, *AXIN2* and *PAX9* play a role in PDC formation in the presence of intact lateral incisors. A minimal number of DNA markers were tested, however, and portions of the genes tested cannot be excluded from involvement as they are not in linkage disequilibrium blocks represented by the markers tested. **This study was supported by the Indiana University Bixler Fund for Research in Genetics, the Southern Association of Orthodontics and the UK College of Dentistry E. Preston Hicks Endowed Chair (JKH).**.

992W

Comparison of neoplastic phenotypes in Cowden/Cowden-like syndrome characterized by germline *SDHx* variants, *PTEN* mutations versus *KLLN* epimutations. *Y. Ni[1,2], MH. Tan[1], K. Bennett[1], J. Mester[1], J. Chen[1], J. Moline[1], M. Orloff[1], C. Eng[1,2,3].* 1) Genomic Medicine Institute, Cleveland Clinic, Cleveland, OH; 2) Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine, Cleveland, OH; 3) Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH.

Cowden syndrome (CS), a Mendelian autosomal-dominant disorder, predisposes to breast, thyroid, and other cancers. As a heterogenous disease, many patients have some features of CS but do not meet diagnostic criteria (CS-like). Therefore, identifying and characterizing susceptibility genes are important for diagnosis, risk assessment, predictive testing and medical management. In addition to germline *PTEN* mutation, variations in mitochondrial complex II succinate dehydrogenase genes (*SDHx*), and germline epimutation in *KLLN* have also been associated with CS/CS-like. In this study, we sought to compare the frequencies and clinical characters of *SDHx* variants to the alterations in *PTEN* and *KLLN* in CS/CS-like patients. We analyzed *SDHB/C/D* genes in 598 *PTEN* mutation negative adult CS/CS-like patients and identified 47 (7.9%) with *SDHx* germline mutations/variants: 15 in *SDHB* [A3G (n=1), R27G (1), H57R (1), N120S (1), and S163P (11)], 1 in *SDHC* [A66V], and 31 in *SDHD* [G12S (18), H50R (12), and H145N (1)]. Among these variant carriers, 27 (57.4%) presented with breast carcinoma, 24 (51.1%) with thyroid cancer, and 4 (6.4%) with renal cancer. Compared to *PTEN* mutation carriers, *SDHx*-related CS/CS-like individuals have increased prevalence of breast cancer (57.4% vs 32.4%, p=0.003) and thyroid cancer (51.1% vs 25.7%, p=0.002). Interestingly, *SDHx*-associated thyroid cancers are papillary (22/24, 91.7%) compared to follicular in *PTEN* mutation carriers (33%, p<0.001). The prevalence of renal cancer in *SDHx* variant carriers is similar to *PTEN* mutation carriers. *KLLN* epimutation were found in 45/123 (36.6%) *PTEN* mutation-negative patients (Bennett et al., JAMA2010). While breast cancer prevalence is much higher compared to *PTEN* mutation carriers (80% vs 32.4%, p<0.001), *KLLN* epimutation positive patients had similar frequencies of thyroid cancer but overrepresentation of renal cancer compared to *PTEN* mutation carriers (4/45 vs 6/155, p= 0.004). In conclusion, *PTEN*, *SDHx*, and *KLLN* germline alterations together account for >50% of CS/CS-like individuals but with different neoplasia risks. In contrast to *PTEN* mutations, *SDHx* variants confer higher breast cancer and papillary thyroid cancer risks, while *KLLN* germline methylation associates with higher breast and renal cancer risks. Therefore, genetic testing for these genes should be prioritized based on patients' specific clinical presentations. Gene-specific surveillance and management should be considered in the future.

993W

Analyzing the contribution of GTF2IRD1 to Williams-Beuren syndrome: mouse knockout phenotypes and molecular mechanisms. *S.J. Palmer[1], J. Widagdo[1], K.M. Taylor[1], S. Bontempo[2], M. Howard[3], A.C. Wong[4], G.D. Housley[4], A.J. Hannan[5], P.W. Gunning[5], E.C. Hardeman[1].* 1) School of Medical Sciences, Neuromuscular and Regenerative Medicine Unit, University of New South Wales, Sydney, Australia; 2) Muscle Development Unit, Children's Medical Research Institute, Sydney, Australia; 3) Howard Florey Institute, University of Melbourne, Melbourne, Australia; 4) School of Medical Sciences, Translational Neuroscience Facility, University of New South Wales, Sydney, Australia; 5) School of Medical Sciences, Oncology Research Unit, University of New South Wales, Sydney, Australia.

Williams-Beuren syndrome (WBS) results from a hemizygous microdeletion within chromosome 7q11.23 involving 28 genes. Its features typically involve characteristic physical abnormalities and a set of cognitive and behavioural symptoms that are collectively called the Williams syndrome cognitive profile (WSCP). The WSCP includes reduced IQ, a severe visuospatial construction deficit, decreased social anxiety, increased non-social anxiety and defects in motor coordination. The high accordance of these features in WBS, irrespective of social or ethnic background, indicates the existence of a consistent biological defect in brain function with an identifiable genetic origin. Genotype/phenotype correlations in patients with atypical deletions have mapped the characteristic craniofacial dysmorphology and the WSCP to a pair of genes that encode the evolutionarily-related transcriptional regulators GTF2IRD1 and GTF2I. We have generated *Gtf2ird1* knockout mouse lines that show some striking similarities to aspects of the human disease, including craniofacial abnormalities, loss of motor coordination, hearing defects and behavioural disturbances. To understand the cellular origin and the molecular basis of these defects, we have conducted detailed studies of *Gtf2ird1* gene expression and analysed the biochemical properties of the GTF2IRD1 protein with respect to its evolutionary conservation, DNA binding properties and its interactions with other nuclear proteins. We have demonstrated that GTF2IRD1 interacts directly with a protein that belongs to a histone deacetylase complex and undergoes post-translational modification through attachment of SUMO via interactions with PIASX and UBC9. These data have begun to reveal a complex interplay between nuclear proteins that explain the observed gene silencing effects of recombinant GTF2IRD1 in vivo. Evidence also suggests that the GTF2IRD1 protein is subject to exquisitely tight control by auto-regulation of gene transcription, SUMOylation and degradation via ubiquitin-mediated proteasomal degradation. Therefore, it is predicted that haploinsufficiency of GTF2IRD1 due to hemizygous loss of one GTF2IRD1 allele would result in unintended increases of transcription from GTF2IRD1 target genes. These data strongly support the genotype/phenotype mapping correlations from human patients and indicate that GTF2IRD1 is a major contributor to the features of WBS.

994W

HoxA9 regulatory SNP is associated with clubfoot and alters gene expression. *T. Powell[1], K.S. Weymouth[1], S. Richards[2], C.V. Patel[3], S.H. Blanton[4], J.T. Hecht[1].* 1) University of Texas Medical School, Houston, TX; 2) Texas Scottish Rite Hospital, Dallas, TX; 3) University of South Carolina School of Medicine, Columbia, SC; 4) University of Miami Miller School of Medicine, Miami, FL.

Clubfoot, a common orthopedic birth defect, is characterized by inward and rigid downward displacement of one or both feet. It occurs in 1/700-1000 newborns and affects 135,000 worldwide each year. Calf musculature is underdeveloped at birth and remains hypoplastic even after corrective treatment. Clubfoot is a complex disease involving both genetic and environmental factors. Despite many studies, the genetic causes of this birth defect are just now being defined. Previously, we found positive associations with multiple SNPs in the HoxA and HoxD gene families. The HoxA family of genes play important roles in limb and muscle development suggesting an etiologic role in clubfoot. One SNP, rs3801776, located in the basal promoter of HoxA9 was significantly overtransmitted in family-based discovery (p= 0.002) and validation (p=0.03) datasets. In silico analysis predicted that the alternate allele of rs3801776 was in a DNA binding site. Since myogenesis occurs in two stages, in undifferentiated and differentiated muscle cells, all expression studies are being performed in these cell types. EMSA analysis in undifferentiated and differentiated mouse muscle nuclear extracts showed that the ancestral allele and not the alternate allele, as suggested by in silico analysis, creates a binding site. Higher gene expression was observed with the ancestral allele in undifferentiated C1C12 mouse muscle cells. These results suggest that variation in HOXA9 may confer differential gene expression during limb and muscle development, which in concert with variants in other genes, may perturb limb and muscle development and contribute to clubfoot. Moreover, these findings suggest that gene dysregulation of limb and muscle-specific genes is a potential common mechanism for clubfoot.

995W

Investigation of molecular basis of X-linked mandibulofacial dysostosis with cleft palate. *N. Sobreira[1], A. Telegrafi[1], L. Cirulli[2], P. Pichurin[3], D. Avramopoulos[1], M. Gunay-Aygun[1], A. Hamosh[1], D. Valle[1].* 1) McKusick Nathans Institute of Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 2) Center for Human Genome Variation, Duke University School of Medicine, Durham, NC; 3) University of Texas Southwestern Medical Center, Dallas, TX.

Clefts of the lip and/or palate (CLP) are common congenital malformations with complex inheritance and affect approximately 1 in 700 live births. Approximately 50% of cleft palate only (CLO) are considered to be non-syndromic and most cases appear to be sporadic. The remaining cases include Mendelian syndromes, chromosomal abnormalities and teratogenic effects. Among the Mendelian syndromes with CPO a few are X-linked with known molecular basis. On the other hand, most of the mandibulofacial dysostosis (MFD) patients present with CLP, but there is no description of an X-linked example. Here we describe a 4 generation X-linked pedigree with 7 males affected with MFD with micrognathia, CPO, malar hypoplasia, and, in some cases, microtia and ear abnormalities. The proband and an affected cousin had normal skeletal survey, echocardiogram, head ultrasonography, karyotype, and SNP genotyping. Despite the X-linked pattern, we sequenced *TCOF1* and found it normal. Using Agilent Sure Select X exome capture and next-generation sequencing, we performed the X chromosome exome sequencing in the proband. Library preparation was performed using SPRIWorks system. We performed a 75 bp paired-end sequencing of the captured fragments in one flowcell lane of an Illumina Genome Analyzer IIx. We aligned sequence reads to the 1000 genomes NCBI36/hg18 human reference using BWA software (version 0.4.9). We found no variants of obvious functional significance (missense, nonsense, splice-site or indels) in the genes associated to X-linked syndromes associated to CLP (*PHF8, BCOR, EFNB1, FLNA, PQBP1, TBX22, and RBM10*). We also identified no functional variants in 3 X-linked genes encoding proteins known to interact to *TCOF1 (OFD1, TBL1X*, and *DCX*). We identified a total of 10 candidate missense variants in 9 other X-linked genes, we determined the segregation of these variants in the family and their frequency in 600 controls. Only one variant segregated with the phenotype and was not present in homozygosity or hemizygosity among the controls. We performed a linkage study (MINX) for this variant in the family (2 affected and 10 unaffected individuals) and found the maximum possible LOD score of 1.93 confirming the complete segregation of this variant with the phenotype. Sequencing of this gene in other families with a similar phenotype and utilization of animal model will confirm if mutations in this gene are responsible for this X-linked MFD with cleft palate.

996W

Increased need for medical intervention in children with 22q11.2 deletion (velocardiofacial) syndrome. *E. King[1], K. Stanford[5], B.J. Leech[1,3], H.M. Saal[1,3], J. James[2], S.R. Callahan[4], S. Geraghty[4], I. Sageser[1], R.J. Hopkin[1,3].* 1) Human Genetics, Cincinnati Children's Hospital, Cincinnati, OH., USA; 2) Cardiology, Cincinnati Children's Hospital, Cincinnati OH, USA; 3) 22q11.2 VCFS Center, Cincinnati Children's Hospital, Cincinnati OH., USA; 4) General and Community Pediatrics, Cincinnati Children's Hospital, Cincinnati, OH., USA; 5) Biostats, Cincinnati Children's Hospital, Cincinnati, OH., USA.

Deletion 22q11.2 or velocardiofacial syndrome causes congenital heart defects, palate abnormalities, learning disabilities and many other medical and developmental problems. Medical interventions in infants and young children with VCFS have been documented, but there is little information on these issues in school age children. **Objective**: to compare the number of surgical procedures and hospitalizations required by children with VCFS to children with isolated congenital heart defects (CHD), isolated cleft palate (CP), and children from the local community (LC) with no evidence of genetic syndromes or birth defects. **Methods**: We conducted a retrospective medical record review of 77 children with VCFS, 35 children with CHD, 30 children with CP, and 32 LC followed in our center from ages 5-10 years. Numbers and types of surgical procedures and hospitalizations were compared among the groups using analysis of variance methods. **Results**: 52 of 77 (68%) with VCFS had a congenital heart defect. 53 of 77 (69%) had a structural or functional palate defect. Children with VCFS required a significantly greater number of surgical procedures than children with CHD, CP, and the LC (adjusted $p<0.0001$ each comparison). Overall, 81% of the VCFS cohort required at least one surgical procedure. There was no difference in cardiac procedures between those with VCFS and CHD. More children with VCFS had palate surgery than children with isolated CP ($p=0.009$). Other (non-palate) ENT surgeries were performed on 47% of the VCFS population compared to 27% with CP, 6% with CHD and 0 from LC ($p <.0001$). The number of hospitalizations in the VCFS cohort was significantly greater than for the CHD, CP, and LC cohorts (adjusted $p<0.0001$ each comparison). Children with VCFS spent an average of 3.10 days hospitalized which was significantly longer than children in the other cohorts. The results of this study document that children with VCFS have an increased need for medical interventions between the ages of 5-10 years compared to children with non-syndromic CP and CHD. The most frequent indications for intervention were palate dysfunction or other ENT problems rather than cardiac defects. Increased medical needs continue to be a major burden for children with VCFS at least through middle childhood.

997W

A complex cardiac abnormality associated with deletion of the NOTCH1 gene. *D. Myles-Reid[1], L. Nield[2], P. Shannon[3], R. Teitelbaum[1], M. Thompson[3], D. Chitayat[1, 4].* 1) The Prenatal Diagnosis and Medical Genetics Program, Department of Obstetrics and Gynecology, Mount Sinai Hospital, Toronto, Ontario, Canada; 2) Division of Cardiology, Department of Pediatrics, The Hospital for Sick Children. Toronto, Ontario, Canada; 3) Department of Pathology and Laboratory Medicine, Mount Sinai Hospital. Toronto, Ontario, Canada; 4) Division of Clinical and Metabolic Genetics, Department of Pediatrics,The Hospital for Sick Children. Toronto, Ontario, Canada.

Mutations in the NOTCH1 gene, coding for a receptor in a developmentally important signaling pathway, are known to be associated with left ventricular outflow tract (LVOT) defects including aortic valve stenosis, coarctation of the aorta and hypoplastic left heart syndrome (HLHS). We report a case with a de novo 614.3 Kb interstitial deletion of 9q34.3 including the NOTCH1 gene among others associated with complex cardiac abnormality including LVOT defects. A stillborn male fetus was born at 26 weeks gestation to 30 years old non-consanguineous and healthy parents following fetal echocardiography findings of complex cardiac abnormality including HLHS. The autopsy showed preductal aortic coarctation, anomalous origin of the right pulmonary artery from the right brachiocephalic artery, perimembranous VSD, dysplastic tricuspid valve leaflets, bicuspid and dysplastic pulmonary and aortic valves. The brain showed periventricuar leukomalacia and the placenta showed areas of chronic villous hydrops with cluster of avascular villi. aCGH showed 614.3Kb interstitial de novo deletion of 9q34.3 with haploinsufficiency of the OMIM Morbid Map genes SEC16A, NOTCH1, EGFL7, MIR126, AGPAT2, LCN10, LCN6, LCN8, C9orf86, PHPT1, EDF1, TRAF2, FBXW5, C8G, LCN12, PTGDS, CLIC3, ABCA2, FUT7, NPDC1, ENTPD2, C9orf140 and MAN1B1genes. Mutations in the NOTCH1 gene have been detected in a few families and in sporadic cases with LVOT defects including bicuspid aortic valve, mitral valve atresia and hypoplastic left ventricle as well as double outlet right ventricle. To our best knowledge, this is the first case with a de novo deletion of the NOTCH1 gene associated with a complex cardiac abnormality as well as brain and placental changes. NOTCH1 mutations were observed in leukemia and have been shown to be associated with gain-of-function mutations. In contrast, where ligand dependent activation is reduced, as in our case, a congenital abnormality develops. These findings are typical of developmentally important genes such as NOTCH1.

998W

R179H mutation in ACTA2 expanding the phenotype to include Prune-Belly sequence and skin manifestations. *J. Richer[1], D.M. Milewicz[2], R. Gow[3], J. de Nanassy[4], G. Maharajh[5], E. Miller[6], L. Oppenheimer[7], M. O'Connor[8].* 1) Med Gen, CHEO, Ottawa, Ontario, Canada; 2) Division of Medical Genetics, Department of Internal Medicine, the University of Texas Health Science Center at Houston, Houston, Tex; 3) Department of Cardiology, CHEO, Ottawa, Ontario, Canada; 4) Department of Pathology, CHEO; 5) Department of Cardiovascular Surgery, CHEO; 6) Department of Diagnostic Imaging, CHEO; 7) Division of Maternal Fetal Medicine, University of Ottawa and the Ottawa Hospital Ottawa,; 8) Ophthalmology Department, CHEO.

Prune-Belly is a rare congenital malformation sequence characterized by loose abdominal musculature, urinary abnormalities and undescended testes. The prognosis is highly variable. The incidence is reported to range between 1 in 35,000 to 1 in 50,000 births; only 3-5% of affected patients are female. The proportion of patients with urethral obstruction remains unclear, and while a small proportion of patients have been diagnosed with rare syndromes such as megacystitis-microcolon, the underlying etiology in a number of patients without an identified mechanical obstruction remains unclear. Mutations in ACTA2 (smooth muscle cell - specific isoform of (-actin) lead to a predisposition for thoracic aortic aneurysms and other vascular diseases. The (-actin isoform is primarily expressed in smooth muscle, but is also expressed in myofibroblasts and patients with this mutation have been described to have bladder dysfunction. We report on a patient heterozygous for the ACTA2 R179H mutation who presented with megacystis at 13 weeks gestational age and, at birth, with Prune-Belly sequence. He also had deep skin dimples and creases on his palms and soles, a finding not previously described but may be related to ACTA2. Moreover, he had pulmonary hypertension, a neonatal aneurysm and areas of hyperintensities in the frontal lobes on MRI. To our knowledge, this is the first report of the R179H mutation in ACTA2 in a child with Prune-Belly sequence and we believe that our patient's Prune Belly sequence is caused by very severe smooth muscle dysfunction with multisystemic involvement. We recommend that molecular testing for this mutation be considered in fetuses presenting with fetal megacystis and a normal karyotype.

999W

Congenital diaphragmatic hernia syndromes and involvement of 1q41. *J. Shieh, A. Slavotinek.* Division of Medical Genetics, Department of Pediatrics, University of California San Francisco, CA.

Congenital diaphragmatic hernia (CDH) is often associated with severe co-morbidities and genetic abnormalities that affect diagnosis and management. Alterations in the 1q41 region have been found in individuals with CDH, and deletions and genes in that region have received recent attention. We found an association of congenital diaphragmatic hernia with an expanded set of anomalies and with new molecular findings that may aid in understanding disease.

We identified a case of Morgagni hernia associated with pericardial absence and bilateral cataracts with severe ophthalmologic complications. The patient also had gallbladder agenesis, an association that appears to be novel. To examine a potential genetic basis for disease, we isolated genomic DNA from peripheral blood and performed 102K CGH microarray analysis (ISCA v2 clinical design) and compared this to pooled reference. Regions of copy number variation were compared with annotated copy number variation to determine polymorphic versus potentially unique variation.

CGH analysis revealed a rare duplication of 1q41 that has not been previously reported with this phenotype to our knowledge. We compare these findings to other individuals with CDH to help refine a critical region. These findings and clinical presentation add further support for the involvement 1q41 and add a unique genomic change. We have identified an area that should be targeted in future studies that focus on disease basis and implications for management.

1000W

FIVE CASES REPORT WITH OPITZ G/B.B.B SYNDROME. TWO PATIENTS WITH CHROMOSOMAL ABNORMALITIES; X CHROMOSOME DUPLICATION (47, XXY) AND TRANSLOCATION 46XX t(3q;4q), IN THE HOSPITAL PARA EL NIÑO POBLANO. *J. Aparicio[1,6], M.D.L. Hurtado[2], I. Marroquin[2], G.A. Rojas[2], p. Sanchez[3], S. Rodriguez[4], R. Zamudio[5], no.* 1) Dept Gen; 2) Cytogenetics; 3) Haematology; 4) Neurosurgery; 5) Cardiology, Hosp para el Nino Poblano, Puebla, Mexico; 6) Estomatology, Benemerita Universidad Autonoma de Puebla, Mexico.

Opitz G/BBB syndrome is a genetic condition that affects several structures along the midline of the body. The most common features of this condition are wide-spaced eyes (hypertelorism) with structural defects of the larynx, trachea, and/or esophagus causing breathing problems and difficulty swallowing (dysphagia). Some times in males shows, the urethra opening on the underside of the penis (hypospadias). Mild intellectual disability occurs in 30 percent approximately of patients with Opitz G/BBB syndrome (GBBBS), most likely caused by structural defects in the brain. About half of affected individuals also have cleft lip with or without a cleft palate as in this study. Some have cleft palate alone. Heart defects, an obstruction of the anal opening (imperforate anus), and brain defects such as an absence of the corpus callosum. Facial abnormalities that may be seen in this disorder include a flat nasal bridge, thin upper lip, and low set ears. There are two forms of Opitz G/BBB syndrome, which are distinguished by their genetic causes and patterns of inheritance. The X-linked form of Opitz G/BBB syndrome is caused by a mutation in a specific gene, MID1, on the X chromosome. Autosomal dominant Opitz G/BBB syndrome is caused by a mutation in an as-yet unidentified gene on chromosome 22. Two chromosomal aberrations in this study were observed in two patients, with chromosome duplication 47,XXY and translocation 46,XX t(3;4). However one patient with normal karyotype 46,XY had an oral encephalocele.

1001W

MECP2 duplication (453 Kb) clinical manifestations in a four generation family. *J. Bartley[1], P. Park[2].* 1) Loma Linda University Children's Hospital, Loma Linda CA; 2) Inland Regional Center, San Bernardino CA.

MECP2 duplication was first identified by SNP micorarray in a two year old child with developmental delay. His brother, mother, grandmother and 75 year old great-grandmother also had the same duplication. All but the great-grandmother were consumers of the Inland Regional Center because of a developmental delay or mental retardation of less than 70. All five of the great-grandmothers boys were mentally retarded and had spastic cerebral palsy. Two died at 18 months of age, one died at 10 years of age, and two died at 15 years of age. One of the great-grandmother's daughters died several years ago prior to MECP2 SNP microararry testing but she must have had the duplication since her daughter has the MECP2 duplication. Another daughter of the great-grandmother also has the duplication since heher son has the duplication and is severely mentaly retarded. Each of the females with the MECP2 duplication were mildly mentally retarded, but enough so that the qualified for becoming a consumer of regional center.

1002W

Genetic Medicine: The NIH Undiagnosed Diseases Program Model. *C.F. Boerkoel III, D. Adams, T. Markello, C. Toro, C.J. Tifft, W.A. Gahl.* Undiagnosed Diseases Program and NHGRI, National Institutes of Health, Bethesda, MD.

In classical evolutionary theory, human disease arises by maladaptation of humans to their ecological niche. By definition common diseases arise in response to abrupt changes in the ecological niche exposing genetic variants underlying susceptibility and resistance to the ecological change. Modifying the environment therefore likely best treats common diseases. In contrast, rare diseases, which affect ~8% of the population, frequently arise from strong genetic and epigenetic mutations causing maladaptation within a stable niche. Therapy for rare diseases therefore targets modification of the dysfunctional physiological pathway. Within this context, a precise diagnosis is the first step to understanding illness and defining appropriate therapies. Focusing on ill individuals without diagnoses, the NIH Undiagnosed Diseases Program (UDP) models a synthetic approach to diagnosis involving participatory care, transdisciplinary clinical evaluations, and integration of basic science tools into the clinical diagnostic paradigm. As a consequence of this “research is care” approach, the UDP has been able to provide diagnoses to 15-20% of the patients seen. As a prelude for export of this model to the wider community, we have defined factors most influencing diagnostic ability, mechanisms allowing efficient integration of basic science tools into clinical care, and possible conceptual constructs for effective therapy in the future. In summary, we find that the infrastructure needed for personalized medicine is achievable despite several remaining conceptual hurdles.

1003W

***Orbicularis Oris* Muscle thickness and area in Cleft Lip with or without Cleft Palate Families versus Controls.** *C.A. Brandon, T. Parsons, J. Joseph, M.E. Cooper, R. DeSensi, S.M. Weinberg, K. Neiswanger, K. Schmidt, J.M. Resick, K. Bardi, M.L. Marazita.* Dept Oral Biology, Center for Craniofacial & Dental Gen, Univ Pittsburgh, Pittsburgh, PA.

Cleft lip with or without cleft palate (CL/P), the most common craniofacial birth defect, is characterized by a wide range of phenotypic variability, and often requires ongoing medical, dental, and speech treatment. Our group has shown that in addition to overt CL/P phenotypes, there are also non-visible discontinuities in the upper lip portion of the *orbicularis oris* muscle (OOM) that are found at a higher rate in unaffected relatives of individuals with CL/P than in controls with no known family history of CL/P. In this study we investigated whether thinning of the OOM should also be considered a subclinical phenotype. Subjects for this study were drawn from a large international consortium (the University of Pittsburgh Orofacial Cleft Study) and comprised 16 “cases” - unaffected parents of individuals with CL/P from multiplex families, and 23 controls with no known family history of CL/P. For each study subject, the OOM was visualized using high-resolution ultrasound, then an area including 1 cm on each side of the mid-line (i.e. the philtrum) was outlined by a blinded assessor using a polygon selection tool in ImageJ. In addition, the thickness of each OOM was measured at every mm across the 2cm span using ImageJ, and the resulting 22 thickness measures were averaged. The mean total area for cases was $0.16cm^2 \pm 0.04$ (range 0.07-0.22), and for controls $0.16cm^2 \pm 0.06$ (range 0.06-0.33). The mean average thickness for cases was 0.09cm±0.02 (range 0.04-0.12) and for controls 0.09cm±0.03 (range 0.04-0.17). No significant differences were found between cases and controls for either area or average thickness for the whole OOM. We also compared the case/control OOM areas and thicknesses separately by left and right sides, and by whether or not the case parents had an additional relative with CL/P. No significant differences were found for any of the comparisons (all p-values>0.50). Therefore although we have previously established that discontinuities in the OOM are part of the phenotypic expression of CL/P risk factors, there is no evidence to add thinning of the OOM as an additional sub-clinical phenotype. Support: DE016148.

1004W

***CYP1B1* mutations in Spanish families with primary congenital glaucoma: identification of six novel mutations and association with early diagnosis.** *J. Escribano[1,2], M.P. Lopez-Garrido[1,2], L. Morales[2,3], J.M. Martinez-de-la-Casa[2,3], M.T Garcia-Anton[2,3], J. Garcia-Feijoo[2,3].* 1) Laboratorio de Genética Molecular Humana, Facultad de Medicina/Instituto de Investigación en Discapacidades Neurológicas, Universidad de Castilla-La Mancha, Albacete, SPAIN; 2) Cooperative Research Network on Age-Related Ocular Pathology, Visual and Life Quality, Instituto de Salud Carlos III, Madrid, SPAIN; 3) Servicio de Oftalmología, Hospital Clínico San Carlos, Madrid, SPAIN.

Primary congenital glaucoma (PCG; MIM# 231300) is usually transmitted as an autosomal-recessive trait with incomplete penetrance and is produced by developmental defects of the trabecular meshwork and the anterior chamber angle of the eye, which leads to aqueous outflow obstruction, elevated intraocular pressure and optic nerve damage. PCG is the most common childhood glaucoma and is a significant cause of visual loss in children. Three loci linked to PCG have been identified, named GLC3A (2p21), GLC3B (1p36) and GLC3C (14q24.3). Mutations in gene *CYP1B1* (MIM# 601771) are the main known genetic cause of this type of glaucoma in different worldwide populations. In a previous study we have shown that approximately 1/3 of Spanish PCG patients carry loss-of-function *CYP1B1* mutations. Here we have extended our initial work to determine the contribution and spectrum of CYP1B1 gene mutations to primary congenital glaucoma (PCG) in Spanish patients and to analyze the genotype-phenotype correlation. We analyzed by PCR DNA sequencing the presence of promoter (-1 to -867) and exon *CYP1B1* mutations in a total of 147 unrelated Spanish families affected by PCG, of which 38 probands were previously reported. The patients were recruited in the Ophthalmology Department of the “Hospital Clínico San Carlos”, Madrid, Spain. A total of 29 different mutations were identified in 52 (35.3%) index cases, most of which were compound heterozygotes (28 probands) and 13 were homozygotes. Interestingly, 8 probands were heterozygotes, suggesting that other genetic or environmental factors determine the disease in these patients. The identified mutations included 16 missense and 4 non-sense nucleotide changes, 9 micro insertions or deletions. To the best of our knowledge, six of the identified mutations were novel. Mutations T404fsX30, E387K, R355fsX69 and A179fsX18 were the most prevalent among index cases, and collectively were present in nearly 90% of patients. Patients carrying 2 mutant alleles showed severe bilateral phenotypes featured by very early PCG onset, usually at birth. Our data corroborate that *CYP1B1* mutations are responsible for approximately one third of PCG cases in Spanish patients. *CYP1B1* associated congenital glaucoma usually presents a severe bilateral phenotype, featured by its frequent diagnosis at birth.

1005W

De novo 5q14.3 deletion in a child with developmental delay, microcephaly, venous malformation and seizures. *A. Guerin[1], R. Babul-Hirji[1], S. Al-Zaidy[2], E. Donner[2], M. Shago[3], D. Chitayat[1,4].* 1) Division of Clinical Genetics, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Division of Neurology, The Hospital for Sick Children, Toronto, Ontario, Canada; 3) Department of Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, Ontario, Canada; 4) The Prenatal Diagnosis and Medical Genetics Program, Department of Obstetrics and Gynaecology, Mount Sinai Hospital; Toronto, ON, Canada.

Microarray technology has been used increasingly in the investigation of cases with unexplained developmental delay, autism spectrum disorders and multiple congenital anomalies and provides a diagnosis in 15-20% of the patients in this group, compared to 3%, using routine chromosome analysis. We present a patient with multiple abnormalities who has been investigated thoroughly since birth and was recently found to have a de novo deletion of 5q14.3. The patient is a 14-year-old girl with developmental delay, microcephaly, venous malformation on the dorsum of the nose, and seizures. She was born to a non-consanguineous couple from Pakistan. Pregnancy history was unremarkable. There were no significant issues in early life with early motor milestones being normal. Delay in milestones became apparent around the age of one, with significant delays in fine motor and speech. Currently, she is unable to read or write. Ophthalmology exam and hearing tests are normal. At the age of 12 seizures were noted and her EEG was suggestive of idiopathic generalized epilepsy and is currently on oral anticonvulsant therapy. She is mildly dysmorphic on examination. Microarray analysis showed a 2.559Mb deletion encompassing the RASA1, CCNH, TMEM161B, and COX7C genes. Many of the genes contained within this regions have an unknown function. RASA1 has previously been described in Parkes-Weber Syndrome and Vein of Galen aneurysm; however, it has not been described in association with developmental delay and seizures. This case highlights the difficulties in providing anticipatory guidance and counseling regarding microarray findings, especially when the patient is developmentally challenged.

1006W

A longitudinal analysis of clinical features in patients labeled cerebral palsy and global developmental delay, define Chronic Complex Disorder syndrome: an undefined clinical entity or genetic disorder? *J. Kapalanga[1,2,3,4], Y. Said[2,6], D. Wong[1,2], A. Gandy[1,2], M. Moyo[4,5], N. Nwebube[3], A. Singh[2].* 1) Dalhousie University, Department of Pediatrics, Halifax, NS, Canada; 2) Summerside Medical Centre, Summerside, PE, Canada; 3) Dept Pediatrics, Genetics, Grey Bruce Health Services, Owen Sound, ON, Canada; 4) Cambridge Memorial Hospital, Cambridge, ON, Canada; 5) Department of Pediatrics, McMaster University, Hamilton, ON, Canada,; 6) Allergy and Pediatric Pulmonology, King Fahad Specialist Hospital, Dammam,Saudi Arabia.

A group of patients commonly given the diagnosis of cerebral palsy and global developmental delay present with a constellation of clinical features which are not due to an identifiable disorder or syndrome. This group of patients also does not share the cardinal features of the developmental encephalopathies. This constellation of clinical features in a group of patients has not been reported previously. We report on a consistent constellation of clinical features in a group of patients identified through a multicenter interdisciplinary collaboration. Patients with multiple medical problems who initially were given the diagnosis of cerebral palsy and global developmental delay were identified through an analysis of clinical records from birth to age10. A total of 38 patients were initially identified. The entire spectrum of clinical features found in each patient over this period was analyzed and clinical features that have been persistent or recurrent beyond the first 3 years of life were recorded. A group of 15 patients aged 5 to 10 years were found to have a consistent constellation of clinical features that included; onset of symptoms and signs during infancy, no identifiable perinatal or prenatal insult, delivery after 35 weeks of gestation, developmental delay, mental retardation, feeding difficulties, gastroesophageal reflux disease, seizures, neurological deficit, no specific brain imaging findings, behavioral difficulties, absence of dysmorphic features, and normal routine clinical laboratory tests, chromosome studies and biochemical studies. Infrequent clinical features include recurrent respiratory difficulties, growth retardation, chronic constipation, sleep difficulties and musculoskeletal problems. We propose that this constellation of clinical features is unique and represent a distinct disorder caused by a mutation in a specific gene or genes at different loci. Patients with these clinical features are suitable for whole genome analysis. We further propose that patients with this constellation of clinical features should be given the diagnosis of Chronic Complex Disorder (CCD) syndrome. We suspect that certain infants who are often given multiple diagnoses that include cerebral palsy and or developmental delay, have CCD syndrome and suggest a systematic analysis of their clinical features for rendering the diagnosis of CCD syndrome.

1007W

Consanguinity as a means to identify pathogenic recessive mutations. *P. Makrythanasis[1], M. Nelis[1], M. Guipponi[2], F. Béna[2], S. Gimelli[2], S. Temtamy[3], A. Megarbane[4], M. Aglan[3], M. Zaki[5], S. Fokstuen[2], L. Gwanmesia[2], A. Masri[6], S. Psoni[7], S. Kitsiou[7], H. Frissyra[7], E. Kanavakis[7], N. Al-Allawi[8], N. Akarsu[9], S. AlHait[10], S. Al-Arrayed[11], I. Al Rashdi[12], H. Hamamy[1], S.E. Antonarakis[1,2].* 1) Dept Genetic Medicine & Dev, University of Geneva, Geneva, Switzerland; 2) Service of Genetic Medicine, University Hospitals of Geneva, Geneva, Switzerland; 3) National Society of Human Genetics , Cairo, Egypt; 4) Université Saint Joseph Campus des Sciences Médicales Unité de génétique médicale , Beirut, Lebanon; 5) Human Genetics and Genome Research Division, National Research Centre, Cairo, Egypt; 6) Pediatric Department. Jordan University Hospital, Amman, Jordan; 7) Laboratory of Medical Genetics, University of Athens Medical School, Athens, Greece; 8) Hematology, Dohuk Faculty of Medicine, Dohuk, Iraq; 9) Genetics, Hacettepe University Medical Faculty, Turkey; 10) Medical Genetics, Al-Amal Hospital, Amman, Jordan; 11) Medical Genetics, Salmaniya Hospital, Bahrain; 12) Clinical Genetics Service, The Royal Hospital, Sultanate of Oman.

Study aim: To define a strategy for the identification of pathogenic variants in patients of consanguineous families. Patient Collection: We have initiated a project to collect samples from families with seemingly recessive phenotypes in consanguinity families. 22 families of different ethnic background (mainly from countries where consanguineous marriages are common) are already participating in the study. From each family, DNA from the patient(s), all their unaffected siblings, and the parents is extracted from blood samples. Any phenotype and family history compatible with autosomal recessive disorder is candidate for participation in the study, which has been approved by the local ethics committee and all participants or their legal representatives have signed an appropriate consent form. Strategy for the detection of the pathological mutation(s): Samples from one or more of the affected individuals per family are examined by aCGH 244K chips for the identification of homozygous deletions and/or pathogenic heterozygote deletions/insertions. All samples per family are genotyped with a dense SNP array (720K) in order to identify the Runs of Homozygosity (ROH), follow the chromosomal haplotypes and define chromosomal regions likely to contain the responsible genes. Exome sequencing (96% of CCDS) is performed in one of the affected individuals. Variants are called genome wide but particularly in the ROH regions and filtered according to polymorphic SNVs deposited in public databases; variants' pathogenicity is also scored using several publicly available software. A step-wise approach has been selected in order to diminish the total number of probable variants. ROH found only in the patients and no other family member are screened first, followed by different comparisons of ROH between the affected individuals and the other family members. Results: The causative variation has been identified in one family (VLDLDR gene, causing disequilibrium syndrome). More likely pathogenic variants will be presented. Consanguineous families provide an opportunity to identify genes responsible for recessive phenotypes and rapidly fill in the space of genotype-phenotype links.

1008W

Transient neonatal diabetes: case report of a long term follow-up. *M.C. Moreira[1], C.R.D.C Quaio[1], I. Gomy[1], A. Ino[2], M.E. Ceccon[2], T. Della Manna[3], A.V. Morgante[4], D.R. Bertola[1], C.A. Kim[1].* 1) Genetics Unit - Pediatrics, Faculdade de Medicina da Universidade de São Paulo, São Paulo - SP, SP, Brazil; 2) UCINE - Pediatrics, Faculdade de Medicina da Universidade de São Paulo, São Paulo - SP, SP, Brazil; 3) Endocrinology Unit - Pediatrics, Faculdade de Medicina da Universidade de São Paulo, São Paulo - SP, SP, Brazil; 4) Biology Department, Universidade de São Paulo.

Transient neonatal diabetes (TND) is a rare condition affecting neonates within the first weeks of life, with an estimated incidence of 1 in 400,000 neonates. Evidence has suggested that overexpression of imprinted and paternally inherited genes within the TND critical region at 6q24 may develop TND, as well as other imprinting defects, duplication of this region and mutations in genes as *KCNJ11* and *ABCC8*. We report on long term follow-up of a case presenting TND with documented paternal uniparental disomy (pUPD) of chromosome 6. The Caucasian male patient was born at gestational age of 36 weeks, weighting 2060g and with unremarkable family history for diabetes mellitus (DM). At initial evaluation, he did not present any sign of perinatal asphyxia, demonstrated good suck, normal tonus and had no dysmorphic facial features except for macroglossia. After six hours from birth, he presented an isolated episode of hypoglycemia, thus glucose infusion was started. Following this, he developed hyperglycemia refractory to the suspension of glucose infusion and plasma glucose levels remained high (range: 302-558 mg/dl) for the next 15 days. Normal glycemic control was only achieved by insulin therapy. The treatment was based on a mixed regimen with NPH insulin (0.2-0.7 U/Kg) plus Lispro (0.08 U/Kg), which was needed for the subsequent three months. Thereafter, glycemic control normalized and insulin was discontinued. Nowadays, the patient, a healthy eleven-year-old boy with normal anthropometric measurements and development, has no signs of hyperglycemia or macroglossia. Molecular analysis demonstrated homozygosity in all of the paternally inherited markers of chromosome 6, confirming pUPD. Progress has been made in establishing the etiology of TND, accounting for 50% of neonatal DM. The 6q24 region, that harbors genes enrolled in regulating the secretion of insulin by) -cell, plays a key role in approximately 70% of cases with TND. Suspicion of TND must be raised when growth restriction and hyperglycemia are present. Rehydration and insulin therapy are usually required at the time of diagnosis. Although remission may occur spontaneously after some months of life, as described here, there is a tendency for the child to develop diabetes afterward. Therefore, in spite of our patient has not presented any signs of recrudescence of diabetes after eleven years of follow-up, close surveillance will be maintained.

1009W

Fetal Akinesia in Metatropic Dysplasia: Combination between Chondrodysplasia and Neuropathy? *S. Unger[1], E. Lausch[2], F. Stanzial[3], G. Gillesen-Kaesbach[4], C. Di Stefano[5], E. Bertini[6], C. Dionisi-Vici[7], B. Nilius[8], B. Zabel[2], A. Superti-Furga[9].* 1) Service de Génétique Médicale, CHUV, Lausanne, Switzerland; 2) Centre for Pediatrics and Adolescent Medicine, Freiburg University Hospital, Freiburg, Germany; 3) Servizio Multizonale di Consulenza Genetica, Bolsano, Italy; 4) Institut für Humangenetik, Lübeck, Germany; 5) Ospedale Umberto I Nocera Inferiore, Salerno, Italy; 6) Molecular Medicine, Institute from Hospital Care to Science (IRCCS), Rome, Italy; 7) Division of Metabolism, Bambino Gesù Children's Hospital, Rome, Italy; 8) Department of Molecular Cell Biology, Leuven, Belgium; 9) Department of Pediatrics, Lausanne, Switzerland.

Heterozygous mutations in the TRPV4 gene have been associated with a family of skeletal dysplasias (metatropic dysplasia, Pseudomorquio type 2, SMD Kozlowski, and brachyolmia) as well as with dominantly inherited neuropathies (hereditary motor and sensory neuropathy 2C,scapuloperoneal spinal muscular atrophy (SMA), and congenital distal SMA). While there is phenotypic overlap between the various members of each group, the two groups were considered to be separate with the former being strictly a structural skeletal condition and the latter group being confined to the nervous system. We report here on fetal akinesia as the presenting feature of severe metatropic dysplasia,suggesting that certain TRPV4 mutations can cause both a skeletal and a neuropathic phenotype. Case 1 was diagnosed at week 20 with short limbs, absence of limb movements, elbow pterygia, and finger contractures. Cases 2 and 3 (twins) were observed to have short limbs with arthrogryposis and akinesia at week 20. Both pregnancies were interrupted and fetal radiographs suggested metatropic dysplasia. Case 4 presented with joint contractures and absent limb movements at birth; skeletal survey showed typical features of metatropic dysplasia. The baby died of respiratory complications at age 4 months. Sequencing of the TRPV4 gene confirmed the presence of de novo heterozygous mutations predicting G78W (case 1), T740I (cases 2 and 3), and K276E (case 4). Although some degree of restriction of movements is not uncommon in fetuses with skeletal dysplasia, akinesia as leading sign is unusual and may indicate that certain TRPV4 mutations produce a severe “combinatorial” phenotype.

1010W

A novel SHH missense mutation causing variable phenotypes in familial holoprosencephaly. *D. Wang, M. Huggins, P. Mohide, C. Li.* McMaster University Medical Center, Hamilton, Canada.

Holoprosencephaly (HPE) is incomplete separation of the forebrain during early embryogenesis, resulting in craniofacial anomalies, midline defects, and neurocognitive delay. Clinical phenotypes vary from very mild to embryonic lethal. The underlying etiologies for HPE include chromosomal anomalies, teratogens and monogenic disorders. One of the genes associated with monogenic HPE is the Sonic hedgehog (SHH) gene whose protein product has been shown to play a crucial role in CNS development. Here we report a novel missense mutation in the SHH gene in a family with strikingly variable phenotypes. The proband was a 38-year-old gravida 4 at 19 weeks of gestation with a pair of fraternal twins. Her first pregnancy resulted in a live born female who became an adult and accompanied the proband to this visit. The second baby was a male with facial clefting, hypotelorism and holoprosencephaly. He expired at 11 months of age. The third pregnancy was terminated after ultrasound detected alobar holoprosencephaly in a female fetus at 13 weeks of gestation. On inspection, the proband and her 20 year-old daughter were of short stature (149.86cm and 139.7cm, respectively). Both appeared to be borderline hypoteloric. The proband also had questionable single central incisor although the daughter did have two incisors and the central frenulum. Genetic testing was discussed but they elected not to proceed as the proband's presenting twin pregnancy had normal ultrasound findings at 19 weeks of gestation. The daughter subsequently became pregnant, and ultrasound revealed semilobar holoprosencephaly and midline facial cleft at 19 weeks of gestation. Following pregnancy termination, genetic testing was undertaken. A novel missense mutation [c.494C>A, A165E] in the SHH gene was found in the fetus. This missense mutation represents amino acid substitution of a large glutamic acid residue for a smaller alanine residue at a position in the SHH protein that is highly conserved across species, and is therefore very likely to be the disease causing mutation. Subsequent testing of the proband, her fetus and the daughter proved that the mutation tracked with HPE phenotype. Our report draws attention to this novel mutation and highlights the extremely variable phenotypes associated with this mutation, from the very mild subclinical to lethal alobar HPE.

1011W

Clinical and molecular characteristics of Noonan syndrome among Arabs. *D.M. AlJeaid, J.Y. Al-Aama.* Department of Genetic Medicine, King Abdulaziz University, Jeddah, Saudi Arabia.

Noonan syndrome is a clinically and genetically heterogeneous multisystem autosomal dominant disorder. It is relatively common with a prevalence of 1 in 1000 to 1 in 2500 live births. It is characterized by short stature, distinctive facial dysmorphism, congenital heart defects, most commonly pulmonary stenosis and cardiomyopathy, and developmental delay of variable degrees. Other findings can include broad or webbed neck, chest deformity, cryptorchidism, varied coagulation defects, lymphatic dysplasias, and ocular abnormalities. So far, heterozygous mutations in nine genes (PTPN11, SOS1, RAF1 KRAS, NRAS, BRAF, MEK1, MEK2, HRAS and SHOC2) have been documented to underlie this disorder or clinically related phenotypes. We believe that Noonan syndrome is somewhat unrecognized and under diagnosed in Saudi Arabia. Also,almost nothing is published about the characteristics of Noonan syndrome in Arabs. Although the majority of patients with Noonan syndrome have an autosomal dominant mode of inheritance, there is thought to be a sub group of patients with the so-called type II Noonan syndrome that is believed to be autosomal recessive. The high rate of consanguinity in the Arab world makes it plausible that the second type of Noonan syndrome may actually be more common. Here, we provide an overview of clinical and molecular aspects of this disorder among Arab patients and major genotype-phenotype correlations. In this study, we aim to characterize the phenotype in Arab patients with Noonan syndrome and identify the most common mutations causing Noonan syndrome and compare it with the world literature. Also, to search for candidate gene for those in which no mutation is found.

1012W

Autoimmune diseases and multiple autoantibodies in a cohort of 43 molecularly proven RASopathy patients. *D.R. Bertola[1], C.R.D.C. Quaio[1], J.F. Carvalho[2], C.A.A. Silva[2, 3], C. Bueno[2], A.S. Brasil[1], A.C. Pereira[4], A.L.L. Jorge[5], A.C. Malaquias[5], C.A. Kim[1].* 1) Pediatrics, Instituto da Criança da Universidade de São Paulo, São Paulo, SP, Brazil; 2) Rheumatology Division - Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo - SP, Brazil; 3) Pediatric Rheumatology Unit, Instituto da Criança - Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo - SP, Brazil; 4) Incor - Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo - SP, Brazil; 5) Endocrinology-Genetics Unit - LIM/25, Discipline of Endocrinology - Faculdade de Medicina da Universidade de São Paulo, São Paulo - SP, Brazil.

Association of RASopathies (Noonan syndrome and Noonan-related syndromes) and autoimmune disorders was sporadically reported. However the concomitant evaluations of autoimmunity diseases and assessment of multiple autoantibodies in a large population of RASopathy with proven mutations were not performed. Clinical and laboratorial features were analyzed in a cohort of 43 RASopathy patients. The following autoantibodies were measured: anti-nuclear antibodies, anti-double stranded DNA, anti-SS-A/Ro, anti-SS-B/La, anti-Sm, anti-RNP, anti-Scl-70, anti-Jo-1, anti-ribosomal P, IgG and IgM anticardiolipin, anti-b2GPI, thyroid, anti-smooth muscle, anti-endomysial, anti-liver cytosolic protein type 1, anti-parietal cell, anti-mitochondrial antibodies and anti-liver-kidney microsome type 1 antibodies, and lupus anticoagulant. At least one autoimmune disease complaint was found in 35 (81%) patients, with 14% of autoimmune diseases (systemic lupus erythematous, polyendocrinopathy - autoimmune thyroiditis and celiac disease, primary antiphospholipid syndrome, autoimmune hepatitis, vitiligo and autoimmune thyroiditis). Autoimmune antibodies were observed in 51%. Remarkably, 3 (7%) of them had specific gastrointestinal and liver autoantibodies without clinical findings. Autoimmune diseases and autoantibodies were frequent in Noonan and Noonan-related syndromes. The careful anamnesis and physical examinations for rheumatic symptoms should be seriously considered and followed with proper laboratory investigations. Importantly, the presence of gastric and liver antibodies in RASopathy may predict the occurrence of future autoimmune gastritis and hepatitis, thus requiring a closer surveillance. Supported by FAPESP (08/50184-2), CNPQ (300248/2008-3 and 300665/2009-1) and Federico Foundation.

1013W

Atypical 7q11.23 deletion in a boy with the facies and behavioral phenotype of Williams syndrome but typical overall cognitive ability: phenotype-genotype correlation. *R.D. Clark[1], B. Haas[2], A.L. Reiss[2].* 1) Division of Medical Genetics, Department of Pediatrics, Loma Linda University School of Medicine, Loma Linda, CA; 2) Center for Interdisciplinary Brain Sciences Research, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Palo Alto, CA.

Williams syndrome (WS) is a microdeletion syndrome characterized by deletion of about 28 genes on 7q11.23 (WSCR) including the elastin gene (*ELN*), which is responsible for the vascular and connective tissue abnormalities in this syndrome. The molecular basis of the facial, behavioral and neurocognitive phenotypes in WS is less clear. One of two recurrent deletions (1.5 and 1.8 Mb) are seen in ~98% of WS patients. Atypical deletions are present in 2-3% of WS patients. In 2007, a 21 month-old male of Indonesian descent presented with fevers, absolute neutropenia, chronic Staph infections, short stature and developmental delay. He walked at 17 months and said his first words between 17 and 21 months. He had a wide mouth, elfin facies, poor dental enamel, small widely spaced teeth, hoarse voice and a friendly disposition. Echocardiogram and calcium were normal. He had microcytic anemia and a low MCV (68). He and his father have) thalassemia trait with a heterozygous mutation for Hemoglobin Malay. At age 6 years, his height is above the 25th percentile. He reads, writes sentences and draws integrated figures. He speaks 2 languages well. His neutropenia persists but he has had no infections for 3 years. Both FISH for the WSCR and gene analysis for *NCF1*, also at 7q11.23 and responsible for autosomal recessive chronic granulomatous disease, were normal. An oligo-SNP microarray revealed a 963 Kb deletion (73,568,190-74,531,569, hg 19) at the telomeric end of 7q11.23. The deleted segment lies within the WSCR and includes *GTF2I*, *GTF2IRD1*, *NCF1* and *CLIP2*. Among reports of patients with atypical deletions in the WSCR, this patient is unique as his deletion is telomeric and excludes the *ELN* gene. His facial features, dental anomalies, hoarse voice and sociable personality are typical for WS but other characteristic features, including significant overall intellectual disability are absent. His features and gene deletion segment will be compared to others with atypical WSCR deletions reported in the literature. Brain MRI and neurocognitive studies on this patient will be presented and compared to children with typical WS deletions as well as healthy controls. The few patients with WS whose deletions do not span the entire WSCR provide a valuable opportunity to advance the current state of knowledge of the genetic map for the clinical, bahavioral and neurocognitive phenotypes in WS.

1014W

POLR1D and POLR1C sequencing in a series of 33 patients without TCOF1 mutations and study of their profile expression during human development. *C. Collet[1], M. Vincent[2], S. Thomas[3], S. Marlin[4], E. Sanchez[2], A. Harroche[3], K. Dahan[5], T. Attie[3], C. Herlin[6], Y. Chabbi[1], G. Captier[6], C. Blanchet[7], A. Ermakov[8], M. Bigorre[6], M. Mondain[7], M. Willems[2], B. Doray[9], P. Sarda[2], F. Djouad[8], D. Geneviève[2,8].* 1) Service de Biochimie et de Biologie Moleculaire, H, Paris, France; 2) Departement de Genetique Medicale, CHRU Montpellier, Université Montpellier 1, Centre de reference anomalies du developpement et syndromes malformatifs, Montpellier, France; 3) Unite Inserm U781, Departement de Genetique, Hopital Necker-Enfants Malades, France; 4) Centre de Reference des Surdites Genetiques, Service de Genetique, Hopital d'Enfants Armand Trousseau, Paris, France; 5) Universite Catholique de Louvain, Genetique clinique, Bruxelles, Belgique; 6) Chirurgie plastique infantile, CHRU Montpellier, Universite Montpellier 1, Montpellier, France; 7) Service d'ORL, CHRU Montpellier, Universite Montpellier 1, Montpellier, France; 8) Unite Inserm U844, Institut des Neurosciences de Montpellier, France; 9) Service de genetique medicale, Hopital Hautepierre, Strasbourg, France.

Treacher-Collins/Franceschetti syndrome (TCFS, MIM 154500) is a disorder of craniofacial development characterized by a facial gestalt including micrognathia, microtia, hypoplastic zygomatic arches with antimongoloid slant of the palpebral fissures and coloboma of the lower lid. TCFS is genetically heterogeneous with heterozygous mutation in the TCOF1 gene in approximately 70% of the patients. An array-CGH strategy recently identified POLR1D and POLR1C as others disease-causing genes in about 4% of the TCFS patients negative for the TCOF1 gene. POLR1D mutations are associated with an autosomal dominant of inheritance in TCFS patients whereas an autosomal recessive mode of inheritance is described in TCFS patients with POLR1C mutations. Here we report the identification of new POLR1D mutations in 4/33 patients without TCOF1 mutations. The clinical features of the patients are consistent with the TCFS criteria. Among the patients, 2 were sporadic and 2 were familial cases. Interestingly, we identified homozygous POLR1D mutation in one patient and heterozygous status in the healthy parents suggesting an unexpected autosomal recessive mode of inheritance in this family. In addition we studied the TCOF1 mRNA expression in blood sample from patients from our series. We also studied the expression of SOX9, TCOF1, POLR1D and POLR1C in craniofacial structures during human development. In conclusion, we identified mutations in the POLR1D in a series of 33 TCFS patients negative for the TCOF1 gene and study the expression of the SOX9, TCOF1, PORL1D and POLR1C in human craniofacial development.

1015W

A further case of Coffin-Siris syndrome with additional findings: Acanthosis nigricans and obesity. *B. Durmaz[1], E. Karaca[1], O. Cogulu[2], F. Ozkinay[2].* 1) Department of Medical Genetics, Ege Univ, Izmir, Turkey; 2) Department of Pediartic Genetics, Ege Univ, Izmir, Turkey.

Coffin-Siris syndrome is a rare congenital disorder also known as `fifth digit syndrome'. The disorder is characterized by mental retardation and developmental delay, a coarse facial appearance, short fifth digits and hypoplastic or absent nails. The underlying cause of Coffin-Siris syndrome is unknown and most cases are sporadic. Familial cases have also been reported that suggest autosomal dominant or autosomal recessive inheritance. Here we report a 11 year-old girl, the first child of non-consanguineous parents. She was referred to the Pediatric Genetics Department with multiple congenital anomalies and developmental delay. On physical examination, she was seen to have a coarse face, puffy eyes and a depressed nasal bridge. She also had a short stature (<3p) and was overweight (>95p, BMI: 39 kg/m2). She had acanthosis nigricans on both the neck and axillary regions, previously not described before. Her fifth fingers were short, especially on the right hand. The fingernails and toenails were hypoplastic. X-ray of the right hand revealed a hypoplastic fifth metacarpal. Additionally, her bone age was advanced (14 years) which is unusual for this disorder. She also had high insulin level and impaired glucose tolerance. Cranial MRI and abdominal ultrasonography revealed no pathology. Her cardiological and opthalmological examinations were normal. Karyotype and FISH analysis for Prader-Willi Syndrome were normal. Metabolic diseases such as hypothyroidism, lysosomal storage disorders, biotinidase deficiency were excluded. In conclusion, all presented features apart from advanced bone age, acanthosis nigricans and obesity were typical of Coffin-Siris syndrome. Therefore, this patient's additional clinical features may represent a new variant of Coffin-Siris syndrome which may contribute to the understanding of this genetic disorder.

1016W

Richieri-Costa-Pereira syndrome. *T.M. Felix[1], I.G. Mazzucco[1], G. Carvalho[1], J.C.L. Leite[1], L.Z. Bonilha[2], M.V. Collares[2].* 1) Medical Genetics Service, Hosp Clinicas Porto Alegre, Porto Alegre, RS, Brazil; 2) Plastic Surgery Service, Hosp Clinicas Porto Alegre, Porto Alegre, RS, Brazil.

Richieri-Costa and Pereira described in 1992 an autosomal recessive acrofacial dysostosis syndrome. The clinical findings include short stature, Robin sequence, cleft mandible, pre and post axial limb anomalies and club foot. Thirty one cases had been described in the literature and all of them except one are from Brazil. The majority of the Brazilian cases are from a region in São Paulo called Ribeira Valley. This data suggest that this disorder is caused by an unknown rare mutation due to a founder effect. We report on a case of a female newborn, born in the Southern state of Brazil. She is the third child of a young and non-consanguineous couple. She was born by cesarean section due to breech presentation at 38 weeks of gestational age. Her birth weight was 2350g. At the physical examination we noticed micrognathia, cleft palate, hypoplasic of the fifth fingers and toes and calcaneus hyploplasia. She was submitted to the following exams: Karyotype (46,XX), limb x-rays showed hyploplastic fifth fingers, short fibulas and ulnas, hypoplastic calcaneus and club feet. A face bone CT scan showed agenesis of the mandible, micrognathia, zygomatic arch and paramentonian region hypoplasia. She evolved with respiratory distress and nasofibrobroncoscopy showed grade III glossoptosis leading to tracheostomy procedure. She was submitted to mandible osteoplasty with double layer parietal bone graft.

1017W

Clinical review of 21 patients with chromosome 22q11.2 deletion syndrome. *J. Garcia-Sagredo, J. Lopez-Ribera, P. Cabello, E. Garcia-Galloway, C. Villalon, J. Cabrejas, M. Talavera, M.T. Ferro.* Dept Med Gen, Univ Hosp Ramon y Cajal, Madrid, Spain.

22q11.2 deletion syndrome is the most frequent microdeletion disorder. This syndrome is associated to a wide variety of phenotypes including Di George syndrome (88%), velocardiofacial syndrome (75%), and conotruncal anomaly face syndrome (72%). We report a review of 75 patients with congenital heart diseases (CHD) referred from the Cardiology Department during the last 4 years to analyze the presence of 22q11.2 deletion using the Di George/VCFS probe (Vysis). Twenty one patients out 75 (28%) showed the 22q11.2 microdeletion. Among the 21 patients, no differences were found in sex ratio. All had CHD, mainly conotruncal malformations, 14 (66.6%) complex CHD, 11 (52.3%) Fallot tetralogy, 7 (33.3%) right aortic arch, 2 truncus arteriosus, 2 with pulmonary atresia and ventricular septal defect, and 2 with auricular septal defect associated to valvular disorders. The majority of the patients (95.2%) showed facial or skeletal malformations. By frequency, the most frequent dysmorphic findings were: (1) Oral malformations (66.6%) including lips malformations, bifid uvula, short and high arched palate, micrognathia, and prognathism. (2) Ear malformations (66.6%), including tags; no hearing loss was found. (3) Eye malformations (52.3%) including eyelids, epicanthic folds, strabismus and myopia. (4) Nose malformations (38.1%). Additionally there were 5 patients (23.8%) with skeletal malformations including polydactyly, clinodactyly, and scoliosis. Furthermore, 57.1% showed psychomotor retardation or cognitive defects ranging from mild to moderate. Two patients have behavioral disorders. For the contrary, more than 50% of the evaluated patients without 22q11.2 deletion had normal phenotype. Our results, similar to previous reports, showed that the most frequent CHD, Fallot tetralogy, are not the most predictive of 22q11.2 deletion syndrome. Fallot tetralogy is associated with this syndrome in one out 8 patients with CHD, while the right aortic arch is associated in 1 out 2 patients. On the contrary, the association of CHD with facio-skeletal malformations is the best predictor of this syndrome. In conclusion, the clinical heterogeneity of 22q11.2 deletion syndrome makes FISH analysis the choice test among the patients with CHD, but the efficiency of this test is low using only CHD as the only indication. Otherwise, association of facial malformations with CHD or neurocognitive disorders is a better indication of carrying out 22q11.2 FISH microdeletion test.

1018W

A seven years follow-up of a new case of Ehlers Danlos Syndrome type VIIB. Major problems are represented by bilateral, extremely severe, hip dislocation. *M. Giovannucci Uzielli[1, 5], C. Giunta[4], G. Scarselli[2], N. Dayan[5], E. Lapi[3], L. Di Medio[5].* 1) Dept Pediatrics, Univ Florence, Florence, Italy uzielli_ml@unifi.it; 2) Dept Pediatric Neurology, Univ Florence, Florence, Italy; 3) Azienda Ospedaliera Universitaria "A. Meyer", Florence, Italy; 4) Div. of Metabolism and Molecular Pediatrics, University Children Hospital, Zurich, Switzerland; 5) Genetica Science - Piazza Savonarola 11 - 50132 Florence, Italy.

The Ehlers-Danlos Syndrome (EDS) is a heterogeneous group of heritable connective tissue disorders whose primary clinical features include marked joint hypermobility, soft hyperextensible skin, dystrophing scarring, easy bruising. An initial classification defined 10 distinct types of EDS, but with recent advances in our understanding of the molecular pathology, a revised nosology has been proposed. In the original classification, EDS type VII, a subgroup characterised by extreme joint laxity, was divided into three subtypes, EDS VIIA, VIIB, and VIIC. In the revised nosology, EDS VIIA and B,(both AD disorders) are combined as the arthrochalasia type of EDS. EDS VIIB, (harbouring mutations in the COL1A2 gene) shows peculiar aspects of the collagen fibrils in skin, bone and fascia, that are near circular in cross - section. Congenital hip dislocation, often bilateral, is described in all patients with EDS VIIB. We report the clinical phenotype and the natural history of a girl, firstly evaluated at birth, because a generalised joint hypermobility, especially expressed at feet and hands level, with the so-called "swan neck deformities of the fingers". Soft skin, umbilical hernia, blue sclerae were also obvious. Direct sequencing of a 358 bp by PCR fragment encompassing intron 5 to 6 of the COL1A2 gene has shown, at the first position G+1 of the donor 5' splice site, a heterozygous G to A (IVS6+1G>A) substitution which alters the obligatory GT nucleotide to AT. The mutation is known to cause skipping of exon 6 in COL1A2 and to be responsible for EDS VIIB. From the periodical, follow-up controls, we especially underline that the bracing was unsuccessful in stabilizing the hips, but the patient is able to walk, jump, cycle, without any pain, or apparent limitation. The legs are now well developed with normal muscular component. The stature is a little below the 3rd centile. The X-rays revealed verticalized acetabulum, marked hip dislocation with valgism of femoral neck and "climbing up" of proximal femoral epiphyses with a "new-joint" with the iliac hip-bones. Surgical correction of the hip situation was considered but rejected by the orthopaedic specialists on the basis of the presently good quality of the patient's life, and due to the limits and risks of the currently offered techniques.

1019W

The Floating-Harbor syndrome: report of the first case series in South America. *I. Gomy, C.R.D.C. Quaio, D.R. Bertola, C.A. Kim.* Pediatrics, Genetics Unit, Instituto da Criança - Universidade de Sao Paulo, Sao Paulo, São Paulo, Brazil.

The Floating-Harbor syndrome (FHS) is characterized by facial gestalt, typical body habitus, short stature with delayed bone age and both speech and language disorders with a peculiar voice. To date, more than 40 FHS patients were reported worldwide and its etiology remains unknown, although either autosomal dominant inheritance or microdeletion have been suggested. Associated abnormalities include congenital heart defects, genitourinary anomalies, precocious puberty and celiac disease. Growth hormone therapy has been used with discrepant outcomes. We report a series of six unrelated patients aged from 12 to 28 years (mean of 18) with typical features of FHS and some individual peculiarities. Archetypal facial dysmorphisms, intellectual disability, short stature, language delay and normal motor development were universal findings. Their height varied from -2 to -4.3SD, with an average Z score of -3.1. Low birth weight (4/6), delayed bone age (3/6), microcephaly (3/6), hypothyroidism (2/6), trigeminal neuralgia (1/6), inguinal hernia (1/6), conductive hearing loss (1/6), posterior urethral valve (1/6) and strabismus (1/6), brachydactyly (3/6) clinodactyly(4/6) digital clubbing (2/6) were also remarkable findings. GH therapy was tried in two patients but only one responded. All patients presented normal karyotype, while three had normal studies for the 22q11 region and one normal CGH array. We described the first South American case series of FHS. The recognition of FHS remains a challenge mainly because it depends upon the recognition of the facial gestalt that changes over time. Furthermore, consensus diagnostic criteria have not been established yet. Whether the association of FHS, non-immune hypothyroidism and trigeminal neuralgia is a mere casualty or expands the phenotype remains uncertain. Therefore, we emphasize the need for prompt recognition and better delineation of this rather underdiagnosed syndrome.

1020W

Autosomal dominant natal teeth with selective tooth agenesis. *J. Graham[1], V. Funari[1], O. Klein[2], K. Seidel[2], P. Kantaputra[3], K. Taylor[1].* 1) Medical Genetics Institute, Cedars Sinai Medical Center, Los Angeles, CA; 2) Dept Orofacial Sciences, University of California, San Francisco, CA; 3) Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand.

We report a 5-generation family with multiple natal teeth and selective tooth agenesis segregating in an autosomal dominant fashion. Natal teeth are usually a sporadic isolated finding in an otherwise normal infant, and familial occurrence is uncommon. Selective tooth agenesis is usually genetic and not associated with natal teeth. In affected persons in the family we report, at least 6-8 natal teeth are usually present at birth, and this is followed by selective tooth agenesis that results in absence of as many as 16 permanent teeth. Natal teeth with hypodontia occurs in some ectodermal dysplasia syndromes. In the family we report, natal teeth with selective tooth agenesis did not include ectodermal dysplasia or any other problem. DNA from 28 family members was analyzed on the Illumina OMNI-express chip using 733,120 SNPs and mapped to an approximately 2Mb segment on chromosome 1q36.11 with LOD score 2.97 at 23.8 Mb to 25.8 MB (GRCh37/hg19; MERLIN). By dividing the pedigree into three 3-generation families, a region of association was found located between LOC284632 and GRHL3 (parenTDT, p=0.005 for rs11249039, rs11249045, or rs7526505). GRHL3 is a gene expressed exclusively in surface ectoderm in drosophila, where it plays an essential role in cuticle formation. Expression of the murine Grhl3 gene is evident in ectodermally derived tissues, including the oral epithelium. We speculate that variation in the regulation of this gene may play a role in the phenotype we describe in this family.

1021W

Congenital neutropenia with retinopathy, a new phenotype without mental retardation caused by VPS13B mutations. *L. Gueneau[1], L. Jego[1], P. Sarda[2], C. Hamel[3,4], B. Aral[1,5], E. Lopez[1], N. Gigot[1,5], MC. Lavigne[5], A. Donzel[5], P. Callier[1,6], A. Masurel-Paulet[8], JR. Teyssier[5], N. Droin[7], S. El Chehadeh[1], L. Faivre[1,8], C. Thauvin[1,8].* 1) Equipe émergente GAD (Génétique et Anomalies du Développement), IFR 100 Santé STIC, Université de Bourgogne, Dijon, France; 2) Service de Génétique Médicale, Hôpital Arnaud de Villeneuve, CHU Montpellier, France; 3) Service d'ophtalmologie, Hôpital Gui de Chauliac, CHU Montpellier, France; 4) Département de génétique et thérapie des cécités rétiniennes, INSERM U583 - Institut des Neurosciences de Montpellier, France; 5) Laboratoire de Génétique Moléculaire, Plateau Technique de Biologie, CHU Dijon, France; 6) Laboratoire de Cytogénétique, Plateau Technique de Biologie, CHU Dijon, France; 7) Inserm UMR 1009, Integrated Research Cancer Institute Villejuif (IRCIV), Institut Gustave Roussy, Villejuif, France; 8) Centre de Génétique et Centre de Référence (Anomalies du Développement et Syndromes Malformatifs) du Grand Est, Hôpital d'Enfants, CHU Dijon, France.

Cohen syndrome, a rare autosomal recessive disease, presents with a highly homogeneous phenotype, including developmental delay, microcephaly, characteristic facial dysmorphism, pigmentary retinopathy, intermittent isolated neutropenia and cheerful disposition. Major clinical criteria with high sensibility have been published. This disorder is due to mutations in the VPS13B gene with over hundred different previously reported mutations including mostly frameshift, leading to truncated protein and predicting to non-functional allele. Here, we report on an adult patient carrying two VPS13B heterozygous splice mutations (IVS34+2T_+3AinsT and IVS57+2T>C) with an atypical phenotype including microcephaly, retinopathy diagnosed at age 22 years and congenital neutropenia diagnosed at age 2 years, but neither obesity nor mental retardation. Indeed, she weighted 43 kg for 153 cm, BMI 18.4 (normal 18.5-25), and her IQ was evaluated at 100. Each parent carried one mutation. In silico analyses predicted abnormal splicing. RNA RT-PCR sequencing analysis up- and downstream IVS57+2T>C mutation showed three abnormal splice isoforms plus the normally spliced one. RNA RT-PCR sequencing analysis around the IVS34+2T_+3AinsT mutation failed to reveal any abnormal spliced fragments. First results of the quantitative RT-PCR in patient's lymphoblastoid cell line showed decrease of the VPS13B mRNA expression on three different fragments, suggesting nonsense-mediated mRNA decay process. These results lead us to suspect the expression of residual normal VPS13B mRNA and normal protein activity, responsible for partial phenotype in this patient, in contrast to patients with Cohen syndrome who present only with expression of abnormal VPS13B mRNA and truncated protein.

1022W

Novel *GJA1* mutation in a patient with Oculo-Dento-Digital Dysplasia and evolving basal ganglia abnormalities. *E.M. Honey, E.J. van Rensburg.* Department Genetics, University of Pretoria, Pretoria, Gauteng, South Africa.

Oculo-dento-digital dysplasia [ODDD, OMIM 164200] is an autosomal dominant disorder characterized by facial dysmorphisms (thin nose with hypoplastic alae nasi) , ocular defects (microphthalmia, microcornea, cataract, glaucoma and optic atrophy), enamel hypoplasia, syndactily of fingers and toes and dry lusterless hair that fails to grow. Neurological abnormalities can also be present and mild mental retardation is commonly noted. Diffuse white matter abnormalities and calcifications of the basal ganglia have been described. Mutations in the human gap junction alpha 1 (*GJA1*) gene on chromosome 6q22-23 coding for Connexin 43 are associated with ODDD.

We report a 14 year old girl who presented with syndactily between the 4th and 5th fingers (surgically corrected) and facial features suggestive of ODDD. She had a thin tapered nose with hypoplasia of the alae nasi, a prominent nasal bridge, micro-opthalmia and abnormally shaped small teeth. Her psychomotor development was in the low normal range and she had to attend a special school. An MRI of her brain showed significant signal changes in the lentiform nucleus in the area of the globus pallidus on the T1 and T2 weighted images at the age of 8 years. The white matter showed a normal signal pattern. These signal changes was more limited on an MRI repeated at 11 years of age. Sequencing of the *GJA1* gene revealed a novel missense mutation, p.L228P (c.683T > C), located in the fourth transmembrane region (TM4) of Connexin 43. She is currently at the age of 21 years in a full-time occupation living with her parents.

The basal ganglia abnormalities in the absence of white matter changes have not been described before in association with ODDD. Could this be the preceding changes to the basal ganglia calcification seen in older patients with ODDD?

1023W

5q31.3 microdeletion syndrome is a clinically discernible new syndrome characterized by severe neonatal hypotonia, feeding difficulties, respiratory distress, and severe developmental delay. *K. Hosoki[1], T. Ohta[2], J. Natsume[3], S. Imai[4], A. Okumura[5], T. Matsui[6], N. Harada[6], F. Scaglia[7], C.A. Bacino[7], N. Niikawa[2], S. Saitoh[8].* 1) Department of Pediatrics, Hokkaido University Graduate School of Medicine; 2) The Research Institute of Personalized Health Science, Health Science University of Hokkaido; 3) Department of Pediatrics, Nagoya University Graduate School of Medicine; 4) Department of Pediatrics, Sagamino Social Insurance Hospital; 5) Department of Pediatrics, Juntendo University School of Medicine; 6) Nagasaki Laboratory, Cytogenetics, 2nd Group Molecular Genetics Testing Department, Clinical Laboratory Center Mitsubishi Chemical Medience Corporation; 7) Department of Molecular and Human Genetics, Baylor College of Medicine and Texas Children's Hospital, Clinical Care Center; 8) Department of Pediatrics and Neonatology, Nagoya City University Graduate School of Medical Sciences.

Chromosomal microarray technology has led to the identification of many novel microdeletion/microduplication syndromes demonstrating multiple congenital anomalies and intellectual disability (MCA/ID). We have applied microarray analysis to the patients with MCA/ID and/or neonatal hypotonia. Three overlapping *de novo* microdeletions at 5q31.3 with the shortest region of overlap (SRO) of 370kb were detected in unrelated patients. These patients showed similar clinical features including severe neonatal hypotonia, feeding difficulties and respiratory distress followed by severe developmental delay. They presented with facial dysmorphisms with narrow forehead with metopic prominence, lateral sparseness of the eyebrows, hypertelorism, depressed nasal bridge, open and tented mouth, marked philtrum and micrognathia. Recently, Shimojima et al. [2011] reported two patients with 5q31.3 microdeletions in which the 370kb SRO was completely included, and these patients demonstrated strikingly similar clinical features to our patients. The 370kb SRO encompasses only four RefSeq genes including *NRG2* and *PURA*. As *NRG2* is a member of the neuregulin family related to growth and differentiation of neurons and development of glial cells, *NRG2* may be a good candidate for this phenotype. *PURA* also remains as a good candidate because *Pura* deficient mice demonstrate postnatal neurological manifestations. We conclude that the 5q31.3 microdeletion syndrome is clinically discernible, and hypothesize that *NRG2* and *PURA* are good candidates to cause this clinical phenotype.

1024W

A novel autosomal dominant Pierre-Robin microtia type I syndrome. *A. Hunter[1], K. Boykott[1], N. Roy[2].* 1) Genetics, Ottawa, ON, Canada; 2) Genetics Service, Porcupine Health Unit, Timmins, Ontario.

In this paper we describe mother and son with an apparently new syndrome, whose predominant signs include type I microtia and Pierre-Robin syndrome. The patients shared normal size, length and OFC at birth but both demonstrated postnatal growth failure that included the OFC. By late childhood the mother showed remarkable catch-up with adult height at the 10th centile and OFC at the 5th. The microtia, hearing loss and middle ear changes were variable; scalp hair was sparse with a high anterior hairline. Childhood photographs of mother and child were remarkable similar; with time the mother's face has become very long and narrow with some asymmetry and a very deep lower jaw that remains retrognathic. Other shared characteristics include a prominent wide nasal bridge, bow-shaped upper lip with some asymmetry of movement, mild clinodactyly and smallish nails. The mother's developmental disabilities appear milder than those of her son who currently shows marked developmental delays. The mother's adult radiographs showed fusion of C4-C5 and an increased height to AP ratio of the lumbar vertebrae so that mild anomalies of the spine may also be part of this syndrome. Given that the mother's parents are normal, we hypothesize that this is an autosomal dominant syndrome which has arisen de novo in the mother and been passed on to her son. Chromosomal microarray analysis was normal suggesting that this syndrome is caused by a de novo change in a single gene. Although there are almost 30 syndromes that include Pierre Robin as a finding most can be distinguished easily on the basis of other associated findings and/or autosomal recessive inheritance. Those few that bare some resemblance will be discussed. The dorsal component of the first pharyngeal arch appears as the embryological site of the two most characteristic components, that is the Pierre-Robin anomaly and microtia. We will use comparative exome sequencing of the mother and her normal parents to attempt to reveal the genetic basis of this syndrome.

1025W

A *de novo* 10.6 Mb tandem duplication of 17q11.2-q12 in a 1 year old boy: Expanding the phenotype. *S. Jamal[1,2], R. Basran[1,2], T. Maher[1], Y. Zou[1,2], X. Huang[1], J. Milunsky[1,2,3].* 1) Ctr Human Genetics, Boston Univ Sch Medicine, Boston, MA; 2) Department of Pediatrics, Boston Univ Sch Medicine, Boston, MA; 3) Department of Genetics and Genomics, Boston Univ Sch Medicine, Boston, MA.

The use of whole genome microarray technologies has the ability to identify novel microdeletion and microduplication syndromes. We describe a 21 month old male with a history of delayed tooth eruption, bilateral hip dislocation, persistent generalized hypotonia, developmental delay, and dysmorphism. His dysmorphic features include a long midface, deep set eyes, thin eyebrows, tubular nose, anteverted nares, thin upper lip, unusual dentition, relative brachydactyly, tapered digits, and a shawl scrotum. In pursuing a syndromic work-up, a renal ultrasound at 22 months was normal; a transthoracic echocardiogram at 23 months revealed a possible anomalous accessory pulmonary vein; a head MRI at 27 months revealed possible mega cisterna magna and hypoplastic cerebellar vermis. Affymetrix 6.0 SNP array revealed a 10.6 Mb interstitial duplication of chromosome 17q11.2-q12. BAC FISH analysis determined the duplication to be in tandem. Subsequent BAC FISH testing of maternal and paternal blood samples were negative for this duplication (paternity confirmed), thus determining the *de novo* nature of this alteration. This duplication contains over 100 annotated genes, and includes 2 well known micro*deletion* syndromes: Neurofibromatosis type 1 contiguous gene syndrome (*NF1* gene [MIM 613113], [MIM 613675]) and Renal Cysts And Diabetes syndrome (*HNF1B/TCF2* gene [MIM 189907], [MIM 137920]). Both recurrent genomic disorders are thought to arise from NAHR due to the presence of flanking segmental duplications, resulting in either duplication or deletion of the intervening segment. In 2008, *NF1* micro*duplications* were observed in patients with intellectual disability, early baldness, and dental enamel hypoplasia (Grisart et al, 2008). Similarly, *HNF1B/TCF2* micro*duplications* were reported in patients with renal disease, diabetes, epilepsy and/or intellectual disability (Mefford et al, 2007; Nagamani et al, 2009; Morena-De-Luca et al, 2010; Faguer et al, 2011). Caselli *et al* (2010) was the first to describe a 12.6 Mb *de novo* duplication of 17q11.2-q12 in a 6 year old boy with developmental delay and minor anomalies. To our knowledge, our patient is the second report of a large sized duplication involving 17q11.2-q12 that encompasses two recently elucidated genomic disorders. As our patient is quite young and the phenotype corresponding to this large duplication continues to be delineated, providing anticipatory guidance remains challenging.

1026W

Phenotypic features of the 14q deletion syndrome in balanced translocation carrier family members. *N.J.B. Kapalanga[1, 2, 3], D. Wong[2,3], A.C. Gandy[2,3].* 1) Pediatrics/Genetics, GBHS, Owen Sound, Ontario, Canada; 2) Pediatrics Dept, Dalhousie University, Halifax, NS, Canada; 3) Summerside Medical Center, Summerside, PEI, Canada.

The clinical phenotype of the majority of cases with terminal 14q deletion syndrome is characterized by distinctive craniofacial features, feeding difficulties, hypotonia, developmental delay in motor skills and speech, communication and mental function difficulties. The clinical phenotype of terminal 14q deletion syndrome has been described in about 22 reports in the literature. About 13 of these cases are pure terminal deletions and only 4 cases involved autosomal translocations and only one of these 4 cases involved a deleted derivative 14 chromosome from a 14;17 balanced paternal cryptic translocation; t(14;17)(q32.32;qter). None of the published reports describe phenotypic features of the terminal 14q deletion syndrome in balanced translocation carriers with a chromosome 14q32.3 breakpoint. We report on a family with a child with 14q32.3 terminal deletion syndrome and balanced translocation carrier family members who share certain phenotypic features with the proband. The shared phenotypic features include telecanthus, epicanthic folds, prominent nose, broad philtrum and mild speech difficulties. The distinctive facies were observed in all family members who are balanced translocation carriers and in none of the family members with normal karyotypes. All these phenotypic features have previously been reported in other cases with the terminal 14q deletion syndrome. As these features are present only in family members with a 14q32.3 breakpoint it is tempting to speculate that these facies have some association with the chromosome 14 breakpoint involved in the translocation in this family. It was also observed that all offsprings of the father carrying the t(14:17) translocation have a 14q32.3 breakpoint. We hypothesize that the 14q32.3 breakpoint results in haploinsufficiency of a gene(s) involved in facial morphogenesis. Further several imprinted genes have been reported in this region and it has been suggested in a previous report that imprinted genes could be involved in the terminal 14q deletion phenotype. Thus, the combination of maternally imprinted genes on 14q and inactivation of functionally active, paternally derived alleles at the 14q32.3 breakpoint could explain the 14q terminal deletion features also observed in translocation carriers.

1027W

Aicardi Goutiere Syndrome- first molecularly confirmed case in Malaysia. *W.T Keng[1], G.I Rice[2], H. Gornall[2], Y.J Crow[2], V. Ganesan[3], R. Ariffin[1].* 1) Deparment of Genetic, Hospital Kuala Lumpur, Kuala Lumpur, Wilayah Persekutuan, Malaysia; 2) Academic Unit of Medical Genetics, School of Medicine, The University of Manchester; 3) Paediatric Dept, Hospital Pulau Pinang.

Aicardi Goutiere syndrome is a rare, early but typically subacute onset encephalopathy characterized by severe learning difficulties, irritability, intermittent fever, developmental regression, seizures, chilblain skin lesions and microcephaly. Mutations in 5 genes [TREX1, RNASEH2B, RNASEH2C, RNASEH2A and SAMHD1] are associated with AGS. Our patient who has Indian parentage, presented with severe learning difficulties and epilepsy in association with profound microcephaly. She cried a lot and fed poorly since birth but there was no documentation of microcephaly at birth, abnormal liver function or abnormal blood count. She did not have scoliosis, cardiomegaly or hypothyroidism. Lumbar puncture showed leucocytosis while the brain CT scan showed basal ganglia, dentate nuclei and periventricular white matter calcifications. CSF interferon and neurotransmitter were not assayed. Sequence analysis showed homozygous R69W mutation in RNASEH2C gene, which interestingly is a recurrent mutation found in many Pakistani families due to founder effect.

1028W

A clinical description of an adult patient with Wolf-Hirschhorn syndrome caused by the unbalanced translocation t(4;8)(p16.1;p23.1). *J. Liu, M. Thomas.* Dept of Medical Genetics, University of Calgary, Calgary, Alberta, Canada.

We describe a 36-year-old woman with Wolf-Hirschhorn syndrome (WHS) associated with the relatively common translocation t(4;8)(p16.1;p23.1). The monosomy 4p in the patient is due to the unbalanced de novo translocation between 4p and 8p. This translocation is likely the second most common recurring translocation and mediated by olfactory receptor gene clusters. Therefore, this particular imbalance is a recurring abnormality in WHS patients, warranting further clinical description. This need in the literature has previously been raised from the 2008 workshop “WHS and Related 4p- Conditions: Defining a Research Agenda”. Conventional cytogenetic analysis identified an abnormal chromosome 4 with additional material on the distal short arm - add(4)(p16.1), with an otherwise normal female karyotype. Metaphase subtelomere FISH studies demonstrated that the additional chromosome segment is derived from chromosome 8p and the distal 4p is deleted, including the WHS critical region (D4S96-). Therefore, this is a derivative chromosome 4 from a translocation between 4p and 8p. By G-banding, the breakpoints correspond to t(4;8)(p16.1;p23.1). As both parental karyotypes were normal, the chromosome rearrangement is presumed to be de novo in this patient. Our patient has been in relatively good health but has significant intellectual disability and lives in a group home. She is able to walk, participate in physical activities, and can feed herself. Although she does not use words, she manages to communicate with those who know her with specific sounds and basic sign language. She is social and interactive and enjoys many activities, including shopping and office work. She had seizures as a child and has not had one in years, although recently had a probable recurrence. She is otherwise in good healthy. Her growth parameters are well below the 3rd percentile. She has distinct facial features, as will be shown with pictures, some in keeping with WHS. Overall, she is not on the severe end of the spectrum of WHS patients. This derivative 4 from a de novo translocation between 4p and 8p has been reported in several large scale WHS studies. There are two different breakpoints which are recurrent on 4p: the common one located at about 9 Mb, and the less common one located at about 4.5 Mb from the telomere. Array comparative genomic hybridization will be performed to determine the precise breakpoints and to further delineate the genotype-phenotype correlation.

1029W

Delineation of the interstitial 6q25 Microdeletion Syndrome: refinement of the critical causative region. *M. Michelson-Kerman[1,2], C. Vinkler[1,2], I. Nezer[1,2], M. Yanoov-Sharav[1,2], T. Lerman-Sagie[2,3], D. Lev[1,2].* 1) Inst Med Genetics, Wolfson Medical Ctr, Holon, Israel; 2) Metabolic Neurogenetic Clinic, Holon, Israel; 3) Pediatric Neurology Unit, Holon, Israel.

Cryptic chromosomal aberrations are associated with various syndromes. Microdeletion of the long arm of chromosome 6q is rare but specific clinical entity. Phenotypic presentation includes mental retardation, acquired microcephaly, dysmorphic features, structural brain malformations and other features. The smallest reported region of microdeletion responsible for the phenotype was mapped between 6q24 and 6q27 and spans >3MB with more than 12 coding genes. A 2 years old boy presented with mental retardation, acquired microcephaly, dysmorphic features and dysgenesis of corpus callosum. He has deep set eyes, prominent forehead, posteriorly rotated ears, short neck, short proximal phalanges, transverse creases, fetal pads and hypoplastic scrotum. Neurological examination revealed mixed axial hypotonia with limbs hypertonia. He is the fourth child of an unrelated generally healthy couple. The mother is carrier of a Robertsonian translocation (14;15). Our patient was found to be a carrier of the same translocation and his karyotype is 45 XY,der(14;15)(q10;q10) Affymetrix Wnole-Genome 2.7M Array Chip revealed deletion of 1191 kbp at 6q25.3. The deleted region contains 2 coding genes: ARID1B and ZDHHC14. These genes are highly expressed and evolutionarily conserved. ARID1B encodes a subunit of chromatin-remodeling complex. The encoded protein is expressed in different brain regions during fetal development suggesting its important role in CNS development. ZDHHC14 belongs to a palmitoylating enzyme family proteins. Protein palmitoylation is crucial in CNS development and plays an important role in neurite outgrowth and synaptogenesis. We found two attractive genes, ARID1B and ZDHHC14 whose haploinsufficiency caused the distinct phenotype of 6q deletion syndrome with dysgenesis of corpus callosum.

1030W

Toriello-Carey Syndrome: First brazilian case with unusual manifestation phenotype. *M.P. Migliavacca[1], T.A. Zanolla[1], N.L.M. Sobreira[2], A.B.A Perez[1].* 1) Medical Genetics Center (Federal University of São Paulo Brazil); 2) Mckusick-Nathans Institute of Genetic Medicine (Johns Hopkins University).

BACKGROUND: Toriello and Carey first described in 1988 four patients comprising with agenesis of corpus callosum, unusual facial appearance, Pierre Robin sequence, and others anomalies. Since then, 16 patients were described with a similar phenotype. PURPOSE: Describe a patient with an addition finding in order to try to further delineate the syndrome. PATIENT AND METHODS: TMS, 7 years age, first child of unrelated parents. No family history, uneventful pregnancy, born by cesarean section at term. Presented at birth congenital skin atrophy, poor sucking reflex, club foot and cardiac anomaly (atrioventricular septal defect), cryptorchidism and pyloric stenosis. Progressed with physchomotor development delay. Physical examination: weight, stature and head circumference at mean percentile ; autistic behaviour; arching of eyebrows, ocular hypertelorism, ptosis of eyelid, downslanted palpebral fissures, short columella, upturned nares, long and smooth philtrum, micrognathia, short neck, clinodactyly of fifth fingers in both hands, camptodactyly of left fifth finger, partial cutaneous syndactyly, small penis, limited atrophic scar area in limbs. Complementary evaluation: Karyotype 46, XY; normal T4/TSH testing; normal transfontanel and abdominal Ultrasound; Brain Magnetic Resonance Imaging: delayed myelinization; Audiometry: bilateral hearing loss; BERA: conductive hearing loss. CONCLUSION: Autosomal recessive inheritance has been hypothesized and chromosome abnormalities have been reported but the etiopathogenesis remains unknown. The phenotype is not completely delineated yet. Our patient presents with a similar phenotype of those already described and in addition with a rare skin anomaly: congenital skin atrophy localized in the limbs, that we believe it is part of the syndrome. We describe findings in a patient, presumed to be another case of the Toriello-Carey syndrome, which extend the phenotype of the syndrome.

1031W

Polymorphism of MTHFR A1298C, a marker in northern Indian mothers with DS babies and its association with biochemical risk factors and CHD. *P.K. Mohanty, S. Kapoor, A.P Dubey, R. Saha, S.K Pandey, S.K Pollipali.* Department of Pediatrics, Maulana Azad Medical College, New delhi, India.

Background:-Down syndrome is a chromosomal disorder due to trisomy 21which is the most common aneuploidy at birth and occurs as a result of nondisjunction in maternal meiosis. Maternal genetic polymorphism of methylene tetrahydrofolate reductase due to low folate, high homocysteine metabolism has been implicated a risk factor.Objective: To evaluate A1298C polymorphism as a risk factor in DS babies and its association with biochemical parameters and congenital heart disease. Methods: Eighty one mothers (mean age 24.9±3.2yrs) with babies having free trisomy 21 of north Indian ethnicity and 99 mothers (mean age 26.9±4.6yrs) who had no children with Down syndrome were evaluated after an informed consent. Fasting blood was collected to determine plasma homocysteine, folate (serum and RBC) and for PCR amplification for MTHFR gene. The MTHFR A1298C polymorphisms were done by allele specific polymerase reaction and enzyme digestion. Homocysteine quantification was done by liquid chromatography-tandem mass spectrometry.Cardiovascular system examination and Echocardiography was done to delineate CHD.Results:The prevalence of A1298C polymorphism with trisomy 21 DS in cases and controls were 66.7% vs. 39.4% respectively. The heterozygous and homozygous genotype frequencies at 1298 (AC and CC) among case and control were (38.3% vs. 22.2% and 28.4% vs. 17.25% respectively, OR= 3.10, 95% CI 1.6-5.79, P= 0.002)Low serum folate in 33.3% % of cases vs.8.0%% in controls (OR= 5.68, CI 95% 2.41-13.4, P= 0.0001).Low RBC folate was found in 28.3% of cases vs. 11.1% in controls,(OR= 3.172, CI 95% 1.43-6.99 P= 0.005).High serum homocysteine was found in 9.8% of cases vs. 2.0% in controls. The median intraquartile range serum homocysteine in cases was 10.2(Q1-Q3, 8.2-12.0) vs. 7.6(Q1-Q3, 5.7-10.1) in controls. No relationship was observed among A1298C polymorphism and CHD(Pvalue=0.601).Conclusion:MTHFR A1298C polymorphism associated with 3 times more risk of developing DS. Low serum, RBC folate and high serum homocysteine are significantly associated with MTHFR polymorphism and therefore the risk of occurrence of DS. Homocysteine levels were higher in DS mothers as compared to controls. Maternal A1298C is associated with an increased incidence of CHD in absolute number. Peri or preconceptional folate supplementation may therefore lead to a decline in DS births and CHD. Key words: Down syndrome, serum and RBC folate, serum homocysteine, MTHFR gene,CHD.

1032W

New mutations in Brazilian patients with overgrowth syndromes. *D. Moretti-Ferreira[1], G.H. Vieira[1], C.E.F. Domingues[1], R.L.L.F. Lima[2].* 1) Genetics, São Paulo State University - UNESP, Botucatu, São Paulo, Brazil; 2) General Biology - Federal University of Bahia - Salvador - Bahia- BRAZIL.

Overgrowth syndromes are a heterogeneous group of disorders resulting from the dysfunction of various processes involving cell proliferation, cell growth or apoptosis. The NSD1 gene (nuclear receptor-SET-domain-containing protein) was isolated by Kurotaki et al. in 2002. Its function are associate with enhances androgen receptor (AR) transactivation. Haplosufficiency of the NSD1 gene has been described as the major cause of both Sotos Syndrome and Weaver Syndrome. NSD1 microdeletions at the 5q35 region have been detected in 10% of the patients with Sotos Syndrome from the United Kingdom, France, Germany and Italy and about 90% of the patients of those countries showed NSD1 gene point mutations while in the japonese population these frequencies are in opposition. In Brazil, Fagali et al in 2009 screened 30 Brazilian patients with a clinical diagnosis of Sotos syndrome using Multiplex ligation-dependent probe amplification (MLPA) and they had been described a total NSD1 deletions and two new partial deletions in three individuals. In this study we screened a cohort of 49 patients with a suggestive diagnosis of overgrowth syndromes and included patients of Sotos Syndrome (21), Weaver Syndrome (6), Bannayan-Zonnana Syndrome (3), Beckwith-Wiedemman Syndrome (3), MOMO syndrome (3) and cases with mental retardation + overgrowth (13). These cases were analyzed using MLPA for the 5q35 region and sequencing of NSD1 gene. We didn't detect deletions in 5q35 region in this cohort. Four different mutations were found (c.1414C>T; c.4779C>T; c.5279delTCTG; c.5950C>T). The mutation c.5950C>T are described previous by Cecconi et al. in 2005, the others mutations are no described. Since NSD1 alterations were not seen in all patients, our study shows that, in addition to the genetic heterogeneity observed in the etiology of SS and SW, ethno-geographical differences in NSD1 alterations are evidenced when patients with SS or SW from Asia, Europe, and North America are compared to South American patients.Finacial support by FAPESP#2005/01880-8.

1033W

Clinical and molecular study of a cohort of 28 patients with suspicion of Rothmund-Thomson, Baller-Gerold and RAPADILINO syndrome referred for RECQL4 analysis. *J. Piard[1, 2], M. Holder[3], C. Baumann[4], G. Baujat[5], L. Martorell[6], F. Boralevi[7], N. Philip[8], B. Aral[9, 2], N. Gigot[9, 2], P. Callier[2, 10], M. Payet[10], C. Ragon[10], L. Van Maldergem[1], P. Vabres[2, 11], L. Faivre[2, 12], C. Thauvin-Robinet[2, 7].* 1) Centre de Génétique Humaine, CHU Besançon, France; 2) Equipe émergente GAD (Génétique des Anomalies du Développement), IFR Santé - STIC, Université de Bourgogne, Dijon, France; 3) Service de Génétique, CHU Lille, France; 4) Service de Génétique, Hôpital Robert-Debré, APHP Paris, France; 5) Service de Génétique, Hôpital Necker, APHP Paris, France; 6) Service de Génétique, CHU Sant Joan de Déu, Espagne; 7) Service de Dermatologie, CHU Bordeaux, France; 8) Service de Génétique, CHU Marseille, France; 9) Service de Génétique Moléculaire, Plateau technique de Biologie, CHU Dijon, France; 10) Service de Cytogénétique, Plateau technique de Biologie, CHU Dijon, France; 11) Service de Dermatologie, CHU Dijon, France; 12) Centre de Référence Maladies Rares (Anomalies du Développement et Syndromes Malformatifs) de la région Grand Est, Centre de Génétique, Hôpital d'Enfants, CHU Dijon, France.

Three autosomal recessive overlapping conditions have been attributed to RECQL4 mutations: Rothmund-Thomson syndrome (RTS), Baller-Gerold syndrome (BGS) and RAPADILINO syndrome. RTS is characterized by congenital poikiloderma associated with extracutaneous symptoms such as juvenile cataract, short stature, dysplastic hair and nails, skeletal anomalies, radial ray defects, gastrointestinal problems, premature aging and an increased risk for osteosarcoma. The association of craniosynostosis and radial ray hypoplasia characterizes BGS and the acronym RAPADILINO refers to the association of radial ray defect, patella hypoplasia and cleft or arched palate, diarrhea and dislocated joints, little size and limb malformation, nose slender and normal intelligence. Differential diagnoses of RTS include Clericuzio-type Poikiloderma with Neutropenia (CPN) due to mutations in the C16orf57 gene. We report here on the clinical and molecular data in a series of 28 patients referred to the laboratory for ReCQL4 analysis with a clinical suspicion of RTS (17 patients), BGS (10 patients) or RAPADILINO syndrome (1 patient). One or two deleterious ReCQL4 mutations were found in 7 patients with RTS and 1 patient with BGS. Clinical features between positive and negative ReCQL4 patients are discussed. Sequencing of the C16orf57 gene was performed in 10 patients with poikilodermia. Two C16orf57 heterozygous nonsense mutations (p.W81X/p.Y89X) were identified in a 5-year-old female child presenting with generalized poikiloderma, dental dysplasia, gingivitis, nail dystrophy, palmoplantar keratoderma and pachyonychia of the first toenails. Previously undetected and silent neutropenia was evidenced after the molecular analysis. This result allows us to confirm that neutrophil count should be performed in all patients with poikiloderma to target the C16orf57 gene sequencing analysis, prior to RECQL4 analysis. Array-CGH did not identity any genomic rearrangements in 8 patients with poikiloderma, and is currently in progress in the rest of the series.

1034W

Variable features of dominantly inherited Cornelia deLange syndrome (CdLS). *M.E. Pierpont[1,2], B. Hall[1,3], R. Temme[1].* 1) Dept Gen, Children's Hospital of Minnesota, St Paul, MN; 2) Dept Pediatrics, University of Minnesota, Minneapolis MN; 3) Minnesota Perinatal, United Hospital, St Paul, MN.

CdLS is characterized by typical facial features, hirsutism, small hands/feet, limb reduction anomalies, cardiac defects and growth/cognitive delays. CdLS is genetically heterogenous, caused by mutations in the NIPBL gene on chromosome 5p13 or by mutations of SMC1A on the X chromosome. We report here a family with CdLS caused by an NIPBL mutation in a father and his 5 offspring. The first child, a female has IUGR, feeding difficulties and clinical findings of CdLS. Physical exam reveals microcephaly, synophrys, arched eyebrows, long flat philtrum, downturned mouth with thin lips, high palate, micrognathia, upturned nose, thick lowset ears, bilateral clinodactyly/ syndactyly with small hands/feet. This child also had severe reflux requiring a gastrostomy tube. The father has microcephaly, synophrys, arched eyebrows, short stature, hirsutism, small hands/feet and moderate learning disability. The next child, a male full sibling, has similar facial/physical features, but also had a VSD and severe myopia. The third child, a female paternal half sibling, has similar facial features, growth retardation, bilateral peripheral pulmonary artery stenosis and persistent 5th aortic arch. She also has bilateral hearing loss requiring aids. The fourth child of this father, a female half sib, has IUGR, characteristic facial/physical findings, mild peripheral pulmonary artery narrowing and small kidneys with pelviectasis. The next half sibling, a female, has facial features of CdLS, IUGR, and unilateral oligodactyly of the right hand with single digit and short forearm. The remaining extremities are small with syndactyly of 2nd- 3rd toes on right foot. All 5 offspring have varying degrees of developmental delay. Other familial cases of CdLS with dominant inheritance have been described in the literature. This is the first known family of 5 children with CdLS from the same affected father, and significant intrafamilial variability in CdLS is demonstrated.

1035W

LEOPARD syndrome in Finland. *M. Poyhonen[1,2], K. Avela[3], J.S. Moilanen[4], O. Saksela[5], S-L. Sallinen[6], M-L. Vaisanen[7], S. Kivirikko[2].* 1) Dept Med Gen, Univ Helsinki, Helsinki, Finland; 2) Dept Clin Gen, HUSLAB, Helsinki Univ Hospital, Helsinki, Finland; 3) Vaestoliitto, The Family Federation of Finland, Dept of Med Gen, Helsinki, Finland; 4) Dept of Clin Gen, Oulu Univ Hospital and Univ of Oulu, Oulu, Finland; 5) Dept of Dermatology, Helsinki Univ Central Hospital (HUCH), Helsinki, Finland; 6) Dept of Dermatology Tampere Univ Hospital, Tampere, Finland; 7) Dept of Clinical Chemistry, Oulu Univ Hospital and Univ of Oulu, Oulu, Finland.

LEOPARD syndrome (multiple **L**entigines, **E**lectrocardiographic conduction abnormalities, **O**cular hypertelorism, **P**ulmonary stenosis, **A**bnormal genitalia, **R**etardation of growth, and sensorineural **D**eafness) [MIM 151100] is a Noonan-like syndrome caused by mutations in *PTPN11*, *RAF1*, and *BRAF* genes. Mutations in exons 7, 12, and 13 of *PTPN11* gene account for 90% of LEOPARD syndrome. We describe two sporadic patients and one two-generation family with LEOPARD syndrome. The first patient was the second child of healthy, non-consanguineous Caucasian parents. He was born at full term and his birth measurements were 3640 g/ 50 cm/ 34,5 cm. A VSD and hypertrophic cardiomyopathy were diagnosed after birth. The patient had hypotonia and a delay in psychomotoral development. He was initially referred to a clinical geneticist at the age of 11 years for evaluation of suspected neurofibromatosis type 1 (NF1). On examination he had Noonan-like facial features, 5 CALM over 1 cm in diameter and freckling in axillary and inguinal area, no neurofibromas, and no iris Lisch nodules. His length was -1.4 SD, BMI 18.9, and OFC -1.9 SD. *PTPN11* gene mutation analysis revealed c.1528C>G (p.Gln510Glu) mutation in exon 13. The second patient was referred to a clinical geneticist at the age of 8 years for evaluation because of several CALM and lentigines. *PTPN11* gene mutation analysis revealed c.1403C>T (p.Thr468Met) mutation in exon 12. In the two-generation family both mother and daughter had hypertrophic cardiomyopathy, prominent lentigines, short stature (-3SD in the mother) and hypertelorism. *PTPN11* gene mutation analysis re-vealed the same p.Gln510Glu mutation in exon 13 as in patient one. The families are not related to each other. According to our knowledge these are the first Finnish patients with LEOPARD syndrome.

1036W

Orofacial phenotype of van der Woude and Poplyteal Pterygium Syndrome patients with IRF6 mutations. *M. Quentric[1,2], A. Petraud[3], M. Legendre[4], M. Ghassibé[1], L. Desmyter[1], I. Jéru[4], S. Marlain[4], M. Gonzales[5], S. Amselem[4], M.P. Vazquez[2,3], A. Berdal[2], M. Vikkula[1], A. Picard[2,3].* 1) Laboratory of Human Genetics, de Duve Institute, Université catholique de Louvain, Brussels, Belgium; 2) Laboratoire de Physiopathologie Orale Moleculaire, Centre de Recherche des Cordeliers, Université Pierre et Marie Curie-Paris 6, INSERM, UMRS 872, Paris Cedex06, France; 3) AP-HP, Hôpital d'enfants Armand-Trousseau, Service de Chirurgie Maxillo-Faciale et Chirurgie Plastique, Paris, F-75012, France; Université Pierre et Marie Curie-Paris 6, UFR de Médecine Pierre et Marie Curie, Paris, F-75005, France; 4) Institut National de Santé et de Recherche Médicale (INSERM) U.933, Université Pierre et Marie Curie-Paris 6 and Assistance Publique-Hôpitaux de Paris, Hôpital Armand-Trousseau, 75571 Paris cedex 12, France; 5) AP-HP, Hôpital d'enfants Armand-Trousseau, Unité de Génétique du Développement Embryo-Foetal, Paris, F-75012, France.

Van der Woude syndrome (VWS, OMIM #119300) is a dominantly inherited developmental disorder characterized by pits and/or conical elevations of the lower lip, cleft lip and/or palate, and hypodontia. Popliteal pterygium syndrome (PPS, OMIM #119500) shares the clinical features of VWS, with the addition of other signs such as popliteal webs (pterygia), synechiae connecting the upper and lower jaws, ankyloblepharon, syndactyly and genital anomalies. Since many signs occur in the orofacial region and the penetrance is incomplete, it is of great interest to better characterize VWS/ PPS patients to see if an oral phenotype-genotype correlation exists. For this, nineteen families (17 VWS + 2 PPS) from Trousseau hospital (Paris) were seen by a multidisciplinary staff, focusing on the orofacial phenotype. Panoramic imaging were used to check for dental agenesis. The coding exons of the IRF6 gene, including intron-exon boundaries, were sequenced, and Multiplex Ligation-dependent Probe Amplification was performed to test for intragenic deletions or amplifications. We identified a mutation in IRF6 in 82% (14/17) of VWS and 100% (2/2) of PPS families. Affected individuals had clefts (79%), lower lip pits (78%), dental agenesis (72%), and abnormal dental morphology (60%). The upper lateral incisors and the second upper premolars were the most affected. This prospective study demonstrates the high frequency of dental anomalies in VWS/PPS patients, affecting mostly the upper lateral incisors and the second upper permanent premolars. This may be a useful clinical clue for correct diagnosis, as lips pits are not always present.

1037W

Chromosome 19p13.3 Deletion in a Child with Peutz-Jeghers Syndrome, Congenital Heart Defect, High Myopia, Learning Difficulties and Dysmorphic Features: Clinical and Molecular Characterization of a New Contiguous Gene Syndrome. *S. RASKIN[1], F. Faucz[1], V. Sotomaior[1], AF. Bonalumi[1], J. Rosenfeld[2], J. Souza[1].* 1) Group for Advanced Molecular Investigation (NIMA), Graduate Program in Health Sciences (PPGCS), Center for Biological and Health Sciences (CCBS), Pontificia Universidade Catolica do Parana, Parana, Brazil; 2) Signature Genomics, Spokane, Washington, USA.

Peutz-Jeghers syndrome (PJS) is an autosomal-dominant hamartomatous polyposis syndrome characterized by mucocutaneous pigmentation, gastrointestinal polyps and increased risk of multiple cancers. The majority of patients have a causative mutation in the STK11 gene, whereas partial and complete gene deletions account for about 30% of the cases. We report a girl with features of PJS, learning difficulties, dysmorphic features and cardiac malformation, with a 19p13.3 deletion. The deletion size and location was defined using an oligonucleotide-based whole-genome microarray. The patient has a novel, apparently de novo, 1.1 Mb deletion at 19p13.3. The deleted area encompasses at least 47 genes, including the STK11 gene responsible for PJS. This patient is the first report of a 19p13.3 deletion detected by array CGH who exhibits features of PJS and other minor and major malformations, suggesting a new contiguous gene syndrome.

1038W

Hearing impairment data analysis in clients of genetic counseling network of State Welfare Organization in Iran. *S. Akbaroghli[1,2], H. Masoudi Farid[2], P. Jamali[16], N. Sorkhkooh Azari[3], B. Azadeh[6], N. Jianabed[11], G. Mohammadian[14], M. Taghdiri[30], M. Borujerdi[19], F. Habibi[25], M. Mohammadi Sarband[8], J. Malbin[4], P. Nikui[29], H. Khodai[30], F. Sabbagh Kermani[21], Z.T. Zand[13], J. Rezazadeh Varaghchi[10], H. Yazdan[22], G. Fatemi[15], G. Karbasi[20], S.M. Rajabi[27], R. Torabi[28], H. Dehghan[24], A. Moshtaghi[18], M. Kasiri[9], S.F. Hejazi[12], M. Sohrabjaidari[7], N. Janmohammadi[17], F. Ebadi[23], S.H. Mussavi[26].* 1) Dr. Akbaroghli Genetic counseling Center, Tehran, Tehran, Iran; 2) Preventing Disabilities Office of State Welfare Organization, Tehran, Iran;; 3) Tabriz Genetic Counseling Center, Azarbayejan Sharghi Province Welfare Organization, Tabriz, Iran;; 4) Orumiyeh Genetic Counseling Center, Azarbayejan Gharbi Province Welfare Organization, Orumiyeh, Iran;; 5) Ardabil Genetic counseling Center, Ardabil Province Welfare Organization ,Ardabil, Iran;; 6) Isfahan Genetic Counseling Center, Isfahan Province Welfare Organization,Isfahan, Iran;; 7) Ilam genetic Counseling Center, Ilam Province Welfare Organization, Ilam, Iran;; 8) Tehran Genetic Counseling Center, Tehran Province Welfare Organization, Tehran, Iran;; 9) Shahrekord Genetic Counseling Center, Chaharmahal and Bakhtiari Province Welfare Organization, Shahrekord, Iran;; 10) Birjand Genetic Counseling Center, Khorasan Jonubi Province Welfare Organization, Birjand, Iran;; 11) Mashhad Geneti Counseling Center, Khorasan Razavi Province Welfare Organization, Mashhad, Iran;; 12) Bojnord Genetic Counseling Center, Khorasan Shomali Province Welfare Organization, Bojnord, Iran;; 13) Bushehr Genetic Counseling Center, Bushehr Province Welfare Organization, Bushehr, Iran;; 14) Ahvaz GeneticCounseling Center, Khuzestan Province Welfare Organization, Ahvaz, Iran;; 15) Zanjan Genetic Counseling Center, Zanjan Province Welfare Organization, Zanjan, Iran;; 16) Semnan genetic Counseling Center, Semnan Province Welfare Organization, Semnan, Iran;; 17) Zahedan Genetic Counseling Center, Sistan and Baluchestan Province Welfare Organization, Zahedan ,Iran;; 18) Qazvine Genetic Counseling Center, Qazvine Province Welfare Organization, Qazvine, Iran;; 19) Qum Genetic Counseling Center, Qum Province Welfare Organization, Qum, Iran;; 20) Sanandaj Genetic Counseling Center, Kordestan Province Welfare Organization, Sanandaj, Iran;; 21) Kerman Genetic Counseling Center, Kerman Province Welfare Organization,Kerman, Iran;; 22) Kermanshah Genetic Counseling Center, Kermanshah Province Welfare Organization, Kermanshah, Iran;; 23) Yasuj Genetic Counseling Center, Kohgiluyeh and Boyer-Ahmad Province Welfare Organization,Yasuj, Iran;; 24) Gorgan Genetic Counseling Center, Golestan Province Welfare Organization,Gorgan, Iran;; 25) Rasht Genetic Counseling Center, Gilan Province Welfare Organization, Rasht, Iran;; 26) Khorramabad Genetic Counseling Center, Lorestan Province Welfare Organization, Khorramabad, Iran;; 27) Sari Genetic Counseling Center, Mazandaran Province Welfare Organization, Sari, Iran;; 28) Arak Genetic Counseling Center, Markazi Province Welfare Organization, Arak, Iran;; 29) Banarabbas Genetic Counseling Center, Hormozgan Province Welfare Organization, Bandarabbas, Iran;; 30) Yazd Genetic Counseling Center,Yazd Province Welfare Organization, Yazd, Iran; Shiraz Genetic Counseling Center, Fars Province Welfare Organization, Shiraz, Iran;.

The importance of genetic and congenital factors which causes many diseases and disabilities is obvious over the world. In Iran prevention of genetic disabilities program has been planned with the aim of implementation of a genetic counseling network. For implementing this program The network has gradually expanded since the year 1997. Now we have 169 genetic counseling centers throughout the country. One of the chief complaints of clients of these centers has been the history of Hearing Impairment. In this study we have gathered the data from 1999 until 2009 about Hearing Impairment in our provincial referral centers in the genetic counseling network. The total number of files having deafness history in pedigrees among 118786 files are 6521(5.5%).The total number of files having deafness history in each of spouses is 960(14.7%), in both spouses is 625(9.6%), in the offsprings is 1460(22.4%), in one offspring is 957(14.7%)& in two or more offsprings is 503(7.7%). The total number of files which are positive for Connexin 26 is 168(2.58%), Connexin 26 & 30 is 8(0.12%), other nonsyndromic AR genes is 7(0.11%), other syndromic AR genes is 26(0.4%), nonsyndromic AD genes is 1(0.015%) and syndromic AD genes is 5(0.08). The total number of Deaf people is 9825 : 5313(54%) males and 4512(46%) females. The number of different types of consanguinity between Deaf people's parents are: double cousins: 87(1.33%), first cousins: 2803(42.98%), first cousins once removed: 310(4.75%), second cousins:720(11.04)& nonconsanguineous: 2839(43.54%). The total number of different modes of inheritance in the pedigrees of clients with Hearing Impairment chief complaint are: environmental factors: 87(1.33%), nonsyndromic AR: 2961(45.41%), syndromic AR: 269(4.13%), nonsyndromic AD:400(6.13%), syndromic AD: 70(1.07%), nonsyndromic X-linked: 32(0.49%), syndromic X-linked: 9(0.14%), mitochondrial: 3(0.05%) & unknown causes: 1171 (17.96%).

1039W

DFNB49 is an important cause of non-syndromic deafness in Czech Gypsy patients but not in the general Czech population. *D. Brožková[1], J. Laštůvková[2], H. Štěpánková[3], M. Krůtová[1], M. Trková[4], P. Seeman[1].* 1) DNA laboratory, Department of Child neurology, Charles University, Prague, Czech Republic; 2) Department of Medical Genetics, Masaryk Hospital, Regional Health Corporation, Ústí nad Labem, Czech Republic; 3) Department of Clinical Genetics, Hospital České Budějovice, České Budějovice, Czech Republic; 4) Centre for Medical Genetics and Reproductive Medicine GENNET, Prague, Czech Republic.

Non-syndromic hearing loss is genetically extremely heterogenous, but in vast majority of cases follows autosomal recessive (AR) inheritance. Mutations in the GJB2 gene are the most common cause of AR non-syndromic hearing loss named as DFNB. The Gypsies have due to their known endogamy a higher risk for autosomal recessive disorders. In most recessive disorders Gypsy patients are homozygous for causal mutations. We used homozygosity mapping in a Czech Gypsy family with a deaf child and a known consanquinity of parents where mutations in the GJB2 gene were previously excluded. The genomic DNA samples from the family were hybridized on the Affymetrix GeneChip Human Mapping 250K NspI arrays. Data were analysed in the Affymetrix software - Genotyping Console. We searched for homozygous segments in the affected patient which were heterozygous in the parents. The 20 homozygous regions of 2Mb size or larger were found in the patient and compared to known loci or genes connected with the non-syndomic hearing loss (DFNB loci). The second largest homozygous region mapped to previously reported DFNB49 region, with the Marveld2 gene. Sequencing of all 6 coding exons and flanking intron parts of Marveld2 gene revealed the splice site mutation c.1331+2 T>C (IVS 4+2 T>C). This mutation was found in the homozygous state only in the patient, the parents and patient's sister were heterozygous. Testing of further 19 Czech apparently unrelated Gypsy deaf patients without GJB2 mutations revealed the same mutation in further two Gypsy patients, showing that DFNB49, namely the IVS 4+2 T>C Marveld2 mutation is probably an important cause of deafness in gypsy population. The same mutation IVS 4+2 T>C in the Marveld2 gene was previously described in six Pakistani families with nonsyndromic hearing loss.Further testing of 40 deaf non-Gypsy patients without GJB2 mutation did not revealed any Marveld2 mutation, showing that DFNB49 is rare cause of deafness in general Czech -Slavic population. Homozygosity mapping using SNP chips followed by sequencing of candidate genes within the largest homozygous intervals proved to be a powerful tool for finding causal mutations in Gypsy families with autosomal recessive disorders. Our results show that the mutations in the Marveld2 gene are important cause of non-syndromic hearing loss in Czech Gypsies and similar may apply for Gypsies also in other countries. Supported by GAUK 309 and IGA MH CZ 10552-3.

1040W

Recurrent children with TRPS I due to germline or low grade somatic mosaicism in healthy mothers. *H.J. Luedecke[1], S. Gkalympoudis[1], M. Heitmann[1], M. Krajewska-Walasek[2].* 1) Human Genetics, University Clinic, Essen, Germany; 2) The Children's Memorial Health Institute, Warszawa, Poland.

The tricho-rhino-phalangeal syndrome type I (TRPS I) is a dominantly inherited syndrome with craniofacial and skeletal anomalies. It is caused by deletion or mutation of the *TRPS1* gene on 8q23.3. Here, we present the first two families with multiple children with TRPS I born to apparently healthy parents. Especially, the characteristic facial features and anomalies of the hand skeleton were absent in all four parents. Family 1 has three healthy children and three children affected with TRPS I. Family 2 has one healthy daughter and three affected children, two of which are identical twins. Initial mutation and deletion screening was done on DNA isolated from leukocytes. All affected members of family 1were found to have an intragenic deletion comprising exons 2-5 of the TRPS1 gene. This generates an abnormal transcript with exon 1 spliced to exon 6, coding for a supposedly non-functional protein because the 887 N-terminal aa are replaced by 22 novel aa. Interestingly, the dosage for the seven MLPA probes (Kit P228, MRC Holland) for exons 2-5 appeared slightly reduced in the mother's DNA from leukocytes. Segregation analyses with polymorphic markers from the *TRPS1* region revealed that all affected children inherited the same maternal haplotype whereas the healthy children inherited the other. This suggested that the deletion is of maternal origin, and that the mother is a somatic mosaic. Dosage analyses with markers from the deletion region confirmed presence of the deletion in approx. 30 % of leukocytes and 9 % of fibroblasts of the mother. All affected members of family 2 are heterozygous for a c.1831C>T substitution in exon 4 that generates a premature translation stop codon (p.Arg611X). Again, the segregation analysis suggested maternal origin of the familial mutation. However, the point mutation could not be detected in DNA from the mother's leukocytes and fibroblasts. This indicates that the mother either has a germline mosaicism for the mutation or that the fraction of cells carrying the mutation in the analyzed tissues is below the detection threshold of sequence analysis. Although the reports of patients with TRPS II and mosaic deletions suggested that the normal function of the TRPS1 protein is extremely dosage sensitive, our results show that a low grade of somatic mosaicism is compatible with a normal phenotype. This should be considered when counseling healthy parents of patients regarding the recurrence risk for TRPS I.

1041W

GENECA: a French regional network for multidisciplinary management of patients at risk of inherited cancers. *C.M. Maugard[1,2,3,4,5], D. Lejri[1,3,6], M. Voltzenlogel[1,3,6], A.-L. Wetzel[1,3], L. Gilleron[1,6], L. Haar[1,3,6], M. Langeois[1,6], H. Nehme-Schuster[6], J.M. Limacher[3], J.-P. Fricker[6], C. Cordier[1,3,6].* 1) Unité d'Oncogénétique, Service d'Hémato-oncologie, Hôpitaux Universitaires de Strasbourg, Strasbourg France; 2) Laboratoire de diagnostic génétique, Nouvel Hôpital Civil, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; 3) Unité d'Oncogénétique, Service d' Hémato-oncologie, Hôpital Pasteur, Colmar, France; 4) Département de Médecine, Université de Montréal, Montréal (QC), Canada; 5) CR-CHUM, Montréal (QC), Canada; 6) Unité d'Oncogénétique, CRLCC Paul Strauss, Strasbourg, France.

Recently, the French national cancer institute (INCa) launched an extramural program, the main goal of which was to integrate, facilitate and improve global risk management for patients at very high risk of cancers, based on their family history and/or genetic testing result. Seven pilot experiments were supported in five different geographic areas of France and received the following mandates: (1) to offer an individualized cancer screening and preventive recommendations program to patients at high risk of cancer, based on their familial and individual medical history using the latest knowledge in the field, (2) to coordinate the introduction of the new screening and preventive option procedures in the province, (3) to insure access to multidisciplinary services, since patients should be offered adequate screening strategies, and all necessary information and psychological support for prophylactic surgery when indicated, (4) to offer specialized expertise when difficult cases have to be discussed. Our pilot experiment takes place in Alsace province (Eastern part of France). First of all, with the support of the INCa grant, we created a regional network, which was named GENECA for «Genetic of Cancer in Alsace». Our network links three familial cancer clinics located in different hospitals with a total annual turnover of around 1100 consultations per year. Between June 2010 and January 2011, we recruited a core team dedicated to these new missions consisting of a genetic counsellor, an assistant-coordinator, two research assistants, and two psychologists coordinated by a medical geneticist. Since October 2010, individuals at high risk of cancer are invited to participate prospectively in the program. Our efforts are currently directed towards carriers of a *BRCA1* or *BRCA2* or a *MMR* (mismatch repair) gene mutation, as well as members of familial cancer aggregation at high risk of breast, ovarian, colonic or endometrial cancers as computed with Boadicea, BRCAPro and/or PREMM models of genetic susceptibility. We will show the information and tracing notebook and the personalized follow-up agenda we have developed to inform patients of the more recent national recommendations to be applied for follow-up of at risk patients. Finally, we will present new tools under development to coordinate the management of this program and assess the compliance of each participant as well as medical community's one to the national recommendations.

1042W

***BRCA1/2* testing, genetic counseling referral, risk knowledge, and use of guidelines among US primary care clinicians, 2010.** *C. Bellcross[1], K. Kolor[2], M.J. Khoury[2].* 1) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) Office of Public Health Genomics, Centers for Disease Control and Prevention, Atlanta, GA.

Purpose: *BRCA1* and *BRCA2* (BRCA) testing for hereditary breast/ovarian cancer susceptibility has been available since 1996, though few recent studies have evaluated applications in primary care. The purpose of this study was to examine BRCA ordering and genetic counseling referral patterns, use of referral/testing guidelines and ability to identify high/low risk patients among primary care clinicians in the United States. **Methods:** Data from the 2010 DocStyles national survey (1504 respondents, 49% response rate) were used to assess parameters related to BRCA testing among 539 family practitioners (FP), 461 internists, 250 obstetricians/gynecologists (Ob-Gyn) and 254 nurse practitioners (NP). **Results:** Almost all clinicians (1484) were aware of BRCA testing. Many (64%) had referred a patient for BRCA genetic counseling in the last year, and 19% had referred 5 or more. Almost half (45%) had ordered a BRCA test in the last year, and 73% indicated they referred these patients for genetic counseling all or most of the time. When asked which of four clinical scenarios implied the highest likelihood for a healthy 30-year-old woman to carry a BRCA mutation, 59% correctly chose “a first-degree relative with a known *BRCA1/2* mutation”, with age less than 41 ($p=0.001$) the only predictor of a correct response in multiple logistic regression analysis. Predictors of correctly identifying the lowest risk scenario (88%) included practice specialty of Ob-Gyn or NP and female gender ($p\leq0.001$). Practice specialty and gender were similarly predictive of having referred 5 or more patients for genetic counseling in the last year, as was patient SES in the mid-upper range ($p\leq0.02$). The same practice specialties and high patient SES were also associated ($p<0.001$) with having ordered BRCA testing for 5 or more patients in the last year. Among clinicians (1202) who either referred or ordered testing, 80% used one or more guidelines, most commonly ACOG (39%), USPSTF (30%) and NCCN (20%). **Conclusions:** Compared to a related DocStyles survey we conducted in 2007, there was a significant increase in ordering BRCA testing among primary care clinicians (45% vs. 30%, $\chi2$ $p<0.0001$). In addition, it appears that clinicians are utilizing available guidelines and frequently referring patients for genetic counseling. Further study of clinician knowledge regarding patient risk status may be warranted along with efforts to increase access to services for low SES patients.

1043W

Germline *PTEN* and *SDHx* mutations and *KLLN* epimutation and thyroid cancer incidence and phenotypic characteristics in prospective series of individuals with Cowden and Cowden-like syndromes. *J. NGEOW[1,2], J. Mester[1,2,5], L. Rybicki[2,3,4], Y. Ni[1,2], K. Milas[5], C. Eng[1,2,3,5].* 1) Genomic Medicine Institute, Cleveland Clinic, Cleveland, Ohio 44195, USA; 2) Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio 44195,USA; 3) Taussig Cancer Institute, Cleveland Clinic, Cleveland, Ohio 44195, USA; 4) Department of Quantitative Health Sciences, Cleveland Clinic, Cleveland, Ohio 44195, USA; 5) Thyroid Cancer Center, Endocrinology and Metabolism Institute, Cleveland Clinic, Cleveland, Ohio 44195, USA.

Thyroid cancer is an important component of Cowden Syndrome (CS) and is the fastest rising incident cancer in women in the US. The only population-based clinical epidemiologic study suggested that 2/3 of CS patients have benign thyroid disease and 10% malignant thyroid neoplasias. However, systematic study of thyroid neoplasms from a prospectively accrued series of individuals with CS and CS-like features has never been performed. Germline *PTEN* and *SDHx* mutations, and *KLLN* epimutation cause CS and CS-like phenotypes. Thus, we sought to compare the incidence of and clinical characteristics of epithelial thyroid cancers in a large prospectively accrued series of CS/CS-like individuals, in the context of PTEN, SDHx and KLLN status. All probands had comprehensive *PTEN* analysis; *SDHx* mutation analysis occurred in those without *PTEN* mutations/variations and elevated MnSOD; *KLLN* epimutation analysis was performed in the subset without any *PTEN* or *SDHx* mutation/deletion/variant/polymorphism. Gene-specific thyroid cancer histologies, demographic and clinical information and adjusted standardized incidence ratios (SIR) were analyzed. Of 2723 CS/CS-like patients, 664 had thyroid cancer. *PTEN* pathogenic mutations were seen in 36 (5.4%) patients. SIRs for thyroid cancer were 72 (95%CI51-99, $p<0.001$) for pathogenic *PTEN* mutations, 63 (42-92, $p<0.001$) for *SDHx* variants and 45 (26-73, $p<0.001$) for *KLLN* epimutations. Men with *PTEN* pathogenic mutations had a higher risk for thyroid cancers (SIR183) as compared to women (SIR57). Interestingly, all 6 (16.7%) cases diagnosed under aged 18yr carried pathogenic *PTEN* mutations. Follicular thyroid cancer (FTC) was over-represented in *PTEN* mutation positive cases compared to those with *SDHx* and *KLLN* alterations. *PTEN* frameshift mutations were more common in individuals with thyroid cancer (31%) than those without thyroid cancer (17%). We saw an over-representation of *PTEN* mutations in exon 4 ($p=0.05$) and in the promoter ($p=0.03$) in individuals with thyroid cancer compared to those with thyroid cancer but without mutations. Our data show that CS/CS-like patients have elevated sex-specific risks of FTC with earlier onset (median 37yr) due to *PTEN* pathogenic mutations and of PTC with later onset (median 52yr) from *SDHx* and *KLLN* alterations. Clinicians should consider evaluating all children presenting with thyroid cancer for *PTEN* mutation. Current surveillance guidelines for thyroid cancer in CS patients may miss pediatric cases.

1044W

MTHFR gene polymorphisms and their association with isolated cleft lip palate in Mexican children. *A. Gonzalez-del Angel[1], B. Estandía Ortega[1], J. Velázquez Aragón[1], MA. Alcántara Ortigoza[1], M. Reyna Fabian[1], C. Sabas Cruz Fuentes[3], M. Diaz Morales[1], S. Villagómez Martínez[2].* 1) Laboratorio Biología Molecular, Instituto Nacional de Pediatría, Mexico City DF, Mexico; 2) Unidad de Apoyo a la Investigación Clínica,Instituto Nacional de Pediatría; 3) Departamento de Genética, Instituto Nacional de Psiquiatría.

In Mexico, the incidence of CLP is 1.1 per 1000 live births. Many reports support a protective effect of folic acid (FA) intake during pregnancy on the risk for CLP. Also several studies have analyzed the association of MTHFR polymorphisms and CLP; in most of them, c.677C>T and c.1298A>C in both mothers and children were not associated with CLP. However, few studies support the association between the presence of the MTHFR polymorphisms and CLP. The aim of our work was to identify if there is an association between the presence of MTHFR c.677C>T and c.1298A>C with CLP and if FA supplement intake has a protective effect on the risk of CLP. A case-control study was carried out with 132 patients with CLP and 269 controls, all were from Mexico City between 0-18 years old. Mothers answered a questionnaire about FA supplement consumption before and during pregnancy. PCR-RFLP assay was used to detect polymorphisms c.677C>T and c.1298A>C in MTHFR gene. Results: The average age was 5.7 years in the children with CLP and 1.27 years in the healthy group. 46.5% mothers of the CLP group used a FA supplement compared with mothers of the control group (74.5%). Mothers who consumed a FA supplement had a lower risk of delivering a CLP child compared with mothers who did not use FA (OR 0.29, 0.19-0.44 p< 0.0001). Frequency of the 677T allele was higher in control infants (0.62) than in CLP cases (0.51), the frequency of the 1298C allele was similar among both groups (0.17 vs. 0.14). Subjects who carried the MTHFR 677TT genotype showed a lower risk for CLP (OR 0.39, CI 95%: 0.23-0.68, p: 0.00076) compared with those who carried the genotype 677CT OR 0.46, CI 95%: 0.27-0.77, p: 0.00293). When mothers consume FA supplement in combination with the presence of 677CT or 677TT genotypes in the children the OR observed was 0.32 (0.19-0.51, p <0.0001). We did not see an association of CLP with c.1298A>C polymorphism. Analysis of 10 ancestry informative markers did not reveal population stratification in case and control groups. In our study, although a small number of mothers consumed a FA supplement, we observed as described before, a lower risk for CLP with folic acid supplement intake during pregnancy. The only report performed in Mexican subjects did not demonstrated an association between MTHFR polymorphisms and CLP, however our results suggest that MTHFR 677TT genotype conditioned a lower risk for CLP, as mentioned before in few studies in the literature.

1045W

Homozygosity Mapping to Guide Clinical Molecular Testing in Consanguineous Families: A Pilot Study. *R.E. Lamont, I. Gjata, C. Beaulieu, R. Casey, R. Ferrier, R.B. Lowry, D.R. McLeod, A.M. Innes, F. Bernier, J. Parboosingh.* Alberta Children's Hospital, Calgary, AB, Canada.

Within the diagnostic setting, genetically heterogeneous disorders pose a distinct challenge requiring prioritization of multiple candidate genes for mutational analysis. If these disorders are identified in consanguineous families the likelihood of an autosomal recessive condition due to homozygosity for a causative mutation inherited from a common ancestor increases significantly. An approach that identifies regions of genomic material that are identical by descent in affected individuals can be used to narrow the number of candidate genes requiring sequencing. Thus, we sought to determine the clinical utility of using identity by descent mapping by SNP microarray to direct mutation screening in consanguineous families with heterogeneous presumably autosomal recessive disorders. To date, we have identified a probable pathogenic mutation in a family with a single affected individual with congenital muscular dystrophy, where commercial sequencing identified a P303L variant in POMGNT1, a known congenital muscular dystrophy gene. We have also identified a causative mutation in a family with mitochondrial complex I deficiency, where a homozygous frameshift mutation in NDUFS4 was identified in three affected individuals. Lastly, we have identified a likely mutation responsible for non-syndromic hearing loss in an extended pedigree where the entire first coding exon of TMC1, a previously identified non-syndromic hearing loss gene, has undergone a complex rearrangement. Here we present the approach to screening used in these families, additional disorders currently being investigated using this technique and a cost-benefit analysis of utilizing this method were it to be taken up in routine clinical testing.

1046W

Australian relatives' experiences of communicating genetic information following the unexpected death of a family member due to an inherited cardiac condition. *J. Hodgson[1, 2], L. Gallacher[1,2], N. Morgan[3], I. Macciocca[4], S. Metcalfe[1,2].* 1) Genetics Education and Health Research, MCRI, Royal Children's Hospital, Melbourne, Victoria, Australia; 2) Department of Paediatrics University of Melbourne, Victoria, Australia; 3) Genetic Health Services Victoria, Royal Children's Hospital, Melbourne, Victoria, Australia; 4) Victorian Institute of Forensic Medicine, Kavanagh St, Southbank, Victoria, Australia.

The sudden death of a relative is a traumatic event that is likely to disrupt the equilibrium in a family. The genetic implications of sudden death, particularly when it raises the possibility of a cardiac genetic condition in the family, are increasingly being recognised and clinical screening of relatives is being recommended with the autopsy findings. In Victoria, Australia the senior next of kin of the deceased is usually contacted and informed of these familial implications. However the communication of genetic information in families can often be a difficult process, even when a death has not occurred. This research aimed to explore the experiences of family members, where the death of a relative raised the possibility of a genetic condition within the family. Participants discussed the processes of hearing about the death, the legal and medical findings, how familial health implications are shared with surviving relatives, and available supports. Seventeen relatives of individuals who died suddenly between the years 2004-2009 were interviewed in-depth. Interviews were audio-recorded and qualitative thematic analysis was conducted on transcripts. A number of important themes emerged. Participants described in detail how the shock and grief associated with the loss impacted on their ability to process complex information at the time. They expressed frustration with the lengthy forensic and genetic investigations, and explained the struggle to make meaning of their loss when a cause of death or specific diagnosis could not be made. While there was confusion surrounding the multiple processes and roles of different health professionals following the death, individuals were highly appreciative when appropriate care was provided. Participants lamented that the opportunity to be forewarned had not been available for the deceased, and this subsequently provided strong motivation for individuals to disseminate relevant health information within the family. These findings highlight the importance of collaboration between forensic and genetic services to provide streamlined care and facilitate communication among at-risk relatives.

1047W

HAPLOTYPE FREQUENCIES LINKED TO S HEMOGLOBIN IN PATIENTS WITH SICKLE CELL ANEMIA AND SICKLE TRAIT IN THE ATLANTIC AND THE PACIFIC COLOMBIAN COAST. *G. Barreto Rodriguez, M. Lizarralde Iragorri, C. Fong Reales.* Universidad del valle, Cali-Colombia, Colombia.

Sickle cell anemia, a monogenic disorder caused by homozygosity for a single) -globin gene (HBB) mutation (HbS;) 6 GAG→GTG; Glu→Val; glu6-val), has a high incidence in afro-descendant people. It's characterized by a deformation in the red blood cells, generating vaso-occlusions that may result from very mild to very severe symptoms. This condition has being associated with five haplotypes (Bantu, Benin, Senegal, and Cameroon Arab-Indian) which have been linked with the severity of sickle cell anemia , also, these haplotypes linked to the HbS can establish the origin of an individual depending of the haplotype, because they are specific to certain areas of Africa. With the objective to establish and compare the frequencies of the haplotypes linked to the HbS in patients with sickle cell anemia and sickle trait between the Colombian population of the Pacific Coast and the Atlantic Coast we studied 60 individuals from the Atlantic Coast (15 from Monteria, Córdoba, and 45 from Cartagena, Bolivar), and 26 from the Pacific Coast (15 from Buenaventura, 11 from Cali; Valle del Cauca). With relation to Atlantic Coast 53.3% from the studied individuals were homozygous and 46.7% heterozygous for the mutation that causes HbS while for Pacific Coast these values were 38.5% and 61.5% respectively. The observed frequencies of haplotypes associated with HbS for Atlantic Coast (N=92 chromosomes) were: 31.5% Benin, 30.6% Atypical (-++- 16.3% and -+--14.3%), 13.0% Bantu, 9.8% Senegal, 2.1% Arab-Indian and 1.1% Cameroon. In the Pacific Coast (N= 36 chromosomes) the Benin haplotype was the most frequent with 75%, then was the Bantu 14%, Senegal 3%, and in low raises the atypical ones (9%). These results show the Benin haplotype has the high frequency in the patients studied from both coasts (although more frequent in the Atlantic Coast by a 2.3 factor) and permit to infer that the slaves brought to each of the coasts, came from the same locations, the Gulf of Guinea. Atypical haplotypes present on the Atlantic Coast can be explained by recombinations between chromosome) S, either with a common haplotype) S or a less common haplotype associated with a) A chromosome, or mutations in the) -globin gene cluster. The high frequency of the atypical haplotypes may be caused by high levels of inbreeding.

1048W

Development of characterized genomic DNA reference material panels for clinical chromosomal microarrays. *L. Kalman[1], H. Kearney[2], L. Toji[3], D. Berlin[3], C. Carmack[4], L. Conlin[5], J. Gastier-Foster[6], N. Gerry[3], L. Jennings[7], V. Jobanputra[8], C. Lee[9], J. Leonard[3], B. Levy[8], C. Shaw[10], R. Shippy[11], S.B. Fulmer-Smentek[12], S. South[13], N. Spinner[5], J. Stavropoulos[14], Z. Tang[3], H. VanSteenhouse[15], D. Wolff[16], A. Yesupriya[1], S. Kulkarni[17].* 1) Centers for Disease Control and Prevention, Atlanta, GA; 2) Fullerton Genetics, Asheville NC; 3) Coriell Institute for Medical Research, Camden NJ; 4) Baylor College of Medicine, Houston TX; 5) Children's Hospital of Philadelphia, Philadelphia PA; 6) Nationwide Children's Hospital, Columbus OH; 7) Children's Memorial Hospital, Chicago IL; 8) Columbia University, New York NY; 9) Brigham and Women's Hospital and Harvard Medical School, Boston MA; 10) Roche Nimblegen, Madison WI; 11) Affymetrix, Santa Clara CA; 12) Agilent, Santa Clara CA; 13) ARUP Laboratories, Salt Lake City UT; 14) Hospital for Sick Kids, Toronto Canada; 15) Illumina, San Diego CA; 16) Medical University of South Carolina, Charleston, SC; 17) Washington University, St. Louis MO.

Chromosomal microarray analysis (CMA) is used to detect chromosomal variations throughout the human genome. Although this technology has become the standard of care, there are no commercially available, well-characterized genomic DNA reference materials (RM) for this technology. RMs are required for assay development and validation, quality control, and proficiency testing. To address this need, the CDC's Genetic Testing Reference Materials Coordination Program (GeT-RM), is collaborating with clinical cytogenetics experts, microarray manufacturers, and the National Institute of General Medical Sciences (NIGMS) Human Genetic Cell Repository (HGCR) at the Coriell Institute for Medical Research to develop genomic DNA RMs for CMA. The NIGHS HGCR includes hundreds of cell lines with chromosomal abnormalities. Many of these samples have been karyotyped or analyzed by fluorescence in situ hybridization (FISH). Additionally, more than 700 cell lines have been genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0. Using these Coriell cell lines, we have created a genomic DNA RM panel consisting of 96 characterized genomic DNA samples with specific chromosomal abnormalities typically detected in clinical cytogenetics laboratories. These include microdeletion and microduplications, subtelomeric abnormalities, uniparental disomy and other genetic variations. Each DNA sample was characterized using four separate CMA platforms to determine consensus genomic coordinates of the copy number alterations. The raw data was analyzed by 10 clinical cytogeneticists to establish the consensus breakpoints, the copy number status of each genomic imbalance and to assess each sample's suitability as a RM. New cell lines derived from patients with recently described novel microdeletion/ duplication syndromes will also be established and DNA from those samples will be used to develop RMs to supply additional chromosome variants absent from the panel of 96 Coriell cell lines. We are also developing a probe evaluation panel to characterize the performance of CMA oligonucleotide probes utilized in any CMA. This tool will include approximately 200 genomic DNA samples which contain large del/dups that collectively encompass most of the genome. We will present a summary of cell line selections showing the coverage of the genome, characterization of chromosomal abnormalities, and describe our progress to date. These characterized genomic DNA RMs are available from the NIGMS HGCR.

1049W

The 22q11.2 Duplication Syndrome - A Genetic Counseling Conundrum! *D.M. McDonald-McGinn, S. Saitta, A. Kohut, A. Bailey, M.D. Ha, B. Emanuel, E.H. Zackai.* Div Human Gen, Children's Hosp Philadelphia, Philadelphia, PA. and The Univeristy of Pennsylvania School of Medicine.

Background: 22q11.2 CNV's are well described entities involving deletions and duplications. Both are the result of unequal meiotic crossovers & thus aberrant interchromosomal exchanges (non-allelic homologous recombination) due to the presence of low copy repeats which flank the deletion/ duplication and define the breakpoints. Although expected to occur with equal frequency based on this common mechanism, we have previously found the deletion to occur approximately twice as often as the duplication. Moreover, in several instances we have identified the duplication in seemingly unaffected adults, raising important questions regarding: management; recurrence risk counseling; and prenatal diagnostic options. Methods: In an attempt to provide such anticipatory counseling, we reviewed our own database of 31 individuals with a 22q11.2 duplication specifically evaluating the size of the duplication; clinical findings; and familial variability. Results: 73% of patients had a standard A-D duplication. Clinical findings included: CNS abnormalities (8); GERD (7); congenital heart disease (4); craniofacial anomalies (4); ENT abnormalities (3); endocrine issues (3); FTT (3); hypospadias; development delay (12) & autism (5). Several patients were thought to have mild dysmorphic features but there was no consistent gestalt. 71% of families where both parents were available for testing were found to be familial. Features in affected relatives varied from developmental disabilities to completely normal intelligence including two unrelated parents with doctoral degrees. Conclusions: In summary, the 22q11.2 duplication is similar to the 22q11.2 deletion in many ways: the mechanism for the occurrence is the same; the majority of individuals have a standard A-D CNV (83% dup v. 85% del); and the clinical features are relatively similar when examining probands. However, the difference in familial cases is notable (71% dup v. 7% del); and despite the common mechanism, the prevalence for the 22q11.2 duplication syndrome is half as common as its counterpart. We believe this may well be explained by under ascertainment due to extreme variable expressivity as evidenced by our two parents with postgraduate degrees. Nonetheless, we caution that this data most definitely supports testing all parents of affected children in order to provide appropriate recurrence risk counseling as well as prenatal diagnostics options despite the perplexing and unpredictable associated phenotype.

1050W

If it's free, do people necessarily agree? Uptake of parental testing after abnormal array comparative genomic hybridization in our clinical genetics experience. *N. Shur[1, 2], J. Bernabe[2], J.T. Machan[1,2,4], K. Hovanes[3], E. Morrow[1,2], D. Abuelo[1,2].* 1) Rhode Island Hospital, Providence, RI; 2) Brown University, Providence, RI; 3) CombiMatrix Diagnostics, Irvine California; 4) University of Rhode Island, Kingston RI.

Array comparative genomic hybridization testing (aCGH) is now considered the gold standard for genetic evaluation of patients with developmental delay, intellectual disability, and/ or multiple congenital anomalies. In our patients whose aCGH testing shows abnormal results or variants of unknown significance (VUS), we routinely offer parental testing. Our purpose is to determine if a parent is likewise affected before providing prenatal counseling. The answer is not always obvious, given variable expression of most microdeletion/ duplication syndromes. It is also important to exclude the possibility of a balanced translocation carrier in one of the parents. In cases when aCGH reveals a VUS, the presence of the same result in an unaffected parent renders the VUS less likely to be diagnostic. Insurance approval for parental testing is not routine, and for most parents, the testing is prohibitively expensive. Therefore, we send aCGH testing to a laboratory that offers free parental testing when indicated. To determine what proportion of parents chose free parental testing, we received institutional review board approval for a retrospective chart review, and a research assistant randomly reviewed 51 charts from 10/27/2010 to 12/22/2010 with the only inclusion criterion that aCGH testing was done. The patients were evaluated as outpatients in Genetics Clinic or as inpatient consults. They ranged in age from 1 day - 10 years, and were evenly split in terms of gender (25 males, 26 females). Out of all patients, 14 patients had abnormal results (27%), 2 patients had VUS (4%), and the remainder were normal (69%). All parents of the 16 patients with abnormal results or a VUS were provided with genetic counseling and offered testing at no cost. Only 3/ 16 (19%) of the parents offered testing chose to complete it. Some laboratories claim that the high cost of parental testing makes it impossible to offer it for all abnormal cases or those with VUS, but in our experience, parental testing is indicated in the minority (31%), and a smaller group of all parents actually completes it (6%). Barriers to completion may include fear of an abnormal result, dislike of blood draws (saliva kits may offer a good alternative for aCGH cases), and lack of understanding regarding future relevance. Alternatively, some parents may not desire future prenatal counseling. Further research should include surveying parents regarding their decisions to accept or decline testing.

1051W

Resolving the genetics of a disease phenocopy by linkage mapping and high-throughput-sequencing. *J.L. Blouin[1], M. Failly[2], A. Letourneau[2], A. Munoz[2], C. Iseli[3,4], D. Robyr[2], C.D. Delozier[1], S.E. Antonarakis[1,2], S. Ala-Mello[5,6], L. Bartoloni[2].* 1) Genetic Medicine, University Hospitals of Geneva, Geneva, Switzerland; 2) Genetic Medicine and Development, University of Geneva School of Medicine; Switzerland; 3) Ludwig Institute for Cancer Research, Lausanne, Switzerland; 4) Swiss Institute of Bioinformatics, Lausanne, Switzerland; 5) Department of Clinical Genetics, Helsinki University Central Hospital, Helsinki, Finland; 6) Rinnekoti Foundation, Espoo, Finland.

In order to identify the genetic etiology of presumed Primary Ciliary Dyskinesia (PCD) in 5 out of 11 children from a consanguineous family, we performed High-Throughput-Sequencing (HTS) targeted to genes located within linkage interval. PCD was strongly suspected because of recurrent respiratory symptoms combined with immotility of nasal cilia and retinal degeneration. Lobectomies of the left lung were performed in 3 brothers due to bronchiectasis. DNA genotypes of 250K SNPs in 8 members of the family were analyzed for linkage, homozygous regions, and CNVs. No novel CNV could be identified. A 14 Mbp region of homozygosity and a highly suggestive linkage, which includes the MHC locus, was identified on chromosome 6p21-p22. Successive mutation searches were then performed on DNA from the index case by sequencing exons and splice sites within the linkage interval: i- in the 4 strong candidate genes; ii- in 24 genes coding for ciliary proteins (www.ciliaproteome.org); iii- in all refseq genes. HTS on microarray-selected DNA for all refseq genes (n= 5570 exons) within the larger linkage interval (Chr6:4'000'000-42'000'000) provided 3 strong homozygous mutation candidates. These 3 missense variants, with potential functional deleterious effects in genes ETV7, UHRF1BP1 and TAP2, segregate perfectly with the phenotype in this family. Mutations in TAP2 have been reported in Bare Lymphocyte syndrome type I (BLSI), an immunodeficient condition characterized by chronic bacterial infections of the respiratory tract. Bronchiectasis, bronchial obstruction and nasal polyps in BLSI are associated with lack of the HLA class I cell surface antigen. In this family, missense variant p.Leu516Arg in TAP2 occurs in a highly conserved 24-residue region and likely affects the conformation of a helix loop of the protein. Clinical presentation of the family is compatible with BLSI since: i- none of the affected individuals has situs inversus, which is expected to occur in about half of patients with PCD; ii- no fertility problems were reported, 3 of the affected men have 1-5 children each; iii- diagnoses of bronchial and lung infections and chronic sinusitis are compatible with diffuse panbronchiolitis. This study demonstrates that massive sequencing approach may help in the resolution of medically-uncertain diagnoses.

1052W

"Not knowing the odds is terrible": Patient and provider experiences with the uncertainties of prenatal microarray testing. *D. Soucier, B. Bernhardt for the NICHD Prenatal Microarray Study Group.* Medical Genetics - Penn CIGHT, University of Pennsylvania, Philadelphia, PA.

Background: Because of incremental yield of important genomic information, prenatal microarray (MA) testing for copy number variants (CNVs) is frequently offered in pregnancies with unexplained fetal anatomic anomalies and increasingly for routine indications. CNVs have variable expressivity and penetrance, complicating prenatal counseling and decision-making. An on-going NICHD-funded multicenter study is comparing the accuracy, efficacy and clinical advantages of prenatal diagnosis using MA analysis with conventional cytogenetic analysis, and has enrolled over 4,000 women having routine amniocentesis or CVS. Results, including potentially important variants of uncertain significance (VUS), are explained to the women by a genetic counselor and/or a clinical geneticist. Methods: We are conducting a qualitative study of the experiences of women in this study who received positive or VUS results, and their providers. To date, we have analyzed interviews with 15 women receiving abnormal results, 10 genetic counselors and 7 physicians. Results: Despite having participated in an informed consent session, few women recalled being told that results could be uncertain. Women reported being blindsided by an abnormal MA result after receiving normal chromosome results. Most women continuing pregnancies remained worried after delivery despite normal follow-up scans and delivering a healthy child. When asked what would have made the experience easier, most women mentioned a need for ongoing support from a knowledgeable and compassionate professional, and some needed more assistance arranging for a pregnancy termination. Several women regretted undergoing MA testing, and suggested that the potential uncertainty of results be highlighted during pre-test counseling. When asked about challenges of prenatal MA testing, nearly all providers first mentioned dealing with uncertain results. Providers expressed frustration with the lack of concrete information about expected fetal outcomes and were often unsure how to assist women making decisions in the face of uncertainty. Conclusions: As MA technology transitions into clinical care, pregnant women and their providers will need tools and support to help cope with uncertain results. Women considering prenatal MA testing need to clearly understand the implications of uncertain findings before being tested. Our results raise important ethical issues relating to reporting uninterpretable prenatal testing results.

1053W

Web-based expert review system of clinical genetics web site. *H. Numabe[1,2], S. Muzuno[2,3], N. Sakai[2,4], N. Okamoto[2,5], T. Ikeda[2,6], Y. Fukushima[2,7].* 1) Dept Med Ethics & Med Genetics, Kyoto Univ Graduate Sch Med, Kyoto, Kyoyo, Japan; 2) The Information and Communication Committee of Jap Soc for Genetic Counseling, Tokyo, Japan; 3) Dept of Pediatrics, Central Hosp, Aichi Human Service Center, Kasugai, Aichi, Japan; 4) Dept of Pediatrics, Osaka Univ Graduate Sch of Med, Suita, Osaka, Japan; 5) Dept of Med Genetics, Osaka Med Center & Res Institute for Maternal & Child Health, Izumi, Osaka, Japan; 6) Dept of Obst & Gyn, Kagoshima Univ, Kagoshima, Kagoshima, Japan; 7) Dept of Med Genetics, Shinshu Univ Sch of Med, Matsumoto, Nagano, Japan.

There are a large number of websites providing information relating to medical genetics or heredity, and more than a few of them are routinely used as reference. In addition, because most of the websites are open to non-healthcare professionals, people with genetic disorders and their families can also refer to them. However, when search engines are used to look for information on medical genetics or heredity, websites found are not always those with information based on evidence-based medicine, EBM, and patients or their families may obtain wrong information. This could, in quite a few cases, bring them a sense of distrust for healthcare professionals or hurt them emotionally. To deal with this issue, we selected some websites providing information relating to medical genetics or heredity and established an online system where several professionals can check some items to evaluate the contents of the websites. What makes this system different from ordinary evaluation systems is that the professionals can give not only a positive rating of 1 to 5 but also a negative rating of -5 to -1 to the websites. Therefore, if a website provides inappropriate information, it will receive negative feedback. We here present some examples of the website evaluation in our pilot system and talk about the challenges of the system we need to deal with.

1054W

Frequency estimation of low-level somatic mosaicism for pathogenic CNVs. *I.M. Campbell[1], S.C.S. Nagamani[1], A. Erez[1], C. Robberecht[2], A. Ester[1], M. Bartnik[3], B. Wisniowiecka-Kowalnik[3], K. Derwinska[3], E. Bocian[3], K.S. Plunkett[1], A.N. Pursley[1], C.A. Shaw[1], S-H.L. Kang[1], W. Bi[1], S.R. Lalani[1,4], C.A. Bacino[1], A. Patel[1], J.A. Veltman[5], L.E. Vissers[5], J.R. Vermeesch[2], S.W. Cheung[1], L.R. Lupski[1,4,6], P. Stankiewicz[1,3].* 1) Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; 2) Centre for Human Genetics, University Hospital, Catholic University of Leuven, Leuven, Belgium; 3) Dept of Medical Genetics, Institute of Mother and Child, Warsaw, Poland; 4) Texas Children's Hospital, Houston, TX, USA; 5) Dept of Human Genetics, Nijmegen Centre for Molecular Life Sciences and Institute for Genetic and Metabolic Disorders, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 6) Dept of Pediatrics, Baylor College of Medicine, Houston, TX.

Only incidental observations of somatic mosaicism for copy-number variations (CNVs) have been reported in the literature, wherein patients or their healthy parents are mosaic for pathogenic genomic deletions or duplications. Recently, we reported a deletion of TGFBR2, in a female with microcephaly and global developmental delay. FISH analysis showed no evidence of this deletion in the parental peripheral blood; however, low-level somatic mosaicism was detected using PCR of the paternal peripheral lymphocyte DNA. We also previously reported low-level somatic mosaicism for a complex PMP22 exon 4 deletion in the mother of two sisters with Charcot-Marie-Tooth disease detected by PCR but not by aCGH; germ line mosaicism could be inferred by recurrence of the CMT causing complex rearrangement. Given the 5-30% sensitivity of diagnostic FISH, aCGH and SNP arrays, and that a significant number of CNVs arise due to microhomolgy-mediated DNA replication errors during mitotic cell divisions, we hypothesized that somatic mosaicism below the detection threshold of FISH and microarray methods in asymptomatic parents of children with genomic disorders may be more common than previously recognized. In support of this contention, we identified a family with two brothers with severe intellectual disability and microcephaly, who both had the same 2 Mb deletion involving AKT3. FISH analysis did not detect this change in either healthy parent. Using multiplex long-range PCR we obtained a patient-specific junction fragment that was subsequently found in the father, albeit with reduced band intensity. To estimate the frequency of this phenomenon, we retrospectively queried our aCGH databases for patients with nonrecurrent interstitial genomic deletions determined to be de novo by FISH or aCGH analyses of the parental DNA samples. We selected over 150 families, narrowed their deletion breakpoints by custom-designed high-resolution oligo aCGH or multiplex PCR, and obtain patient-specific junction fragments. PCR analysis was then performed on parental peripheral lymphocyte DNA with primers specific for their child's deletion. We will present the results of screening over 100 families. Our findings suggest low-level somatic mosaicism can lead to a clinically relevant number of potentially heritable CNV in humans. Moreover, we propose that new methodologies for detecting these low-level changes are needed for appropriate diagnosis, recurrence risk estimation and genetic counseling.

1055W

Trends in genetic counseling in India - experience from one Genetic Center. *S. Bijarnia, R.D. Puri, U. Kotecha, S. Kohli, M. Jain, J. Verma, R. Saxena, M. Lall, I.C. Verma.* Center of Medical Genetics, Sir Ganga Ram Hospital, New Delhi, New Delhi, India.

With the advent of the human genome project, the practice of clinical genetics and prenatal diagnosis in India has changed for the better. A major contribution to the change has also been brought about by advancement in technologies for genetic testing and prenatal screening. The awareness among medical profession is also gradually improving. We analyzed the trends in genetic counseling at Sir Ganga Ram Hospital over the past 13 years. The categories of genetic disorders and reasons for referrals in 3850 patients in whom genetic counseling was provided in 2010 were compared to the initial 1370 patients seen in 1997-98. Majority (n=2234/3850, 58%) of the patients sought advice during pregnancy (or pre-pregnancy stage) for prenatal diagnosis. This is in contrast to 38.4 % patients seeking prenatal diagnosis in the nineties. The screening for chromosomal disorders (first trimester biochemical screen, triple test, ultrasonography) and amniocentesis for advanced maternal age and previous Down syndrome accounted for 1494 (38.8%) cases, compared to 21% in year 2000. Prenatal diagnoses for 329 (8.5%) single gene disorders were also carried out. Other causes for a genetic referral and counseling (with some overlap with prenatal diagnosis) included mental retardation, multiple malformations in child (339, 8.8%), Down syndrome and other chromosomal disorders (319, 8.28%) Thalassemia/hemophilia and other hemoglobinopathies (83, 2.15%), Muscular dystrophies & SMA (194, 5%), Neural tube defects and other birth defects (45, 1.16%), Metabolic disorders (223, 5.79%), Skeletal dysplasias and other endocrine disorders (114, 2.96%), and others. A significant number of previously unrecognized single gene disorders (263, 6.8%) including neurological, hepatic, renal, ear, eye and skin diseases etc were seen in 2010. Conclusion: The awareness of biochemical and ultrasound screening tests during pregnancy and its incorporation into obstetric practice has resulted in significant increase in prenatal diagnosis of chromosomal disorders. The advancement in molecular technology and knowledge of new genes has also increased the diagnosis of single gene disorders helping in accurate prenatal diagnosis and reduction in burden of genetic disorder in the families.

1056W

Characteristics of healthy participants in a whole genome sequencing study. *M.K. Cho, C. Caleshu, L. Hudgins, H. Greely, S.J. Lee, K.E. Ormond.* Stanford University, Stanford, CA.

In 2011, Stanford University began enrollment of up to 40 individuals in a whole genome sequencing (WGS) study that offered clinical interpretation to `genetically educated individuals,' primarily faculty in genetics and genomics. We designed behavioral surveys to assess the participants' characteristics and attitudes at various points in the process; this paper will present the initial enrollment data before and after the provision of pre-sequencing genetic counseling (GC). As of late-May 2011, the participants (N=12) are primarily scientists who self-report that they are non-smokers, moderate exercisers and low alcohol users. 70% have previously undergone genotyping through a DTC company, and most felt they would pay $500-1000 out of pocket for WGS results. Primary reasons for participating in the WGS study were curiosity, that `it is exciting', to improve teaching of genomics, cost (free), to contribute to research, for future clinical use, the potential to make health behavior changes in the future, and the right to know one's genetic data. Participants felt there was currently low clinical validity and utility, and that scientists, doctors and themselves currently have a limited ability to interpret WGS results. Nonetheless, most felt that WGS should be available to the general public. When asked about specific types of conditions they do not want to know about from their genome, participants indicated interest interested in all health information available, and prioritized obtaining ancestry and pharmacogenomic variant information. After GC (N=4), participants reported that the opportunity to receive GC services was an important, and they would pay up to $100. All would recommend GC for individuals with less genomics knowledge, and one participant reported that prior to GC (s)he had not considered the issues related to identifying late-onset and low penetrance alleles. While a biased population, most participants did not report anxiety about the potential information available or the decision to participate, and only one expressed moderate concerns re: confidentiality.

1057W

Evaluation of a molecular genetic testing strategy for autosomal dominant retinitis pigmentosa and its translation into the genetic counselling process. *M.A. Day[1], J.E. Sutherland[1], E. Heon[1,2,3].* 1) Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Genetics and Genome Biology Program, The Hospital for Sick Children, Toronto, Ontario, Canada; 3) Department of Ophthalmology and Vision Sciences, University of Toronto, Toronto, Ontario, Canada.

Retinitis pigmentosa (RP), a genetically heterogeneous retinal condition, affects an estimated 1 in 3500 individuals of which 30-40% are thought to be autosomal dominant (AD). Twenty-one ADRP genes are now clinically available for testing. Rhodopsin is the most commonly involved (up to 30% of ADRP). Purpose: To effectively integrate advances in molecular knowledge into clinical care, we underwent a systematic review of our molecular genetic testing strategy for ADRP at the Hospital for Sick Children (Toronto). Implications for genetic counselling will be discussed using specific cases. Methods: A retrospective review of our ocular genetics database identified probands with a clinical diagnosis of RP and a clear or suspicious AD pedigree, who underwent molecular genetic testing. The family history was classified as “suspicious” when the questionably affected relatives lived geographically far from our proband and were not available. Results: Of the 115 probands identified, close to 70% have undergone some genetic testing. Mutations in the rhodopsin gene were the most common but responsible for only 15% of ADRP. This may reflect Toronto's ethnically diverse population, with 47% of individuals belonging to a visible minority. The *RDS* gene accounted for 5% and another 18 ADRP genes were involved. For probands for whom variants of unknown significance are identified, validating the results requires further testing and examining family members which increases counseling/clinic time and the time to deliver results. Those with a suspicious family history are invariably difficult to interpret. The number of patients with polygenic variants has increased as testing panels include multiple ADRP genes. The causative mutation is important for management, lifestyle choices and eligibility for human clinical trials. Probands who know their causative mutation are faced with the decision of presymptomatic diagnosis for their children. Conclusion: Mutation detection rates for ADRP improved. Periodic review of our testing yield and validation strategies will help us and others to improve upon them and may help guide lab clinicians in the improvement of their services. Our experience also highlights the need for a concerted effort to develop an international web-based mutation database that could be easily accessed and up-dated. Furthermore, the important post-testing genetic counselling issues of patients with ADRP must be highlighted.

1058W

A primary care model for assessing patient disease risk based on family health history: Description and preliminary analysis. *V.C. Henrich[1], E. Hauser[2], L. Orlando[3], C. Christianson[1], A. Buchanan[3], K.P. Powell[1], A. Agbaje[4], G. Ginsburg[5], Genomedical Connection.* 1) Ctr for Biotech, Genomics, and Health Research, Univ North Carolina,Greensboro, Greensboro, NC; 2) Center for Human Genetics, Duke University, Durham, NC; 3) Department of Medicine, Duke University, Durham, NC; 4) Cone Health System, Greensboro, NC; 5) Institute for Genome Sciences and Policy, Duke University, Durham, NC.

The positive impact of genomic medicine on human health will depend largely upon its successful practice in primary care. Yet, research shows several impediments to collecting family health history (FHH) - the most readily available and applicable piece of genomic information - and applying it to manage disease risk in primary care. The Genomedical Connection has developed a FHH-based decision support program to facilitate risk management for for three diseases: colorectal cancer, breast/ovarian cancer, and thrombophilia. An algorithmic computational tool (MeTree) assesses individual patient disease risk from the FHH provided by the patient, and offers risk management recommendations to the practitioner and patient. For this study, patients were recruited prior to a regularly scheduled wellness visit at two community-based primary care practices; the frequency of medical recommendations based on FHH assessments are noted here. Among 523 patients recruited so far, 59.1% were female, 83% were Caucasian; mean age was 58.3±12.3 years. Based on the MeTree analysis of FHH, 26.8% (N=140) of all patients received a recommendation to see a cancer genetic counselor. A larger percentage of females (32.4%, 100 of 309) were recommended to see a cancer counselor than males (18.7%, 40 of 214). Seven female patients (1.3% of all patients) received a recommendation to see a genetic counselor concerning their risk for thrombophilia. An elevated risk for breast/ovarian cancer led to recommendations for 43 female patients (13.9% of females), including chemoprevention (N=32, 10%), breast MRI exams (N=6, 1.9%), and gynecological surveillance (N=5, 1.6%). Early colorectal cancer screening was recommended for 54 patients (10%), with about equal frequency in both sexes. Primary care providers referred 46 patients for genetic counseling, and 20 of them received a counseling session. Whether the risk levels seen in this patient population reflect overall risk levels in the general population cannot be ascertained. However, the percentage of participants for whom some increased-risk management was recommended is similar to findings from earlier research. This supports a conclusion that the use of FHH can be a valuable component of a genomic medicine model in primary care.

1059W

A comparison study of the practices of genetic counsellors between France and Canada. *E. Le Boette[1,2], M. Edmont[2,3], M-A. Voelckel[2,4], J-L. Mandel[5], H. Sobol[6], C. Cordier[2,7].* 1) Dept of medical genetic, University Hospital, Strasbourg, France; 2) AFCG, French Association of Genetic counsellors, Marseille, France; 3) Dept of genetic, University Hospital, Montreal, Canada; 4) Dept of medical genetic, Timone's Hospital, Marseille, France; 5) Laboratory of medical genetic, University Hospital, Strasbourg, France; 6) Dept of oncology, Institut P.Calmettes, Marseille, France; 7) Dept of oncology and haematology, University Hospital, Strasbourg, France.

Genetic counsellors are Health Professionals with specialized training and experience in the areas of clinical genetics and counselling. They did work as members of a multi-disciplinary healthcare team that provides genetic services. In France, the profession of genetic counsellors is relatively recent (2005). This profession has been created due to the increasing number of genetic consultations but also face to the decrease of medical professional in this field. About Canada, the profession has been created, for the same reason, since 1985 by a genetic counsellor graduated in the United-states and by the auspices of geneticists who exercise their profession in Canada. Members of different groups (French and Canadian) have received an electronic survey based on their background,the role and the practice of the profession of genetic counsellors in their own country. The questionnaire was sent to the Association of Genetic Counsellors which transmitted it to all their members. Data were collected during the first quarter of 2011. We are looking to see if there are major differences in the practice of exercising the profession, but also in the education and in the collaboration established between genetic counsellors and medical geneticist.

1060W

Personal genotyping in genetics faculty members considering personal whole genome sequencing. *K.E. Ormond, L. Hudgins, C. Caleshu, H. Greely, S.J. Lee, M.K. Cho.* Stanford Univ, Stanford, CA.

Genetics faculty members who were participating in a whole genome sequencing research project (which plans to enroll up to 40 participants by summer 2011) completed an online behavioral survey that assessed, in part, their personal experiences with genotyping through a DTC company. As of late-May 2011, 8 of 11 (70%) participants reported undergoing genotyping. Of the 3 who had not had genotyping noted cost, lack of clinical utility, and `waiting for whole genome sequencing' as reasons. Of the 8 tested, 5 reported having paid between $100-300 out of pocket for the testing. None shared results with a health care provider, nor planned to. The most common reasons listed for undergoing the testing were: general curiosity, to obtain information that may be clinically useful in the future, because the data is “exciting,” may improve teaching of genetics/genomics, and to inform family members of risks. 5 of the 8 appear to be regularly engaging in research about the meaning of their genotype results, and 4 have shared information with family members. Seven participants felt they would recommend genotyping to others, although only 4 individuals reported that the experience improved their understanding of or teaching of genetics and genomics. Six participants report sharing with students that they have undergone genotyping (4 of whom share their results as part of a lecture), and they used this information primarily as a way to perform additional research for their lectures; 3 participants reported the experience made them consider the implications on individuals undergoing such testing. Views were mixed on whether personal genotyping should be offered to students, and if so whether it should be an optional exercise within a required or an elective course, although only those who had undergone genotyping were supportive of its inclusion in required courses. These experiences should be considered when offering faculty genotyping as an educational tool and when offering whole genome sequencing to `early adopters.'.

1061W

An integrated program for care of children with agenesis of the corpus callosum encompassing caregivers, clinical services and research. *K. Pope[1], M. Spencer-Smith[1], D. Amor[4, 1], M. Delatycki[3, 5], P. Lockhart[5], A. McIlroy[1], G. McGillivray[4], V. Anderson[1], R. Leventer[2].* 1) Murdoch Childrens Research Institute, Parkville, Victoria, Australia; 2) Royal Childrens Hospital, Parkville, Victoria, Australia; 3) Clinical Genetics, Austin Health, Heidelberg West, Victoria, Australia; 4) Victorian Clinical Genetics Service, Parkville, Victoria, Australia; 5) Bruce Lefroy Centre for Genetic Health Research Murdoch Childrens Research Institute Parkville,Victoria Australia.

Purpose: Agenesis of the corpus callosum (ACC) is a common brain malformation diagnosed by neuroimaging. Children present with neurocognitive impairment ranging from severe developmental delay to mild to no learning or behavioural. Although there have been major advances in understanding the structural features and causes of ACC, for many families we are still unable to adequately explain why their child is not developing as expected. The ability to provide accurate prognostic and genetic counselling also remains deficient. We are attempting to address this through the Genetics of Brain Development (GBD) Program at the Murdoch Childrens Research Institute, which is contributing to greater understanding and support for families and children with a brain malformation. This project is multifaceted, with major strengths in 1. Understanding caregiver wellbeing, a crucial factor in ensuring quality of care for families, who provide the majority of care for these patients. 2. Provision of clinical care and genetic counselling 3. The application of new genomic technologies to understanding the underlying molecular basis of ACC. To the best of our knowledge, this approach is unique in its application to the wellbeing of caregivers and children with ACC. ACC Families were recruited through the GBD Clinic. Five families will be described. Caregivers completed: i) a structured interview to examine medical history, interventions, schooling and current concerns about their child; and ii) the Hospital Anxiety and Depression Scale to examine their own anxiety and depression symptoms. Some families were also enrolled in linkage and molecular genetic studies to identify the causative gene associated with ACC. High density SNP CHIP arrays provides data for copy number analysis and linkage analysis. Genes in candidate regions are then analysed by whole exome capture and massively parallel sequencing. Conclusions: There was a wide range in functioning and most children require intervention. Caregivers experienced considerable uncertainty about the health, academic abilities and future for their child; most demonstrated increased levels of anxiety. Uncertainty about possible causes and recurrence risks are also important issues and there was considerable enthusiasm for research aimed at identifying `their' gene. Caregivers reported feeling isolated, and we are planning a support group for families of children with ACC in Australia to address this.

1062W

Utility of Fetal Autopsy Evaluation on Genetic Counseling in Developing Countries. *R.D. Puri, U.K. Kotecha, S.B. Bijarnia, M.L Lall, P.S Savior, R.S Saxena, I.C.V. Verma.* Center of Medical Genetics, Sir Ganga Ram Hospital, New Delhi, INDIA, Delhi, India.

Antenatal ultrasound detection of fetal malformations is increasing with improving technology. The threshold of continuing a pregnancy in the presence of a fetal abnormality is low in India and most of such pregnancies are terminated. The fetal autopsy data was analyzed from April 2003 through May 2011 at a tertiary care genetic referral center. The aim was to evaluate the common indications for referral, autopsy diagnosis and its utility to counsel families. Of 733 fetal autopsies performed from 2003 to date, the indications were intrauterine death and spontaneous abortion, structural malformations, hydrops fetalis, growth retardation and chromosomal disorders. Of the 116 autopsies performed for intrauterine death / spontaneous abortion, a definitive etiology was identified in 47% cases. These were fetal malformations, infections, cord and placental abnormalities and chromosomal disorders. In 274 fetuses with antenatally detected structural malformations, diagnosis was established in 49.6 % cases. Chromosomal defects, rubella infection, isolated malformations / multiple malformation syndromes were seen. In hydrops fetalis, etiology was established in 50% cases, mainly multiple malformations and chromosomal syndromes. In the lethal skeletal dysplasias we demonstrated in 21 fetuses, that a detailed physical examination and radiographs were imperative for diagnosis of the type of dysplasia. Definitive diagnosis was established in 73.2 % of autopsies performed, of which in 43% the diagnosis was revised or further defined. In 26.8% cases no definitive diagnosis could be established after autopsy evaluation Minimum basic investigations essential to establish a definitive diagnosis and improve perinatal care was evaluated. For skeletal dysplasias, fetal photograph and radiographs with DNA storage are imperative. Fetal karyotype is essential for fetuses with multiple malformations. When fetal blood is unavailable or placental tissue culture unsuccessful, parental karyotype for a balanced translocation is done. In fetuses with non immune hydrops fetalis, we found antenatal karyotype, hematological and infection evaluation most rewarding. Placental histopathology is important in unexplained intrauterine death. In developing countries expertise of antenatal ultrasound diagnosis varies greatly and fetal autopsy remains the gold standard for evaluation and appropriate counseling to prevent recurrence.

1063W

UK Recognition of International Genetic Counsellor Training and Registration: A Continuing Dialogue. *B. Stayner[1,4], J. Walford-Moore[2,4], A. Middleton[3,4] on behalf of the Genetic Counsellor Registration Board.* 1) Clinical Genetics, Churchill Hospital , Oxford, Oxfordshire, United Kingdom; 2) West Midlands Regional Genetics Service, Birmingham Women's NHS Foundation Trust, Birmingham, United Kingdom; 3) The Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom; 4) Genetic Counsellor Registration Board (GCRB), Wessex Genetics Service, Princess Anne Hospital, Southampton, United Kingdom.

Internationally, the number of Masters programmes in Genetic Counselling has doubled over the past 10 years to over 60, creating challenges for regulating bodies who receive requests from overseas trained individuals seeking employment abroad. The Genetic Counsellor Registration Board (GCRB) is the independent, not for profit company who registers Genetic Counsellors (GCs) and accredits MSc Genetic Counselling Programmes in the United Kingdom (UK). Registration (certification) began as a voluntary process in the UK in 2002 and today approximately 50% (151/300) of members of the Association of Genetic Nurses and Counsellors (AGNC) are registered with the GCRB, a number of whom were trained overseas. It is generally accepted that most Genetic Counsellors (GCs) in the UK are working towards registration. Increasingly, National Health Service employers are hiring only those GCs eligible to register with the GCRB, as the GC profession draws closer to being formally adopted by the Health Professions Council (HPC), the British Parliamentary regulatory body. The GCRB assesses the registration of overseas trained GCs within the UK, using formalised pathways agreed by a GCRB Working Group in 2006. Recognition is afforded to GCs who have trained and certified in the USA/Canada and Australasia, thus GCs from these countries who have completed a recognised training and certification in their own country are able to complete a reduced UK registration portfolio that takes account of their experience. The GCRB completed a large mapping exercise, which evaluated each country's training, accreditation of training and professional regulation process. There is an increasing demand for registration in the UK by recognised and non recognised overseas trained GCs. In addition to this British registered GCs often wish to work outside of the UK and require that the GCRB act on their behalf to establish reciprocal registration agreements so that their British training and experience is recognised outside the UK. The GCRB present here current pathways for GCs trained outside of the UK to register professionally in the UK, and the evaluation model used for assessing overseas MSc Courses to facilitate this dialogue at the International Congress of Human Genetics.

1064W

Introducing "Lexigene": An Online French-English Lexicon of Terms Related to Genetic Counselling. *R. Vanneste[1], G. Sillon[1], J. Hathaway[2], M. Cloutier[3].* 1) McGill University Health Centre, Montreal, Quebec, Canada; 2) IWK Health Centre, Halifax, Nova Scotia, Canada; 3) Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada.

To facilitate the provision of genetic services in both of Canada's official languages and to contribute to the development of the genetic counselling profession, a French-English lexicon of terms was created by genetic counsellors for the specific use of genetic counselling. Trainees in bilingual genetic counselling training programs, as well as francophones who have access to the mainly anglophone scientific literature are in particular need of such a tool. While there exists a few French-English medical genetics glossaries, none were created specifically for the field of genetic counselling and therefore lack many of the terms relevant to this profession. Lexigene was funded through the Canadian Association of Genetic Counsellors (CAGC)'s Small Project Grant. This bilingual website, www.lexigene.com, allows an individual to search for genetics related terms in either French or English and find the equivalent term in the other language. Terms are also listed by specific category, which may be downloaded as PDF files for offline reference. The terms in the lexicon were gathered through 6 main resources where French-English translations currently exist. Seventy other documents were also used to populate the lexicon and/or confirm a word's translation; however, every translation was also verified for accuracy by the four authors. Following the website's creation, a pilot project was undertaken to ensure that the website was user friendly and contained words relevant for genetic counselling. Eighty percent of terms searched for by respondents were found using Lexigene. All respondents rated the website as "very easy to use" and indicated that they would use it in practice. This online tool boasts over 3500 translated terms, with more being added regularly. Lexigene will be used by genetic counsellors, geneticists, and others who work in the field of genetics in both English and French on an international scale.

1065W

The First Year: Impressions from Clinicians and Families Following Clinical Utilization of Whole Genome Sequencing. *R. Veith[1], S. Dugan[1], A. White[1], A. Laedtke[1], J. Kopesky[1], D. Bick[1,2], D. Dimmock[1,2].* 1) Genetics, Children's Hospital of Wisconsin, Milwaukee, WI; 2) Medical College of Wisconsin, Milwaukee, WI.

Clinical utilization of whole genome sequencing (WGS) faces many challenges from both clinician and patient/family perspectives. Our program utilizes a categorical model of choice related to result(s) disclosure and data analysis. The parents work with the genetics team to create a patient/family specific result(s) disclosure and follow-up plan. Over the past two years we have worked with several families to facilitate informed decision-making, discuss key parental concerns, and begin to explore the patient/family experience surrounding results disclosure and the consent process for whole genome sequencing. Informed by these experiences, our institution has established a clinical WGS program with structured protocols for patient selection, data evaluation, informed consent, results disclosure and follow-up. We have reached the 1 year follow-up for our initial patients. To date, 43 families have been reviewed by our clinical program; of the cases reviewed 13 have been approved for sequencing and are in various stages of consent, analysis, disclosure and follow-up. Of the 13 approved cases, eight have completed counseling/informed consent. After counseling, seven of these cases have elected to pursue WGS, one family remains undecided. Of the seven who have elected to proceed, all wanted additional information beyond the mandatory pathogenic result; seven have asked for non-actionable findings in childhood, seven have asked for actionable results in adulthood, and six have asked for non-actionable adulthood findings--with one not wanting this information. Ongoing discussion with parents suggests consistency over time with types of results they desire returned. Our experiences have provided unique insight into parental impressions and concerns regarding clinical whole genome sequencing. Information will be updated on the other cases at the time of presentation.

1066W

MutaDATABASE, a standardised, centralised, open access database of variants leading to human genetic disease. *P. Willems.* GENDIA-MutaBASE, Antwerp, Belgium.

A small fraction of the variants causing genetic disease have been published in the literature or listed in public DNA variant databases, as most novel disease variants identified over the last decade have been identified in clinical diagnostic laboratories. International journals have published individual or lists of variants without accompanying detailed clinical and/or functional data, which is generally not available to the diagnostic laboratory. Many variants are not introduced into mutation databases because at present there is no easy or efficient way for a busy diagnostic laboratory to submit novel variants into a standardized database platform. Furthermore, there is only limited exchange of information about variants between different diagnostic laboratories. Consequently, the clinical significance of many novel variants remains undetermined. Furthermore, no standardized, centralized, and open access database of variants exists that includes well-vetted information on the common disease genes and variants implicated in human genetic diseases. To address these problems we have developed MutaDATABASE, a standardized, centralized, and free open access database. Included for each disease gene is : i) general information on the disease gene, including genomic sequence, cDNA sequence, amino acid sequence, genomic structure, intron-exon boundaries, functional domains, and function, ii) general information on the diseases associated with variants in that gene, iii) tables of exon-intron sequences and disease-causing variants, and iv) figures of the cytogenetic position, genomic structure of the gene, and disease-causing variants, v) lists of MutaCIRCLES -- labs working on the same disease gene that are linked in community groups. MutaDATABASE currently is supported by hundreds of gene curators and genetic labs. MutaDATABASE has a real-time bidirectional communication with a new software interface, MutaREPORTER, which is designed to define, characterize and archive variants in human disease genes. MutaREPORTER currently provides: • A genome browser • A HGVS nomenclature checker • In silico prediction tools (Splice site prediction, SIFT, POLYPHEN) • Possibility to automatically import variants from in-house databases • Possibility to easily submit variants into MutaDATABASE • Possibility to communicate with other genetic (MutaCIRCLES).

1067W

Application of next-generation sequencing in molecular diagnosis of mitochondrial disorders. *R. Bai, M. saifi, S. Suchy, J. Higgs, M. Knight, S. Warren, J. Compton, F. Gibellini, S. Buchholz, S. Benhamed, B. Boggs, C. Chinault, Y. Shevchenko, G. Richard, S. Bale, J. Compton.* GeneDx Inc, Gaithersburg, MD.

Mitochondrial disorders (MtD) are genetically highly heterogeneous, similar clinical features can be caused by different mutations in the mitochondrial (Mt) genome, or mutations in many different nuclear genes. In the practice of molecular diagnosis of MtD, mutation-by-mutation or gene-by-gene testing has been time-consuming, laborious, costly and inefficient; whole genome or whole exome sequencing is still far from ready to be used for routine clinical molecular diagnosis; we have applied targeted next-generation sequencing technology to significantly increase the efficiency and cost-effectiveness of mutation detection in MtD: Deep-parallel sequencing is used to detect any mutations in the Mt genome (at low levels of heteroplasmy) and mutations in 24 nuclear genes important for normal Mt function (accounting for about 80% of known nuclear gene mutations associated with MtD). Samples of 162 patients suspected of MtD (22 definite, 41 probable, 49 possible, others with clinical information not provided) were sequenced at GeneDx, 73 for Mt genome and 108 for 24 nuclear genes (19 for both). All variants identified were compared with specific mutation databases (Mitomap for MtDNA variants, HGMD for nuclear gene variants), other online resources and GeneDx internal variant databases. All novel variants were thoroughly evaluated and classified as novel mutations (nMut), variants of unknown significance: likely mutation (VLM), undetermined (VOUS), or likely benign (VLB), or as a benign polymorphism (Poly). Some published pathogenic mutations without solid evidences were re-evaluated. Results: For the 73 samples tested for Mt genome sequencing, 11 (15.1%) were positive for a definite mutation (8 for a point mutation and 3 for a large deletion, 4.5kb, 5.0kb, and 5.2 kb each), 8 (11%) positive for a nMut/VLM, 6 (8.2%) for a VOUS, and 8 for a VLB; In the 108 samples tested for defects in 24 nuclear genes, homozygous/hemizygous, or compound heterozygous mutation(s) were identified in one gene and confirmed the diagnosis in 12 (11.1%) of the patients, 5 of them also harbor a heterozygous nMut/VLM/VOUS in 1-2 other genes in the panel; Heterozygous nMut/VLM/VOUS were identified in 1-3 genes in another 16 (15%) patients, subsequent aCGH array analysis identified a heterozygous exon deletion in two of them. Conclusion: Targeted next generation sequencing of the Mt genome and a panel of selected nuclear genes is an efficient way to perform molecular diagnosis of MtD.

1068W

Novel *MLL2* mutations identified in Kabuki Syndrome. *R.K. Basran[1, 2], A. Milunsky[1,2], T.A. Maher[1], J.M. Milunsky[1,2,3].* 1) Center for Human Genetics; 2) Department of Pediatrics; 3) Department of Genetics and Genomics, Boston University School of Medicine, Boston, MA.

Kabuki syndrome (KS, MIM147920) is a rare autosomal dominant genetic disorder characterized by intellectual disability and multiple congenital anomalies including distinctive craniofacial features. Other clinical features include postnatal growth restriction, clefting, hearing loss, skeletal, cardiac and renal anomalies, as well as ophthalmic and immune abnormalities. Recently the gene responsible for KS, myeloid/lymphoid or mixed-lineage leukemia 2, *MLL2*, was identified through exome sequencing. It has been reported that sequence analysis of all coding exons of the *MLL2* gene detects mutations in up to 76% of patients with a clinical diagnosis of KS; suggesting either the presence of other yet unknown loci within the genome or mutations occurring within regions of the *MLL2* gene not included in routine sequence analysis. Herein, we report our clinical experience of molecular testing of the *MLL2* gene. Our laboratory performs sequence analysis of the fifty-four coding exons and intron-exon boundaries of the *MLL2* gene. In addition, we are the only laboratory to offer dosage analysis using a custom design multiplex ligation dependent probe amplification (MLPA) strategy for 10 selected exons spanning the entire *MLL2* gene. Among the 76 individuals referred to our laboratory for clinical molecular testing, we identified 30 alterations including: frameshift (n=12), missense (n=11) termination (n=6) and splice site (n=1). Of the 30 alterations we identified in our laboratory, all were novel except for one mutation which has been previously reported. In addition, the clinical significance of one missense alteration identified in a proband and his mother is currently being investigated through molecular testing of other family members and therefore the pathogenicity of this alteration currently remains unknown. Four previously unreported missense alterations identified in our laboratory were designated polymorphisms/variants either because they were inherited from an asymptomatic parent or co-inherited with a pathogenic alteration. MLPA analysis was undertaken in 40 patients and to date no deletions/duplications have been identified in the selected exons tested for within the *MLL2* gene. Further analysis is currently underway to characterize the clinical phenotype of the patients' harboring a *MLL2* gene alteration. This report expands the spectrum of mutations associated with *MLL2* and reports an additional 24 mutations causing KS.

1069W

Autosomal dominant congenital nonprogressive spinocerebellar ataxia is associated with a missense mutation in the *ITPR1* gene. *L. Huang[1, 2], R. Zou[2, 3], M. Carter[4], D.E. Bulman[2, 3], K.M. Boycott[1, 2].* 1) Children's Hospital of Eastern Ontario Research Institute, Ottawa, Ontario, Canada; 2) University of Ottawa, Ottawa, Ontario, Canada; 3) Ottawa Hospital Research Institute, Ottawa, Ontario, Canada; 4) Hospital for Sick Children, Toronto, Ontario, Canada.

The spinocerebellar ataxias (SCA) are a group of genetically heterogeneous disorders characterized by slowly progressive incoordination of gait and often associated with poor coordination of hands, speech, and eye movements. In contrast to the common SCAs, congenital nonprogressive spinocerebellar ataxia (CNPCA) is characterized by early gross motor delay, hypotonia, gait ataxia, and mild dysarthria and dysmetria. The clinical presentation improves or remains stable throughout the patient's life. Families with autosomal dominant CNPCA have been reported approximately nine times since 1985. Linkage to 3pter has been demonstrated in an Australian family and the locus designated as SCA29.

We present a three-generation family with autosomal dominant CNPCA. The proband is a 3 year old girl with global developmental delay, hypotonia, truncal ataxia, and cerebellar vermis atrophy on MRI. Her father had early gross motor delays and did not walk until the age of 6. He has an ataxic gait, mild dysarthria, saccadic eye movements and poor balance. His sister and mother are less severely affected.

The SCA29 overlaps with the SCA15 locus at 3pter. SCA15 is a slowly progressive cerebellar ataxia with mid-life onset; heterozygous *ITPR1* deletions are disease-causing. Given the significant clinical overlap between the Austrian SCA29 family and our family, we genotyped all the family members with microsatellite makers from 3pter and found the region containing *ITPR1* cosegregated with the disease. Sequencing all of the exons and the exon/intron boundaries of *ITPR1* in one affected individual identified a missense change (c.1804A>G; p.N602D), which cosegregated with the disease phenotype. The change is located at a highly conserved amino acid position, is predicted to be pathogenic and was not found in over 100 normal Caucasian controls.

ITPR1 is an intracellular ligand-gated $Ca2^{+}$; release channel, playing a critical role in modulating intracellular $Ca2^{+}$; signaling. Mutations in *CA8*, encoding an inhibitor of ITPR1, causes a recessive form of CNPCA, supporting our finding that mutations in *ITPR1*, or genes in the *ITPR1*-related pathway, can give rise to CNPCA. Analysis of the original Australian SCA29 family is currently underway.

1070W

Discovery of a probable gene mutation causing mental retardation, microsomia, and signs of skeletal dysplasia in an Arab family with a previously undelineated autosomal recessive disorder. *M. Osman, Y. Al-Saraj, G. Billedo, S. Zaineddin, H. El-Shanti, J. Alami.* Shafallah Medical Genetics Center, Doha, Qatar.

Autosomal recessive diseases are the largest category of single-gene disorders among Arab population. Our goal is to identify the genetic causes of undelineated autosomal recessive disorders among Arab families. We have recruited a family with three siblings with a mental retardation (MR) syndrome who were born to consanguineous Qatari parents. The clinical problems comprised significant mental retardation, microsomia, signs of skeletal dysplasia, and thoracolumbar kyphosis. The oldest patient suffers also from epileptic seizures. The parents and the other three of their six children are healthy. The disease gene mapping strategy focused on large genomic regions demonstrating homozygosity in all of the affected individuals. Homozygosity mapping revealed a single large, shared region of homozygous SNPs on the long arm of chromosome 4 flanked by rs28419770 (4q13.1)and rs 4105671 (4q21.23). This block contains more than 120 genes, none of which has been implicated in MR or any of the above mentioned phenotype so far. Sequencing of candidate genes within the region revealed two novels missense variations in FRAS1 gene; an Arg3099Gln and Thr3149Met. Both variations were found in the three affected siblings in homozygous status, while the parents were heterozygous. Furthermore, these two variations have not been found in 140 individual controls in homozygous pattern, however, a heterozygous pattern of variations were found in three individuals.

1071W

Exome sequencing for noncoding regions : identification of the first branch point deletion in Aarskog-Scott syndrome. *M.H. Breuning[1], Y. Sun[1], E. Aten[1], R. al Momani[1], T. Messemaker[1], J.F.J. Laros[1], S.M. Maas[2], J.T. den Dunnen[1].* 1) Department of Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands; 2) Department of Clinical Genetics, Academic Medical Centre, UVA, Amsterdam, the Netherlands.

Exome sequencing is an attractive method to identify causative mutations in coding regions responsible for Mendelian disorders. Aarskog-Scott syndrome (ASS) is clinically and genetically heterogeneous. It is characterized by short proportionate stature, broad hands and feet, genital hypoplasia (shawl scrotum) and facial dysmorphisms. Defects in FGD1 gene cause the ASS. However, only in ~ 20% of the patients mutations in FGD1 are identified by conventional sequencing methods. Here, we report a Dutch family with ASS with two affected boys where diagnostic Sanger sequencing failed to detect mutations in FGD1. The siblings were sequenced by whole exome sequencing (Sureselect and GAIIX) and a routine analysis for the exonic variants (nonsense, missense, splice site, frameshift) was performed. No obvious candidate was identified using the normal criteria and thresholds. An additional analysis was performed, extending the region to+/-50bp of exons. This revealed an intronic variant, 35 nucleotides upstream of an exon, which could affect splicing. The deletion is in the predicted branch point of an exon in FGD1, important in lariat formation. This variant was not reported in any disease or control database and cosegregated with the disease in this family. RNA isolated from peripheral blood showed a skip of the exon involved. The out of frame product generates a premature stop codon. To establish genotype-phenotype correlations, effects of NMD and X-inactivation were studied. The extended analysis, searching outside the exonic region up to 50bp, increased the number of detected variants by 55%. This may be caused by a high variant rate in introns and by lower coverage and strand bias towards the end of the exons. Minimal future changes in primer design for exome capture will ensure valuable extension of the current sequenced regions. The low mutation detection rates in ASS may be explained by branch point mutations currently missed by conventional diagnostic techniques. The location of the variant is within the region where standard DNA diagnostics protocols allow primer design. Splice site mutations affecting the branch-site might often go unrecognized due to allelic dropout in amplification and this might be the reason why they are rare in published literature. These data demonstrate that whole exome sequencing allows the recognition of mutations outside exonic regions and application in diagnostics might help to uncover more branch point mutations.

1072W

Regulation of *PLP1* alternative splicing by secondary structure. *G. Hobson[1], J. Taube[1], K. Sperle[1], B. Cavan[2], J. Garbern[3].* 1) Nemours Biomedical Res, A I duPont Hosp Children, Wilmington, DE; 2) Cebu Institute of Medicine, Cebu City, Philippines; 3) University of Rochester Medical Center, Rochester, NY.

Alternative splicing is an important mechanism to increase protein diversity. Much attention has been devoted to the role of exon and intron splicing elements (ESEs and ISEs) and their binding factors in regulation of alternative splicing, but several recent discoveries have highlighted the important role of secondary structure. The X-linked proteolipid protein 1 gene (*PLP1*) produces two alternatively spliced transcripts, *PLP1* and *DM20*, due to alternative splice donor sites in exon 3. The *PLP1* transcript is 105 bases longer than *DM20* and gives rise to a protein that is 35 amino acids larger. Mutations that affect the *PLP1/DM20* alternative splice ratio, even without any amino acid changes, result in the leukodystrophy called Pelizaeus-Merzbacher disease (PMD). We reported that an ISE at bases c.453+28_+46 in intron 3 regulates alternative splice site selection. To localize other sequences that regulate alternative splice site selection, we performed deletion scanning of intron 3 using a splicing mini-gene construct of the *PLP1/DM20* alternative splice and transfection into the Oli-neu cell line. Results of these studies demonstrated regulatory sequences throughout the intron, but a particularly strong regulatory sequence that is highly conserved from mouse to man resides between bases c.454 -346 and -307 in intron 3. Two families with PMD and strong X-linked inheritance pattern, but without mutations in the *PLP1* coding region, had mutations within this region, c.454-322G>A and c.454-314T>A. Both of these mutations decreased the *PLP1/DM20* ratio from the mini-gene construct in transfections, providing further evidence of a regulatory sequence in this region. Prediction of secondary structure using mfold revealed that intron 3 bases c.454-323 to -308 can form a secondary structure stem with bases c.454+151 to +166. Both patient mutations are predicted to destabilize the secondary structure. We demonstrated that mutations in each arm of the stem that disrupt the secondary structure also decrease the *PLP1/DM20* ratio, while swap mutations that restore the secondary structure bring the *PLP1/DM20* ratio to near normal levels. Taken together, our data strongly suggest that secondary structure within intron 3 is important in regulating the *PLP1* alternative splice.

1073W

Splice site variants in the Wilson disease gene, *ATP7B*, that affect splicing of an exon 18 minigene. *G. Macintyre[1], A.M.E. Wilson[2], D.W. Cox[2].* 1) Department of Medical Genetics, University of Alberta, Faculty of Medicine & Dentistry, Edmonton, AB, Canada; 2) Department of Medicine, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, AB, Canada.

Wilson disease (WND; OMIM 27790) is an autosomal recessive disorder of copper transport affecting approximately 1 in 30,000 people worldwide. When ATP7B, the protein encoded by the WND gene, is defective, copper overload in the liver, brain and kidney can result. This leads to tissue damage and in some cases organ failure. At present, there are greater than 600 *ATP7B* variants listed in the Wilson Disease Mutation Database (http://www.wilsondisease.med.ualberta.ca/), and 54 are splice site variants. Splicing disruptions can lead to exon skipping, generating aberrant mRNAs and/or proteins that are defective. To functionally characterize these splice site variants, we have developed a minigene approach. A portion of the *ATP7B* gene, exons and introns included, is inserted into a mammalian expression vector. This allows expression in Chinese hamster ovary (CHO) cells, which do not express endogenous *ATP7B*, allowing specific detection and analysis of the splicing products. We have successfully used this approach to identify splice site and exonic variants of *ATP7B* that result in skipping of exon 8. Here, we present a minigene that starts in intron 16 and continues to intron 19, with all introns and exons in between. This was used to characterize five splice site variants adjacent to exon 18. c.3700-2A>T, c.3700-1G>A, c.3700-1delG, c.3903+1delG, and c.3903+6C>T were introduced into a wild-type minigene by site-directed mutagenesis, then transfected into CHO cells. RNA was extracted and exon 18 splicing was analysed by reverse transcriptase PCR. Altered splicing patterns were observed for four of the five *ATP7B* variants. We have categorized these as probable disease-causing variants that are likely to affect the production of normal levels of wild-type ATP7B protein, and/or generate defective truncated proteins. It is important to consider intronic splice-site variants of *ATP7B* as probable disease-causing. We previously identified *ATP7B* exon 8 variants, both synonymous and non-synonymous, that result in altered transcript processing. The construction of the exon 18 minigene will allow similar analysis of exon 18 variants. These studies are essential to the correct interpretation of the contribution of each variant to WND. Functional testing of missense variant proteins may be misleading, as splicing defects may have a significant impact upon the type and stability of ATP7B proteins produced and influence the severity of the WND phenotype.

1074W

Discovery of mRNA Splicing Mutations in the Human Gene Mutation Database by SNP-based Information Theory Prediction Confirmed by Expression Microarray Analysis. *E.J. Mucaki[1], X. Sun[1], P.K. Rogan[1,2].* 1) Department of Biochemistry, University of Western Ontario, London, Ontario, Canada; 2) Department of Computer Science, University of Western Ontario, London, Ontario, Canada.

Mutations affecting mRNA splicing have been associated with more than 15% of all inherited single gene disorders. Potential effects on mRNA splicing may not be recognized because mutation screening is based predominantly on genomic sequence analysis. We predict point mutations which may affect splicing in genetic databases such as the Human Gene Mutation Database (HGMD) using information theory-based methods. We then exploit expression microarray results from published variants at other expressed genomic loci which carry the same sequence to support or refute these predictions. New software was developed for batch analysis of changes in information content at mRNA splice sites that result from any point mutations recorded in HGMD. We previously reported a MySQL database with Affymetrix exon1.0 ST microarray expression data of HapMap individuals which correlated information analysis of splicing changes for all Phase II HapMap SNPs. This database was updated to add the sequences surrounding each SNP. SQL queries identified SNPs and adjacent sequences identical to the predicted splicing mutations in HGMD. Whisker plots and statistical analysis of multiple individuals with the same genotypes revealed differences in expression of HapMap SNP alleles which were concordant with the information theory-based predictions of altered splicing for a number of HGMD variants. We find 2520 (of 51245) point mutations were predicted to affect splicing, of which 1049 occurred at natural splice sites, and 1471 affect the strengths of cryptic splice sites. Exon expression data for sequences circumscribing HapMap SNPs identical in sequence to two natural and 14 cryptic splicing mutations in HGMD were concordant with predictions. The mutations affecting natural sites are F8: c.787G>A and PROS1: c.727G>A. The 14 mutations altering exonic cryptic splice sites include: MATN3: c.361C>T, PHGDH: c.1468G>A, NSD1: c.1357G>T, HADHA: c.1234G>T, BRCA1: c.1141A>T, F8: c.5665C>T, NPHS1: c.1250G>T, TG: c.6461G>A, AVPR2: c.673C>T, PRF1: c.757G>A, SLC45A2: c.298G>A, DSG1: c.430A>T, TLR4: c.2081A>G and SLC4A11: c.478G>A. Many of these HGMD mutations induce aberrant mRNA splicing either alone or in combination with protein coding changes. Information analysis of suspected eSNPs, concordant with allele-specific changes in expression, can provide insight into the pathogenic mechanisms of splicing mutations in unrelated genes with identical splice site sequences and variants.

1075W

Novel MFN2 mutations in two familial cases with Charcot-Marie-Tooth type 2A. *M. Muglia[1], A. Patitucci[1], D. Messina[1], A. Magariello[1], G. Nicoletti[1], R. Mazzei[1], FL. Conforti[1], C. Ungaro[1], L. Citrigno[1], I. Mikerezi[2], W. Sproviero[1,3], A. Gambardella[1].* 1) Institute of Neurological Sciences, Mangone, Italy; 2) Department of Biology, Faculty of Natural Sciences, University of Tirana, Albania; 3) Institute of Neurology, University `Magna Graecia', Catanzaro, Italy.

Charcot-Marie-Tooth type 2A is a dominantly inherited neuropathy characterized by axonal degeneration of sensory and motor nerves. The disease is caused by mutations in the mitochondrial fusion gene (MFN2). Mfn2 is an integral outer mitochondrial membrane protein composed of a large GTPase domain and two heptad repeat domains that face on cytoplasm. The aim of this study was to identify the causative mutation in a cohort of CMT2 patients. We identified two novel variations in two patients originated from Southern Italy and Albania, respectively. The Italian patient is a twenty-four year old man, who, by the age of 18 years, complains muscles weakness in the distal legs, and more recently burning feet. The mother and one brother are also affected, but with a milder phenotype. The second patient is a 64 years old man affected by an axonal neuropathy associated with deafness. His sister reportedly has similar symptoms, but denied neurophysiological testing or genetic analysis. The entire coding region were analyzed by DHPLC and fragments showing altered profiles were directly sequenced. In the Italian patient we identified the variation c.775C>T leading to the p.Arg259Ser amino acid substitution. The analysis of the other family members confirmed the co-segregation of the mutation with the CMT2 phenotype in the affected subjects. In the PolyPhen program, the PSIC (Position-Specific Independent Counts) score difference for the two amino acids R and S was 2.61 (>2.0). The variant is predicted to be probably damaging. The mutation p.Arg259Ser is located in the GTPase domain of the protein, the region of the MFN2 molecule necessary to induce fusion to other mitochondria. Disruption of functional domain of the protein is particularly likely to cause neuropathy. In the Albanian patient, a variation in c.1287+325C->T has been detected. The Splice Site Prediction program predicts that the variation could create a novel accept splice site in the c.1287+277_278AG of MFN2. Based on the results from similar studies, in which mutations occurring in intronic regions have been found to cause various levels of normal and aberrant transcripts, we presume that the c.1287+325C->T mutation affects the splicing of MFN2, and leads to a dysfunctional protein. Further investigation on mRNA will be needed in order to verify the real functional effect of the variation.

1076W

Deep intronic mutations flanking the alternatively spliced *NF1* exon 31/23a cause missplicing preferentially including exon 31/23a in the transcript. *J. Xie, T. Callens, J. Williams, L. Messiaen.* Medical Genomics Laboratory, Department of Genetics, University of Alabama at Birmingham, Birmingham, AL.

Neurofibromatosis type 1 (NF1), a common autosomal dominant disorder affecting 1 in 3000 individuals worldwide, is caused by mutations in the *NF1* gene, which encodes neurofibromin, a negative regulator of the Ras signaling pathway. The *NF1* gene harbours at least 3 alternatively spliced tissue-specific exons (9br, 23a and 48a) and these isoforms have been detected at the protein level. The two major *NF1* isoforms are distinguished by the absence (type I) or presence (type II) of an alternatively spliced cassette exon 23a, located in the GTPase activating protein- (GAP-) related domain (GRD). Type II neurofibromin has higher affinity for Ras-GTP but lower GAP activity compared with the type I isoform. Using comprehensive *NF1* mutation analysis including direct cDNA sequencing of the entire coding region we identified 9 different splice defects in 11 individuals, leading to exonisation of different parts of intron 30 (23.2) or 31 (23a). Analysis of the flanking gDNA sequences allowed identification of the underlying mutations causing the splice defects. Alternative splicing of the in-frame exon 31 (23a) is highly regulated with multiple evolutionary highly conserved intronic sequence blocks flanking this exon. In blood, both isoforms are equally expressed in NF1 patients and controls alike. We investigated if the deep intronic mutations equally affected missplicing of the 2 major isoforms, including and excluding exon 31 (23a). Interestingly, the deep intronic mutations residing either 5' or 3' of exon 31 (23a) were shown, by fragment and cloning analysis, to predominantly affect the isoform 2. Among *NF1* mutations about 27% are splicing mutations. So far only 9 deep intronic *NF1* mutations have been reported in literature. We report a hotspot region for *NF1* deep intronic mutations: 12% of all deep intronic splice mutations detected by comprehensive RNA-based mutation analysis reside in introns 30 (23.2)-31 (23a) comprising only 2% of the total *NF1* intronic sequence. Our results may shed light on the intronic sequences functionally contributing to alternative splicing of the cassette exon 31 (23a).

1077W

The genetic etiology of otosclerosis in the genetic isolate of Newfoundland, Canada. *N. Abdelfatah[1], A. Griffin[2], K. Hodgkinson[1], T. Batten[4], S. Moore[3], J. Houston[1], C. Negrijn[1], T. Young[1].* 1) Discipline of Genetics, Faculty of Medicine, Memorial University, St. John's, NL A1B 3V6; 2) Central Regional Integrated Health Authority, Grand Falls-Windsor, NL A2A 2E1; 3) Department of Pediatrics, Memorial University of Newfoundland, St. John's, NL, Canada; 4) ENT Consultants, 1Paton St, St John'S, NL A1B 4S8.

Introduction: Otosclerosis (OTSC)is the most common cause of hearing loss among adult Caucasians, with an estimated prevalence of 0.3-0.4 worldwide.It is characterized by conductive hearing loss due to fixation of the stapes bone to the oval window. Sensorineural hearing loss may develop later in the disease or rarely presents as first symptoms. To date, 8 OTSC loci have been mapped OTSC1-OTSC10 (OTSC6,9 recalled), however, no genetic cause has been discovered for this disease. Purpose of the study: To identify the disease-causing gene(s) for otosclerosis in NL families Methods: Two large multiplex NL families (2114, 2081) and seven other probands, total of forty eight members; 23 members with otosclerosis were recruited. DNA samples from families 2081 and 2114 were genotyped for microsatellite markers spanning the intervals of all previously mapped OTSC loci, and three associated genes (COL1A1, COL1A2 and NOG). Disease-associated haplotypes were compared within and between pedigrees. DNA samples from family2114 were sent to genome wide scan for SNP genotyping and analysis, and positional candidate genes were sequenced using Sanger sequencing Results: Our previous results suggest that family 2081 is linked to the whole length of OTSC4 (10Mb). Comparing OTSC4 haplotype of family 2081 with haplotypes of four proband that belong to other NL otosclerosis families minimize the linked region from 10Mb into 4Mb. To determine the boundaries of the OTSC4 linked locus in family 2081, haplotypes were extended using microsatellite markers that flank OTSC4. As a result, family 2081 and family 2114 show a suggestive linkage to a region of 7 Mb that located downstream of OTSC4. We are using a combination of next-generation and Sanger sequencing to sequence the otosclerosis exome in order to discover the first-ever causative mutation for this disease. Summary: Families 2114 and 2081 are linked to an expanded region that overlaps with the OTSC4 critical region at chromosome 16q with different haplotype. Furthermore, family 2081 is also linked to the whole length of OTSC4. We hypothesize the gene to be located in the extended locus, and the patients of two families likely harbour a different mutation in the sa gene. To confirm our result, we will use the Illumina 610-quad microarrays to genotype the family members of 2114. We are also currently engaged in sequencing entire exomes from affected and unaffected members of family 2081 and 2114.

1078W

Homozygous chromosomal microdeletion of *NEUROG1* in a consanguineous family: A new human gene for the development of cranial sensory ganglia and inner ear neurons causing syndromic hearing loss, Mondini malformation, and gulp and mouth motor disorder. *O. Bartsch[1], J. Schröder[1], A.K. Lä) ig[2], A. Keilmann[2], W. Müller-Forell[3], D. Galetzka[1].* 1) Institute of Human Genetics, Johannes Gutenberg-University Mainz, Mainz, Germany; 2) Department for Communication Disorders, Johannes Gutenberg-University Mainz, Mainz, Germany; 3) Institute for Neuroradiology, Johannes Gutenberg-University Mainz, Mainz, Germany.

A homozygous chromosomal microdeletion sized > 111 kb, 46,XY.arr 5q31.1(134,764,990-134,875,801)x0 (GRCh37/hg19 assembly), was identified in a boy with profound congenital sensorineural hearing loss, bilateral cochlear hypoplasia with single widened cochlear turn (Mondini malformation), and severe gulp and mouth motor disorder. He also had low-set posteriorly rotated ears, plagio- and scaphocephaly, wide/long palpebral fissures, high and very narrow palate, sacral dimple, and developmental retardation. The parents were first cousins, they were unaffected and heterozygous for the chromosome 5q31.1 deletion. The deletion interval harbored 3 genes, all in full length; *DCNP1* (*C5orf20*), *TIFAB*, and *NEUROG1*. None of these genes was previously associated with deafness or disorders of brain development in humans, but in Xenopus neurogenin-1 (ngn1) was reported to convert ectodermal cells into neurons (Ma et al. Cell 87:43-52, 1996). In mice, *Ngn1* null mutant embryos failed to generate neuronal precursor cells for the proximal cranial sensory ganglia, leading to a loss of the vestibulo-cochlear, trigeminal and other ganglia, and to neonatal lethality by interfering with suckling (Ma et al. Neuron 20:469-82, 1998). *Ngn1* -/- mice showed complete absence of the inner-ear sensory neurons; neither afferent, efferent, nor autonomic nerve fibers were detectable (Ma et al. J Assoc Res Otolaryngol 1:129-43, 2000). In contrast, *DCNP1* and *TIFAB* are not likely candidate genes for deafness. DCNP1 is thought to function in the pathogenesis of depressive disorders and in the hypothalamic stress response, and TIFAB is highly expressed in spleen and inhibits TRAF6-induced cellular functions such as B-cell maturation and maturation of macrophages and dendritic cells. The human *NEUROG1* gene maps within a yet unresolved locus for autosomal recessive non-syndromic hearing loss, DFNB60 on chromosome 5q22-q31, but recent linkage data have excluded *NEUROG1* from being causative in the DFNB60 patients (R. Smith and M. Hildebrand, personal communication). The 5q22-q31 area is large, spanning 35 Mb and > 100 genes, and may well contain more than one deafness gene. Collectively, our results identify a new gene, *NEUROG1*, for autosomal recessive syndromic hearing loss. This is the first report on the human phenotype associated with *NEUROG1* null mutations and there is excellent concordance with experimental findings in *Ngn1* null mutant mice.

1079W

***AQP4* and Syndromic Menière's disease.** *C.A. Campbell[1,3], N.C. Meyer[1,3], L.T. TeGrootenhuis[1], C.C. Della Santina[2], J.P. Carey[2], L.B. Minor[2], M.R. Hansen[3], B.J. Gantz[3], R.J.H. Smith[1,3].* 1) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology - Head and Neck Surgery, University of Iowa, Iowa City, IA; 2) Department of Otolaryngology-Head & Neck Surgery, The Johns Hopkins University, Baltimore, MD; 3) Department of Otolaryngology - Head and Neck Surgery, University of Iowa, Iowa City, IA, 52242, USA.

Menière's disease (MD) is a complex idiopathic disorder of the inner ear characterized by hearing loss, tinnitus, and vertigo (1995). Its histopathologic feature is endolymphatic hydrops or swelling of one of the inner ear compartments. In this study we completed a candidate-gene association study by screening Aquaporin 4 (*AQP4*) for rare and common variants as a test of the Common Disease Rare Variant (CDRV) and Common Disease Common Variant (CDCV) hypotheses. We selected *AQP4* because: 1) this member of the aquaporin family is expressed in multiple cell types in the cochlea and vestibule; 2) its targeted deletion in the mouse is associated with hearing loss; and 3) its function as a water channel suggests a possible role in maintaining endolymph and perilymph homeostasis in the inner ear.

Mutation screening of the coding region of *AQP4* in 124 sporadic MD patients and matched controls identified two cases carrying the same rare variant: M224T. This variant was not identified in an expanded screen of 848 control chromosomes and functional studies have shown that it decreases water permeability. Genetic counseling, pedigree analysis, detailed medical histories and screening of additional family members revealed additional phenotypes in both families, with the *AQP4* M224T variant segregating with a partial MD phenotype and with MD associated with migraine. Haplotype reconstruction surrounding M224T also indicates that the two families may share a small region of autozygosity by descent.

Our data show that rare variants in *AQP4* are causally related to MD and an MD-like phenotype that includes migraine. This finding is significant because it is the first to implicate aquaporins in human hearing loss and identifies for the first time a gene - *AQP4* - that is associated with syndromic MD. (This research was supported in part by a grant from the American Otological Society (RJHS).).

1080W

Rationalization of molecular diagnosis of Usher syndrome in the Spanish population. *T. Jaijo[1,2], E. Aller[1,2], G. Garcia-Garcia[1], MJ. Aparisi[1], JM. Millan[1,2,3].* 1) Grupo de Enfermedades Neurosensoriales, IIS-La Fe, Valencia, Spain; 2) CIBER de Enfermedades Raras (CIBERER), Valencia, Spain; 3) Unidad de Genetica y Diagnostico Prenatal, Hospital Universitario La Fe, Valencia, Spain.

Usher syndrome (USH) is an autosomal recessive inherited disease that associates sensorineural hearing loss with a progressive loss of vision due to retinitis pigmentosa (RP). To date, nine causative genes have been identified for the three clinical subtypes distinguished: USH1 (*MYO7A*, *USH1C*, *CDH23, PCDH15* and *USH1G*) USH2 (*USH2A, GPR98* and *DFNB31*) and USH3 (*USH3A*). Early diagnosis is critical for adapted educational and patient management choices, and for genetic counseling. PURPOSE: The purpose of this study is to determine the genetic epidemiology of USH in Spain and establish an accurate molecular diagnosis algorithm for our population. PATIENTS and METHODS: Currently, our series has 247 USH families collected from several Spanish Hospitals. From these, 70 are classified as USH1, 116 as USH2 and 16 as USH3. 15 families present with atypical Usher features (USHA) and detailed clinical data could not be obtained from 30 patients remaining as non-classified (USHNC). These patients have been screened for mutations in Usher genes using different methods: direct sequencing, the genotyping microarray for Usher syndrome, MLPA and array-CGH. RESULTS: In this study a total of 155 different mutations have been identified. Interestingly, 85 out of these (54.8%) have been detected only in the Spanish population. We could determine the genetic cause of the disease in 193 out of the 247 families studied. Pathologic mutations have been found in 95.7% of USH1 families: 52.9% have mutations in *MYO7A*, 21.4% in *CDH23*, 14.3% in *PCDH15*, 2.9% in *USH1C* and 4.3% in *USH3A*. Regarding USH2 families, mutations have been found in 85.3% of them: 81% have mutations in *USH2A*, 4.3% in *GPR98* and 1.7% in *CDH23*. For USH3 families, mutations have been detected only in 31.25%: 12.5% have mutations in *USH3A*, another 12.5% in *USH2A* and 6.25% in *PCDH15*. Heterogeneous results have been obtained for USHA and USHNC families, pointing out the importance of an accurate clinical diagnosis. No mutations have been identified in *USH1G* and *DFNB31*. CONCLUSION: Our study has allowed us to determine the genetic cause of the disease in 78.1% of USH Spanish families. However, it has been a long and hard task. Currently, there is no satisfactory solution for rapid in-depth and simultaneous mutation screening of the known USH genes. However, next generation sequencing technologies promise to be able to fulfil this task within the next few years.

1081W

Assessment of GJB6, GJB4, and GJC3 genes for double heterozygosity with GJB2 heterozygots in autosomal recessive non-syndromic hearing impairment patients. *D. Kooshavar[1,2], M.R. Noori Daloii[1], M. Hashemzadeh Chaleshtori[2].* 1) Department of Medical Genetics, Tehran University of Medical Science, Tehran, Iran; 2) Cellular and Molecular Research Center, Shahrekord University of Medical Science, Shahrekord, Iran.

Hereditary non-syndromic hearing loss is inherited in autosomal recessive pattern in about 80% of cases. Up to 50 percent of all autosomal recessive nonsyndromic hearing impairment (ARNSHI) cases in different populations are caused by mutations in the GJB2 gene which encodes the gap-junction (GJ) protein connexin (Cx) 26. However 10 to 50 percent of patients with recessive GJB2 mutations carry only one mutant allele. GJB6, GJC3, and GJB4 encoding Cx30, Cx30.2, and Cx30.3 respectively, are expressed in cochlea and the same inner-ear structures as GJB2. GJB6 is shown to be normally co-assemble with GJB2 into GJs in the cochlea. Besides, mixing of different connexins in heteromeric and heterotypic GJ assemblies is also possible. This study aims to determine whether variations in any of the genes GJB6, GJC3 or GJB4 can be the second mutant allele causing the disease in the GJB2 heterozygous cases studied. We examined 44 unrelated GJB2 heterozygous ARNSHI subjects from different geographic and ethnic areas in Iran, for any sequence variations in three genes, using polymerase chain reaction followed by direct sequencing. Sequence analysis of GJB4 showed 3 heterozygous substitutions (c.451C>A, c.507C>G, c.542C>T) also one frameshift caused by 4bp deletion (c.155_158delTCTG) causing the changes (p.Arg151Ser, p.Cys169Trp, p.Thr181Met, p.Ala52ValfsX55) in amino acid sequence. There was one heterozygous substitution (c.173C>T) in GJB6 in one case which leads to the change of (p.Pro58Leu) in amino acid sequence. We found no variations in GJC3 gene sequence. So our detected GJB4 and GJB6 allelic variations can be considered as second allelic variation in patients with only one GJB2 mutant allele but variations of GJC3 was not the second allele in studied samples. Further examinations on control samples and co-segregational family studies are in progress to confirm the pathogenicity of the detected allelic variations as the second complementary mutation responsible for the disease in double heterozygote pattern.

1082W

Examination of Gene Expression Patterns Associated with PMP22-Related Auditory Dysfunction. *M.J. Kovach, T.A. Carver, W.R. Bolus.* Biological & Environmental Sci, Univ Tennessee, Chattanooga, TN.

Characterization of expression patterns of genes responsible for deafness, can help dissect the molecular events underlying both deafness and normal hearing. A variant of Charcot-Marie-Tooth (CMT1E) disease associated with deafness, has been linked to point mutations in the *PMP22* gene. *PMP22* is largely expressed in Schwann cells responsible for nerve myelination, but is also detected in a variety of non-neural tissues, including the cochlea and lungs, at critical times of tissue development. The mouse homolog of *PMP22*, *Gas3*, was first isolated as a Growth Arrest Specific gene. In non-neural tissues, Gas genes are induced during growth arrest and repressed at terminal differentiation. Thus, PMP22 appears to play a role in nerve myelination and cell growth regulation of non-neural tissues. This dual expression is consistent with both neural and cochlear hearing loss in CMT1E patients. We hypothesize that PMP22 expressed in non-neural tissues serves to regulate expression of genes important in normal tissue development. More specifically, PMP22 is regulating genes involved in development of the inner ear, which may explain the deafness phenotype of CMT1E. The Trembler-J mouse was chosen as a model for PMP22-associated auditory dysfunction to study gene expression patterns throughout development. Lung tissue was used as a non-neural surrogate for the cochlear membranous labyrinth. Tissues were collected from WT and TrJ mice throughout development and gene expression profiles were characterized by differential display and qRT-PCR. Thus far, 85 differentially expressed transcripts have been identified, verified and classified according to cellular function, 55% of which are homologs or family members of genes in the human cochlear library. In an effort to understand the molecular pathology of the disease state, these genes have been categorized into groups by cellular function. Major groups include: cell communication and signal transduction (25%), nucleic acid metabolism (22%), and cell growth/maintenance (15%). Ten genes differentially expressed in the TrJ mouse model were identified as candidates for the deafness phenotype. Differential expression patterns of candidate genes were characterized by qRT-PCR throughout embryonic and adult development. Two developmental time points, E11.5 and P14, consistently exhibited divergent expression patterns between WT and TrJ samples, and may indicate critical junctures in non-neural tissue development.

1083W

Pitfalls in the etiological diagnosis of sensorineural hearing loss in Brazil. *P.Z. Ramos[1], A.M. Castilho[2], V.C.S. de Moraes[1], E.L. Sartorato[1].* 1) Centro de Biologia Molecular e Engenharia Genética (CBMEG), Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo, Brasil; 2) Departamento de Otorrinolaringologia, Hospital de Clínicas, UNICAMP, Campinas, SP, Brasil.

Deafness is considered the most prevalent sensory disorder in humans, caused by a variety of environmental and genetics factors. The environmental factors include frequent exposure to high intensity sound, acoustic trauma, infections, and ototoxic drugs, whereas the genetics factors are caused by mutations in different genes or regulatory elements that are involved in proper development, structure, and function of the ear. In developed countries, most of hearing loss cases are due to genetic factors, however, in Brazil, environmental factors are among the major causes. Although a simultaneous testing approach, including clinical exams, audiological, laboratorial, imaging and genetic expands the etiological diagnosis, overloads the healthcare system due to high costs. The goal of the present study was to evaluate the effectiveness of imaging and genetics tests and their impact on public health, aiming to increase efficiency and reduce costs. It was conducted an analysis of 50 patients submitted to cochlear implantation between 2005 and 2010 at our tertiary referral university hospital. Clinical and audiological data were retrieved from patient's charts. Results of computed tomography and magnetic resonance scans were reviewed. In addition, a molecular analysis was conducted, including *GJB2*, *GJB6*, *SLC26A4* genes and A1555G mitochondrial mutation in 12S ribosomal RNA gene. Radiologic abnormalities were identified in 13 patients, while molecular alterations were found in 18 individuals, including 35delG, V27I, M34T, V37I, E47X, L90P, V95M, K168R, W172X in *GJB2* gene and V609G in *SLC26A4* gene. Imaging and genetic findings were important to establish the diagnostic of hearing loss in 22 and 20% of the patients, respectively. After specific tests, the number of individuals with unknown cause was thus reduced from 36 to 22 (39% of reduction). The etiology was environmental in 22% of the cases, genetics in 20% and inner ear malformations in 14%. Data obtained in the present work confirmed the high prevalence of mutations in *GJB2* gene, especially 35delG mutation, in cases of severe to profound bilateral sensorineural hearing loss. This study also demonstrated that both imaging and genetic analysis are important in the identification of the etiology of hearing loss. Moreover, we observed that the unknown etiology prevailed, which points to the need of carrying out detailed genetic studies in order to elucidate the etiological diagnosis.

1084W

Forward genetics to identify novel neuromuscular genes in zebrafish. *V. Gupta[1], S.R. Gundry[1,2], M. Discenza[1], A.T. Chen[3], Y. Zhou[3], L.I. Zon[3,4], A.H. Beggs[1].* 1) Genomics Program and Division of Genetics, The Manton Center for Orphan Disease Research, Children's Hospital Boston, Harvard Medical School, Boston, MA-02115, USA; 2) Behavioral Neurosciences, School of Arts and Sciences, Northeastern University, Boston, MA-02115, USA; 3) Stem Cell Program and Hematology/Oncology, Children's Hospital Boston, Harvard Stem Cell Institute, Harvard Medical School, Boston, MA-02115, USA; 4) Howard Hughes Medical Institute.

Primary inherited disorders of muscle include both muscular dystrophies and non-dystrophic myopathies. These disorders, characterized by muscle weakness and impaired locomotion, form a heterogeneous group of heredity diseases affecting both children and adults. Owing to genetic and clinical heterogeneity, variable penetrance and mortality, genetic studies of some of the more rare myopathies in humans are difficult and studying the pathophysiology can be problematic in the absence of suitable animal models. The zebrafish, Danio rerio, is a powerful developmental and genetic system for dissection of skeletal muscle disorders and diseases. Several features of zebrafish, such as a well-characterized genome with high degree of synteny and sequence homology (50-90% of amino acids) to humans, conserved structure of vertebrate skeletal muscles, transparency at embryonic stages and rapid and ex vivo development, make it ideal for skeletal muscle biology research. We have employed a forward genetics approach in zebrafish as a tool to identify novel genes causing muscular disorders, and have recently identified 12 unique mutants with defective skeletal muscle. One of the mutants identified in this screen, Osoi, displays impaired motility behavior and exhibits skeletal muscle hypotrophy with extensive central nucleation. Ultrastructural studies identified sarcomeric disorganization and prominent chromatin changes in the nuclei of the mutant fish. Genetic mapping of the mutant locus in Osoi has identified a loss of function mutation in a novel RNA binding protein. Preliminary studies suggest a role for this gene in ribosomal biogenesis in skeletal muscles. The central nucleation is reminiscent of centronuclear myopathy, a human congenital myopathy. Through an understanding of the molecular pathways controlling skeletal muscle hypotrophy using this mutant, we hope to gain insights into this and related conditions, and can begin to develop corrective therapies for such muscle defects.

1085W

A multiplex kindred with severe Buruli Ulcer displaying Mendelian inheritance. *Q.B. Vincent[1], A. Chauty[2], M.F. Ardant[2], H. Dokponou[2], J. Gnossike[2], H. Koussemou[2], L. Marsollier[3], J.L. Casanova[1,4], L. Abel[1,4], A. Alcais[1,4].* 1) Laboratory of Human Genetics of Infectious Diseases, Necker Branch, Institut National de la Recherche Medicale U980 (INSERM), Université Paris-Descartes, 156 rue de Vaugirard, 75015 Paris, France; 2) Centre de Détection et de Traitement de l'Ulcere de Buruli (CDTUB), BP 191, Pobe, Benin; 3) Groupe d'Etude des Interactions Hote-Pathogene, Universite d'Angers, Angers, France; 4) Saint-Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, 1230 York Avenue, Box 163, New York, NY 10065, USA.

Buruli Ulcer (BU), caused by Mycobacterium Ulcerans, is the third most common mycobacteriosis worldwide after tuberculosis and leprosy, and has been flagged in 1998 by the World Health Organization as an emerging neglected infectious disease. The physiopathology of Mycobacterium ulcerans infection primarily involves the lipidic toxin mycolactone, a unique feature among mycobacteria. The resulting extensive skin ulcers and osteomyelitis cause pathologic scarring responsible for severe life-lasting functional disabilities in the affected population, mainly composed of children of less than 15 year of age. Buruli ulcer mainly strikes in Western Sub-Saharan Africa but cases have been reported in more than 30 countries worldwide. A common characteristic of the endemic countries consists in the extreme clustering of BU cases in families living in the vicinity of slow-flowing or stagnant waters in rural areas. However, only a fraction of these heavily exposed individuals develop Buruli ulcer, which leads us to hypothesize a genetic etiology accounting for this variability. To tackle this issue, we adopted an extreme-phenotype strategy, which consisted in recruiting the most severe of the >1,500 BU cases diagnosed and treated during the last 7 years at the Centre de Détection et de Traitement de l'Ulcère de Buruli in Pobè, Benin. We report here the analysis of a single highly-informative consanguineous family in which two siblings were affected with exceptionally severe PCR-confirmed BU. The index case suffered from a multifocal edematous form of BU, which disseminated under treatment and involved the four limbs, eventually requiring amputation to heal. Blood was obtained from the 2 parents, 2 affected and 3 unaffected children. DNA was processed for the genotyping of >900,000 Single Nucleotide Polymorphisms by the Affymetrix Genome-Wide 6.0 array. After quality control procedures, 120,156 independent SNPs were used for linkage analysis by homozygosity mapping. Three regions, on chromosome 5 and 8, cosegregated with the affected status following a Mendelian recessive inheritance mode, i.e. were shared homozygous by descent by the 2 affected individuals but not the 3 unaffected siblings, yielding the maximum possible LOD score given the pedigree. Sequencing of genes in these regions is currently ongoing and show promising results. This first description of a genetic etiology for extremely severe BU will have far reaching biological and medical implications.

1086W

Target gene selection and dual genome analysis by Next Generation Sequencing for mitochondrial disorders. *W. Zhang, H. Cui, F. li, l. Wong.* Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Background: Mitochondrial disorders are a group of complex diseases that can be caused by mutations in nuclear or mitochondrial genomes. More than 90% of the proteins involved in mitochondrial biogenesis and function are encoded by the nuclear genome. To capture the full spectrum of genetic defects, multiple different and complementary methods are required. For example, current molecular analytical methods include Sanger sequencing for the detection of point mutations, and qPCR/Southern blot/MLPA/array CGH for large insertion/deletion. These procedures are labor intensive, time consuming, and costly. Methods: A target gene enrichment using RNA probes to capture 360 nuclear genes targeted to mitochondria followed by a high throughput “deep” coverage next generation sequencing (NGS) approach was developed for the application in clinical settings. In addition to the targeted nuclear genes, the capture library also contains probes for the entire mitochondrial genome. Results: With the gene enrichment, about 40% of the sequence reads are mapped to target genes. An average coverage of >500X for targeted nuclear genes and >5000X for each of the 16,569 bases of the mitochondrial genome was achieved. The high coverage allows not only the highly accurate detection of nucleotide changes in both nuclear and mitochondrial genomes, but also the degree of heteroplasmy at every single base of the mitochondrial genome. This method accurately detected a heterozygous c.761T > A (p.I254N) mutation and a heterozygous deletion of exons 1 and 2 of the TK2 gene in a sample with known diagnosis. An average of 20 nonsense and/or novel mutations were identified in each of the 7 samples analyzed. However, all of these mutations occurred as a single heterozygous allele, not consistent with autosomal recessive inheritance. Conclusion: Our results demonstrated the superior sensitivity and specificity of base calls compared to the gold standard Sanger sequencing. With deep coverage, measurement of mitochondrial nucleotide heteroplasmy is easily achieved. This approach can be used for simultaneous sequence analyses of a group of selected genes related to mitochondrial disorders for molecular diagnosis.

1087W

***FKBP10*-null mutation in recessive type XI OI leads to abnormal collagen trafficking to Golgi.** *A.M. Barnes[1], W.A. Cabral[1], E. Makareeva[2], M. Weis[3], W. Chang[1], L. Felley[1], D. Eyre[3], S. Leikin[2], C. Trujillo[4], J.C. Marini[1].* 1) Bone and Extracellular Matrix Branch, NICHD/NIH, Bethesda, MD, United States; 2) Section on Physical Biochemistry, NICHD/NIH, Bethesda, MD, United States; 3) Orthopaedic Research Laboratories, University of Washington, Seattle, United States; 4) Genetics Unit, Dr. Erfan & Bagedo General Hospital, Jeddah, Saudi Arabia.

Osteogenesis imperfecta (OI), or "brittle bone disease", is a genetic disorder characterized by bone fragility and deformity and short stature. Dominant OI (85-90% of cases) is caused by mutations in either of the genes for type I collagen, *COL1A1* or *COL1A2*. Recessive OI is caused by defects in genes whose products interact with type I collagen for modification and/or folding. We identified a 5-generation Palestinian pedigree with both moderate and lethal forms of recessive OI caused by homozygous mutations in *FKBP10* or *PPIB*, which encode the ER-resident peptidyl-prolyl cis-trans isomerases FK506-binding protein 65 (FKPB65) and cyclophilin B (CyPB), respectively. For each mutation, we compared transcripts and protein levels of collagen chaperones, steady state collagen, chain incorporation, differential scanning calorimetry, amino acid analysis, and immunofluorescence (IF) staining in proband and control fibroblasts. One pedigree branch includes a child with moderate type XI OI and a homozygous *FKBP10* mutation (c.1271_1272delCCinsA); both parents and one sibling are carriers. *FKBP10* transcripts in proband fibroblasts are 6% of control; the mutation leads to intronic retention in ¼ of transcripts, but both forms have premature stops. FKBP65 protein is absent in proband cells analyzed by Western blot and IF microscopy. Transcripts for other proteins that interact with collagen are normal for CRTAP and P3H1, while HSP47 is increased approximately 2.5 fold in proband cells. CRTAP, P3H1 and HSP47 protein levels are near normal. Proband collagen gel electrophoresis reveals moderate band broadening, with a 13% increase in hydroxylysine and a 7% increase in hydroxyproline as compared to control, with normal chain incorporation. Proband collagen (1(I)Pro986 3-hydroxylation is normal, as is collagen T_m. Collagen in proband cells is not aggregated on IF and appears to remain in the ER. A second pedigree branch includes two children with lethal OI. Both parents carry a 4 nt deletion in exon 5 of *PPIB* (c.563_566delACAG), predicting type IX OI in offspring. Mutant transcripts would likely escape NMD. Truncated CyPB would be predicted to cause severe dysfunction of the collagen 3-hydroxylation complex, compatible with the lethal outcome. In conclusion, *FKBP10*-null cells have a slight folding delay, with a ~10% increase in hydroxylation. This delay is associated with abnormal collagen trafficking to the Golgi and may lead to a delay in secretion.

1088W

Leri Weill Syndrome caused by a duplication of cis-regulatory DNA elements downstream of SHOX gene. *D. Coviello[1], L. Capone[2], M. Baffico[1], R. Bertorelli[2], L. Iughetti[3], B. Predieri[3], S. Vanelli[4], B. Stasiowska[4], M. Baldi[1], A. Forabosco[2].* 1) Laboratory of Human Genetics, E.O. Ospedali Galliera, Genova, GE, Italy; 2) Laboratory of Molecular Biology and Medical Genetics, Cante di Montevecchio Institute, Fano, Italy; 3) Pediatric Department, University of Modena and Reggio Emilia, Modena, Italy; 4) Auxology Unit, University of Tourin, Tourin Italy.

Leri-Weill syndrome (LWS)(MIM127300) is a dominantly inherited skeletal dysplasia characterized by mesomelic disproportionate short stature and Madelung deformity of the wrist. Heterozygous deletions or mutations of the short-stature homeobox-containing gene (SHOX) (MIM 312865) have been identified as the molecular basis of LWD. Homozygous or compound heterozigous SHOX mutation have been demonstrated to be responsible for the most severe dwarfism known as Langer mesomelic dysplasia (LMD) (MIM 249700). The disturbance of the long range regulation of gene expression, essential for producing the gene product in the proper time and place and in the correct quantity, has been proposed as the cause of a number of diseases in which the suspected target genes appeared to be completely normal. The discovery in the last few years that heterozigous and homozogous DNA deletions downstream the intact SHOX gene cause the LWS and LMD syndrome respectively, led to hypothesize the presence of DNA elements acting as enhancers in the 3' region downstream of SHOX. It has been shown that this enhancer action is due to conserved non coding DNA elements (CNEs). Many authors have identified a number of CNEs, showing enhancer activity both in vitro and in vivo. Recently, LWD has been reported also in subjects with cis-interstitial duplications of the PAR1/SHOX region encompassing entirely or partially the SHOX gene. The precise mechanisms of the pathogenic effect caused by the microduplication of the CNEs-containing region are unclear. It is possible that duplication exerts a negative effect on SHOX transcription. Recently, it has been demonstrated that the proper gene regulation depends not only on the required transcription factors and associated complexes being present but also on the integrity, chromatin conformation and nuclear positioning of the gene chromosomal segments. On the basis of the above considerations it is not unlikely that the duplication alters the three dimensional chromatin spatial organization impeding in some way the enhancer to contact the promoter, resulting in a lack of gene activation resulting in a condition of haploinsufficiency. We report the first cases of "pure" duplications of the region downstream the SHOX gene containing CNEs regulatory elements in two LWS families, suggesting that duplications may represent an additional class of mutations implicated in the molecular etiology of the Leri Weill syndrome.

1089W

Musculo-contractural Ehlers-Danlos syndrome (EDS type VIB): a new EDS-subtype caused by a defect in proteoglycan biosynthesis. *A. De Paepe[1], P. Vlummens[1], D. Syx[1], S. Nampoothiri[2], S. Symoens[1], F. Malfait[1].* 1) Center for Medical Genetics, Ghent University Hospital, Belgium; 2) Amrita Institute of Medical Sciences and Research Center, Cochin, Kerala, India.

Musculo-contractural Ehlers-Danlos syndrome (MC-EDS) is a recently described autosomal recessive form of EDS characterized by craniofacial abnormalities, congenital joint contractures, especially of fingers and toes, talipes equinovarus, tapering fingers and excessive palmar wrinkling, severe progressive muscle hypotonia and multi-systemic tissue fragility with ophtalmological, gastro-intestinal and genito-urinary manifestations, in addition to skin fragility, wound healing problems, bleeding diathesis and joint hyperlaxity. The condition is caused by homozygous mutations in *CHST14*, encoding dermatan-4-sulfotransferase 1 (D4ST-1). The clinical phenotype of MC-EDS strongly overlaps with that of adducted thumb-clubfoot syndrome (ATCS), and with "EDS Kosho type", a Japanese EDS-variant, two conditions that were independently shown to be caused by *CHST14* mutations. Detailed phenotypic analysis of the 20 patients reported with *CHST14* mutations strongly suggests that these conditions form a single entity with multi-systemic, but age-dependent and variable phenotypic features, of which joint contractures and generalized muscle weakness are distinguishing features, hence the name "musculo-contractural EDS". Hitherto reported *CHST14* mutations comprise six missense, two nonsense and one frameshift mutation, some of which were shown to result in loss of function of D4ST-1. This is a key enzyme in the biosynthesis of dermatan sulfate (DS), catalyzing 4-O-sulfation of N-acetyl-galactosamine. Loss of D4ST-1 activity results in the replacement of DS by chondroitin sulfate. The broad array of physiological events in which DS plays a role, including the correct assembly of collagen fibrils mediated by decorin, a DS proteoglycan, explains the multisystemic manifestations of this condition.

1090W

Novel canine chondrodysplasia mutation. *K. Kyöstilä[1,2,3], H. Lohi[1,2,3].* 1) Department of Veterinary Biosciences, University of Helsinki, Finland; 2) Research Programs Unit, Molecular Medicine, University of Helsinki, Finland; 3) Department of Molecular Genetics, Folkhälsan Institute of Genetics, Finland.

Skeletal dysplasias are heterogeneous disorders of bone and cartilage. Chondrodysplasias affect cartilage tissue and often result in disproportionate short stature or dwarfism. We report here the identification of a recessive canine chondrodysplasia mutation in a gene that represents a new candidate for human chondrodysplasias. The novel mutation was identified in Norwegian Elkhounds, a Nordic hunting breed that suffers from hereditary chondrodysplasia. The condition results from generalized disturbance in the endochondral ossification process. Abnormalities are seen in both the arrangement and structure of growth plate chondrocytes. At a histopathological level, the disorder is reported to bear resemblance to human spondylometaphyseal dysplasias. Our pedigree analysis suggested a recessive mode of inheritance for the condition. We applied the genome-wide association method to map the causative mutation to a novel canine chondrodysplasia gene on CFA17. Nine affected dogs and nine control dogs were genotyped using Illumina's canine SNP chip of 22K SNPs. Association analysis revealed a 2 Mb disease associated locus on CFA17 (Praw=6.46E-06, Pgenome= 0.012). Mutation screening of a plausible candidate gene on the locus revealed a homozygous nonsense mutation in all affected dogs, predicted to result in a premature stop-codon in the encoded protein. Segregation of the mutation was studied further in 157 unrelated population controls none of which were homozygous for the mutant allele. To the best of our knowledge, this is the first report of a spontaneous disease causing mutation for this gene which now serves as a plausible candidate gene in human chondrodysplasias as well. We have initiated screens in human patients with related phenotypes. We hope our findings will improve the understanding of the endochondral ossification processes and related molecular pathways in health and disease.

1091W

Identification by exome sequencing of truncating mutations in the last exon of NOTCH2 as a cause of Hajdu-Cheney syndrome, a rare disorder with osteoporosis. *C. Le Caignec[1,2,3,4], B. Isidor[1], P. Lindenbaum[1,2,3], O. Pichon[1], S. Bezieau[1], C. Dina[2,3,4], S. Jacquemont[5], D. Martin-Coignard[6], C. Thauvin-Robinet[7], M. Le Merrer[8], J.L. Mandel[9], A. David[1], L. Faivre[7], V. Cormier-Daire[8], R. Redon[1,2,3].* 1) Service de Genetique Medicale, Institut de biologie - CHU, Nantes, France; 2) INSERM, UMR915, l'institut du thorax, Nantes, France; 3) Université de Nantes, Nantes, France; 4) CNRS, ERL3147, Nantes, France; 5) CHUV, Service de Génétique Médicale, Lausanne, Switzerland; 6) Unité de Génétique Clinique, Centre Hospitalier du Mans, Le Mans, France; 7) Centre de Génétique, Centre de Référence Maladies Rares “Anomalies du développement et syndromes malformatifs de l'interrégion Est” Hôpital d'Enfants, CHU, Université de Bourgogne, Dijon, France; 8) INSERM U781 and Département de Génétique, Université Paris Descartes, Hôpital Necker-Enfants Malades, Paris, France; 9) Translational Medicine and Neurogenetics Program, Institut de Génétique et de Biologie, Moléculaire et Cellulaire (IGBMC), INSERM U964, CNRS UMR 7104, Université de Strasbourg, CHU de Strasbourg, Collège de France, Illkirch, France.

Hajdu-Cheney syndrome is a rare skeletal disorder characterized by the association of facial anomalies, radiological findings (acro-osteolysis, general osteoporosis, insufficient ossification of the skull) and periodontal disease (premature loss of permanent teeth). Although most cases are sporadic, transmission in a few families suggests autosomal dominant inheritance. We sequenced the exomes of six unrelated patients and identified nonsense and frameshift heterozygous mutations in NOTCH2 for five of them. The five mutations are invariably located within the last 3' coding exon of NOTCH2, distal to the ankyrin repeats and NLS necessary for the transcriptional activity of Notch2 intracellular domain, while deleting the PEST domain. These results suggest that mutant mRNA products escape nonsense-mediated decay and that truncated NOTCH2 proteins act in a gain-of-function or dominant-negative manner. This mutational mechanism also suggests that modulating Notch2 signaling using selective gamma-secretase modulators may have therapeutic interest in this severe and progressive disease. Finally, our findings establish an important role for Notch2 signaling in bone homeostasis that may notably be relevant to better understanding of mechanisms leading to osteoporosis.

1092W

Mutations in the TGFβ binding protein-like domain 5 of FBN1 are responsible for acromicric and geleophysic dysplasias. *C. Le Goff[1], C. Mahaut[1], L.W. Wang[2], S. Allali[1], A. Abhyankar[3], S. Jensen[4], L. Zylberberg[5], G. Collod-Beroud[6], C. Bole-Feysot[7], P. Nischtke[8], P. Handford[4], J.L. Casanova[9], C. Boileau[10], S.S. Apte[2], A. Munnich[1], V. Cormier-Daire[1], clinical consortium of gelophysic and acromicric dysplasias.* 1) Dept Gen, INSERM U781, university Paris Descartes Hopital Necker, Paris, France; 2) Department of Biomedical Engineering, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio, USA; 3) St. Giles Lab of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, New York, NY, USA; 4) Department of Biochemistry University of Oxford, South Parks Rd Oxford UK; 5) ISTEP, CNRS UMR71 93, Université Pierre et Marie Curie, Paris, France; 6) INSERM, U827, F-34000 France and Université Montpellier1, UFR Médecine, Montpellier France; 7) Plateforme Génomique, Fondation Imagine Université Paris Descartes, Paris, France; 8) Plateforme de Bioinformatique, Université Paris Descartes, Paris, France; 9) Lab of Human Genetics of Infectious Diseases, University Paris Descartes and INSERM U980, Necker Medical School, Paris, France, and St. Giles Lab of Human Genetics of Infectious Diseases, , The Rockefeller University, New York, NY, U; 10) INSERM U698, Bichat Claude Bernard and Laboratoire de Biochimie et de Génétique Moléculaire, hôpital Ambroise Paré, Boulogne, France.

Geleophysic (GD) and acromicric dysplasia (AD) belong to the acromelic dysplasia group and are both characterized by severe short stature (<-3SD), short hands and feet, joint limitations and skin thickening. GD is distinct from AD by an autosomal recessive mode of inheritance and a progressive cardiac valvular thickening often leading to an early death. AD is transmitted with an autosomal dominant mode of inheritance and is characterized by distinct dysmorphic features, a hoarse voice and an internal notch of the femoral head. We recently identified mutations in A Disintegrin And Metalloproteinase with Thrombospondin repeats-like 2 gene (ADAMTSL2) in GD. We demonstrated an involvement of ADAMTSL2 in TGFβ bioavailability. However, absence of ADAMTSL2 mutations in 19/33 GD patients suggested genetic heterogeneity. AD has unknown molecular basis. We performed exome sequencing in 2 GD cases with no ADAMTSL2 mutations and in 3 AD patients. Based on the phenotypic overlap between AD and GD, we searched for a shared mutated gene among the 5 exomes. We identified changes in three genes: MUC17, HYDIN and FBN1. The presence of tall stature in Marfan syndrome caused by FBN1 mutations prompted us to consider FBN1 as the best candidate gene. We identified 16 heterozygous FBN1 mutations (15 missense and one insertion) all located in exon 41-42, encoding TGFβ binding protein-like domain 5 (TB5) of FBN1 in 19 GD and 10 AD cases. While GD has been described as an autosomal recessive disorder, the identification of heterozygous FBN1 mutations demonstrates a dominant form of GD, strictly fulfilling the diagnostic criteria for GD (including progressive cardiac valvular thickening, and early death in 3/19). Similarly, all AD cases fulfilled the diagnostic criteria of AD and had no cardiac involvement or early death. These data demonstrate that GD and AD are distinct but allelic conditions. Microfibrillar network disorganization and enhanced TGFβ signaling were consistenty observed in GD/AD fibroblasts. Importantly, a direct interaction between ADAMTSL2 and FBN1 was demonstrated suggesting a dysregulation of FBN1/ADAMTSL2/ TGFβ interrelationship as the underlying mechanism of the short stature phenotypes. Our findings support that TB5 mutations in FBN1 are responsible for short stature phenotypes. Ongoing studies will hopefully help to further understand how enhanced TGFβ signaling caused by FBN1 mutations can trigger either Marfan syndrome or GD/AD.

1093W

Sc65 is a novel regulator of bone homeostasis. *R. Morello[1,4], B. Hendrix[1], K. Gruenwald[1], P. Castagnola[2], D. Gaddy[1], L.J. Suva[3].* 1) Dept Physiology & Biophysics, Univ Arkansas, Little Rock, AR; 2) National Institute for Cancer Research, Genoa, ITALY; 3) Dept of Orthopaedic Research and Physiology & Biophysics, Univ Arkansas, Little Rock, AR; 4) Division of Genetics, Univ Arkansas, Little Rock, AR.

Leprecan-like 4 (*Leprel4*, aka Sc65) is a member of the Leprecan family of genes which include *Crtap* (encoding cartilage associated protein), *Lepre1* (*Leprecan* encoding prolyl 3-hydroxylase 1 or P3h1), *Leprel1* (*Leprecan-like 1* encoding prolyl 3-hydroxylase 2 or P3h2) and *Leprel2* (*Leprecan-like 2* encoding prolyl 3-hydroxylase 3 or P3h3). We and others showed that mutations in two Leprecan genes, *CRTAP* and *LEPRE1* cause recessive forms of osteogenesis imperfecta (OI). Such findings shifted much of the current OI interest from matrix components (i.e. type I collagens) to endoplasmic reticulum resident proteins, led to the identification of additional genes (*PPIB*, *FKBP10*, and *SERPINH1*) linked to recessive OI and greatly increased the molecular understanding of this disease. Having previously characterized the collagen prolyl 3-hydroxylation complex, containing Crtap, P3h1 and Cyclophilin B (CypB), we now extended our studies to determine the function of *Sc65*, a poorly described gene encoding the most closely related protein to Crtap. The hypothesis being that Sc65 could also be involved in collagen prolyl 3-hydroxylation and perhaps interacting with the other members of the Leprecan family. Sc65 was originally described as a nuclear epitope and more recently as a cytoplasmic `adaptor protein'. In human fibroblasts we showed that Sc65 localizes to the rER similarly to the other Leprecan proteins. In addition, Sc65 is highly expressed during skeletal formation. Using a gene-trap ES cell clone containing a proviral insertion in exon 5 of the *Sc65* gene we generated homozygous mice. Western blot analyses using osteoblasts from Sc65 mutant and WT mice revealed the absence or markedly reduced Sc65 protein in the mutants. Micro-CT analysis of the appendicular skeleton at 10 weeks of age showed a statistically significant reduction in BV/TV and other static parameters in both the tibia and femur. Scans of the femoral midshaft also demonstrated a significantly reduced cortical cross sectional area (CSA) and thickness, with increased total CSA and endosteal perimeter. Histomorphometric analysis showed no difference in dynamic indices of bone formation or in osteoclast parameters in the tibias of *Sc65* mutant mice compared to controls. Current efforts are aimed at elucidating the cellular and molecular mechanisms responsible for the low bone mass. Our data suggest that impaired *Sc65* function has an impact on bone homeostasis and may be linked to human osteoporosis.

1094W

Whole-exome sequencing in Fine-Lubinsky syndrome reveals a promising candidate gene. *D.R. Murdock[1], P. Campeau[2], J. Lu[1], M-C. Gingras[1], D. Scott[2], R. Gibbs[1], B. Lee[2,3].* 1) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Howard Hughes Medical Institute, Houston, TX.

Fine-Lubinsky syndrome is an autosomal recessive condition characterized by craniosynostosis/abnormal calvaria, prominent frontal bones, a flat facial profile, a small nose, microstomia, hearing loss, developmental delay/mental retardation, and abnormal digits. We investigated by whole-exome sequencing two siblings previously described (Holder AM et al. AJMG 2007). A homozygous missense mutation was identified in *RPA1*, and compound heterozygous missense variants were identified in *ASPM* , *FMN1*, *CAST*, and *DOCK2* in both sibs. In humans, *ASPM* mutations are known to cause non-syndromic microcephaly and CNS malformations, while *FMN1* homozygous deletions are associated with oligosyndactyly and radioulnar synostosis in both humans and mice. The protein encoded by *FMN1*, formin1, inhibits BMP signaling and the mutations identified (p.T42M and p.A440S in isoforms 3 and 5) affect highly conserved amino acids. *Cast* knockout mice do not have an obvious phenotype, *Dock2* knockout mice have an impaired lymphocyte migration and *Rpa1* heterozygous mutations in mice cause a susceptibility to lymphoid tumors. *FMN1* is thus the most likely candidate gene based on its function and deletion effect in mice and humans. We are currently sequencing these genes in other individuals with Fine-Lubinsky syndrome and confirming the effect of the *FMN1* mutations on BMP signaling.

1095W

The ratio of urinary pyridinoline to deoxypyridinoline crosslinks — a promising diagnostic tool in Osteogenesis imperfecta. *M. Rohrbach[1], M. Kraenzlin[2], D. Eyre[3], I. Kennerknecht[4], Ch. Netzer[5], O. Semler[5], F. Rutsch[6], B. Steinmann[1], MR. Baumgartner[1], C. Giunta[1].* 1) Division of Metabolism, University Children`s Hospital and Children's Research Centre, Zurich, Switzerland; 2) Division of Endocrinology, Diabetes, and Clinical Nutrition, University Hospital, Basel, Switzerland; 3) Department of Orthopaedics and Sports Medicine, University of Washington, Seattle, Washington, USA; 4) Institute of Human Genetics, Münster University, Germany; 5) Institute of Human Genetics, University of Cologne, Cologne, Germany; 6) Department of General Pediatrics, Munster University Children's Hospital, Münster, Germany.

Urinary pyridinoline crosslinks, hydroxylysyl-pyridinoline (HP, or pyridinoline PYD) and lysyl-pyridinoline (LP, or deoxypyridinoline DPD) are well characterized markers for bone resorption and collagen degradation, and proven diagnostic tools for genetic disorders of collagen metabolism such as EDS VIA, SCD-EDS and Bruck syndrome. Osteogenesis imperfecta (OI) is a heterogeneous genetic disorder characterized by increased susceptibility to fractures. The majority of OI is inherited as an autosomal dominant trait caused by mutations in *COL1A1* and *COL1A2* resulting in quantitative and/or qualitative alterations of type 1 collagen. A small proportion of OI is inherited in an autosomal-recessive manner due to mutations in seven different genes encoding for proteins of the prolyl 3-hydroxylation complex (*CRTAP, LEPRE1, PPIB*); for collagen chaperones (*SERPINH1, FKBP10*); for a transcription factor (*SP7/OSX*) assumed to regulate the differentiation of preosteoblasts to osteoblasts; and for *SERPINF1*, a secreted glycoprotein of the serpin superfamily, probably involved in bone homeostasis. The aim of this study was to evaluate the ratio of total urinary pyridinolines LP/HP (or DPD/PYD) as a non-invasive, reliable and cost effective screening tool in individual OI patients, prior to collagen biochemical and/or molecular genetic analyses. We analyzed spot urines of OI patients of known genetic background, including three with defects in *LEPRE1*, two with mutations in *CRTAP*, and two with defects in *SERPINF1*, as well as two heterozygous carriers for a *CRTAP* mutation. Compared to control LP/HP ratios (0.20 ± 0.03, n=325), we found markedly decreased LP/HP ratios in OI caused by mutations in *LEPRE1* (mean: 0.078) and in *CRTAP* (mean: 0.117), but normal LP/HP ratios in OI caused by mutations in *COL1A1/COL1A2* (mean: 0.21± 0.028; n=4), in *SERPINF1* (mean: 0.195), and in the two heterozygous carriers for a *CRTAP* mutation (mean: 0.172). Bisphosphonate treatment did not influence the ratio of total urinary pyridinoline crosslinks. Thus, LP/HP ratios have the potential to detect recessive forms of OI caused by mutations in the genes *LEPRE1* and *CRTAP*, and therefore to improve the efficacy of the final diagnosis and reducing the costs of molecular genetic investigations. We expect a similar result in OI cases caused by some mutations in *PPIB*. With this report, we hope to attract more cases of OI with a known genetic defect in order to statistically validate this preliminary study.

1096W

Mutations in NOTCH2 in families with Hajdu-Cheney Syndrome. *M. Samuels[1], J. Majewski[2], J. Schwartzentruber[2], A. Caqueret[1], L. Patry[1], J.P. Fryns[3], K. Boycott[4], L.-G. Ste-Marie[5], F. McKiernan[6], I. Marik[7], H. Van Esch[3], J. Michaud[1].* 1) Centre de Recherche du CHU Ste-Justine, University of Montreal, Montreal, PQ, Canada; 2) McGill University, Montreal, PQ, Canada; 3) Center for Human Genetics, University Hospitals Leuven, Leuven, Belgium; 4) Department of Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada; 5) Centre de Recherche du CHUM, University of Montreal, Montreal, PQ, Canada; 6) Center for Bone Disease, Marshfield Clinic, Marshfield, WI; 7) Ambulatory Centre for Defects of Locomotor Apparatus, Praha, Czech Republic.

Hajdu-Cheney syndrome is a rare genetic disorder whose hallmark is acro-osteolysis, shortening of terminal phalanges and generalized osteoporosis. We assembled a cohort of 7 families with the condition and performed whole exome resequencing on a selected set of affected patients. One protein-coding gene, NOTCH2, carried heterozygous truncating variants in all patients and their affected family members, consistent with being the likely causal gene for the disorder. In total we identified five novel and one previously reported mutation, all clustered near the carboxyl terminus of the gene, suggesting an allele specific genotype-phenotype effect since other mutations in NOTCH2 have been reported to cause a form of Alagille syndrome. Notch mediated signaling is known to play a role in bone metabolism. Our results support a potential therapeutic role for the notch pathway in treatment of osteoporosis.

1097W

A next-generation sequencing approach to diagnosis of a family's skeletal abnormalities and retinitis pigmentosa. *K. Schrader[1,2], A. Heravi-Moussavi[3], P. Waters[4], J. Senz[1], J. Whelan[5], G. Ha[3], P. Eydoux[4], T. Nielsen[6], B. Gallagher[7], A. Oloumi[3], N. Boyd[3], B.A. Fernandez[8], T.L. Young[8], S.J.M. Jones[9], M. Hirst[9], S.P. Shah[3], M.A. Marra[9], J. Green[5,8], D.G. Huntsman[1].* 1) Dept Pathology & Laboratory Medicine, Univ British Columbia, Vancouver, BC, Canada; 2) Dept Medical Genetics, Univ British Columbia, Vancouver, BC, Canada; 3) BC Cancer Agency, Vancouver, BC, Canada; 4) Dept Pathology & Laboratory Medicine, Children's & Women's Hospital, Vancouver, BC, Canada; 5) Dept Ophthalmology, Memorial University of Newfoundland, St John's, Nfld, Canada; 6) Vancouver Hospital and Health Sciences Centre, Anatomical Pathology, Vancouver, BC, Canada; 7) James Paton Memorial Regional Health Centre, Pathology, Gander, Nfld, Canada; 8) Discipline of Genetics, Memorial University of Newfoundland, St John's, Nfld, Canada; 9) Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada.

We used a next-generation sequencing approach to determine the underlying cause of the unusual combination of spondyloepiphyseal dysplasia (SED) and adult-onset retinitis pigmentosa, co-segregating in members of a large family in Newfoundland. Based on the rare presentation and autosomal recessive inheritance pattern with known consanguinity, we predicted the underlying genetic cause would be novel and homozygous in affected individuals and heterozygous in the obligate carriers. To rapidly select candidate variants fulfilling this inheritance pattern and to eliminate potential false positives, we sequenced the exomes of 3 affected individuals and one carrier. This revealed, on average, 412 novel non-synonymous variants, 242 novel indels and 60 splice site variants per case. Of these, only 2 novel variants fulfilled our criteria. Both were validated, and shown to segregate with disease, by Sanger sequencing on 14 further family members. The more promising candidate variant was an in-frame 6bp deletion (p.80K_81Ydel) in GNPTG, which encodes the . -subunit of GlcNAc-1-phosphotransferase. The deletion was predicted to be probably damaging by Polyphen and was absent from 368 ethnically-matched control chromosomes. Loss-of-function mutations in GNPTG are known to cause mucolipidosis III. , a disorder of lysosomal enzyme targeting. Biochemical analysis of serum from 4 affected and 3 unaffected-carrier family members, run in parallel with 4 healthy controls from the same population, showed greater than 10-fold elevations of four lysosomal enzymes in affected individuals compared with controls. These findings confirmed the diagnosis of mucolipidosis III. (MLIII.). The phenotypic spectrum of MLIII is notoriously broad and the clinical presentation in this family was atypical. Rod-cone dystrophy has not previously been reported in MLIII patients, although evidence from null mice models does imply a causal link between loss of phosphotransferase function and retinal degeneration. Variable presentations of MLIII now need to be considered as a possible etiology for other reported syndromes of SED or related skeletal disorders seen together with retinal manifestations. The approach taken has allowed for rapid diagnosis of this family's complex phenotype and further broadening of the phenotypic spectrum of MLIII. . Our study provides support for the utility of next-generation sequencing in diagnosing phenotypically and genetically heterogeneous Mendelian disorders.

1098W

Dominant and recessive forms of fibrochondrogenesis resulting from mutations at a second locus, *COL11A2*. *S.W. Tompson[1,2,3], E. Ali Faqeih[4], L. Ala-Kokko[5], J.T. Hecht[6], R. Miki[1,2,3], T. Funari[3], V.A. Funari[3,7], L. Nevarez[1,2,3], D. Krakow[2,8,9], D.H. Cohn[1,2,8].* 1) Dept of Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA; 2) Dept of Orthopaedic Surgery, University of California, Los Angeles, CA; 3) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 4) Dept of Pediatric Medicine, Children's Hospital, King Fahad Medical Center, Riyadh, Saudi Arabia; 5) Connective Tissue Gene Tests, Allentown, PA; 6) Dept of Pediatrics, University of Texas Medical School, Houston, TX; 7) Dept of Pediatrics, University of California, Los Angeles, CA; 8) International Skeletal Dysplasia Registry, Cedars-Sinai Medical Center, Los Angeles, CA; 9) Dept of Human Genetics, University of California, Los Angeles, CA.

Fibrochondrogenesis is a severe, recessively inherited skeletal dysplasia that can result from mutations in the gene encoding the pro(1(XI) chain of type XI procollagen, *COL11A1*. We studied a case in which sequence analysis of *COL11A1* failed to identify mutations. As the parents were known to be consanguineous, consistent with autosomal recessive inheritance, whole-genome SNP genotyping was performed to identify blocks of homozygosity, identical-by-descent, wherein the disease locus would reside. *COL11A1* was not within a region of homozygosity, excluding it as the disease locus. However, the gene encoding the pro(2(XI) chain of type XI procollagen, *COL11A2*, was located within a large block of homozygosity. Sequence analysis identified homozygosity for a splice donor mutation that was confirmed, by exon trapping, to result in exon 18 skipping and deletion of 54bp of coding sequence. Since homozygosity for null mutations in *COL11A2* is associated with the milder otospondylomegaepiphyseal dysplasia (OSMED) phenotype, we infer that the mutation was not a null allele and that the presence of abnormal chains has a detrimental effect on type XI collagen function. Mutant transcripts are predicted to encode a protein with an 18 amino acid deletion at position 556-573, toward the amino-terminal end of the triple helical domain, disrupting the structure of all type XI procollagen molecules.

In a second case, sequence analysis of *COL11A1* and *COL11A2* revealed heterozygosity for a single mutation, a 9bp deletion in exon 40 (c.2899_2907del9) of *COL11A2*. Analysis of parental DNA showed that neither carried the sequence change, and genotypes at 12 informative pan-genomic microsatellite markers were consistent with parentage. We conclude that the disease was caused by a *de novo* dominant mutation in *COL11A2*, resulting from incorporation of pro(2(XI) chains harboring a 3 amino acid deletion (p.967_969del3) within the triple helical domain. These results demonstrate that *COL11A2* is a second locus for fibrochondrogenesis and that mutations in the gene can result in either dominant or recessive forms of the disorder. Perinatal lethal fibrochondrogenesis can now be recognized as the most severe disease phenotype associated with mutations in *COL11A2*.

1099W

Multiple Synostoses Syndrome Is Due to a Missense Mutation in Exon 2 of FGF9 Gene. *X. Wu[1], M. Gu[1], L. Huang[1], X. Liu[1,2], H. Zhang[1], X. Ding[3], J. Xu[4], B. Cui[5], L. Wang[2,6,7], S. Lu[2,6,7], X. Chen[1], H. Zhang[1], W. Huang[8], W. Yuan[8], J. Yang[9], Q. Gu[9], J. Fei[7], Z. Chen[6], Z. Yuan[10], Z. Wang[1,2,6,7].* 1) Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China; 2) Laboratory of Genetic Engineering, Institute of Health Sciences, Shanghai Institutes for Biological Sciences of Chinese Academy of Sciences and SJTUSM,Shanghai 200025,China; 3) Department of Radiology,Rui-jin Hospital, SJTUSM,Shanghai 200025, China; 4) Department of Orthopaedics,Rui-jin Hospital, SJTUSM,Shanghai 200025, China; 5) Department of Endocrinology, Rui-jin Hospital, SJTUSM,Shanghai 200025, China; 6) State Key Laboratory of Medical Genomics, Rui-jin Hospital, SJTUSM,Shanghai 200025, China; 7) Shanghai Research Centre for Model Organisms, Shanghai 201210,China; 8) Chinese National Human Genome Centre at Shanghai, Shanghai 201203, China; 9) Department of Inspect, Qinghai Chinese Medical Hospital, Xining 810000, China; 10) Radiation Biology Division, Department of Radiation Oncology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA.

Fibroblast growth factors (FGFs) play diverse roles in several developmental processes. Mutations leading to deregulated FGF signaling can cause human skeletal dysplasias and cancer. Here we report a missense mutation Ser99Asp in exon 2 of FGF9 in 12 patients with multiple synostoses syndrome (SYNS) in a large Chinese family. In vitro studies demonstrate that FGF9S99N is expressed and secreted as efficiently as wild-type FGF9 in transfected cells. However, FGF9S99N induces compromised chondrocyte proliferation and differentiation, which is accompanied by enhanced osteogenic differentiation and matrix mineralization of bone marrow-derived mesenchymal stem cells (BMSCs). Biochemical analysis reveals that S99N mutation in FGF9 leads to significantly impaired FGF signaling, as evidenced by diminished activity of Erk1/2 pathway and decreased b-catenin and c-Myc expression when compared with wild-type FGF9. Importantly, the binding of FGF9S99N to its receptor is severely impaired although the dimerization ability of mutant FGF9 itself or with wildtype. FGF9 is not detectably affected, providing a basis for the defective FGFR signaling. Collectively, our data demonstrate a previously uncharacterized mutation in FGF9 as one of the causes of SYNS, implicating an important role of FGF9 in normal joint development.

1100W

Mutations in the NSUN2 gene cause autosomal recessive intellectual disability in Middle Eastern populations with elevated frequency. *L. Abbasi Moheb[1], S. Mertel[2], L. Nouri Vahid[3], K. Kahrizi[3], A. Tzschach[1], D. Wieczorek[4], M. Garshasbi[1], S. Cirak[5], SS. Abedini[3], H. Najmabadi[3], HH. Ropers[1], S. Sigrist[2], AW. Kuss[1,6].* 1) Human Molec Gen, Max Planck Inst Molec Gen, Berlin, Germany; 2) Genetics, Institute for Biology, Freie Universität Berlin, Berlin, Germany; 3) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran; 4) Institut für Humangenetik, Universitätsklinikum , Essen, Germany; 5) The Dubowitz Neuromuscular Centre, University College London Institute of Child Health London, UK; 6) Current address: Institute for Human Genetics, Interfaculty Institute for Genetics and Functional Genomics, Ernst Moritz Arndt University, Greifswald, Germany.

During the course of our investigations into the autosomal recessive causes of intellectual disability (ARID) we have previously identified numerous new loci for this condition.Interestingly, so far no more than six hotspot loci for unspecific or non-syndromic autosomal recessive intellectual disability (NS-ARID) have been identified (Kuss et al. 2011, Human Genetics Vol.129, 141-148). In this study we now resolved the underlying gene defect of MRT 5 and report three deleterious mutations in NSUN2. These were found in two independent consanguineous Iranian families and one Turkish family with several patients suffering from non-syndromic ARID. NSUN2 encodes a methyltransferase, which catalyzes the intron-dependent formation of 5-methylcytosine at C34 of tRNA-leu(CAA). Two of the observed changes were nonsense mutations (p.Q227X and p.Q372X), which cause a complete loss of NSUN2 transcripts in the patients. In the third family we found an intronic exchange of an adenosine for a guanine 11 nucleotides upstream of exon 6. This change causes exon 6 to be skipped during splicing and results in the loss of the main transcript. Hence all mutations lead to a loss of NSUN2 protein function in homozygous mutation carriers and thus in all likelihood cause the patient phenotype. In order to gain further evidence for an involvement of NSUN2 in cognitive functions, we studied fruit fly mutants that lack the NSUN2 ortholog. These experiments revealed a marked learning impairment in mutant flies, which clearly underscores the relevance of NSUN2 in higher brain functions.

1101W

Identification and Mapping of locus on the long arm of chromosome 4 that is associated with mental retardation and optic atrophy in a Qatari family with undelineated autosomal receive disease. *Y. Al-Saraj, M. Osman, Y. Bejaoui, G. Billedo, E. Abuazab, H. El-Shanti, J. Alami.* Molecular Genetics, Shafallah Medical Genetics Center, Doha, Qatar.

Autosomal recessive diseases are the single largest category of single-gene disorders among Arab population. Our goal is to identify undelineated autosomal recessive disorders among families in the Arab world especially in the gulf region and discovery the genetic causes of these recessive disorders. A Qatari family includes 6 individual from 3 related consanguineous sibships, with mental retardation. The clinical picture comprised significant mental retardation, retinal degeneration, Optic nerve atrophy, and ataxic gait. They suffer also from oedemal puffiness of hands and feet. All parents and the unaffected family members are healthy. Homozygosity mapping revealed a 19.6 MB segment in the long arm of chromosome 4 flanked by rs4345237 (4q12) and rs422140 (4q13.3). This interval contains more than 100 genes, none of which has been implicated in any of the above mentioned phenotype so far. Candidate genes were selected and we are in the process of sequencing them.In addition, whole exome sequencing will be perfomed to expedite the mutation identification.

1102W

The Syndrome of Mental Retardation, Epilepsy and Cerebellar Atrophy Maps to Chromosome 20p11.21-q11.23. *F. BAYRAKLI[1], M. CANPOLAT[2], H. PER[2], S. KUMANDAS[2], E. PINARBASI[3], I. GUNEY[4].* 1) NEUROSURGERY, CUMHURIYET UNIVERSITY SCHOOL OF MEDICINE, SIVAS, Turkey; 2) CHILD NEUROLOGY, ERCIYES UNIVERSITY SCHOOL OF MEDICINE, KAYSERI, Turkey; 3) MEDICAL GENETICS, CUMHURIYET UNIVERSITY SCHOOL OF MEDICINE, SIVAS, Turkey; 4) MEDICAL GENETICS, MARMARA UNIVERSITY SCHOOL OF MEDICINE, ISTANBUL, Turkey.

Aim To locate chromosomal locus which is possibly disease causative gene lie in Methods The family was identified in Eastern Turkey after the index cases presented to medical attention with symptoms of mental retardation, epilepsy and gait disorders. Genotyping and linkage analysis was done using the GeneChip Mapping 250K Nsp I Array (Affymetrix Inc., Santa Clara, CA) containing nearly 250000 SNP markers for genome wide linkage analysis, according to the company's protocols using affected children and their parents. Multipoint linkage analysis was performed using the Allegro software. We assumed an autosomal recessive inheritance pattern and assigned a penetrance of 0.999. Results SNP allele frequencies were estimated from Affymetrix data on the Caucasian population. Using these parameters, a theorical maximum logarithm of the odds (LOD) score of 2.69 was identified at chromosome 20p11.21-q11.23. Interpretation Parametric linkage analysis of consanguineous pedigrees has proven a powerful means for disease gene discovery in Mendelian disorders and an important approach for identifying biological pathways relevant in common, complex phenotypes. Here we demonstrated the power of linkage analysis to identify possible causative gene's chromosomal locus which is novel in a family presenting with epilepsy, mental retardation, and cerebellar atrophy.

1103W

Genetic basis of a new form of hereditary hyperekplexia. *J. Capochichi[1,2], L. Patry[1,2], M.E Samuels[1,2], J.L Michaud[1,2].* 1) Centre de Recherche du CHU Sainte-Justine, Montréal, Québec, Canada; 2) Centre of Excellence in Neuroscience of Université de Montréal (CENUM).

Hyperekplexia (HK; [MIM 149400]) is a rare genetic disorder characterized by neonatal hypertonia and startle reflex induced upon tactic or auditory stimuli. HK is associated with the disruption of glycine signaling, which inhibits synaptic activity in motor systems. In humans, mutations in the genes coding for the presynaptic glycine transporter (GLYT2), the glycine receptor (1 (GLYRA1) and) 1 (GLYRB1) subunits and the receptor clustering proteins Gephyrin (GPHN) and Collybistin (ARHGEF9) are known to cause HK. We observed a new form of HK in four female siblings from a consanguineous family from Cambodian origin. In addition to severe HK, all four girls showed small birth weight, microcephaly with simplified gyral structure, and burst suppression on the electroencephalogram. They could not breathe by themselves and died a few days after birth. Sequencing of known HK genes did not reveal any pathogenic mutation. To study the molecular basis of this new phenotype, we performed whole genome SNP genotyping of all four siblings using the Illumina 610 Quad panel. Using Plink software, we identified 12 regions of homozygosity (RH) composed of at least 25 consecutives SNP that are shared by the 4 siblings. The largest RHs (/ 2 Mb) were: 1) chr11:41 539 163-93 695 503 (~52 Mb); 2) chr22: 45 396 830-49 562 479 (~ 4 Mb); 3) chr18: 1 458 409-4 439 084 (~3 Mb); 4) chr10: 132 462 359-135 372 744 (~3 Mb).None of the known HK genes lie in the candidate RHs reinforcing our conclusion that the cause of this phenotype is due to an as yet unidentified gene. These RHs contain 8 genes (ACP2, ALG8, DHCR7, NDUFS3, NDUFS8, NDUFV1, RNASEH2C and SLC35C1) that have been associated with severe neonatal encephalopathy. Sanger sequencing of these genes did not reveal any pathogenic mutation. We are currently performing whole-exome sequencing in one of the affected siblings using the 50 Mb Agilent SureSelect biotinylated RNA library. We will establish a list of rare variants that are located in the candidate RHs. We will prioritize the validation of variants in genes that may play a role in inhibitory systems such as those mediated by GABA and glycine.

1104W

VCP gene analysis in sporadic ALS patients. *F.L. Conforti[1], W. Sproviero[1,2], I.L. Simone[3], G. Logroscino[3], P. Valentino[2], M.R. Monsurrò[4], V. La Bella[5], C. Rodolico[6], F. Bono[2], A. Patitucci[1], A. Magariello[1], L. Citrigno[1], M. Muglia[1], A. Chiò[7], A. Gambardella[1], R. Mazzei[1].* 1) Institute of Neurological Sciences, CNR Mangone, Cosenza, Italy; 2) Institute of Neurology,University Magna Graecia, Catanzaro, Italy; 3) Department of Neurological and Psychiatric Sciences, University of Bari, Italy; 4) Second Division of Neurology, Second University of Naples, Italy; 5) ALS Clinical Research Center, Bio.Ne.C, University of Palermo, Italy; 6) Department of Neurosciences, Psychiatric and Anaesthesiological Sciences, University of Messina, Italy; 7) ALS Center, Deaprtment of Neurosciences, University of Turin, Italy.

Valosin containing protein (VCP) is a highly conserved member of the AAA+ATPase family. It is highly expressed in all tissues where it has a variety of cellular functions, including protein homeostasis through the ubiquitin proteasome system (UPS) and autophagy pathways. It has been proposed that VCP mutants lead to TDP-43 mislocalization from the nucleus to the cytoplasm of transfected cells and transgenic mice; this results in neurodegeneration within motor neurons in the spinal cord. In accordance with these experimental data, it has been shown that mutations in the TDP-43 gene may cause Amyotrophic Lateral Sclerosis (ALS) in 2% of familial cases (FALS) and 1% of sporadic cases (SALS). The recent finding of mutations in the VCP gene as the cause of familial ALS, prompted us to investigate this gene in our cohort of SALS patients to better define the role of VCP as cause of neurodegeneration. Two hundred fifty-seven unrelated patients from South Italy with adult-onset ALS were diagnosed according to the El Escorial criteria. Following informed consent, DNA was extracted from whole blood using standard procedure. Molecular screening of the exons 2, 3, 5, 6, 10 and 14 of the VCP gene (NM_007126.3) has been performed by DHPLC and direct sequencing of the whole cohort of DNA samples. The DNA of all patients have been previously tested negative for mutations in SOD1, ANG, VAPB, TDP-43 and FUS genes. Genetic analysis of the VCP gene showed four intronic variants in 120 patients. One of these, c.129+47G>A has been found in 55 patients and has been already described (rs10972300; http://browser.1000genomes.org). The other substitutions detected represent three new viariants: c.576+10C>G was found in intron 5 in one patient, c.1194+38T>C found in intron 10 in 14 patients, and c.1082-21INS[TTGTGTACTGT] located in intron 9 and detected in 50 patients. Moreover, two silent mutations have been identified in exons 14, p.Q568Q, in four patients, and p.L574L, in one patient. Carrying any of these variants did not have any impact on ALS phenotype. Despite the biological evidence that VCP could be implicated in ALS, the current study suggests that VCP mutations are not a common cause of adult-onset sporadic ALS. However, it remains to be elucidated the role of the silent VCP variants, since these variations might affect mRNA's shape, its translation and degradation, as well as led to errors in the splicing process.

1105W

Spatacsin gene mutations are a frequent cause of recessive spastic paraplegia but are rare in juvenile recessive forms of amyotrophic lateral sclerosis. *C. Gellera, B. Castellotti, V. Pensato, R. Fancellu, E. Salsano, D. Pareyson, D. Di Bella, C. Mariotti, F. Taroni.* Unit of Genetics of Neurodegeneration, IRCCS - Istituto Neurologico Carlo Besta, Milano, Italy.

Mutations in the spatacsin gene are the major cause of spastic paraplegia with thin corpus callosum (HSP-TCC). The gene has been mapped to chromosome 15q21. It encodes a predicted protein of 2,443 amino acids, and has been associated to the phenotype classified as SPG11 (Stevanin G. et al, 2007). Moreover, a new form of recessive amyotrophic lateral sclerosis (ALS) was mapped to the same locus designated ALS5 (Hentati A. et al, 1998). More recently, mutations in the spatacsin gene have been found in association to recessive forms of ALS linked to 15q21 (Orlacchio A. et al, 2010). We have studied spatacsin gene in two different groups of patients affected by juvenile forms of HSP and ALS. Methods: Direct sequencing of spatacsin gene (40 exons) in i) 48 index cases presenting a sporadic (27 patients) or recessive (21 patients) juvenile form of lower limb spasticity, negative for paraplegin (SPG7) mutations. In this cohort TCC was present in 43% of cases (21 patients) and mental retardation in 35% (17 patients); ii) 45 index cases presenting a sporadic (18 patients) or recessive form (27 patients) of juvenile onset ALS. Results: i) In the HSP cohort we found 12 out of 48 patients positive for spatacsin mutations (25%). Three index cases, from healthy consanguineous parents, were homozygous for three different frame-shift mutations. Nine patients were compound heterozygous either for nonsense, splicing, frame-shift and missense mutations. Overall, we have identified 21 different pathological mutations that are predicted to cause absence of the spatacsin protein. ii) In the ALS cohort we found only one positive patient presenting as a compound heterozygous for two different missense mutations in exon 1. Conclusions: We have identified spatacsin gene mutations in 25% of patients with HSP(±TCC) and in 2% of juvenile ALS. The large majority of mutations are novel and they predicted to cause absence of the spatacsin protein. Our results favor the hypothesis that spatacsin mutations have a large prevalence in patients presenting a phenotype characterized by spastic paraplegia. We should also take in consideration that ALS5 is an intermediate form between ALS and spastic paraplegia, and although classified by some as ALS, it most closely resembles spastic paraplegia with atrophy of the lower extremities without bulbar involvement.

1106W

Identification of ANK3 as a novel gene for autosomal recessive intellectual disability. *Z. Iqbal[1], A. Razzaq[2], M. Shahzad[2], K. Neveling[1], L. Vissers[1], M. Yasir Zahoor[2], L. Tomas Roca[5], C. Gilissen[1], A. T. Vulto-van Silfhout[1], A. Schenck[1], J. A.Veltman[1], A. P.M. de Brouwer[1,3], S. Riazuddin[2,4], H. van Bokhoven[1,3].* 1) Department of Human Genetics, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; 2) National Centre of Excellence in Molecular Biology, University of the Punjab, Lahore 53700, Pakistan; 3) Department of Cognitive Neurosciences, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, Nijmegen, The Netherlands; 4) Allama Iqbal Medical College, Lahore 54550, Pakistan; 5) Department of Human Anatomy and Psychobiology, School of Medicine, University of Murcia, Murcia, Spain.

Identification of the causative genes of autosomal recessive intellectual disability (ARID) has been challenging due to the extreme heterogeneity of the condition. Homozygosity mapping in consanguineous families has been proven to be very helpful in these type of disorders. Here, we have ascertained 60 ARID families from the Pakistani population in which there is a high degree of consanguineous marriages (>70%). Homozygosity mapping data revealed one or more homozygous regions per family ranging in size from 1-51Mb. No pathogenic copy number variations (CNVs) were found. On the basis of homozygosity mapping, we selected seven families with novel ARID loci for targeted next generation sequencing (NGS) of the specific homozygous regions, and six families for exome sequencing. Our NGS data revealed a number of potential mutations that are validated at the moment. In one family, PKMR14, this strategy has proven to be successful as we have identified a homozygous frameshift mutation in the ANK3 gene, affecting the largest brain specific isoform 1. A cohort of ID patients (from different ethnic backgrounds) with shared homozygous intervals encompassing ANK3 gene is being tested to identify additional mutations in this gene. Ankyrin 3 is an adaptor protein that links spectrin cytoskeleton to the integral membrane proteins. It has a crucial role in maintaining the neuronal polarity and molecular organization of the axon initial segments (AIS). Recently, in several independent genome wide association (GWAS) studies, ANK3 has strongly been associated with bipolar disorder, and also with schizophrenia, suggesting an important role of ANK3 in psychiatric and cognitive disorders. This result confirms that homozygosity mapping together with NGS is a powerful method to locate the genetic defect in consanguineous families.

1107W

Massively parallel sequence analysis reveals the causative gene of an autosomal recessive neurodegenerative disorder, posterior column ataxia with retinitis pigmentosa. *H. Ishiura[1], Y. Fukuda[1], J. Mitsui[1], Y. Nakahara[1], B. Ahsan[1], Y. Takahashi[1], Y. Ichikawa[1], J. Goto[1], T. Sakai[2], S. Tsuji[1].* 1) Dept Neurology, Univ Tokyo, Tokyo, Japan; 2) Dept Neurology, Himeno Hospital, Fukuoka, Japan.

[Background] Posterior column ataxia with retinitis pigmentosa (PCARP) is an autosomal recessive neurodegenerative disorder characterized by early-onset retinitis pigmentosa and slowly progressive sensory ataxia. Previous studies showed a linkage to 1q31-q32. However, detailed investigations on the clinical presentations as well as molecular genetics of PCARP have been limited. [Method] DNA samples from two affected siblings, an unaffected sibling, and consanguineous parents were obtained. Parametric linkage analysis was carried out under an autosomal recessive model using SNP arrays, a pipeline software SNP-HiTLink, and Allegro. After removal of repetitive regions, target capture with Sequence Capture 2.1M array (NimbleGen) was performed, followed by massively parallel sequence analysis (Illumina GAIIx). Short reads were aligned to a reference genome (hg18) with BWA and variant calling was performed with SAMtools. [Results] The patients suffered from childhood-onset retinitis pigmentosa and slowly progressive sensory ataxia. They also showed mild mental retardation, which had not been described in patients with PCARP. Linkage analysis revealed a highest LOD score of 1.93 in chromosome 1 and 20, spanning 30.3Mb. Candidate region on chromosome 1 was overlapped with known PCARP locus, refining the minimum candidate region to 7.1Mb. Massively parallel sequence analysis produced 37M reads with an average coverage of 89X. More than 10X coverage was obtained in 99.2% of bases with designed probes. Five novel nonsynonymous SNVs were found, two of which were called heterozygous and not confirmed by direct nucleotide sequence analysis. In one of the error site, the coverage was only 4X. In the other error site, biased strand balance was observed. Of the remaining three SNVs, only a novel homozygous missense mutation (c.1477G>C, p.G493R) in *FLVCR1* (feline leukemia virus subgroup C recepter 1) was located in the minimum candidate region. The mutation was not observed in 192 control chromosomes and G493 is evolutionally well conserved amino acid located in a transmembrane domain. [Conclusions] A recent study has also identified three independent mutations in *FLVCR1* in the original and other families. Our results further confirmed that mutations in *FLVCR1* cause PCARP. Our experience showed that high-throughput sequencing analysis along with SNP-based linkage analysis and target capture efficiently identified the causative gene even with a small family.

1108W

Novel disease mechanisms underlie ATP7A-related distal motor neuropathy. *S. Kaler[1], L. Yi[1], A. Donsante[1], P. Steinbach[2], J. Hicks[1].* 1) Unit on Human Copper Metabolism, Molecular Medicine Program, NICHD, NIH, Bethesda, MD; 2) Center for Molecular Modeling, Center for Information Technology, National Institutes of Health, Bethesda, MD.

ATP7A is a P-type ATPase that regulates cellular copper homeostasis via activity at the trans-Golgi network (TGN) and plasma membrane (PM). Alterations in ATP7A lead to Menkes disease, or its variants occipital horn syndrome and distal motor neuropathy, a newly discovered condition for which the precise pathophysiology is obscure. We characterized two ATP7A-related distal motor neuropathy mutations (T994I, P1386S). Confocal imaging of patient fibroblasts showed diffuse ATP7A signal. Total internal reflection fluorescence microscopy (TIRFM) indicated a shift in steady-state equilibrium of ATP7A-T994I and ATP7A-P1386S with increased plasma membrane (PM) localization. Transfection of 293T cells with the mutant alleles tagged with Venus fluorescent protein showed higher PM localization. Endocytic retrieval of the mutant alleles to the TGN was delayed. Sucrose gradient experiments suggested no abnormal association with PM lipid rafts. Transfection of NSC34 motor neurons indicated PM and axonal membrane localization of ATP7A-T994I and ATP7A-P1386S. In pull-down assays, we identified an abnormal interaction between ATP7A-T994I and VCP97, a TGN-resident protein implicated in the causation of two other inherited motor neuropathies. Co-transfections with a dominant negative VCP97 mutant and siRNA knock-down of VCP each resulted in correction of defective ATP7A-T994I localization. In flow cytometry experiments, we documented that non-permeabilized ATP7A-P1386S fibroblasts bound a C-terminal ATP7A antibody, consistent with intermittent relocation of the 8th transmembrane segment and a di-leucine endocytic signal to the extracellular from the cytoplasmic face of the PM. Based on these findings, we propose two hypotheses: 1) ATP7A-T994I causes disease via abnormal interaction with VCP9 and 2) ATP7A-P1386S induces motor neuron Cu deficiency by constitutive PM localization via partial loss of an endocytic signal. Our findings provide insights about ATP7A structure, function, and trafficking, and highlight the importance of copper homeostasis in the peripheral nervous system.

1109W

Why X? super X-ome sequencing for monogenic neuronal disease classes: intellectual disability and chemosensory impairment. *D. Lancet[1], D. Oz-Levi[1], T. Olender[1], E. Ben-Asher[1], I. Keydar[1], M. Khen[1], E. Ruzzo[2], K. Pelak[2], H. Resnik-Wolf[3], B. Ben-Zeev[3], Y. Anikster[3], D. Goldstein[2], E. Pras[3].* 1) molecular genetics, Weizmann Institute of science, Rehovot, Israel; 2) Molecular genetics and microbiology, Duke university, Durham, NC, USA; 3) The Genetic Institute, Sheba medical center, Ramat-Gan, Israel.

Malfunctioning of the central nervous system may result in diverse phenotypes, from significant intellectual disability (ID) to congenital general anosmia (CGA), a complete inability to perceive smells. We maintain that such broadly-defined disease classes, with equal probability of gene disposition on all chromosomal genome territories, are statistically more likely to be visible in an X-linked disposition. A very large number of underlying genes are known for ID, of which nearly 200 are found on human chromosome X (a large number, as argued). In contrast, precious little is know about the genetic underpinning of CGA. However, we predict that for CGA, with expected highly heterogeneous etiology, X chromosome excess will be observed too. ID still constitutes a major challenge, as it seems that the gamut of causative mutations is far from being exhausted, leaving numerous families without prenatal diagnosis. We have recruited a dozen Israeli families of different ethnic origins, showing both syndromic and non-syndromic ID, as well as other X-linked neuronal disorders. In parallel, we identified 10 families and ~60 sporadic cases of CGA. In at least one case, the identified syndrome includes both ID and CGA. Among syndromic families, ID is also accompanied with neurological anomalies causing a neurodegenerative disease and early death. In a preliminary study, we have identified by standard whole exome-sequencing two causative genes for rare neurodevelopmental autosomal recessive diseases. Based on the above statistical argument, we now focus on generating an enhanced capacity for mutation detection on chromosome X. To this end, we designed a novel X chromosome `super X-ome' capture kit. This includes all coding and non-coding exons, with extended splice junction regions for protein-coded genes. We have also included MiRNAs and other RNA genes, regulatory regions, promoters and enhancers, as well as highly conserved genomic regions. Also included are the entire genomic regions for 93 known XLMR related genes (Chiurazzi et al. EJHG 16:422-34 ,2007). For the relevant analyses, we are developing next-generation-sequencing bioinformatics tools suitable for super X-ome. Deciphering the disease-causing mutations will shed new light on the mechanisms of the ID and CGA disease classes, with implications to other neuronal deficits, and would provide the relevant families with accurate genetic testing and prenatal diagnosis.

1110W

Mechanism of recurrent expansion of a 700kb deletion on chromosome 14q causing dopa-responsive dystonia and ptosis. *K. Lohmann[1], H. Tönnies[2], S.B. Bressman[3,4], I. Nagel[2], A. Rakovic[1], A. Haake[2], J.I.M. Subero[2], K. Wiegers[1], F. Hinrichs[5], Y. Hellenbroich[5], D. Raymond[3], L.J. Ozelius[6], E. Schwinger[5], R. Siebert[2], R. Saunders-Pullman[3,4], C. Klein[1].* 1) Section of Clinical and Molecular Neurogenetics at the Department of Neurology, University Lübeck, Lübeck, Germany; 2) Institute of Human Genetics, Christian-Albrechts-University Kiel and University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany; 3) Department of Neurology, Beth Israel Medical Center, New York, NY, USA; 4) Department of Neurology, Albert Einstein College of Medicine, New York, NY, USA; 5) Institute of Human Genetics, University Lübeck, Lübeck, Germany; 6) Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY, USA.

DNA rearrangements are increasingly recognized as disease causes. We previously identified a patient with dopa-responsive dystonia (DRD) who carried a heterozygous deletion of all six exons of the GTP cyclohydrolase 1 (GCH1) gene on chromosome 14q22. The deletion not only comprised the GCH1 gene but also 23 additional annotated genes in a 3,370kb region. Mutational analyses in his family members revealed the same mutation in the three affected relatives (paternal aunt and two of her sons) tested. All mutation carriers also presented with ptosis, and two had congenital digit abnormalities. Surprisingly, the father of the index patient who was considered an obligate carrier of the deletion did not carry this deletion, despite confirmed paternity. This person and his mother carried a smaller 670kb-deletion downstream of GCH1 as demonstrated by haplotype analysis. Notably, this deletion expanded twice independently in this family to the same deletion of ~3,370kb. All deletions were confirmed by quantitative PCR, by array comparative genomic hybridization (aCGH), and by fluorescence in situ hybridization (FISH). The expansion was mediated by a 13kb-duplication at the centromeric breakpoints of both deletions. This suggests that intrachromosomal nonallelic homologous recombination between low-copy repeats is the underlying cause for the recurrent expansion. Our data demonstrate that (1) co-occurrence of apparently unrelated disorders (in this case DRD and ptosis) may suggest a polygenic deletion, and (2) that the elucidation of the genetic mechanism leading to recurrent expansion of a deletion contributes to a better understanding of the origin of large deletions, an important type of mutation.

1111W

A patient affected by CADASIL carrying a novel mutation on the exon 7 of the NOTCH3 gene. *R. Mazzei[1], C. Ungaro[1], M. Muglia[1], F. Ruscica[2], W. Sproviero[1], A. Patitucci[1], L. Citrigno[1], A. Magariello[1], A. Gambardella[1], F.L. Conforti[1].* 1) Institute of Neurological Sciences CNR, Mangone (CS), Cosenza, Italy; 2) Fondazione Istituto San Raffaele - G. Giglio, Cefalù, Palermo, Italy.

CADASIL is an autosomal dominant disorder leading to cognitive decline and dementia. Mutations in the NOTCH3 gene, encoding a transmembrane protein involved in cellular signalling and cell differentiation, are responsible. These highly stereotyped mutations are located within the 22 exons, encoding for the 34 Epidermal Growth Factor (EGF)-like repeats of the extracellular domain of the Notch3 receptor, all mutations resulting either in a gain or loss of a cysteine residue. Therefore it has been suggested that the unpaired cysteine residues, generated by these mutations, may cause aberrant interaction of the Notch3 receptor with its ligand. In the present study we examined the NOTCH3 gene exons in a patient with clinical and radiological findings consistent with CADASIL. After clinical evaluation the patient underwent MRI investigation, and then she was analyzed for mutations in the NOTCH3 gene using the DHPLC analysis and direct sequence. The DHPLC analysis revealed a variant profile in exon 7. Sequencing of the exon 7 showed a nucleotide change leading an amino acid substitution at position 379 (C379G), resulting in a loss of a cysteine residue in the 9th EGF-like domain. This is the first time that we find a mutation in the exon 7 in more than 700 patients recruited in our Institute with clinical suspect of CADASIL. We believe that C379G is pathogenic due to four main points: 1) It is a typical mutation of CADASIL patients, involving a cysteine residue substitution. 2) No other pathogenic mutation previously reported was seen by direct sequencing of exons 2-23 of NOTCH3 gene in this patient. 3) This mutation was not seen in more than 100 population-matched controls. 4) A mutation involving the same cysteine residue (C379S) was reported as pathogenic.

1112W

iPS cells to model Rett spectrum disorders. *I. Meloni[1], M. Amenduni[1], R. De Filippis[1], A.Y. Cheung[2], F. Ariani[1], F. Mari[1], M.A. Mencarelli[1], Y. Hayek[3], J. Ellis[2,4,5], A. Renieri[1].* 1) Medical Genetics, Department of Biotechnology, University of Siena, Siena, Italy; 2) Developmental & Stem Cell Biology Program, SickKids, Toronto, Ontario, Canada; 3) Child Neuropsychiatry, University hospital of Siena, Siena, Italy; 4) Ontario Human iPS Cell Facility, SickKids, Toronto, Ontario, Canada; 5) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.

Rett syndrome (RTT) is a progressive neurologic disorder that represents one of the most common causes of mental retardation in females. Mutations in 3 genes have been associated with this condition. Classic RTT is caused by mutations in the *MECP2* gene, while variants can be due to mutations in either *MECP2*, *FOXG1* or *CDKL5.* In particular, mutations in *CDKL5* gene have been identified in females with the early onset seizure variant of RTT but also in males with X-linked epileptic encephalopathy. CDKL5 is a kinase protein highly expressed in neurons, but its exact function inside the cell is unknown. To address this issue we employed the recently developed technology of genetic reprogramming that allows to reprogram patients fibroblasts into induced Pluripotent Stem Cells (iPSCs); these cells can be subsequently used to model the disease in vitro by inducing neuronal differentiation. We successfully derived iPSCs from two patients with CDKL5 mutations: one female with p.Q347X and one male with p.T288I mutation. Analysis of CDKL5-mutated clones confirmed that female iPSCs retained X-chromosome inactivation and we could identify clones expressing either the mutant or the wild type CDKL5 allele. These last clones represent the ideal experimental control since they are genotypically identical to those expressing the mutant allele and only differ for CDKL5 expression. The clones could be successfully differentiated into neurons in vitro with variable but reproducible efficiency. Interestingly, clones derived from the same fibroblasts but expressing different alleles (either wild type or mutant) differentiated with comparable efficiency. Results of comparison of neuronal differentiation in wild type and mutant CDKL5 expressing clones will be presented. Future experiments comparing neuronal differentiation in clones derived from patients with mutations in *MECP2*, *CDKL5* and *FOXG1* will allow to define the functional role of the three genes and to check whether they might participate to common pathways, as suggested by the similar phenotypes resulting from their mutations. These cells will also represent an invaluable tool for in vitro testing of new therapeutic compounds.

1113W

A novel *SACS* mutation in a family with atypical phenotype of autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS). *S. Miyatake[1,2], N. Miyake[1], K. Ogata[2], M. Kawai[2], N. Matsumoto[1].* 1) Department of Human Genetics, Yokohama City University, Yokohama, Kanagawa, Japan; 2) Department of Neurology, National Hospital Organization Higashi-Saitama Hospital, Saitama, Japan.

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is an inherited neurodegenerative disorder with the clinical features of early-onset spastic ataxia, nystagmus, axonal and demyelinating neuropathy, pyramidal tract signs, finger and foot deformities, and hypermyelination of retinal nerve fibers. We encountered a patient with spinocerebellar ataxia from a Japanese consanguineous family. He first walked after two years of age. Walking unsteadiness was obvious in his first decade, and was slowly progressive. He became wheelchair-bound at 44 years of age. On neurological examination at 54 years of age, he had cerebellar ataxia, positive Babinski sign, diminished or disappeared deep tendon reflexes except for patellar tendon reflex, muscle atrophy and weakness of lower extremities without spasticity, decreased deep sensation. Interestingly, he showed the autonomic symptoms such as urinary urgency, impotence, and severe constipation. Pes cavus and hammer toe deformity were noticed. He had normal intellectual ability. Hypermyelination of retinal nerve fibers was not observed. There were no abnormal biochemical findings. MRI findings revealed cerebellar atrophy, especially in the upper vermis, linear pontine hypointensities, and cervicothoracic spinal atrophy. Based on their consanguinity suggesting that this disease inherits in autosomal recessive fashion, we performed homozygosity mapping using a proband and three unaffected siblings by GeneChip Human Mapping 10K XbaI (Affymetrix) and Allegro version2.0. We identified 10 informative homozygous regions. Within these regions, *SACS*, which was located on 10.3-Mb homozygous region at chromosome 13 with the maximum LOD score 1.55, was the only known causative gene for spinocerebellar ataxia. Although he lacks a few major symptoms of ARSACS such as spasticity and hypermyelination of retinal nerve fibers, other clinical symptoms were similar to those of ARSACS. By direct sequencing, a novel homozygous missense mutation was identified in the patient. In this pedigree, cosegregation of this homozygous mutation with the disease in the autosomal recessive manner was confirmed. This missense mutation occurred at the evolutionally conserved amino acid. Four of 5 computational prediction algorithms predicted this mutation to be pathogenic. This mutation was not detected in 186 normal Japanese chromosomes. This patient was diagnosed as atypical ARSACS, demonstrating the clinical diversity of ARSACS.

1114W

Aniridia and autism in a Greek male child with a de-novo heterozygous PAX6 (Arg317X) mutation. Is there a linked etiopathogenesis? *A. Psychogios.* Department of Pediatrics, Quillen College of Medicine , Johnson City, TN.

PAX6 gene, a member of the paired box gene family, encodes a transcriptional regulator involved in early human developmental processes, predominantly in the eye, brain and pancreas. PAX6 mutations primarily cause aniridia characterized by iris hypoplasia, foveal hypoplasia and nystagmus presenting in early infancy. Different types of mutations lead to different ocular phenotypes in later life including cataract, glaucoma, keratitis, Peter's anomaly, microphthalmia, and coloboma. Autism is a highly variable brain developmental disorder with a strong genetic basis. There is emerging evidence of the roles of PAX6 mutations in behavioral and neurodevelopmental phenotypes such as autism spectrum disorders and mental retardation. We describe a 3 ½ -year-old Greek male child with almost total bilateral aniridia, foveal hypoplasia, pendular nystagmus, and keratopathy. The maternal family history is positive for bilateral 2-3 syndactyly and grandfather's colon cancer at age 39. There was no history of consanguinity, chromosomal abnormality, inborn error of metabolism, brain, eye, pancreatic, renal, genital, or mental disease. The proband gradually acquired symptoms of autism-spectrum disorder since the age of 2 years with predominant language and developmental delay,and a peculiar food aversion to all but milk. On examination, his weight was 12.6 kg (<2%), his height 86 cm, had broad forehead, bilateral aniridia, horizontal nystagmus, low set ears with stenotic external canals, round nasal tip, anteverted nares, short neck, inverted nipples, 2-3 toe syndactyly, mild hypotonia, ataxia, and tip-toe walking. Blood karyotype and FISH testing were normal. Sequencing of PAX6 gene revealed an exon 11, c.1311C>T (p.Arg317X) mutation. Parental mutation analysis and eye examination were normal. Brain MRI identified a Chiari I malformation. In conclusion, patients with aniridia or other eye-brain abnormalities and autism may share a common etiopathogenesis. Detailed ophthalmologic evaluation for subtle abnormalities, targeted imaging and functional brain studies, family history interpretation, careful genetic clinical examination and testing for PAX6 mutations may be considered in selected cases in the clinical research setting.

1115W

The Genetic Basis of SPG36 - a Novel Disease Mechanism. *R. Schüle[1], N. Schlipf[2], F. Schiele[1], C. Beetz[3], A. Dürr[4, 5], S. Klimpe[6], A. Lossos[7], S. Otto[8], F. Santorelli[9], L. Schöls[1], P. Bauer[2].* 1) Dpt. of Neurodegenerative Disease, Hertie Institute for Clinical Brain Research, Tuebingen, Germany; 2) Dpt. of Medical Genetics, University of Tuebingen, Tuebingen, Germany; 3) Institute for Clinical Chemistry and Laboratory Diagnostics, University Hospital Jena, Jena, Germany; 4) Departmente of Genetics and Cytogenetics, Groupe Hospitalier Pitié-Salpêtrière, Paris, France; 5) INSERM U975, Federation de Neurologie, Paris, France; 6) Department of Neurology, University of Mainz, Mainz, Germany; 7) Department of Neurology, Hadassah University Hospital, Jerusalem, Israel; 8) Department of Neurology, Ruhr-University Bochum, Bochum, Germany; 9) Department of Molecular Medicine, IRCCS Bambino Gesu Hospital, Rome, Italy.

Objective: To identify the gene responsible for autosomal dominant HSP (SPG36) Background: Hereditary Spastic Paraplegias are genetically exceedingly heterogeneous. We have recently mapped a new locus for autosomal dominant HSP (SPG36) in a German family to chromosome 12q23-24. SPG36 is characterized by an adult onset and peripheral nerve involvement. The SPG36 locus comprises 13.09Mb and contains >210 genes. Design/Methods: targeted exon enrichment (NimbleGen Sequence Capture Array), next generation sequencing (454 FLX Genome Sequencer, Roche), high resolution comparative genomic hybridization (CGH custom array, NimbleGen) Results: No pathogenic variations were identified in 29 candidate genes examined by conventional Sanger sequencing. Therefore next generation sequencing was performed after targeted exon enrichment of the whole SPG36 locus. 97.4% coverage of the region yielded 98 variants but no pathogenic variant shared by the two affected family members examined. Screening for genomic deletions by HR-CGH identified a 27kb-deletion that co-segregates with the disease. This deletion partially deletes one gene and the adjacent intergenic region. Loss of the poly-A signal of this gene leads to formation of a fusion mRNA containing parts of two neighboring genes. The fusion transcript was verified by RT-PCR in blood and fibroblasts of affected family members. Conclusions: By applying state of the art genetic techniques we were able to identify a genomic deletion to be the cause of autosomal dominant HSP SPG36. A fusion mRNA is formed, containing parts of two adjacent genes and presumably leading to a combined hyploinsufficiency/overexpression phenotype. Pathophysiological characterization of this unique disease mechanism is underway. Study supported by: Deutsche Forschungsgemeinschaft (grant SCHO 754/4-1), E-Rare program of the European Union (grant to EUROSPA 01GM0807).

1116W

Targeted Resequencing for Gene Discovery in Joubert Syndrome. *P. Taylor[1], H. Lee[1], SF. Nelson[1,2].* 1) Department of Human Genetics, University of California, Los Angeles, California, USA; 2) Department of Pathology and Laboratory Medicine, and Psychiatry, David Geffen School of Medicine, University of California, Los Angeles, California, USA.

Background: Next generation sequencing (NGS) builds upon the successes of linkage for disease gene discovery and is particularly powerful for disorders where large pedigrees are not available. In fact, NGS approaches make it possible to identify variants that underlie rare diseases by sequencing just a few cases, and in some instances single individuals suffice. This approach is therefore very promising for the analysis of Mendelian disorders and has already proven to be successful in a number of studies. Methods: We applied this approach to discover novel genes in patients affected with Joubert Syndrome (JBST), a rare brain malformation disorder belonging to the growing class of disorders known as ciliopathies. Although JBST is inherited in an autosomal recessive fashion, it shows marked genetic and phenotypic heterogeneity as several genes have been identified in recent years. There are more than 10 genes that are mutated in patients with JBST, however these mutations account for only about 50% of all known cases. The genes for other forms of the disease have not yet been found. All known JBST disease genes affect the structure and/or function of primary cilia and it is likely that other primary cilia genes are causal for JBST but have yet to be discovered. To maximize the success of gene discovery, we created a custom capture array designed to pull down key cilia genes. The array targets the protein-coding sequence of 169 candidate genes amounting to 1Mb of target. We made libraries for 14 individuals affected with JBST, barcoded each using in-house barcodes, hybridized the pool of libraries to the custom capture array and finally sequenced the pool on a single lane of Illumina HiSeq. Results: We generated 1.5Gb of paired-end sequence data with a mean coverage of 90X per sample. The sequencing data was aligned using Novoalign and variants were called using the Genome Analysis Toolkit. Variants were filtered against common variants found in dbSNP and 1000 genomes. Because JBST is a recessive disorder, we focused on variants causing changes in the final protein product. Conclusions: Targeted sequencing of these 14 individuals has provided a great opportunity for gene discovery by scanning functionally relevant candidate genes to identify novel causal genes in JBST. This approach could also be applied to efficient rare variant detection on more commonly diagnosed disorders such as ADHD and autism.

1117W

A Novel STXBP1 Mutation in Genetic Focal Seizure with Neonatal Onset. *M. Vatta[1], M.B. Tennison[2], A.S. Aylsworth[3], C.M. Turcott[3], M.P. Guerra[4], C. Eng[1], Y. Yang[1].* 1) Dept Molecular Human Genetics, Baylor Col Medicine, Houston, TX; 2) Department of Pediatric Neurology,University of North Carolina, Chapel Hill, NC; 3) Department of Pediatric Genetics/Metabolism, University of North Carolina, Chapel Hill, NC, USA; 4) Department of Pediatrics/Child and Adolescent Neurology, University of Texas-Houston Medical School, Houston, TX.

Mutations in the STXBP1 gene have been mainly associated with early infantile epileptic encephalopathy (EIEE), also known as Ohtahara syndrome, which is characterized by suppression-burst pattern on surface electroencephalogram (EEG). In addition to EIEE, mutations in STXBP1 have been identified in subjects presenting with West syndrome, which demonstrates hypsarrhythmia pattern on EEG, and early-onset epileptic encephalopathy (EOEE), a severe form of epilepsy with EEG features diverging from a specific epilepsy syndrome, although mostly evolving to West syndrome within the first five months of age. More recently, the spectrum of phenotypes associated with STXBP1 gene mutations has included non-syndromic intellectual disability (NSID) with or without nonsyndromic epilepsy. So far, 15/24 (62.5%) reported STXBP1 mutations have been associated with EIEE, 2/24 (8.33%) with West syndrome, 4/24 (16.7%) with EOEE, 2/24 (8.3%) with NSID + Epilepsy and 1/14 (4.16%) with isolated NSID. The STXBP1 mutations reported thus far include gross deletions, nonsense mutations, splice site mutations, small deletions and insertions and missense mutations, indicating that haploinsufficiency is the disease-causing mechanism for STXBP1. Here we present a de novo, novel single-nucleotide duplication mutation, c.931dupT (p.S311fsX3), in exon 11 of STXBP1 in a four month-old Caucasian infant who developed focal seizures at two weeks of life. The EEG demonstrated normal background frequency content with multifocal sharp waves and no evidence of suppression-burst pattern or hypsarrhytmia. Therapy with Levetiracetam and Oxcarbazepine effectively managed the seizure episodes. Previously normal testing includes brain magnetic resonance imaging (MRI) and Computerized Axial Tomography (CT) scan and SCN1A, SCN1B and ARX gene testing. In conclusion, we have identified a novel mutation in STXBP1 in a subject with neonatal-onset genetic focal seizures, which broadens the phenotypic spectrum of STXBP1 mutations. STXBP1 gene sequence testing and copy number analysis may be considered for patients with EIEE, EOEE, other neonatal or infantile-onset epilepsies, as well as NSID with or without epilepsy.

1118W

A Nijmegen breakage syndrome family with four affected members and three carriers having malignancies. *F. Ozkinay[1], E. Karaca[2], H. Onay[2], N. Karaca[1], G. Aksu[1], B. Erturk[2], N. Kutukculer[1].* 1) Dept Pediatrics, Ege Univ, Izmir, Turkey; 2) Department of Medical Genetics, Ege Univ, Izmir, Turkey.

Nijmegen breakage syndrome (NBS) is a rare autosomal recessive chromosomal instability disorder characterized by progressive microcephaly, “bird-like” facial appearance, short stature, mental retardation, severe combined immunodeficiency, recurrent infections, radiosensitivity and a predisposition to malignancy. Here we present the clinical and pedigree analysis of a large family with Nijmegen breakage syndrome. The proband was a 13 year-old male with typical dysmorphological features of NBS and combined immunodeficiency. His parents were first cousins. They had two previous children having the same clinical findings, both of whom had died from leukemia at an early age. The paternal grandmother and grandfather were also consanguineous and had a child with similar features who died of leukemia at the age of 7.5. Detailed pedigree analysis revealed three different cancers (uterus, breast and thyroid cancers) in three phenotypically normal family members, two surviving, one deceased (uterus cancer). Molecular analysis showed a homozygous 657del5 mutation in the proband's NBN gene. The surviving two family members with cancer were heterozygous for the same mutation. This family successfully demonstrates that homozygous NBN 657del5 mutation causes classical NBS phenotype whereas heterozygous NBN 657del5 mutation appears to be associated with an increased risk of cancer in phenotypically normal carriers.

1119W

Study of undelineated autosomal recessive disorder among Arabs. *J. Alami, Y. Al-Saraj, Y. Bejaoui, M. Osman, E. Abuazab, M. El-Dow, H. El-Shanti.* Shafallah Medical Genetics Center, Doha, Qatar.

The aim of this study is to map loci and identify genes that play a role in autosomal recessive disorders among Arab families and to examine their role with the final aim of outlining novel genes and pathways. The investigation capitalizes on utilizing large inbred families, as well as smaller inbred families, by employing homozygosity approaches for mapping the etiologic genes. It includes the recruitment of families and obtaining detailed clinical, genealogical and genotypic data. This is followed by examining these families for linkage and association Families with pedigrees that provided convincing evidence of autosomal recessive mode of inheritance and with consanguinity were selected. We performed homozygosity mapping on one recruited family to seek a region of homozygosity shared by affected individuals. This family includes eight individuals from 4 related sibships in an extended Palestinian family who suffer from isolated congenital cataract. Homozygosity mapping revealed a region flanked by rs4276160 (3p22.1) and rs749512 (3p21.31) on on the short arm of chromosme 3. This interval contains 92 genes, none of which has been implicated in eye disease. Further investigation for this family is currently underway using whole exome sequencing to identify the causative gene mutation.

1120W

Familial Alzheimer's disease with amyloid precursor protein D678N mutation: a case report. *V. Andreoli[1], F. Trecroci[1], A. La Russa[1], M. Liguori[1], P. Spadafora[1], G. Di Palma[1], A. Gambardella[1-2], R. Cittadella[1].* 1) Institute of Neurological Sciences-National Research Council, Pianolago di Mangone, Cosenza, Italy;; 2) Institute of Neurology-Campus di Germaneto, University “Magna Graecia”, Catanzaro, Italy.

Alzheimer's disease (AD) is the most common cause of dementia and may be either familial or sporadic. Genetically, AD is heterogeneous, as it displays no single or simple mode of inheritance. Notably, the clinical presentation of familial forms of AD (FAD) is more complex, and mutations of the Presenilin 1 (PSEN1) gene on chromosome 14, Presenilin 2 (PSEN2) on chromosome 1 and Amyloid-beta precursor protein (APP) on chromosome 21 have also been described in these patients. Overall, mutations in these genes result in an increased amyloidogenic processing of APP: this seems to be the key pathological mechanism of FAD. Nowadays, 24 different missense mutations located in exons 16 and 17 of APP have been reported. Here, we describe a subject bearing the APP D678N mutation who developed a peculiar early-onset (EO) FAD phenotype. The patient was enrolled at the Institute of Neurology, University “Magna Graecia” in Catanzaro, and informed consent was obtained for genetic study at the Institute of Neurological Sciences, National Research Council, Cosenza, Italy. The exonic regions 4-13 of PSEN1, 3-12 of PSEN2 and 16-17 of APP genes were PCR amplified by specific primers, and a mutational screening was done by DHPLC and direct sequencing. The patient is a 71-year-old man who developed, since the age of 58 years, progressive memory loss and visuospatial problems. At follow-up, he became disoriented in time and space, apathic, with reduction in verbal fluency, attention and planning. At the age of 66 years, the result of MMSE was 17. In five affected individuals of the same family, the age at onset of cognitive disorders started from 50 to 65 years, while the age at death ranged between 62 and 80 years. Genetic analysis of the proband's PSEN1 and PSEN2 genes disclosed no abnormalities, whereas analysis of the APP gene showed a GAC to AAC nucleotide substitution in codon 678 EX 16 of this gene, causing an amino acid substitution of aspartic acid to asparagine in heterozygosis. This pathogenic D678N mutation of APP gene was already reported in a Japanese family, in which mean age at onset clustered around the age of 60 years. Our data confirm that APP is actually an EO-AD gene, in which onset occurs primarily prior to 65 years of age and often much earlier; and for a given mutation, ages at onset seem to be tightly clustered.

1121W

Mutations in *RAB40AL* cause Martin-Probst syndrome, an X-linked disorder characterized by sensorineural hearing loss, cognitive impairment, short stature, and craniofacial dysmorphisms. *J.K. Bedoyan[1], V. Schaibley[2], W. Peng[2], Y. Bai[3], K. Mondal[4], A.C. Shetty[4], M. Durham[1], A. Dhiraaj[1], J.M. Skidmore[1], J.B. Kaplan[1], C. Skinner[5], R.E. Stevenson[5], C.E. Schwartz[5], A. Antonellis[2], M.E. Zwick[4], J.D. Cavalcoli[3], J. Li[2,3], D.M. Martin[1,2].* 1) Department of Pediatrics, University of Michigan, Ann Arbor, MI; 2) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 3) Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI; 4) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA; 5) Greenwood Genetic Center, Greenwood, SC.

Martin-Probst syndrome (MPS; MIM 300519) is a rare inherited disorder characterized by sensorineural hearing loss, cognitive impairment (CI), short stature (SS), and distinct craniofacial dysmorphisms (CDs). Interestingly, MPS has some characteristics similar to Costello and Noonan syndromes, including SS, CI, and specific CDs. Previously, microsatellite marker analysis identified a common haplotype region on the X chromosome spanning 68 Mb and containing 683 genes. In this study, SNP array comparative genomic hybridization excluded deletions or duplications in the common region with an average resolution of 1.5 kb. Whole-genome and exome sequencing in two affected males from the same family with MPS yielded 100% sequence coverage for 96.4% of exons and identified a D59G variant in the *RAB40AL* gene. The remaining genes expressed in the fetal nervous system were analyzed by direct sequencing and no additional potentially damaging or pathogenic variants were identified. D59G lies within a highly conserved region of RAB40AL and segregates with the phenotype in the family. We identified, in an unrelated male with clinical features identical to those reported in MPS, two variants (G47A and E113D) in *RAB40AL*. All three *RAB40AL* variants were absent by direct sequencing of 363 control males obtained from the Greenwood Genetic Center and Coriell, and in 206 exomes. *RAB40AL* has been implicated previously in severe CI in a male with an inversion disrupting the *RAB40AL* promoter region. We found that *RAB40AL* is expressed in MPS relevant fetal and adult human tissues, including brain. *RAB40AL* (previously *RLGP*) encodes a distinct Ras-like GTPase protein with one SOCS box. SOCS box-containing proteins act as part of a multiprotein complex and interact with Cullin-RING E3 ubiquitin ligase complexes which polyubiquitinate target proteins for subsequent degradation. HeLa cells expressing RAB40AL-, RAB40AL D59G-, or RAB40AL G47A/E113D-GFP fusions have been constructed to examine the effects of these variants on cell viability, protein localization, and downstream ERK signaling in the Ras/MAPK pathway. We hypothesize that the SOCS box in RAB40AL promotes modulation of extracellular stimuli in signaling pathway(s) by selectively degrading target substrates. Identification of *RAB40AL* as the gene mutated in MPS allows for detailed investigations into the molecular mechanism(s) for this disorder, and may improve diagnosis and treatment of individuals with related phenotypes.

1122W

Exome sequencing reveals new gene for autosomal recessive congenital ichthyosis in a consanguineous German family. *K.M. Eckl[1], A. Önal-Akan[1], R. Casper[1], H. Thiele[2], I. Hausser[3], M.L. Preil[4], J. Altmüller[2], G. Nürnberg[2], F. Stock[5], P. Nürnberg[2,7,8], H. Traupe[6], H.C. Hennies[1,7,8].* 1) Div of Dermatogenetics, Cologne Center for Genomics, Univ Cologne, Cologne, Germany; 2) Cologne Center for Genomics, University of Cologne, Cologne, Germany; 3) Dept. of Dermatology, University Hospital of Heidelberg, Heidelberg, Germany; 4) Practice for Dermatology Dres. Krnjaic, Merk und Schäfer, Ansbach, Germany; 5) Inst. of Human Genetics, University Hospital of Leipzig, Leipzig, Germany; 6) Dept. of Dermatology, University Hospital of Münster, Münster, Germany; 7) Center for Molecular Medicine, University of Cologne, Cologne, Germany; 8) Cluster of Excellence on Cellular Stress Responses in Aging-associated Diseases, University of Cologne, Cologne, Germany.

Autosomal recessive congenital ichthyosis (ARCI) is a rare and severe genodermatosis characterized by extensive scaling of the entire skin, variable erythema, and a collodion encasement at birth. ARCI shows clinical and genetic heterogeneity, as mutations in six genes, involved in different pathways, have been known so far. A patient was presented to our outpatient special clinics showing all signs for ARCI. She was born as collodion baby with ectropium and eclabium. In infant life the patient showed a whitish, mild scaling on the entire body but rather sparsely on face, hands and feet. Hair, nails and teeth were normal. The ability to sweat was reduced; allergies and secondary skin infections have not been reported so far. Ultrastructural analysis of skin biopsy specimens showed regular epidermis, mild acanthosis and orthohyperkeratosis with only a slight thickening of the stratum corneum. Mutation analyses in the six known ARCI genes were negative. Family history revealed an extended pedigree of German descent, with a total of six affected members including the great-grandfather of the index patient, and multiple consanguinity. Subsequent linkage analysis and homozygosity mapping using chip-based SNP analysis resulted in a LOD score of 4.5 and a candidate interval of 3.4 Mb in length. By whole-exome sequencing using samples from two patients we identified one single homozygous mutation in the candidate region with a >100-fold coverage. The missense mutation completely co-segregates in the pedigree. It affected a highly conserved residue of predicted structural importance, represented by maximum probability of pathogenicity as determined by various algorithms such as ConSeq, PMut or Polyphen. We have modelled the disease in vitro using 3D full-skin equivalents showing lack of the protein in the epidermis. Disease models demonstrated mild hyperkeratosis and a slightly reduced epidermal barrier function compared to wild-type using Lucifer Yellow penetration assays. This is the first identification of a new gene for a severe genetic skin disorder using the combined methods of whole-genome linkage analysis and exome sequencing with only one, consanguineous family demonstrating again the feasibility and stringency of the approach.

1123W

The *MYH9* and *APOL1* genes are independently associated with Sickle Cell Disease nephropathy. *M.E. Garrett[1], E.C. Okocha[1,2], K. Soldano[1], L.M. De Castro[3], J.C. Jonassaint[3], E.P. Orringer[4], J.R. Eckman[5], M.J. Telen[3], A.E. Ashley-Koch[1].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Institute for Genome Sciences and Policy, Duke University, Durham, NC; 3) Division of Hematology, Department of Medicine, Duke University Medical Center, Durham, NC; 4) Department of Medicine, University of North Carolina, Chapel Hill, NC; 5) Department of Hematology and Medical Oncology, Emory University Medical Center, Atlanta, GA.

Background: Renal failure occurs in 5-18% of sickle cell disease (SCD) patients and is associated with early mortality. At risk SCD patients cannot be identified prior to the appearance of proteinuria and the pathobiology is not well understood. The *MYH9* and *APOL1* genes have been associated with risk for focal segmental glomerulosclerosis and end-stage renal disease in African Americans without SCD and are excellent candidate genes for SCD nephropathy. **Methods:** We genotyped 26 SNPs in *MYH9* and 2 SNPs in *APOL1* (G1 and G2 from Genovese et al, 2010) in 521 unrelated adult (18-83 years) SCD patients screened for proteinuria. To assess population substructure, we used 392 Ancestry Informative Markers (AIMs) and excluded individuals who were outliers. Using logistic regression, SNPs were evaluated for association with proteinuria, adjusting for important covariates such as age and hypertension. **Results:** Thirty-six percent of our patients had proteinuria and those individuals were on average 6 years older than the patients without proteinuria ($p<0.0001$). Seven SNPs in *MYH9* and one in *APOL1* remained significantly associated with proteinuria after multiple testing correction ($p < 0.0025$). An *MYH9* risk haplotype ($p=0.001$) and the *APOL1* G1/G2 recessive model ($p<0.0001$) were significantly associated with proteinuria, both remaining significant when including them simultaneously in the model. Glomerular filtration rate was negatively correlated with proteinuria ($p < 0.0001$), and nominally associated with *MYH9* and *APOL1* in age-adjusted analyses. **Conclusion:** Our data provide insight into the pathobiology of renal dysfunction in SCD, suggesting that *MYH9* and *APOL1* are both independently associated with risk. *MYH9* is a stronger functional candidate, encoding the heavy chain of non-muscle myosin IIA and in combination with actin, talin, (-actin-4, and vinculin, makes up the cytoskeleton of the podocyte - the cells lining the visceral surface of Bowman's capsule in the kidney- foot process (Drenckhahn and Franke 1988). *APOL1* is associated resistance to *Trypanosoma brucei rhodesiense*, a common African parasite, and this role in host immunity likely contributed to selective pressures on that gene and the surrounding region which includes *MYH9*. Further, *APOL1* has been implicated more strongly in renal disease among non-SCD patients. Thus, functional work will be needed to disentangle this complex genomic region implicated now in multiple types of nephropathies.

1124W

Molecular Genetic Characterization of SMAD Signaling Molecules in Pulmonary Arterial Hypertension. *R.D. Machado[1], M.T. Nasim[1], T. Ogo[1], M. Ahmed[1], R. Randall[1], H.M. Chowdury[1], K.M. Snape[1], T.Y Bradshaw[1], L. Southgate[1], G.J. Lee[1], I. Jackson[2], G.M. Lord[2], J.S.R. Gibbs[3], M.R. Wilkins[4], K. Ohta-Ogo[5], K. Nakamura[5], B. Girerd[6], F. Coulet[7], F. Soubrier[7], M. Humbert[6], N.W. Morrell[8], R.C. Trembath[1].* 1) Medical and Molecular Genetics, King's College London, London, United Kingdom; 2) MRC Centre for Transplantation, King's College London, School of Medicine, Guy's Hospital, London, UK; 3) National Heart & Lung Institute, Imperial College London, UK; 4) Division of Experimental Medicine, Imperial College of Medicine, London, UK; 5) Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan; 6) Univ Paris-Sud, INSERM U999, Service de Pneumologie, Hôpital Antoine Béclère, Assistance Publique-Hôpitaux de Paris, Clamart, Paris, France; 7) Laboratoire d'Oncogénétique et d'Angiogénétique Moléculaire, Departement de Génétique, Groupe Hospitalier Pitié-Salpêtrière, Paris, France; 8) Department of Medicine, University of Cambridge, Cambridge, UK.

Pulmonary arterial hypertension (PAH) is a progressive vascular disorder characterized by the occlusion of pulmonary arterioles as a result of the uncontrolled proliferation of endothelial and smooth muscle cells. It is a devastating disease that, in the absence of treatment, is fatal due to right heart failure. PAH may occur in a familial setting (FPAH) but, more commonly, is idiopathic (IPAH) or associated with other disorders including congenital heart disease, thromboembolic disease and HIV infection (APAH). Heterozygous germ-line mutations of BMPR2, encoding a type II receptor of the TGF-) signaling pathway, represent the principal genetic predisposition to the familial form of PAH. By contrast, BMPR2 mutations are observed at a significantly lower frequency in idiopathic and associated forms of PAH. BMPR-II triggers signal transduction through the receptor SMADs (R-SMADs) 1, 5 and 8, upon ligand binding and complex formation with a type I receptor, namely BMPR-1A, -1B or ALK1. R-SMADs translocate to the nucleus in complex with co-SMAD4 to regulate transcription of target genes. To further explore the molecular genetic basis of PAH we adopted a candidate gene approach to identify deleterious variation predisposing to the disorder. Specifically, members of the canonical BMP pathway, namely SMADs 1, 4, 5 and 8, were analyzed for mutations capable of disrupting BMPR-II signaling. In the largest study of its kind, four variants were identified in SMADs 1, 4 and 8 amongst a cohort of 324 PAH cases and excluded in over 900 matched controls, 60 fully exome sequenced samples and publicly available variation databases. Of the three amino acid substitutions detected, two demonstrated reduced signaling activity when tested by reporter assays. A putative splice site mutation proximal to the terminal exon of SMAD4 resulted in moderate transcript loss in patient cells due to defects in splicing efficiency. These results emphasize the central role of BMPR2 mutation in PAH pathogenesis and, importantly, indicate that potentially deleterious variation within the SMAD family is likely to be an infrequent cause of disease.

1125W

Examination of Fuchs endothelial corneal dystrophy (FECD) in African-Americans. *M.A. Minear[1], J. Rimmler[1], S. Watson[1], E. Balajonda[2], Y.-J. Li[1], G.K. Klintworth[2], N.A. Afshari[2], S.G. Gregory[1].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Department of Ophthalmology, Duke University Medical Center, Durham, NC.

Fuchs endothelial corneal dystrophy (FECD) is a late-onset disorder of the corneal endothelium that is a leading indication for corneal transplantation. Much progress has been made in recent years to identify the genetic architecture of FECD, with both linkage and association studies identifying mutations in the *COL8A2*, *SLC4A11*, and *TCF8* (*ZEB1*) genes in FECD patients. The literature on FECD genetics is very biased in that only Caucasian patients have been included in linkage and association studies. Even though a handful of studies have made an effort to include other racial and ethnic groups by screening selected Asian FECD patients for mutations in *COL8A2* and *SLC4A11*, nobody has analyzed African-American patients with FECD. The only published report of an African-American FECD patient was in 1924, and yet questions remain as to whether African-Americans are even susceptible to FECD. The purpose of our study was to perform a comprehensive examination of the clinical, histological, and genetic features of African-American FECD patients we have recruited at the Duke University Eye Center. We have enrolled 70 African-Americans, comprising 32 singleton cases and nine multiplex families. The affected subjects have been examined both clinically and histopathologically (when tissue specimens were available following a corneal transplant) to verify a FECD diagnosis, including the presence of central corneal guttae. In 38 of our 41 singletons/probands, we are sequencing *COL8A2*, *SLC4A11*, and *TCF8* to determine whether or not mutations in these genes are present in African-American FECD cases. Preliminary sequencing in *TCF8* has detected two novel missense mutations in exon 7, p.L708Q and p.P559S, as well as novel intronic mutations and known polymorphisms in dbSNP. Additional sequencing will be needed to confirm these mutations and to confirm their absence in control samples. Our study is the first comprehensive clinical report of FECD in African-Americans and is also the first to examine the genetic basis of FECD in this population.

1126W

A Novel Mutation in *PRPF31* Linked with Autosomal Dominant Retinitis Pigmentosa in a large Indian Family. *S. Saini[1], V. Vanita[1], D. Singh[2], J.R. Singh[3], P.N. Robinson[4].* 1) Department of Human Genetics, Guru Nanak Dev University, Amritsar, India; 2) Dr. Daljit Singh Eye Hospital, Sheranwala Gate, Amritsar, India; 3) Central University of Punjab, Bathinda, India; 4) Institute of Medical & Human Genetics, Charité Campus Virchow-Klinikum, Berlin, Germany.

Purpose: To localize and identify the disease linked gene for non-syndromic autosomal dominant retinitis pigmentosa (ADRP) in a large Indian family. Methods: Genome-wide linkage analysis using highly polymorphic fluorescently labeled microsatellite markers was carried out in an ADRP family with 14 living members in 3-generations affected with non-syndromic retinitis pigmentosa. Genotyping was carried out on a DNA sequencer ABI-377, data collected and analyzed by GENESCAN version 3.1.2, and genotyping was done using GENOTYPER 2.5.1 software. 2-point and multipoint linkage analysis were performed by means of the program packages linkage v.5.2, Genehunter v.2.1 and simwalk2 v.2.82. Mutation screening in the potential candidate gene at the mapped interval was carried out by bi-directional sequence analysis of the amplified products. Results: In a genome-wide search, significant positive 2-point lod scores indicative of linkage were obtained with markers D19S921, D19S572, and D19S927 at 19q13.4. Two-point linkage data with these markers was further supported by multipoint lod scores and haplotype analysis. Mutation screening in the candidate gene pre-mRNA processing factor 31 homolog (S. cerevisiae) (*PRPF31*) at the mapped interval resulted in identification of a novel 7 bp deletion i.e. c.59-65del7 (p.Gly20AlafsX43) which leads to premature stop codon in the next exon 43 amino acids downstream. Conclusion: The present study maps the locus for non-syndromic autosomal dominant retinitis pigmentosa in a large Indian family at 19q13.4. The identified novel deletion in *PRPF31* was identified in all the tested 14 affected members and in one asymptomatic individual, consistent with a high, but not complete penetrance of c.59-65del7 (p.Gly20AlafsX43). This deletion was not observed in any other tested unaffected family members or in 100 ethically matched control (200 chromosomes) subjects. The present findings further substantiate the role of *PRPF31* that encodes a component of the spliceosome complex in relation to ADRP.

1127W

Familial amyloid polyneuropathy (ATTRV30M): what can we learn from the study of families of this AD disorder in which the proband had no affected parent at the time of diagnosis? *A. Sousa[1, 2], C. Lemos[1, 2], A. Martins-da Silva[3], R. Magalhães[1], J. Neto[2], I. Alonso[1,2], J. Pinto Basto[1,2], J. Sequeiros[1,2], T. Coelho[3].* 1) ICBAS, Instituto Ciências Biomédicas Abel Salazar, Univ. Porto, Portugal; 2) UnIGENe, IBMC, Porto, Portugal; 3) Unidade Clínica de Paramiloidose, Centro Hospitalar do Porto (CHP), Porto, Portugal.

Familial amyloid polyneuropathy (FAP-ATTRV30M) is an autosomal dominant (AD) systemic amyloidosis manifesting as severe peripheral neuropathy, due to a point mutation in the transthyretin (TTR) gene (chr18q12.1). It was first described by Andrade in Northern Portugal (1952) as a disease occurring between 25-35 yrs. This AD disorder was later shown to present remarkable differences in mean age-at-onset between clusters (Portugal: 35,1; Sweden: 56,7 and Baleares Islands: 45,7). Emphasis is often put in differences between clusters, but a large variability can also be found within each one. Portuguese focus has been characterized by its early onset (o.<40yrs) and assumed to reach full penetrance. However, reality is not that simple: more and more late-onset (o⁄ 50yrs) cases have been ascertained, often descending from old asymptomatic carriers. Moreover, when analyzing the families ascertained over the past 70 yrs, around 40% of probands had no affected parent at the time of the diagnosis (60% of families recently ascertained). We have a unique opportunity to perform a longitudinal study: in CHP (Porto), 2440 patients belonging to 566 different families were diagnosed 1939-2010. Mean age-at-onset is 35,1 yrs (SD 10,7), women having a later onset - 37,4 (SD 10,3) - than men - 33,1 (SD 10,7). This study concentrates on 216 families whose proband, at the time of his/her diagnosis, ignored the existence of the disease in previous generations. The most intriguing aspect is that in those probands, mean age-at-onset was 46,2 (SD 12,5), vs. 33,5 (SD 8,8) in probands of families where the disease was already known (P<0,001). Going further in the analysis of those 216 families, no gender differences were found between male - 45,9 (SD 13.0) and female probands - 46,7 (SD 11,6). However, when considering the 415 non-proband patients belonging to these families, men had a lower onset - 36,7 (SD 12,9) than women - 41,8 (12,5), P<0,01). In these families, when we put the emphasis on whether the patient had one affected parent at the time of diagnosis (which excludes the probands and some others) gender differences are present again, whereas they are not in the group of patients (probands and some others) with no affected parent. Thus, our conclusion is that gender differences in age-at-onset are mostly due to the affection status of the transmitting parent. This reinforces the hypothesis of modifiers close to or within the TTR gene, on which we are currently working.

1128W

Identification of an interaction between LIX1, a feline SMA candidate gene, and DACHSOUS1. *E.N. Wakeling[1], J.C. Fyfe[2,3].* 1) Genetics Program, Michigan State University, East Lansing, MI; 2) Department of Microbiology & Molecular Genetics, Michigan State University, East Lansing, MI; 3) College of Veterinary Medicine, Michigan State University, East Lansing, MI.

The spinal muscular atrophies (SMA) are a group of inherited disorders distinguished by proximal muscle weakness and atrophy due to spinal cord lower motor neuron degeneration. Autosomal recessive SMA is the leading genetic cause of infant mortality and is divided into four subtypes based on severity, age of onset and survival time. In humans, ~97% of autosomal recessive SMA is due to mutation of the survival of motor neuron (SMN) gene. Despite intensive research, the molecular mechanism by which SMN depletion results in motor neuron death remains poorly defined. Feline SMA is an autosomal recessive, juvenile onset lower motor neuron disease that resembles Type 3 human SMA. Molecular analysis identified a 140 kb deletion on feline chromosome A1q that abrogated expression of limb expression 1 (LIX1), a gene of unknown function that is highly expressed in spinal cord. We conducted a yeast two-hybrid screen of a human fetal brain cDNA library to identify putative LIX1 interacting partners and thus assign a function for LIX1. This screen of 2 x 10^7 clones identified 12 unique preys, some of which were identified multiple times. Confirmation of the protein interactions was accomplished by in vitro co-immunoprecipitation and band shift assays. The cytoplasmic domain of DACHSOUS1 (DCHS1) was identified 20 times and was the only prey that interacted with LIX1 in vitro. DCHS1 is a transmembrane protein that contributes to the planar cell polarity of the wings, legs, and eyes of Drosophila. In transfected Cos7 cells, GFP-tagged Lix1 and Lix1alt, a murine specific transcript with an alternative promoter, both localized throughout the cytoplasm and nucleus. When co-expressed with DsRed-tagged Dchs1, GFP-Lix1 was entirely cytoplasmic and co-localized strongly with DsRed-Dchs1. GFP-Lix1alt also co-localized with Dchs1-DsRed, however, GFP-Lix1alt was not excluded from the nucleus. In NSC34 cells, a motor neuron-like cell line, GFP-Lix1 localized throughout the cell body, neurites and growth cones and co-localized with DsRed-Dchs1. GFP-Lix1alt also co-localized with DsRed-Dchs1 in the cell body, neurites, and growth cones. As was observed in Cos7 cells, co-expression with DsRed-Dchs1 was not sufficient to exclude GFP-Lix1alt from the nucleus. These in vitro experiments and in vivo co-localization support an interaction between LIX1 and DCHS1. As a neuronal function for DCHS1 has yet to be identified, the importance of this interaction in motor neurons remains unclear.

1129W

NPHP4 Variants are Associated with Congenital Heart Malformations and Heterotaxy. *V. French[1], I. van de Laar[1], M. Wessels[1], C. Rohe[1], J. Roos-Hesselink[2], I. Frohn-Mulder[3], L-A. Severijnen[1], B. de Graaf[1], G. Wang[4], R. Schot[1], G. Breedveld[1], E. Mientjes[1], M. van Tienhoven[1], E. Jadot[5], Z. Jiang[6], A. Verkerk[7], S. Swagemakers[7], H. Venselaar[8], Z. Rahimi[9], H. Najmabadi[10], H. Meijers-Heijboer[11], E. de Graaff[1], W. Helbing[3], R. Willemsen[1], K. Devriendt[12], J. Belmont[6], B. Oostra[1], J. Amack[4], A. Bertoli-Avella[1].* 1) Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, Netherlands; 2) Department of Cardiology, Erasmus Medical Center, Rotterdam, The Netherlands; 3) Department of Pediatric Cardiology, Erasmus Medical Center-Sophia, Rotterdam, The Netherlands; 4) State University of New York Upstate Medical University, Department of Cell and Developmental Biology, Syracuse, New York, United States; 5) Lille University of Sciences and Technologies, Lille, France; 6) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, United States; 7) Department of Bioinformatics, Erasmus Medical Center, Rotterdam, The Netherlands; 8) Center for Molecular and Biomolecular Informatics (CMBI) and Nijmegen Center for Molecular Life Sciences (NCMLS), Radboud University, Nijmegen, The Netherlands; 9) Medical Biology Research Center and Biochemistry Department, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran; 10) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran; 11) Department of Clinical Genetics, VU Medical Center, Amsterdam, The Netherlands; 12) Department of Clinical Genetics, University Hospital Leuven, Leuven, Belgium.

Congenital heart malformations are a major cause of morbidity and mortality especially in newborns and young children. Failure to establish normal left-right (L-R) asymmetry often results in cardiovascular malformations and other laterality defects (heterotaxy). Using SNP arrays genome-wide linkage analysis and gene sequencing, we identified mutations in NPHP4 in patients with cardiac laterality defects from a consanguineous family. The patients had various combinations of defects that included dextrocardia, transposition of great arteries, double outlet right ventricle, atrio-ventricular septal defects and caval vein abnormalities. To replicate this finding we investigated 146 unrelated individuals with similar cardiac laterality defects originating from United States, Belgium and The Netherlands. Forty-one percent of these patients also had laterality defects of the abdominal organs, including abdominal situs inversus, asplenia or polysplenia, midline liver and intestinal malrotation. Sequence analyses of the 30 exons of NPHP4 in this cohort identified eight additional missense variants that were absent or very rare in ethnically matched control populations. To study the role of nphp4 in establishing L-R asymmetry, we used antisense morpholinos to knockdown nphp4 expression in zebrafish. Depletion of nphp4 disrupted L-R patterning as well as cardiac and gut laterality. We show in vivo that nphp4 knockdown results in shorter Kupffer's vesicle cilia and compromises asymmetric fluid flow that is necessary for normal L-R patterning. The linking of NPHP4 to L-R axis determination and laterality defects will help dissect the complex genetic composition of heterotaxy and related cardiovascular malformations.

1130W

Exome Sequencing of Distantly Affected Relatives Effectively and Efficiently Identifies Causative Genes for Familial Thoracic Aortic Aneurysms and Dissections. *D.C. Guo[1], E.S. Regalado[1], R. Zhao[1], C.E. Kibler[1], G. Chang[1], J. Shendure[2], M.K. Rieder[2], D.A. Nickerson[2], D.M. Milewicz[1].* 1) Dept Internal Med, Univ Texas/Houston Med Sch, Houston, TX; 2) Dept Genome Sciences, Univ Washington, Seattle, WA.

Thoracic aortic aneurysms leading to acute aortic dissections (TAAD) can be inherited in families in an autosomal dominant manner with variable expression and decreased penetrance. Familial TAAD demonstrates genetic heterogeneity, with six genes identified to date for familial TAAD that are responsible for 20% of the disease (MYH11, ACTA2, MYLK, FBN1, TGFBR1, TGFBR2). To identify causative mutations in 7 large TAAD families with variable clinical presentations suggesting underlying genetic heterogeneity, exome sequencing was performed on DNA from two distant relatives with TAAD sharing 1/8th or 1/16th of their genome from each family. Approximately 230 unique heterozygous missense, splice-site, or indel variants were identified on each individual that were not present in public databases or in-house controls. For each family, 12 - 29 heterozygous rare variants were shared between the affected relatives of each family; relatives that shared 1/8th of the genome had average 27 candidate variants and relative with 1/16th of genome had average 14 candidate variants ($P = 0.006$). Sanger sequencing was used to validate the shared rare variants and only 2 out of 159 variants failed to validate. Segregation of the rare variants with TAAD in other family members narrowed the candidate rare variants for familial TAAD to between 3 to 7 per family in 5 families and 15 to 16 per family in 2 families. Variants under genome-wide linkage peaks for TAAD in 3 families also significantly narrowed the list of variants ($P < 0.001$). PolyPhen-2 analyses predicted that 42% of candidate variants are probably damaging and 19% are possibly damaging. Amino-acid conservation of the altered amino acid and tissue specific expression of candidate genes were also analyzed. Finally, we sequenced genes with candidate variants in an additional 188 affected probands from unrelated TAAD families to confirm genes as disease-causing. Using these criteria, SMAD3 was identified as the cause of disease in a family with TAAD and intracranial aneurysms; 2% of 188 affected probands also had SMAD3 mutations. Using these same criteria, additional novel genes for TAAD were identified in a family with TAAD associated with decreased penetrance in women and a family with TAAD associated with BAV. In conclusion, exome sequencing on two distantly affected family members and linkage analysis of large TAAD families can rapidly decrease the number of potential rare variants causing disease.

1131W

Novel Insights Regarding the Mechanism and Treatment of Stress-Induced Cardiomyopathy in Marfan syndrome. *R. Rouf[1], E. Takimoto[1], D. Bedja[1], N. Koitabashi[1], D.P. Judge[1], D.A. Kass[1], H.C. Dietz[1, 2].* 1) Johns Hopkins School of Medicine, Baltimore, MD; 2) Howard Hughes Medical Institute.

Marfan syndrome (MFS) is a systemic connective tissue disorder caused by a deficiency of extracellular fibrillin-1, a core component of extracellular microfibrils that negatively regulates transforming growth factor) (TGF)) bioavailability by sequestering latent TGF) to the matrix. Common cardiovascular manifestations of MFS include mitral valve prolapse and aortic root aneurysm and dissection. Many manifestations of MFS are attenuated or prevented by TGF) antagonists, including the angiotensin II type 1 receptor blocker losartan. Heart failure is the leading cause of death in severely affected children and many patients with more typical MFS show myocardial dysfunction out-of-proportion to any volume overload imposed by valve regurgitation. On this basis, we hypothesized that the myocardium in MFS may be more sensitive to cardiovascular stress, and that this may relate to excessive TGF) signaling. Adult male wild-type (WT) and fibrillin-1-deficient mice (Fbn1C1039G/+) underwent either sham operation or transverse aortic constriction (TAC) to induce mild pressure overload. WT mice remained well compensated after TAC, with chronic preservation of heart size and function. This correlated with an induction of fibrillin-1 expression in the myocardium. In contrast, Fbn1C1039G/+ mice failed to upregulate fibrillin-1 deposition and showed a significant increase in end-systolic (4.7 vs. 1.5 mm) and end-diastolic (5.4 vs. 4.28 mm) LV dimensions and a decline in fractional shortening (12.9 vs. 54.23 %) within 48 hours of TAC, when compared to WT littermates; the majority of Fbn1C1039G/+ died secondary to heart failure within 3 weeks. Compared with WT mice, MFS mice had increased LV mass (LV/BW 14.11 vs.6.25 mg/g), myocyte hypertrophy, and perivascular and interstitial fibrosis. Fibrillin-1-deficient mice also showed activation of both the canonical (pSmad2) and noncanonical (pERK1/2) TGF) signaling pathways in the myocardium in response to pressure overload. Finally, in vivo treatment with losartan normalized TGF) signaling and completely rescued cardiac decompensation, myocyte hypertrophy and fibrosis despite an ongoing pressure overload imposed by a fixed obstruction (TAC). These data suggest a primary regulatory role of fibrillin-1 in cardiac tissue homeostasis. Abrogation of TGF) -dependent signaling emerges as a therapeutic strategy for MFS-associated cardiac failure and perhaps in other presentations of pathologic LV remodeling.

1132W

The Profession as a Cause for Mutation Identification. *H. Yonath[1,2], H. Reznik-Wolf[1], L. Zeler[3], E. Pras[1,2], M. Frydman[1,2].* 1) Inst Human Gen, Sheba Med Ctr, Ramat Gan, Israel; 2) Sackler Medical School, Tel Aviv University. Israel; 3) Ben Gurion University of the Negev, Israel.

The majority of Marfan syndrome patients are ascertained by their Marfanoid phenotype, though there are some familial cases that have minimal manifestations. We present a sporadic patient with minimal and atypical features which was ascertained following a routine echocardiography. A 30 year old professional soccer player was referred for genetic evaluation due to a dilatation of the proximal ascending aorta identified on routine echocardiography. His past medical history included repair of bilateral inguinal hernia, right undescendent testis and left hydrocoele, and an operation for abdominal muscle strengthening. His parents, two siblings and only child were normal. On physical examination: Height 182 cm, arm span: 186.5 cm, arm span/height 1.024, upper/lower segment 0.89. Normal palate and uvula were obserbved without dental crowding, normal chest wall, no joint laxity, negative wrist and thumb signs, normal scars and no striae. Ophthalmological evaluation was normal and there was no evidence of dural ectasia on lumbar CT. Though he did not have sufficient criteria to establish the clinical diagnosis of Marfan syndrome (MIM # 154700), molecular testing was recommended due to the impact on his profession. Sequencing of FBN1 identified a c.3509G>A missense mutation resulting in Arg1170His substitution. The combination of the dilatation of the aorta, and a known, amino acid altering mutation, inguinal hernia and probably incisional hernia established the diagnosis of Marfan syndrome, followed by a recommendation to discontinue professional soccer. This case illustrates that occasional patients may have minimal manifestations, that might not meet the diagnosis based on the Ghent Nosology, and argues in favor of mutation analysis in atypical cases.

1133W

Acid fatty metabolism: a new therapeutic target for Epidermolysis Bullosa Simplex. *M. Bchetnia[1], M. Tremblay[1], G. Leclerc[2], A. Dupérée[2], J. Powell[3], C. McCuaig[3], C. Morin[2], V. Legendre-Guillemin[1], C. Laprise[1].* 1) Dept sciences fondamentales, Université du Québec à Chicoutimi, Saguenay, Canada; 2) Centre de santé et de services sociaux de Chicoutimi, Saguenay, Canada; 3) Hôpital Sainte-Justine, Montréal, Canada.

Epidermolysis bullosa simplex (EBS) is a skin disorder resulting from a weakened cytoskeleton and caused by mutations in keratin 5 (KRT5) or keratin 14 (KRT14) genes which encode for the major keratins of the basal cell layer of the epidermis. The altered cellular mechanisms underlying this disease are still unclear and effective therapies are still lacking. Therefore, we propose to sequence KRT5 and KTR14, to compare the gene expression profile of EBS and healthy controls and to test several therapeutic strategies for this disease in vivo. We performed direct sequencing of KRT5 and KRT14 genes in six EBS patients (2 EBS Dowling Meara (EBS-DM), 4 EBS localised (EBS-loc)) in order to identify the pathological mutations. These patients were paired to six healthy individuals according to age and gender. RNA was extracted from skin biopsy specimens and analyzed with HuGene-1_0-st-v1 GeneChip microarrays. Real-time PCR of five selected genes were performed to confirm GeneChip results. Next, we evaluate the therapeutic efficiency of three chemical chaperone molecules: sodium 4-phenylbutyrate (4-PBA), trimethylamine N-oxide (TMAO), and sulforaphane (SF) in attenuating keratin aggregation observed in heat stressed immortalized EBS cells. We identified six pathogenic mutations from which two are novel R125S (K14) and R559X (K5). Expression profiling comparisons show that 28 genes are differentially expressed in all the EBS patients compared to control subjects and 41 genes in EBS-DM compared to their paired controls. Nine genes involved in fatty acid metabolism (AWAT2, DGAT2L6, ELOVL3, THRSP, FADS2, FAR2, ACSBG1, AADACL3, and CRAT) and 2 genes in epidermal keratinisation (SPRR4 and KRT79) are common higher expressed genes between the two subgroups. Pre-treatment of EBS cells with the low molecular weight fatty acid 4-PBA, TMAO, and SF show variable effects in reducing the load of keratin aggregates in heat stressed EBS cells. Taken together, our results show that fatty acid metabolism and epidermal keratinisation pathways are higher expressed in EBS-DM patients. These two biological pathways, which might be involved in EBS disease, contribute both to the formation of the cell envelope barrier. Our findings on fatty acid metabolism support the therapeutic efficiency of 4-PBA chaperone molecule that seems, with TMAO, and SF, to be promising therapeutic tools for EBS and other protein misfolding disorders.

1134W

Combined malonic aciduria and methylmalonic aciduria (CMAMMA): exome sequencing reveals mutations in the *ACSF3* gene in patients with a non-classical phenotype. *L. Dempsey Nunez[1, 2], A. Alfares[1, 2], K. Al-Thihli[1, 2], J. Mitchell[3], S. Melançon[1, 2, 3], N. Anastasio[1], J. Majewski[1], K.C.H. Ha[1], D.S. Rosenblatt[1, 2, 3], N. Braverman[1, 2, 3].* 1) Department of Human Genetics, McGill University, Montreal, Canada; 2) Division of Medical Genetics, McGill University Health Centre, Montreal, Canada; 3) Department of Paediatrics, McGill University Health Centre, Montreal, Canada.

Combined elevated malonic acid (MA) and methylmalonic acid (MMA), or CMAMMA, is a rare autosomal recessive condition that is classically characterised by metabolic acidosis, developmental delay, seizures, and cardiomyopathy. Most cases of CMAMMA are due to malonyl-coenzyme A decarboxylase (MCD) deficiency. Two patients with CMAMMA, one of French Canadian and one of Ashkenazi Jewish origin, were identified by newborn screening for elevated MMA. Unlike the classical form, these patients had a higher level of MMA than MA and have been clinically asymptomatic. MCD activity was found to be normal in patient fibroblast extracts and no mutations were found in *MYLCD*. To identify the underlying molecular cause of the non-classical form of CMAMMA seen in these patients, we performed exome capture and sequencing. This powerful technique has been successfully used to identify the genetic basis of rare Mendelian conditions with only one or few affected individuals. Exome sequencing was performed in the French-Canadian proband from a consanguineous family and revealed a homozygous missense mutation, c.1075G>A (p.E359K), in exon 5 of the *ACSF3* gene, a member of the acyl-CoA synthetase (ACS) family. Members of the ACS class of proteins catalyze the activation of fatty acids by forming a thioester bond between a CoA and a fatty acid, to form acyl-CoA. A different homozygous missense mutation in *ACSF3*, c.1411C>T (p.R471W), was identified in the Ashkenazi Jewish patient. Both substitutions were in residues that are highly conserved within signature domains of the ACS family and the changes were predicted to be damaging. Segregation analysis of family members further supported these findings. Sequencing of both mutations in ethnically matched controls revealed one carrier in 139 French Canadian controls and one carrier in 176 Ashkenazi Jewish controls. This represents an allele frequency of less than 0.5% of their respective control populations. These results suggest that *ACSF3* is a candidate gene for the non-classical form of CMAMMA seen in these two patients and reinforces the value of exome sequencing of a limited number of patients for the identification of novel disease genes.

1135W

Mitochondrial mistargeting causes autosomal dominant renal Fanconi syndrome. *E. Klootwijk[1], M. Reichold[2], A. Helip-Wooley[3], H. Stanescu[1], J. Reinders[2], D. Bockenhauer[1], W.A. Gahl[3], R. Warth[2], R. Kleta[1].* 1) University College London, London, United Kingdom; 2) University Regensburg, Regensburg, Germany; 3) NHGRI, NIH, USA.

Renal Fanconi syndromes are characterized by a generalized renal proximal tubular dysfunction leading to aminoaciduria, glucosuria, phosphaturia, small molecular weight proteinuria, and metabolic acidosis. The clinical consequences in childhood include rickets and growth problems. We investigated the genetic basis and the underlying molecular pathology in an extended family with autosomal dominantly inherited renal Fanconi syndrome without kidney failure. Whole genome multipoint parametric linkage analysis was performed, revealing a significant LOD score (> 3) for a single locus. All genes in the linked area were sequenced resulting in the identification of a heterozygous mutation in a gene, which we call Fanconin; the mutation leads to de-novo formation of a mitochondrial targeting motif. This mutation was not found in ethnically matched controls. To assess its functional impact, a stable permanently transfected inducible renal proximal tubular cell model was generated. Immunohistochemical analysis using these cells showed appropriate intracellular localization of the wild type human Fanconin, but mitochondrial mistargeting of the mutant human Fanconin. Immunohistochemical studies of mouse kidney showed that the Fanconin protein is expressed in renal proximal tubules, consistent with the proximal tubular dysfunction observed in our patients. Knockout mice for Fanconin did not exhibit a proximal tubular transport defect, as assessed by aminoacid analysis using GC-MS. This was consistent with the hypothesis that the renal Fanconi phenotype in our family is not caused by haploinsufficiency, but rather by a dominant negative effect of the Fanconin protein. Since Fanconin is mistargeted to mitochondria, we studied the impact of this mutation on mitochondrial function. Oximetric analyses showed a complex coupled ATP synthase deficiency (oxidative phosphorylation) in cells expressing the mutant Fanconin. Transport studies documented abrogated transepithelial water transport. To our knowledge this is the first description of a genetic defect leading to intracellular mistargeting of a mutant protein resulting in mitochondrial pathology.

1136W

Novel recurrent CFTR mutation in Chilean cystic fibrosis patients. *G. Lay-Son[1,3,6], M. Vasquez[1], F. Vial[1], M.L. Boza[2], I. Contreras[3], J. Lozano[4], D. Zenteno[5], F. Bello[5], G.M. Repetto[1,3].* 1) Human Genetics Center, Faculty of Medicine, Clinica Alemana-Universidad del Desarrollo, Santiago, Chile; 2) Hospital San Borja-Arriaran, Santiago, Chile; 3) Hospital Padre Hurtado, Santiago, Chile; 4) Hospital Roberto del Rio, Santiago, Chile; 5) Hospital Clinico Regional Dr. Guillermo Grant Benavente, Concepcion, Chile; 6) Hospital Dr. Luis Calvo Mackenna, Santiago, Chile.

Cystic fibrosis (CF) affects about 1 in 10,000 newborns in Chile. A 36 most frequent mutation panel has an 80-85% detection rate in North American and Northern European CF patients but a lower detection rate of approximately 50% in Chile. This suggests that some of the so-far unidentified mutations in Chilean patients may be of local/regional origin and raises a challenge to certify the diagnosis and for the genetic counseling. We carried out sequencing analysis of select exons of the CFTR gene by PCR amplification and direct sequencing in Chilean patients with CF and incomplete allele identification by 36 CFTR mutation panel. Four unrelated patients had a previously unreported deletion of two nucleotides (c.2462_2464delGT/p.Ser821ArgfsX4), which is predicted to cause a frameshift at 821 codon with a subsequent premature stop codon at codon 824. This mutation is located in exon 14 at the regulatory domain of the CFTR protein. These four patients were heterozygous for p.F508del and they have pancreatic insufficiency. Parental studies in one of the patients confirmed that these 2 mutations were in trans. Prevalence in Chilean patients from the National Program of CF is currently being evaluated. This mutation should be included in current studies in CF patients with Chilean origin. Further analyses are necessary to determine prevalence and geographic distribution in other Latin Americans and Hispanics populations.

1137W

Contribution of *GH* an *GHRHR* mutations to isolated growth hormone deficiency - Identification and functional characterization of new variants. *S. Amselem[1,2,3], ML. Sobrier[2], F. Dastot-Le Moal[1,2], N. Collot[1], S. Rose[1,2], A. Soleyan[1], P. Duquesnoy[2], B. Copin[1,2], M. Legendre[1,2].* 1) Génétique clinique et moléculaire, Hôpital Trousseau, APHP, Paris, France; 2) Inserm U933, Hôpital Trousseau, Paris, France; 3) Université Pierre et Marie Curie, Paris, France.

Growth is a complex phenomenon that depends on several factors including growth hormone (GH), the GH releasing hormone (GHRH) and its receptor (GHRHR). Although GH and GHRHR have been recognized as key etiologic factors in non-syndromic forms of isolated growth hormone deficiency (IGHD), a small number of mutations have been identified in this rare condition. Depending on the studies, *GH* and *GHRHR* defects would account for 6-12.5% and 0-6.7% of IGHD cases. So far, as for GHRHR, very few functional studies have been performed in order to assess the consequences of the identified variants.

With the aim to assess the contribution of the *GH* and *GHRHR* genes in the pathogenesis of IGHD, we screened for mutations all coding exons and flanking intronic sequences of these two genes in a large cohort of patients presenting with a non-syndromic form of IGHD characterized by a small or normal anterior pituitary and an eutopic posterior pituitary.

The *GH* gene was first analyzed in a total of 191 independent patients. Mutations were identified in 28 patients (15%), 10 of them (10/191, 5%) representing familial forms of IGHD. These include 8 novel mutations, among which 2 frameshift mutations, 1 splicing defect and 5 missense mutations. The *GHRHR* gene was subsequently analyzed in the remaining 144 independent patients in whom no *GH* defect was identified and for whom DNA was available. This led to the identification of mutations in 22 patients (15%), 7 of them (7/144, 5%) representing familial cases. The *GHRHR* mutation spectrum, which comprises 7 novel mutations, consists of 2 nonsense mutations, 4 frameshifts, 1 splicing defect and 10 missense mutations. In vitro functional studies of several missense mutations, which are underway, include the assessment of GHRH response through a CRE-dependent luciferase assay. The first results are consistent with a loss of function associated with 4 of these missense variations.

Noteworthy, up to 66% (33/50) of the patients with a *GH* or *GHRHR* germline mutation represent sporadic cases. Overall, this study performed in a large cohort of patients, which identified molecular defects in the *GH* or *GHRHR* gene in 50 out of 191 independent patients (26%), reveals the importance of those two genes in the pathogenesis of non-syndromic IGHD with a normal location of the posterior pituitary.

1138W

A novel spontaneous autosomal recessive mutation/deletion that causes a multifarious alopecia-like phenotype in mouse. *H. Beauchemin[1,2], C. Polychronakos[1,2].* 1) Pediatric Endocrinology, Montreal Children's Hospital-RI/MUHC, Montreal, Quebec, Canada; 2) McGill University, Montreal, Quebec, Canada.

Alopecia Areata is an autoimmune disease characterized by temporary local hair loss. Here we report a yet to be identified spontaneous mutant (*Alo1*) that exhibits an elaborate and very consistent complex phenotype reminiscent of Alopecia Areata along abnormal sebaceous gland/hair follicle morphology. This *Alo1* spontaneous mutation occurred in a male NOD mouse and is now currently being transferred into the C57Bl/6 background, showing an autosomal recessive pattern of transmission with 100% penetrance, regardless of the genetic background. Interestingly, this mutation that involves a single genetic locus, leads to a complex phenotype progression: Young mice (<6 weeks) showed a sparse abnormal-looking fur often associated with lacrymating/purulent/bulging eyes. At six weeks of age, mice enter a remission phase as from then, mice looked fairly normal, showing only temporary patch-like hair losses that were very reminiscent of Alopecia Areata in humans. However, after several months, mice showed systematically greasy fur and an ill-looking general appearance but no skin injuries. In addition, these older mice developed chronic eye abnormality. Histological analysis revealed impaired hair growth associated with hyperplasic sebaceous glands. Because Alopecia Areata is a CD8-mediated autoimmune disease, we looked at the cellular compartment of the immune system in *Alo1* mice by multiparametric flow cytometry. Surprisingly, we found no deficiency in either the CD8 or the CD4 T-cells as they present normal CD4/CD8 ratios in thymus, spleen and in lymph nodes. Furthermore, no evidence of impairment of any other cellular compartment of the immune system was found (including in the memory/naive CD8 or CD4 T-cells, regulatory T-cells, natural killer cells, B-cells, macrophages and monocytes), suggesting that the phenotype observed does not have an autoimmune component. Mapping of the *Alo1* mutation is currently underway. In conclusion, we report here a novel mutation affecting integument that exhibits a complex phenotype reminiscent of a juvenile non-T-cell mediated alopecia associated with sebaceous gland hyperplasia.

1139W

Exome Sequencing strategy in a family with Congenital Fiber-Type Disproportion and Insulin resistance reveals an unexpected excess of non synonymous variants. *T. Esposito[1], D. Formicola[1,2], S. Magliocca[1], M. Simonetti[2], O. Farina[2], F. Cipullo[2], S. Sampaolo[2], F. Gianfrancesco[1], G. Di Iorio[2].* 1) Inst Gen & Biophysics, Italian Natl Res Council, Naples, Italy; 2) Department of Neurological Sciences, Second University of Naples, Italy.

Whole-exome sequencing is a new strategy that enables to rapidly identify rare variants in about 1% of the proteins coding genome and to detect pathogenic variants in families not informative for linkage analysis. We chosen to use this approach to define the complex genetic substrate of a family, with consanguineous parents, affected from an unusual syndrome characterized by insulin resistance, left ventricular noncompaction (LVNC), long QT, and congenital fiber type disproportion (CFTD) in the index case. This latter died when she was 11 years old by sudden cardiac death. The exome sequencing was performed on DNA obtained from two affected members of the family using SureSelect Exome target enrichment technology, followed by paired-end sequencing on a Solid 4 System. 11,432 synonymous changes, 13,089 non-synonymous changes and 181 frameshifts were identified in each patient. Using both dbSNP and HapMap exomes as filters to remove common variants, we disclose that 200 novel heterozygous coding variants were shared between two patients. Using a recessive model of transmission we were able to reduce this list to 50 genes. At least 10 of these genes are involved in determining muscle and cardiac phenotypes (ACTA1, ACTC1, SEPN1, MYH11, MYH7B, etc). Missense variants in this subset of genes were analysed for segregation with the disease phenotype. We hypothesize that novel missense variants detected in ACTA1 gene combined with novel missense variants in ACTC1 and MYH7B could explain the complex phenotype observed in our family. Functional studies are ongoing to verify their role in the etiopathogenesis of the disease.

1140W

The novel M1304R mutation in the von Willebrand factor gene (VWF) causes defective VWF multimerization and storage and results in type 2 von Willebrand disease (VWD). *J. Hinckley[1], J. Chen[2], R. Montgomery[3], S. Haberichter[3], R. Wong[4], J. Lopez[2], J. Di Paola[1].* 1) Human Medical Genetics Program and Department of Pediatrics, University of Colorado Anschutz Medical Campus, Aurora, CO; 2) Puget Sound Blood Center, Seattle, WA; 3) Medical College of Wisconsin, Milwaukee, WI; 4) Barbara Davis Center for Childhood Diabetes, Aurora, CO.

VWD, the most common genetic bleeding disorder, is characterized by incomplete penetrance and variable expressivity. Clinically, it presents with mucocutaneous bleeding which is often not concordant with VWF protein levels or activity. Qualitative defects in the VWF protein define type 2 VWD. We characterized a 24-member pedigree with VWD type 2 that demonstrates autosomal dominant inheritance and exhibits clinical and biochemical phenotypic variability. Focused mutational analysis of exon 28 of VWF, which harbors most type 2 mutations, was performed in all individuals. A novel T>G missense mutation at position 3911 that predicts a methionine to arginine (M1304R) change in the A1 domain of the VWF peptide segregated in all members with VWD. Exonic sequencing of VWF in 1 affected and 1 non-affected member did not identify additional mutations or VWF polymorphisms previously reported to alter VWF levels or activity. Evaluation of allele-specific mRNA levels by a novel primer extension assay demonstrates consistent mRNA ratios that cluster within wild-type (WT) relatives (95% WT allele) and VWD patients (76% WT allele), ruling out a hypomorphic WT allele, and ratios are stable within individuals over eight years. Protein analysis by Western blot demonstrates variability of VWF multimers among individuals with the M1304R allele. By ELISA, a nanobody that detects the mutated A1 domain binds with a wide range of variability among affected members, suggesting VWF multimers contain different amounts of mutant protein. In vitro expression of the M1304R allele shows decreased VWF secretion and lack of formation of storage granules. When co-expressed with the WT allele, total VWF secretion is also decreased and multimerization is nearly normalized but no storage granules are formed, suggesting that this particular mutation affects multimerization and trafficking. In summary, we characterized a family with VWD caused by a novel VWF mutation. Phenotypic variability does not appear to be due to polymorphisms in VWF or allele-specific mRNA expression, suggesting the importance of other modifier genes that interact with VWF through biosynthesis, clearance, or hemostatic pathways. In order to further understand the clinical and laboratory variability of VWD, it is important to map genetic variants that influence VWF levels and activity and develop new technologies to quantify the amount of mutant VWF in plasma from VWD patients.

1141W

CHD7 mutations causing CHARGE syndrome are predominantly of paternal origin. *J. Kohlhase[1], N. von Velsen[2], P. Burfeind[2], M. Steckel[2], J. Maenz[2], A. Buchholz[1], W. Borozdin[1], S. Pauli[2].* 1) Center for Human Genetics Freiburg, Germany; 2) Institute for Human Genetics, University of Goettingen, Goettingen, Germany.

CHARGE (coloboma, heart defects, atresia of the choanae, retarded growth and development, genital hypoplasia, ear anomalies and deafness) syndrome is a congenital malformation syndrome caused by mutations in the *CHD7* gene in approximately 2/3 of cases. In the vast majority of cases, CHARGE syndrome is sporadic. There are only a few reports of parent-to-child transmission and somatic or gonadal mosaicism. To determine the parental origin of *CHD7* mutations in sporadic CHARGE syndrome, we screened 30 families for informative exonic or intronic polymorphisms located near the detected *CHD7* mutation. An informative polymorphism could be identified in 13 out of 30 families. Linkage analysis was performed between the *CHD7* mutation and the polymorphism in the child. In 12 out of 13 families, the mutation affected the paternal allele (92.3%). In our cohort, the mean paternal age at birth was 32.92 years. Comparing the age of fathers of an affected CHARGE patient with the paternal age of the German population in general, we could not observe any paternal age effect. Taken together, we show in this study that *de novo CHD7* mutations occur predominantly in the male germ line, and we present a series of new *CHD7* mutations in CHARGE syndrome.

1142W

Mutations in MAP3K1 cause 46, XY DSDs by shifting the balance in downstream signaling pathways. *J. Loke[1, 2], A. Pearlman[1, 2], S. Blais[3], T. Neubert[3], H. Ostrer[1,2].* 1) Human Genetics Program, NYU School of Medicine, New York, NY; 2) Pathology and Genetics, Albert Einstein College of Medicine, Bronx, NY; 3) Proteomics Core, NYU School of Medicine, New York, NY.

Mutations in MAP3K1 are a common cause of 46,XY disorder of sex development (DSD), accounting for up to 18% of cases (Pearlman, et al. AJHG 87:898-904, 2010). All of the mutations identified to date are non-truncating and are found near the N-terminus, a region that harbors binding domains to a number of co-factors. To determine the effects of these mutations, we performed immunoprecipation of lymphoblastoid cell lysates using an anti-MAP3K1 antibody, then identified the interacting proteins by tandem mass spectrometry (MS/MS) and standard Western blots or flow cytometry-based Western (Flow Western). By MS/MS, we observed increased binding of WNT-signaling proteins, including FRAT1 and PKCalpha, to the MAP3K1 complex in mutants from one familial case (p.Gly616Arg) and in two of eleven sporadic cases (p.Leu189Pro, p.Leu189Arg) versus wildtype cell lines. The increased binding of the interacting proteins was confirmed by Western blot analysis. Furthermore, we have demonstrated that these mutants enhanced the binding of RHOA to the MAP3K1 complexes, an effect that is known to inhibit SOX9 transcription. Thus, these results demonstrate that MS/MS and/or Flow Western co-IP analysis represent a rapid means for vetting new gene variants that are identified by sequencing the genomes of patients with genetic disorders by using readily accessible primary lymphoblastoid cells. In the case of 46,XY DSD, these methods and experiments demonstrated that the identified mutations enhanced the binding of factors that promote the WNT/beta catenin signaling of ovarian development and that suppress SOX9 transcription of testicular development. This provides a mechanism to account for the 46,XY DSD phenotypes observed in individuals with MAP3K1 mutations.

1143W

Identification of a Novel Missense Mutation in Exon 4 of the Human Factor VIII Gene Associated with Sever Hemophilia A Patient. *H. Onsori[1], M.A. Hosseinpour Feizi[2], Sh. Mintasser Kouhsari[3], A.A. Hosseinpour Feizi[4].* 1) Department of Biology, Islamic Azad University, Marand, Iran; 2) Faculty of Sciences, University of Tabriz, Tabriz, Iran; 3) Department of Biology, Faculty of Sciences, University of Tehran, Tehran, Iran; 4) Tabriz University of medival sciences, Tabriz, Iran.

Hemophilia A is an X-linked congenital bleeding disorder affecting approximately 1 in 10000 males, and is caused by deficiency of factor VIII (FVIII). The FVIII gene is on the long arm of the X chromosome at Xq28, spans 186 kb, and consists of 26 exons. In this research, we report a new mutation in FVIII gene in Hemophilia A patient recourse to Tabriz children's hospital in 2006. The patient was a boy with 6 years old (HA10) that for the reason of gum of the teeth bleeding recourse to Tabriz children's hospital in 2006. Sever bleeding, low rate of FVIII and medical inspections showed probability of sever hemophilia A. So, for detection of causal mutation, we used PCR-SSCP method in this research. In this study we report a novel missense mutation in exon 4 of FVIII gene that had not been reported in the list of FVIII gene mutations. The novel mutation due to T→C transition at codon 153 (TGC) of the factor VIII gene, which replaces a cysteine with an arginine residue. This mutation was recorded in GenBank with accession number EF581382. In this research observed that a point mutation in FVIII gene can be cause of sever hemophilia A. So, this study shows that the methodology of PCR-SSCP may be useful in detecting most of the genetic defects of hemophilic patients. Also, disease screening and genetic consulting before marriage are necessary for inhibition of the birth of affected Childs.

1144W

Histone acetylation deficits in lymphoblastoid cell lines from Rubinstein-Taybi syndrome patients. *C. Gervasini[1], J.P. Lopez-Atalaya[2], S. Spena[1], F. Mottadelli[1], M. Piccione[3], A. Selicorni[4], A. Barco[2], L. Larizza[1].* 1) Medical Genetics, University of Milan Milano, Italy; 2) Instituto de Neurociencias de Alicante (Universidad Miguel Hernández-Consejo Superior de Investigaciones Científicas), Alicante, Spain; 3) Dipartimento Materno Infantile, Università di Palermo,Palermo, Italy; 4) Ambulatorio Genetica Clinica Pediatrica Clinica Pediatrica Università Milano Bicocca Fondazione MBBM A.O S Gerardo Monza, Italy.

Rubinstein-Taybi syndrome (RSTS, #180849) is a congenital neurodevelopmental disorder characterized by postnatal growth deficiency, characteristic skeletal abnormalities and mental retardation caused by mutations in either of the genes encoding for the transcriptional co-activators with intrinsic histone acetyltransferase (HAT) activity CBP and p300. Recent studies have shown that neuronal histone acetylation is reduced in mouse models of RSTS. We selected nine patients, who carry mutations at the CREBBP locus that differ in the strength of the predicted consequences at the protein level and recapitulate different grades of the clinical variability in the spectrum of the syndrome, to generate lymphoblastoid cell lines. The patients were sorted out from a large cohort of 110 RSTS-diagnosed patients recruited from different Italian Clinical Units and screened for mutations in the entire coding sequence and intronic flanking regions of CREBBP gene by DHPLC (Denaturing High Performance Liquid Chromatography) analysis and direct sequencing. The group comprises one whole gene deletion, one truncating mutation, four missense mutations affecting the HAT domain, and three splicing mutations, all characterized at DNA, RNA and protein level. We assessed histone acetylation in the lymphoblastoid cell lines to correlate the possible deficits with the type of mutation in the CREBBP gene and with the severity of the phenotype, focusing on mental impairment. The comparison of RSTS and control cell lines revealed significant deficits in the acetylation of histones H2B and, in lower degree, in histone H2A. The most severe defects were observed in the cell lines carrying CREBBP whole gene deletion and the truncating c.2356delC mutation that lead to a haploinsufficiency state. Interestingly, the treatment with an inhibitor of acetytransferases rescued the histone acetylation deficit observed in the cell line showing the most pronounced impairment. Our results validate the seminal observations in mouse models of the disease and identify molecular markers of the disease such as specific acetylation deficit.

1145W

Identification of a new gene involved in IHH and ataxia by positional cloning of a patient with a balanced translocation t(3;12)(p13;p13). *H. Kim[1], R. Ullmann[2], H. Ropers[2], V. Kalscheuer[2], L. Layman[1].* 1) Dept OB/GYN, Georgia Health Sciences University, Augusta, GA.USA; 2) Department of Human Molecular Genetics, Max Planck Institute for Molecular Genetics, Ihnestrasse 63-73, Berlin, 14195 Germany.

Patients with idiopathic hypogonadotropic hypogonadism (IHH) present with absent puberty due to a hypothalamic-pituitary defect, and may be either normosmic (nIHH) or anosmic -Kallmann syndrome (KS). Although mutations in genes such as FGFR1, KAL1, CHD7, PROKR2, and GNRHR constitute the most commonly encountered etiologic genes in IHH/KS, the molecular basis for most patients remains unknown. Approximately 1/2,000 liveborns possess a de novo balanced translocation, and about 6% of these patients manifest the phenotype of a significant, developmental disorder. These rare, but exceedingly informative patients provide the unique opportunity to identify new genes causing birth defects. We were able to ascertain a male patient with a mos46,XY,t(3;12)(p13;p13)[18]/46,XY[3] with nIHH and cerebellar ataxia, which affords the opportunity to identify a gene important in pubertal development. Array Comparative Genome Hybridization (CGH) analysis of patient DNA was consistent with a balanced translocation and excluded Copy Number Variation (CNV) as the cause of the IHH phenotype. In this patient with the mosaic 3;12 translocation, homogeneous lymphoblastoid cell lines with the balanced translocation were successfully transformed from peripheral white blood cells. IHH and cerebellar ataxia often occur together and they are seen in Gordon Holms syndrome and Boucher-Neuhauser syndrome. We hypothesize that one of the breakpoints of this translocation case is likely to harbor a gene responsible for this phenotype. A positional cloning technique was applied to clone each of the breakpoints. FISH mapping for a breakpoint at 12q13 and array painting in this patient is underway. This chromosome translocation affords the potential to identify a gene involved in IHH and ataxia.

1146W

Microarray-based copy number analysis can identify genomic disruptions of PHEX causing X-linked hypophosphatemic rickets. *S. Mumm[1,2], D. Wenkert[2], M. Huskey[1], V.A. Wollberg[1,2], M.P. Whyte[1,2].* 1) Division of Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, MO, USA, 63110; 2) Center for Metabolic Bone Disease and Molecular Research, Shriners Hospital for Children, St. Louis, MO, United States, 63131.

Heritable forms of hypophosphatemic rickets (HR) include X-linked dominant HR (XLH, caused by de-activating mutations in the PHEX gene), autosomal recessive HR (ARHR, caused by de-activating mutations in the DMP1 gene), and autosomal dominant HR (ADHR, caused by activating mutations in the FGF23 gene). Over the past 27 years, we have cared for 268 pediatric HR patients from throughout the United States. After excluding 12 patients with HR from McCune-Albright syndrome, epidermal nevus syndrome, tumor-induced rickets, or cystinosis, etc., there were 72 sporadic cases diagnosed clinically with XLH. We are investigating 30 of these 72 sporadic patients (those for whom DNA is available) to identify their genetic defect causing rickets. In 2009, we reported that no mutations in the DMP1 or FGF23 genes were identified among these 30 sporadic HR cases (Mumm et al, 2009 ASHG meeting). We have now sequenced the PHEX gene in all 30 cases and identified mutations in 8 patients, (including splice site and nonsense mutations, and small insertion/deletions) and used microarray-based copy number analysis using Affymetrix SNP 6.0 arrays to identify apparent genomic disruptions in the PHEX gene for another 4 patients. Genomic DNA was isolated from blood leukocytes. All 22 coding exons and adjacent mRNA splice sites of the PHEX gene were amplified by PCR and sequenced. The patients' DNA sequences were analyzed by alignment to a control sequence using VectorNTI AlignX software, and by examining DNA sequence electropherograms. Microarray data analysis was performed with Partek Genomics Suite. Copy number changes, indicating genomic disruption of the PHEX gene, were found in 4 spordic HR patients. One girl had a large heterozygous deletion that removed exons 13-22 and extended beyond the 3' end of the gene, but did not appear to disrupt neighboring genes. Two unrelated male patients had an apparent copy number amplification of exons 13-15, and another boy had an apparent copy number amplification of exons 6-9. All of these disruptions must be confirmed using a complementary method. No copy number changes in PHEX, DMP1, or FGF23 were seen in the remaining sporadic HR patients. After a search for genetic defects in PHEX, DMP1, and FGF23, only 40% of our sporadic HR patients have PHEX mutations (27% identified by PCR/sequencing - 13% identified by microarray). For 60% of our sporadic HR patients, their underlying genetic defect remains unknown.

1147W

Approach to the genetic dissection of Primary Ciliary Dyskinesia (PCD) in the Qatari population. *A.S. AL-DOSARI[1], Y.A. ALSARRAJ[1], I.A. JANAHI[2], A. Saadoon[2], S.F. MOHAMMED[1], J. ALAMI[1], H.I. ELSHANTI[1].* 1) Molecular genetics, shafallah Medical Genetics Center, DOHA,Qatar; 2) Department of Pediatrics, Hamad Medical Corporation, Doha, Qatar.

Primary ciliary dyskinesia (PCD) is a heterogeneous autosomal recessive genetic disorder that leads to ultra-structural and functional defects in cilia and is characterized by recurrent and chronic respiratory infections, male infertility and occasionally situs inversus. PCD can result from mutations in at least 12 genes; however, these mutations explain the disease in only 40% of patients. We identified several extended Qatari families with multiple individuals affected by PCD according to clinical criteria. We performed whole-genome SNP genotyping assays using an Illumina platform for all families, after the development of a homozygosity map of the regions harboring genes known to play a role in PCD. If the affected individuals show homozygosity in a region identified by the homozygosity map, we resequenced that gene first. Families showing homozygosity in regions outside the regions identified by the homozygosity map, are further studied by linkage analysis and candidate gene positional cloning approach. However, in these families, we resequenced all known genes to exclude compound heterozygotes. We examined the homozygous region of each family and compared it to the developed homozygosity map. In one family, we encountered homozygosity in the region harboring DNAH11 on chromosome 7. Mutation analysis by direct resequencing of DNAH11 exons, flanking intronic sequences and splice sites showed a novel splice site mutation (c.5945+1 G>C) in exon 34, which produces a truncated protein. The mutation segregates with the disorder in this family, is not present in 338 control chromosomes and is inherently a deleterious mutation. We are currently studying the frequency of this mutation in the Qatari population and its effect on the transcription and translation of the protein. In another family, we identified a 35 Mb homozygous region on the long arm of chromosome 3. While examining candidate genes in the region guided by the human celiome, a recently published study identified CCDC39 as a PCD gene through studying an animal model and currently we are examining this gene in the family by resequencing. In a third family, we identified a 46 Mb homozygous region on chromosome 5. Candidate genes were prioritized based on conservation through evolution and expression in cilia and are being examined by resequencing. In conclusion, we have developed a population specific tailored approach to identify the genetic cause of PCD in families in Qatar.

1148W

The ciliopathy gene cc2d2a controls zebrafish photoreceptor outer segment development through a role in Rab8-dependent Vesicle trafficking. *R. Bachmann-Gagescu[1, 4], I.G. Phelps[1], G. Stearns[2], B.A. Link[3], S. Brockerhoff[2], C.B. Moens[4], D. Doherty[1].* 1) Pediatrics, Univ Washington, Seattle, WA; 2) Biochemistry, Univ Washington, Seattle, WA; 3) Cell Biology, Neurobiology & Anatomy, Medical College of Wisconsin, Milwaukee, WI, USA; 4) Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.

Ciliopathies are a genetically and phenotypically heterogeneous group of human developmental disorders whose root cause is the absence or dysfunction of primary cilia. Joubert syndrome is characterized by a distinctive hindbrain malformation variably associated with retinal dystrophy and cystic kidney disease. Mutations in CC2D2A are found in ~10% of patients with Joubert syndrome. Here we describe the retinal phenotype of cc2d2a mutant zebrafish consisting of disorganized rod and cone photoreceptor outer segments resulting in abnormal visual function as measured by electroretinogram. Our analysis reveals trafficking defects in mutant photoreceptors affecting transmembrane outer segment proteins (opsins) and striking accumulation of vesicles, suggesting a role for Cc2d2a in vesicle trafficking and fusion. This is further supported by mislocalization of Rab8, a key regulator of opsin carrier vesicle trafficking, in cc2d2a mutant photoreceptors and by enhancement of the cc2d2a retinal and kidney phenotypes with partial knockdown of rab8. We demonstrate that Cc2d2a localizes to the connecting cilium in photoreceptors and to the transition zone in other ciliated cell types and that cilia are present in these cells in cc2d2a mutants, arguing against a primary function for Cc2d2a in ciliogenesis. Our data support a model where Cc2d2a, localized at the photoreceptor connecting cilium/transition zone, facilitates protein transport through a role in Rab8-dependent vesicle trafficking and fusion.

1149W

Mutational Analysis of *SDCCAG8* in Bardet-Biedl Syndrome Patients with Severe Renal Disease. *G. Billingsley[1], C. Deveault[1], E. Héon[1, 2].* 1) Genetics and Genome Biology Program; 2) Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, Toronto, Ontario, Canada.

Ciliopathies are a heterogeneous group of diseases that present overlap in both clinical features (retinal degeneration, obesity, renal abnormalities, digit anomaly, cognitive impairment) and genetic basis. Mutations of *SDCCAG8* (serologically defined colon cancer antigen 8, OMIM 613524) were recently identified as the cause of a retinal-renal ciliopathy which included both Senior-Loken syndrome (SLSN) and Bardet-Biedl syndrome (BBS) patients. All patients had end stage kidney failure, retinal degeneration and no polydactyly. In our BBS cohort (105 cases), mutational analysis of *BBS1-BBS12* identified disease-causing mutations in 76% of families. We selected a subset of BBS cases with no disease causing gene presently identified, to undergo mutational analysis of *SDCCAG8*. Seven BBS cases (6 probands) with severe renal disease and retinal dystrophy were selected for mutational analysis of *SDCCAG8* (NM_006642.3). Five patients had no digit anomaly, 4 patients had some degree of cognitive impairment and 3 patients were obese. Direct sequencing of PCR amplified coding exons (n = 18) and flanking introns was performed. Family segregation and control screening (n=150) confirmed the pathogenicity of novel sequence changes. Two East Indian sibs were found to carry previously published (Indian) compound heterozygous mutations in exon 12 (c.1444delA, p.Thr482LysfsX12) and exon 14 (c.1627-1630 delATAG, p.Asp543AlafsX24) which segregated with the disease phenotype. These sibs underwent kidney transplant at ages 11 and 9. The family also carried a novel polymorphism (c.1473 + 48 insA, MAF = 0.3) in cis with the exon 12 mutation. In addition, the following novel BBS variants were observed in one sib: *BBS5* c.751 A>G, p. N251D and *BBS11* c.896 G>A, p.R299Q. The phenotype information collected did not support a significant functional influence of these *BBS* changes on phenotype severity. In conclusion, results to date show that *SDCCAG8* may play an important role in “BBS patients” with severe renal disease and no digit anomaly. Further screening is ongoing. Comprehensive phenotyping of ciliopathy-related diseases will help direct the prioritization for genetic screening of newly identified patients.

1150W

A Critical Review of the Human Ciliopathy-Related Literature Reveals a Need for Revision of Diagnostic Criteria. *C. Deveault[1], G. Billingsley[1], E. Héon[1,2].* 1) Department of Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, Toronto, Ontario, Canada.

Introduction Ciliopathies are an emerging group of disorders, often pleiotropic, characterized by significant genetic heterogeneity and clinical overlaps. Bardet-Biedl syndrome (BBS) is a pleiotropic ciliopathy disorder that has been shown to overlap clinically and genetically with other ciliopathies such as McKusick-Kaufman syndrome (MKKS), Alström syndrome (ALMS), Meckel-Gruber syndrome (MKS) and Joubert syndrome (JBTS). The diagnostic features of these conditions are evolving with our increasing understanding of their molecular background. For these 5 ciliopathies, we performed a comprehensive literature review of cases published with two principal mutations and their reported clinical features.

Methods Publications up to May 2011, discussing the molecular characterization of those five related ciliopathies (BBS, JBTS, MKS, MKKS and ALMS) which presented cases or families with two principal mutations were included. For every case, the mutations and complete clinical features provided were tabulated and compared with the proposed current diagnostic criteria.

Results The literature review of JBTS syndrome showed strong and well defined clinical data for each of the genes in which principal mutations are found. Correlations of certain features with specific genes were observed and the molar tooth sign was consistently present However, for the BBS literature, over half (54.66%) of the cases with mutations reported did not show detailed phenotype information and in some cases was limited to a mention of whether or not the patients fit the published criterias. Trends are observed but correlations are not possible due to the inconsistent clinical phenotype information provided. A diagnostic algorithm is presented.

Conclusions Phenotype-genotype correlation in JBTS now enables a strategized mutational analysis based on the phenotype of the patient. This is not possible for BBS. This highlights the need for a more systematic clinical characterization of BBS cases in order to determine potential correlations, guidelines for genetic screening and optimize patient management.

1151W

Genetic and phenotypic characterisation of an ovine large animal model of the MKS/JBTS/NPHP ciliopathy, Meckel-Gruber syndrome. *M.R. Eccles[1], B. Lett[1], C. Stayner[1], A. Wiles[2], L. Slobbe[1], K. Parker[2], A.C. Johnstone[3], R.J. Walker[2], A.C. Poole[2], J. McEwan[4], D. Markie[1].* 1) Dept Pathology, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand; 2) Dept Medicine, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand; 3) Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand; 4) Invermay Agricultural Centre, AgResearch, Mosgiel, Otago, New Zealand.

Background: Cystic renal diseases are among the most common genetically inherited disorders in humans, and frequently cause end-stage renal failure. All are associated with defects in primary cilia function. Autosomal recessive polycystic kidney disease (ARPKD) mainly affects infants and young children, and may be present in a number of different syndromes, including Meckel-Gruber Syndrome (MKS). We have identified a unique ovine model of MKS, and are characterising the ovine phenotype and genotype. Methods: New Zealand inbred Coopworth breed sheep carrying the disease allele were maintained for research purposes. Homozygosity mapping was chosen to map candidate disease loci. DNA isolated from 14 affected lambs and 1 carrier ram was analyzed using a recently developed single nucleotide polymorphisms (SNPs) Beadchip (SNP50; International Sheep Genomics Consortium / Illumina Inc, USA). Results & Discussion: Three candidate chromosomal regions were identified by homozygosity mapping, involving chromosomes 4, 9, and 11. The strongest candidate region on chromosome 9 contains the *TMEM67*/*MKS3* gene, which has been implicated in many ciliopathies, including Meckel-Gruber syndrome. The characteristic abnormalities of human infants with Meckel-Gruber syndrome match the phenotype in the affected sheep, except for absence of encephalocoele and polydactyly more commonly associated with *MKS1* mutations. Sequencing of all 28 exons of the ovine *TMEM67*/*MKS3* gene revealed two novel missense variants, both in exon 20, which were co-inherited in every affected lamb and known carrier sheep examined, but not observed in >30 unrelated Coopworth sheep. The exon 20 variants led to amino acid substitutions at two closely spaced isoleucine residues (I680N and I686S) in the predicted MKS3 protein, meckelin, at positions conserved across all vertebrate species, suggesting that the isoleucines play an important role in meckelin structure/function. Modelling of these sequence variants using structure/function prediction programmes (A-GVGD, SIFT, Polyphen) uniformly predict that either variant would have a deleterious effect on protein function. Meckelin is now thought to form part of a protein complex responsible for delivery and `gate keeping' of proteins destined for the primary cilium, and our on-going studies are aimed at determining whether a shift in the localization of meckelin from the plasma membrane has occurred in affected cells when compared with wild-type cells.

1152W

***NEK1* IS NOT RESPONSIBLE FOR ALL SHORT RIB POLYDACTYLY, MAJEWSKI TYPE OR FOR BEEMER LANGER CASES.** *J. EL HOKAYEM[1], C. HUBER[1], G. BAUJAT[1], C. BAUMANN[2], D. CALVACANTI[3], F. COLLINS[4], AL. DELEZOIDE[5], M. GONZALES[6], D. JOHNSON[7], M. LE MERRER[1], A. LEVY-MOZZICONACCI[8], D. MARTIN-COIGNARD[9], G. MORTIER[10], MJ. PEREZ[11], L. OLIVIER-FAIVRE[12], J. ROUME[13], G. SCARANO[14], C. THAU-VIN-ROBINET[12], A. MUNNICH[1], V. CORMIER-DAIRE[1].* 1) Université Paris Descartes, Inserm U781, Department of Genetics, Hôpital Necker, Paris, France; 2) Unité fonctionnelle de génétique clinique, Département de Génétique, CHU de Paris Hôpital Robert Debré, France; 3) Perinatal Genetic Programs, Department of Medical Genetic, FCM, UNICAMP, Campinas, SP, Brazil; 4) Western Sydney Genetics Program, Dept of Clinical Genetics, Children's, Hospital at Westmead, Sydney, Australia; 5) Service de Biologie de Développement, Université Paris Diderot, Hôpital Robert Debré, Paris, France; 6) Service de Génétique et d'Embryologie Médicales, Hôpital Armand Trousseau, Assistance Publique-Hôpitaux de Paris (AP-HP), Paris, France; 7) Sheffield Clinical Genetics Service, Sheffield Children's NHS Foundations Trust, Western Bank, England; 8) Laboratoire de Biochimie et Biologie Moléculaire, Hôpital Nord, Marseille, France; 9) Pôle de biopathologie, UF 3162, Centre hospitalier, LE MANS CEDEX,FRANCE; 10) Department of Medical Genetics, Antwerp University Hospital, Antwerp, Belgium; 11) Service de Génétique Médicale et Chromosomique, Centre de Référence Maladies Rares Anomalies du Développement et Syndromes Malformatifs Sud-Languedoc Roussillon, Hôpital Arnaud de Villeneuve, CHRU Montpellier, Université Montpellier 1, Faculté de Médecine; 12) Centre de Référence Anomalies du Développement et Syndromes Malformatifs Grand Est, Centre de Génétique, Hôpital d'Enfants, CHU Dijon, France; 13) Service de génétique médicale, centre hospitalier Poissy-Saint-Germain, France; 14) Department of Genetics, Hôpital du Mans, France.

The short rib-polydactyly (SRPS) group belongs to the ciliopathy spectrum and includes four distinct lethal autosomal recessive conditions (type I-IV) characterized by short ribs, inconstant polydactyly and variable malformations. Among them, Majewski syndrome or SRP type II (MIM 263520) is characterized by short tubular bones with smooth ends, pre- and postaxial polysyndactyly, and characteristic short ovoid tibiae. Other features include cleft lip/palate, malformed epiglottis and larynx, renal cysts, genital, cardiac and intestinal abnormalities. It is closely related to Beemer-Langer syndrome or SRP type IV (MIM 269860) which is distinguished by inconstant pre-axial polydactyly, short and often bowed long bones, tibia longer than fibulae and high frequency of brain defects. Recently, mutations in *NEK1* have been identified in three families with SRP type II. *NEK1* encodes a serine/threonine kinase with proposed function in DNA-double strand repair, neuronal development and coordination of cell-cycle-associated ciliogenesis. The aim of our study was to screen *NEK1* in SRP type II or type IV either by linkage analysis in consanguineous families or by direct sequencing in sporadic cases. Among the SRP type II cases, five were from consanguineous families originating from Lebanon, India, Madagascar, Tunisia, and six were non-consanguineous cases originating from Europe and Brazil. Among the SRP type IV cases, two were consanguineous originating from Tunisia and two were non-consanguineous cases originating from Europe. In three consanguineous SRP type II families, we identified three distinct *NEK1* mutations present in the homozygous state in the affected children including two missense mutations (c.433G>A; p.G145R) and (c.758T>C; p.L253S), and one frameshift mutation (c.2846_2847insGG-2847delT; p.D949EfsX6) respectively. These mutations cosegregated with the disease, were located in regions encoding conserved kinase and C domains and were not observed in 200 control chromosomes. Furthermore, *NEK1* was excluded in the other SRP II families and in all SRP type IV cases. A genome wide search is currently performed for two consanguineous families with SRP type II and type IV respectively. We conclude that *NEK1* is not the only gene responsible for SRP type II and is not involved in SRP type IV supporting locus heterogeneity in this group of disorders. Ongoing studies will hopefully identify other genes presumably also involved in ciliogenesis.

1153W

Strain-specific differences in perinatal viability support genetic modifiers in Bardet-Biedl syndrome. *R.L. Gottlieb[1], D. Nishimura[2,3], C. Searby[2,3], K. Bugge[2,3], V.C. Sheffield[2,3].* 1) Internal Medicine, University of Iowa Hospitals and Clinics, Iowa City, IA; 2) Pediatrics, University of Iowa Hospitals and Clinics, Iowa City, IA; 3) HHMI.

Bardet-Biedl Syndrome (BBS) is an autosomal recessive disorder caused by mutations in as many as 16 different genes. The cardinal feature of BBS are central obesity, retinal degeneration, developmental delay, renal defects, and polydactyly. BBS phenotypic modification via either complex inheritance or modifier loci has been suggested. Low weights at weaning precede progressive adult-onset obesity in several murine models of BBS. We report highly skewed non-Mendelian ratios of BBS2 knock-out mice ($Bbs2^{-/-}$) following seven generation backcrossing of heterozygous carriers to the C57/Bl6 murine strain, in contrast to nearly Mendelian ratios present in litters of $Bbs2^{-/-}$ mice on the 129 inbred murine strain, and intermediate skewing on the mixed background. The litters display Mendelian ratios late in utero, and non-Mendelian skewing occurs predominantly due to the neonatal death of $Bbs2^{-/-}$ mice between P0-P3. Together, these data indicate that the fitness of $Bbs2^{-/-}$ mice is dependent on genetic background effects consistent with inheritance of one or more modifier alleles. Our current data indicate that that the decreased viability of $Bbs2^{-/-}$ mice on the C57/Bl6 genetic background is neither due to an overt cardiopulmonary defect nor due to an overt defect in lateralit We have also begun similar experiments using a BBS1 knock-out model ($Bbs1^{-/-}$). We have previously created a knock-in mouse that harbors the BBS1 M390R mutation ($^{Bbs1M390R/M390R}$). Crossing of heterozygous mice Bbs1+/- was found to produce homozygous knockout offspring ($Bbs1^{-/-}$) at a level far below expected values (2.9%, n=173, $p=2e^{-8}$) at weaning. Necropsy of $Bbs1^{-/-}$ mice with spontaneous neonatal demise has not revealed any overt cardiovascular defect to-date. However, several mice have survived the first two weeks of life by which point a failure-to-thrive phenotype becomes overt with acquired microsomia. Echocardiography of a live microsomic pup indicates a structurally normal heart. We hypothesize that understanding the mechanism of the failure-to-thrive phenotype in these mice will lead to a better understanding of BBS related pathophysiology.

1154W

***PKD1* hypomorphic alleles act as major modulators of disease severity in Autosomal Dominant Polycystic Kidney Disease (ADPKD).** *K. Hopp[1], S. Rossetti[2], C.J. Ward[2], C.M. Heyer[2], J.L. Sundsbak[2], V.E. Torres[2], P.C. Harris[2].* 1) Dept. of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN; 2) Nephrology and Hypertension Research, Mayo Clinic, Rochester, MN.

ADPKD is a dominantly inherited kidney condition normally caused by single inactivating mutations to *PKD1*/*PKD2*. End stage renal disease (ESRD) typically occurs at 54y in PKD1 and 74y in PKD2. However, we recently described *PKD1* hypomorphic alleles that result in very mild PKD in heterozygotes and modify the typical disease to *in utero*/early onset disease when found in *trans* with a truncating mutation or a second pathogenic missense allele. To further investigate the role of hypomorphic alleles, we screened additional ADPKD families with atypical presentations (*in utero*, early, or late onset disease). This analysis identified a number of distinct *PKD1* genotypic patterns that likely explain the phenotype. In mild cases we found one or two likely hypomorphic alleles segregating with the disease, but rarely resulting in ESRD even in old age. Mutations in this group include L2816P and co-segregation of I3167F/R3277H. Two alleles in *cis*, such as V609G and N970K, can also result in typical ADPKD. In two *in utero* onset cases the likely hypomorphic alleles (S4189F or R4276W) were found in *trans* with a truncating mutation. A similar arrangement with a weaker hypomorphic allele, G2452C, resulted in ESRD at 25y. These studies indicate that hypomorphic alleles either as pathogenic mutations or modifiers often significantly modulate ADPKD severity. To further validate the concept and to understand the mechanism of disease, we generated a mouse knock-in model of one of the initially identified hypomorphic alleles, *PKD1* R3277C. Compound heterozygous animals ($Pkd1^{C/-}$) mimic *in utero* ADPKD, presenting with progressive cystic disease initiated by E16.5d and resulting in grossly enlarged kidneys by 1 month. In contrast, homozygous mice ($Pkd1^{C/C}$) present with mild disease including slightly but significantly enlarged kidneys with a few medium sized cysts at 3 month of age. Ultrasound analysis of these animals highlights the slow but progressive disease development, similar to typical ADPKD patients. We further developed an *in-vitro* system to study the pathobiology of these alleles in respect to protein folding, function and localization. Initial analysis shows that the pathobiology of the *PKD1* R3277C allele is not associated with disrupted membrane integration or protein cleavage. Together, our data highlights that the level of functional protein is key to disease development/severity. This is of clinical importance for the molecular diagnostics and prognostic of ADPKD.

1155W

Next-generation exon re-sequencing of 86 ciliopathy candidate genes in 480 individuals using a DNA pooling strategy. *E.A. Otto[1], J.L. Innis[1], F. Hildebrandt[1,2,3].* 1) Dept Ped, Univ Michigan, Ann Arbor, MI; 2) Dept Hum Genet, Univ Michigan, Ann Arbor, MI; 3) Howard Hughes Medical Institute.

Background: Nephronophthisis-related ciliopathies (NPHP-RC) comprise a group of autosomal recessive cystic kidney diseases that include nephronophthisis (NPHP), Senior-Loken-(SLS), Joubert-, and Meckel syndrome. 23 causative genes have been identified, rendering Sanger re-sequencing tedious and expensive. To overcome this problem we devised a DNA pooling and NextGen sequencing (NGS) strategy. **Methods:** We performed mutation analysis in 480 individuals with NPHP-RC by exon re-sequencing on a Illumina HighSeq next generation sequencer platform. 1,400 exons derived from 23 known NPHP-RC genes (*NPHP1-NPHP11, AHI1, ARL13B, TTC21B, TMEM216, TMEM138, CC2D2A, INPP5E, TCTN1, TCTN2, ATXN10, XPNPEP3, MKS1*) and 63 ciliopathy candidate genes were resequenced. We generated 5 equimolar DNA-pools derived from 96 patients each, and 1 pool of 96 control individuals as PCR templates. PCR products were enzymatically processed using DNaseI- and exonuclease BAL-31 to generate small random fragments followed by library preparation and paired-end sequencing of 100 bases on 6 lanes of an Illumina HighSeq platform. Alignment of sequence reads to the human reference genome and variant/mutation calling were performed using CLC Genomics-Workbench™ software. A mutant frequency below 0.3% or a nucleotide coverage below 1000X were used as a cutoff. Potential mutation carriers were identified by MALDI-TOF analysis (Sequenom™) or by direct Sanger sequencing of all 96 patients from the respective DNA-pool. **Results:** NGS (6 lanes) generated about 1 billion reads (100 Gb). The median coverage per exonic nucleotide was 27,183 (mean: 38,873). We identified 25 loss-of-function mutations e.g. in *NPHP1* (M1V, E38X), *INVS* (2069-1G>T), *NPHP3* (R1207X, R1259X), *NPHP4* (R59X, 1763+1G>A), *IQCB1* (R332X, R461X, R364X, W444X, R445X), *CEP290* (Q662X, R751X, Q1268X, K1575X, 3103-2A>G), *RPGRIP1L* (A1183G), *SDCCAG8* (E262X, Q371X), *TMEM67* (R198X), *AHI1* (R891X), *CC2D2A* (337-2A>G), *XPNPEP3* (793-2A>G), and *TTC21B* (W119X). Furthermore, we found 108 different missense variants of unknown significance. Mutation analysis in 63 additional candidate genes revealed further nonsense- (3), frameshift- (2), obligatory splice site- (3), and start codon mutations (2) in seven of these novel ciliary/centrosomal candidate genes. **Conclusions:** The combined approach of DNA pooling followed by NGS facilitates mutation analysis in broadly heterogeneous single-gene disorders and accelerates identification of novel disease genes.

1156W

Primary Ciliary Dyskinesia Caused by Homozygous Mutation in DNAL1 Encoding Dynein Light Chain 1. *R. Parvari[1,3], M. Mazor[1], S. Alkrinawi[2], V. Chlifa-Caspi[3], E. Manor[4], V.C. Sheffield[5], M. Aviram[2].* 1) Department of Virology and Developmental Genetics, Ben Gurion University of the Negev, Beer Sheva, Israel; 2) Division of Pediatrics, Soroka University Medical Center, Beer-Sheva, 84101 Israel; 3) National Institute of Biotechnology in the Negev, Ben Gurion University of the Negev, Beer Sheva 84105, Israel; 4) Institute of Genetics, Soroka Medical Center, Beer Sheva 84105 and Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva 84101, Israel; 5) Department of Pediatrics - Division of Medical Genetics, Howard Hughes Medical Institute, University of Iowa, Iowa City, IA 52242, USA.

Genetic defects affecting motility of cilia and flagella cause chronic destructive airway disease, randomization of left-right body asymmetry, and, frequently, male infertility in primary ciliary dyskinesia (PCD). The most frequent defects involve outer and inner dynein arms (ODAs and IDAs) that are large multiprotein complexes responsible for cilia-beat generation and regulation, respectively. Although long suspected that mutations in DNAL1 encoding the outer arm dynein light chain1 may cause PCD such mutations were not found.We demonstrate here that a homozygous point mutation in this gene is associated with PCD with absent or markedly shortened outer dynein arms. The mutation (NM031427.3: c.449A>G; p.Asn150Ser) changes the Asn at position150 which is critical for the proper tight turn between the beta strand and the alpha helix of the leucine rich repeat in the hydrophobic face that connects to the dynein heavy chain. The mutation reduces the stability of the axonemal dynein light chain 1 and damages its interactions with dynein heavy chain and with tubulin. This study adds another important component to understanding the types of mutations that cause PCD and provides clinical information regarding a specific mutation in a gene not yet described to be associated with PCD.

1157W

Mutations of NEK1 cause short rib-polydactyly syndrome type Majewski with defective Ciliogenesis. *C.T. Thiel[1], K. Kessler[1], A. Giessl[2], A. Dimmler[3], S.A. Shalev[4, 5], S. von der Haar[6], M. Zenker[7], D. Zahnleiter[1], H. Stoess[8], E. Beinder[9], R. Abou Jamra[1], A.B. Ekici[1], N. Schroeder-Kress[2], T. Aigner[10], T. Kirchner[11], A. Reis[1], J. Brandstaetter[2], A. Rauch[12].* 1) Institute of Human Genetics, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany; 2) Animal Physiology, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany; 3) Institute of Pathology, St. Vincentius Hospital, Karlsruhe, Germany; 4) Genetic Institute, Emek Medical Center, Afula, Israel; 5) Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel; 6) Private Clinic Kossakiewicz, Nuremberg, Germany; 7) Institute of Human Genetics, Otto-von-Guericke University Magdeburg, Magdeburg, Germany; 8) Institute of Pathology, St. Johannisstift, Paderborn, Germany; 9) Department of Obstetrics, University Hospital, Zurich, Switzerland; 10) Institute of Pathology, University of Leipzig, Leipzig, Germany; 11) Institute of Pathology, Ludwig-Maximilians University Munich, Munich, Germany; 12) Institute of Medical Genetics, University of Zurich, Schwerzenbach-Zurich, Switzerland.

Defects of ciliogenesis have been implicated in a wide range of human phenotypes and play a crucial role in different signal transduction pathways and cell cycle coordination. We thoroughly delineated the clinical, radiographic, and histological phenotype of the autosomal-recessive short-rib polydactyly syndrome Majewski type (SRPS II). Parametric multipoint linkage and haplotype structure analysis confirmed a linked interval with a maximum LOD score of 2.95 on chromosome 4. Assuming an overlap of the phenotypic spectrum of our patients with other ciliopathies, we used known genes of the cilia proteome database and compared them with the genes in our candidate interval. The NIMA-related kinase 1 (NEK1) gene was highlighted as homozygous mutant mice show polycystic kidney disease, craniofacial anomalies, and growth reduction. Sequencing of NEK1 identified nonsense, splice-site and frame shift mutations in patients with SRPS II. NEK1 encodes a serine/threonine kinase with proposed function in DNA double-strand repair, neuronal development, and coordination of cell cycle-associated ciliogenesis. With immunofluorescence analyses we confirmed that the absence of functional NEK1 significantly decreases the number of ciliated fibroblasts and alters the structural morphology of the primary cilium in vivo as well. Transmission electron microscopy indicated a defect in progression from stage I after vesicular accumulation of ciliogenesis to stage II and subsequent failure of axoneme growth. Furthermore, the effect of the known SRPS genes, DYNC2H1 and IFT80, and NEK1 on the cilia associated pathways in siRNA experiments explained the phenotypic overlap of different SRPSs. We further substantiate a proposed digenic diallelic inheritance of ciliopathies by identification of heterozygous mutations in NEK1 and DYNC2H1 in a further non-consanguineous family.

1158W

***KIF7* (*Costal2*) mutations cause fetal Hydrolethalus and Acrocallosal syndromes.** *S. Thomas[1,2], A. Putoux[1,2], K.L.M. Coene[3], E.E. Davis[4,5], Y. Alanay[6], G. Ogur[7], A. Munnich[1,2,8], V. Cormier-Daire[1,2,8], R. Hennekam[9], E. Colin[10], N.A. Akarsu[11], S. Lyonnet[1,2,8], F. Encha-Razavi[1,2,8], J.P. Siffroi[12,13], M. Winey[14], N. Katsanis[4,15], M. Gonzales[12,13], P.L. Beales[16], M. Vekemans[1,2,8], T. Attié-Bitach[1,2,8].* 1) INSERM U781, Paris, France; 2) Université René Descartes, Paris, France; 3) Radboud University Nijmegen Medical Centre, Department of Human Genetics, Nijmegen, The Netherlands; 4) Departments of Cell Biology and Pediatrics, Center for Human Disease Modeling, Duke University, Durham, NC, USA; 5) Department of Pediatrics, Duke University Medical Center, Durham, NC, USA; 6) Pediatric Genetics Unit, Department of Pediatrics, Hacettepe University Medical Faculty, Ankara, Turkey; 7) Ondokuz Mayis University, Department of Medical/Pediatric Genetics, Samsun, Turkey; 8) Département de Génétique, Hôpital Necker-Enfants Malades, AP-HP, Paris, France; 9) Department of Pediatrics, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands; 10) Service de Génétique, Hôpital d'Angers, France; 11) Gene Mapping Laboratory, Department of Medical Genetics, Hacettepe University Medical Faculty, Ankara, Turkey; 12) Service de Génétique et d'Embryologie Médicales, Hôpital Armand Trousseau, Paris, France; 13) Université Pierre et Marie Curie, Paris 6, France; 14) MCDB, UCB 347, University of Colorado at Boulder, Boulder, CO, USA; 15) Departments of Cell Biology and Pediatrics, Center for Human Disease Modeling, Duke University, Durham, NC 27710, USA; 16) Molecular Medicine Unit, University College London (UCL) Institute of Child Health, London, UK.

KIF7, the human ortholog of *Drosophila Costal 2*, represents a key component of the Hedgehog (Hh) signaling pathway which in vertebrates is dependent upon primary cilium. Here we report mutations in *KIF7* in 11 unrelated individuals with two overlapping disorders, the rare Hydrolethalus (HLS, OMIM 236680) and Acrocallosal syndromes (ACLS, OMIM 200990) sharing polydactyly, midline brain and facial abnormalities confirming these two syndromes as members of the ciliopathy group, and extending the phenotypic spectrum of ACLS. Transcriptome-wide analysis on *KIF7* mutated patient RNA showed a specific up-regulation of most of the known Gli transcription factor target genes associated with impaired GLI3 processing and consistent with a major role of KIF7 in the transduction of the Hh signaling pathway in humans. *KIF7* is also a likely contributor of alleles across the ciliopathy phenotypic spectrum; sequencing of a cohort of patients with diverse ciliopathies revealed several heterozygous missense changes which, upon *in vivo* testing in zebrafish were shown to be detrimental to protein function. Furthermore, consistent with an epistatic role for such alleles, *in vivo* genetic interaction studies in zebrafish indicated that *KIF7* hypomorphic alleles could exacerbate the phenotype induced by the loss of other ciliopathy loci. Our data demonstrate the conserved role of KIF7 in human primary cilia to ensure the transduction of the Hedgehog signaling pathway through the regulation of Gli transcription factor activity and expand the clinical spectrum of ciliopathies.

1159W

The MDM2/MDMX complex is required for control of p53 activity in vivo. *L. Huang[1], Z. Yan[1], X.D. Liao[1], Y. Li[1], J. Yang[1], Z.G. Wang[1,2], Y. Zuo[3], H. Kawai[4], M. Shadfan[5], S. Ganapathy[5], Z.M. Yuan[5].* 1) Dept Med Gen, Shanghai Jiaotong Univ Sch Med, Shanghai 200025 , China; 2) Shanghai Research Centre for Model Organisms, Shanghai 201203, China; 3) Department of Biochemistry and Molecular and Cell Biology, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China; 4) Institution of Radiation Research, Hiroshima University, Hiroshima 732-0068, Japan; 5) Department of Radiation Oncology, UTHSCSA, Texas 78229 USA.

There are currently two distinct models proposed to explain why both MDM2 and MDMX are required in p53 control, with a key difference centered on whether these two p53 inhibitors work together or independently. To test these two competing models, we generated knockin mice expressing a point-mutation MDMX mutant (C462A) that is defective in MDM2 binding. This approach allowed a targeted disassociation of the MDM2/MDMX heterocomplex without affecting either the ability of MDMX to bind to p53 or the MDM2 protein itself at all. Significantly, MdmxC462A/C462A homozygous mice died at around day 9.5 of embryonic development, resulting from a combination of apoptosis and decreased cell proliferation, as shown by TUNEL and BrdU incorporation assays, respectively. Interestingly, even though the MDMX mutant protein abundance was found elevated in the MdmxC462A/C462A homozygous embryos, both the abundance and activity of p53 were markedly increased. A p53-dependent death was demonstrated by the finding that concomitant deletion of p53 completely rescued the embryonic lethality in MdmxC462A/C462A homozygous mice. Our data demonstrates that MDM2 and MDMX function as an integral complex in p53 control, providing new insights into the nonredundant nature of the function of MDM2 and MDMX.

1160W

Analysis of splice variants of the *FANCC* gene and their impact in FANC-BRCA DNA repair pathway. *S. Bélanger, C. Joly Beauparlant, C.S. Pedneault, N. Litim, O. Ouellette, Y. Labrie, F. Durocher, INHERIT.* Cancer Genomics Laboratory, CHUL Research Centre, CHUQ, Laval University, Quebec, Canada.

Recent attention and research efforts have been drawn toward the Fanconi Anemia gene family due to their implication in what is known as the FANC-BRCA DNA repair pathway. Until now, 15 genes have been identified in the FA family and they are all essential for its proper functioning. Regarding breast cancer, three FA genes are now known to be responsible for an increase in the risk of developing breast cancer : *FANCD1/BRCA2*, FANCJ/BRIP1 and *FANCN/PALB2*. Following the sequencing of the complementary DNA (cDNA) of the gene *FANCC* in high-risk French-Canadian women and unrelated, unaffected women from the same population, at least three distinct splicing events were observed. Of these transcript variants, one is the skipping of exon 7 (*FANCCdel7*) and another is the insertion of an intronic fragment between the exon 4 and 5 (*FANCCins4a*). RT-PCR demonstrated the presence of these variants in the majority of tested tissues and cell lines. Moreover, none of these splicing events seemed to be affected by nonsense-mediated mRNA decay. Since the *FANCCdel7* variant does not alter the open reading frame, localization testing was performed with the vector pEGFP and demonstrated that contrary to the wild type FANCC protein, the variant FANCCdel7 is not recruited to the nucleus following treatment with a potent DNA crosslinker like mitomycin C. The optimization of the minigene technique allowed to clarify the impacts of different genomic variants in the skipping event of the 7th exon of *FANCC*. Monoubiquitylation experiments were performed with the same variant in order to evaluate its implication in the DNA repair pathway. Other experiments such as coimmunoprecipitation and colony survival assays are currently underway and will help to unravel the different effects that the different alternative transcripts of *FANCC* may have on the FANC-BRCA DNA repair pathway and in the maintenance of genomic integrity.

1161W

Analysis of FANCA sequence and splicing variants in French Canadian women with high risk of breast and ovarian cancer. *F. Durocher, Y. Labrie, S. Desjardins, N. Litim, G. Ouellette, INHERIT BRCAs.* Cancer Genomics Lab, CHUQ Research Center (CHUL) and Laval University 2705, Laurier Blvd Quebec, PQ, Canada.

Sequence alterations in FANCA gene account for approximately 60-70% of Fanconi Anemia (FA) cases, a rare recessive disease characterized by bone marrow failure, genomic instability and predisposition to cancer. The majority of genes associated with breast cancer susceptibility, including BRCA1 and BRCA2 genes, are involved in DNA repair mechanisms. Moreover of the four genes recently associated with an increase susceptibility to breast cancer, three are FA genes: FANCD1/BRCA2, FANCJ/BACH1/BRIP1, and FANCN/PALB2. FANCA is implicated in DNA repair and has been shown to directly interact with BRCA1. It has been proposed that the formation of FANCA/G (regulated by the phosphorylation of FANCA) and FANCB/L sub-complexes altogether with FANCM, represent the initial step for DNA repair activation and subsequent formation of other sub-complexes leading to ubiquitination of FANCD2 and FANCI. As only approximately 25% of inherited breast cancers are attributable to BRCA1/2 mutations, FANCA therefore becomes an attractive candidate for breast cancer susceptibility. We thus analyzed FANCA gene in high-risk French Canadian non-BRCA1/2 breast cancer individuals as well as in healthy control individuals from the same population. Among a total of 85 sequence variants found in both series, 26 are coding variants and 17 are missense variations leading to amino acid changes. Three of the amino acid changes, namely Thr561Met, Cys625Ser and particularly Ser1088Phe, which has been previously reported to be associated with FA, are predicted to be damaging by the SIFT and PolyPhen softwares. Haplotype analyses using variants having a frequency >5% or coding variants, identified 9 and 7 haplotypes, respectively, with a frequency >3%. In both analyses, specific haplotypes were significantly over-represented in one sample set while global haplotype analyses led to significant Fisher's p-values (0.01 for MAF>5%; 0.0006 for coding variants). cDNA amplification revealed significant expression of 4 alternative splicing events (ASEs): one leading to the insertion of an intronic portion of intron 10, and the skipping of exons 11, 30 and 31. In silico analyses of relevant genomic variants have been performed in order to identify potential variations involved in the expression of these ASEs. Sequence variants in FANCA could therefore be potential spoilers of the Fanconi-BRCA pathway and as a result, they could in turn have an impact in non-BRCA1/2 breast cancer families.

1162W

Functional implications of the FancLdel4 splice variant on DNA repair in the FANC-BRCA pathway. *C.S. Pedneault, S. Belanger, G. Ouellette, K. Plourde, N. Litim, Y. Labrie, F. Durocher, INHERIT.* Cancer Genomics Laboratory, CHUL Research Center, CHUQ, Laval University, Quebec, Canada.

The 15 known Fanconi proteins (*FANCA, B, C, D1, D2, E, F, G, I, J, L, M, N, O, P*) function altogether to repair DNA interstrand cross-links (ICLs) in response to DNA damaging agents. Biallelic mutation carriers for a single FANC gene suffer from Fanconi Anemia, while a monoallelic mutation can confer breast cancer susceptibility. This type of association has already been demonstrated for the *FANCD1*/*BRCA2*, *FANCJ*/*BRIP1*, *FANCN*/*PALB2* and *RAD51C*/*FANCO* genes. FANC2 monoubiquitilation activates the recruitment of the DNA repair proteins at the ICL lesions, thus making this step the key event of the Fanconi pathway. The monoubiquitilation is catalyzed by FANCL, which is the only known Fanconi protein to possess an ubiquitin ligase activity. A *FANCL* splicing variant showing exon 4 skipping has been isolated and is expressed in several breast cancer and Fanconi-deficient cell lines. The deletion does not alter the reading frame, which most probably makes the transcript unaffected by nonsense-mediated decay (NMD) and can indeed be over-expressed in human cells by transfection. In silico analysis shows that 4 of the 19 amino acids are perfectly conserved in all species down to the drosophilia, and 5 others are semi-conserved, thus suggesting an essential function to this portion of the protein. To this day, results obtained with FANCL-GFP fusion proteins show that the alternative form loses its capacity to translocate to the nucleus, where it normally performs its activities. A minigene assay will allow to evaluate the contribution of certain sequence variants to exon skipping in the exon 4 region. Co-immunoprecipitation experiments and monoubiquitilation studies are in progress to better understand the functions of this new splicing variant.

1163W

Molecular signatures of Tissue Inhibitor of Metalloproteinases-1 over-expression in breast cancer cells. *K.C. Belling[1], C.A. Bjerre[2], L. Vinther[2], L. Fogh[2], K. Do[1], A.S. Rasmussen[2], U. Lademann[2], W. Jun[3], L. Bolund[3], N. Brünner[2], J. Stenvang[2], R. Gupta[1].* 1) Center for Biological Sequence Analysis, Technical University of Denmark, Kgs Lyngby, Denmark; 2) Sino-Danish Breast Cancer Research Centre at Faculty of Life Sciences, University of Copenhagen, Copenhagen, Denmark; 3) Beijing Genomics Institute, Shenzhen, China.

Tissue Inhibitor of Metalloproteinases-1 (TIMP-1) is present in many breast tumour cells and possesses several cancer-promoting functions such as stimulation of growth and inhibition of apoptosis. Lack of TIMP-1 has in clinical studies been shown to predict increased sensitivity to the topoisomerase 2 inhibitor epirubicin in adjuvant treatment of breast cancer. Also, low plasma TIMP-1 levels have been shown associated with increased benefit from the topoisomerase 1 inhibitor irinotecan in patients with metastatic colorectal cancer. The molecular reasons behind these observations are still unclear. In order to gain detailed insight into the molecular mechanisms of TIMP-1 interference with benefit from these drugs, we transfected MCF-7 human breast cancer cells with a plasmid conferring TIMP-1 expression. Twelve clones representing different levels of TIMP-1 expression and protein levels were applied to Agilent whole human genome oligo microarrays 4x44K. The data were analyzed using the limma R/Bioconductor package. Transcripts with expression correlating either negatively or positively with TIMP-1 expression were identified using linear regression. A subset of transcripts highly correlated with TIMP-1 expression has been identified. A systems biological analysis of these genes is currently ongoing, including analysis of pathways and protein-protein interaction complexes. The combined analyses should provide insights into how TIMP-1 affects cancer cells and response to topoisomerase inhibitors. Data presented here will focus on the transcriptomics and its network biology interpretation.

1164W

Detection of viral sequences in cancer transcriptomes using high-throughput sequence analysis. *I. Borozan[1], S. Wilson[1], A.M.K. Brown[1], P. Laflamme[1], S. Gnaneshan[2], R. Rottapel[1], J.D. McPherson[1], V. Ferretti[1].* 1) Ontario Institute for Cancer Research, Toronto, Ontario, Canada; 2) Ontario Agency for Health Protection and Promotion, Toronto, Ontario, Canada.

In a recent worldwide study by the World Health Organization it was estimated that 18% of human cancers were caused by infection, of these 12% have been shown to be of viral origin. For example, the papillomavirus HPV is now known to be responsible for 70% of cervical cancers, which represents 500,000 new cases per year worldwide. The discovery of new human cancer viruses is still challenging despite the advance of molecular technologies. Recently novel computational methods have been successfully applied to the discovery of new pathogens. Here we present an innovative genome-wide computational pipeline for identifying both exogenous and endogenous viral nucleotide sequences in tumor genomes and transcriptomes with the ultimate goal to elucidate new viruses that may cause human cancer.

The pipeline is composed of four main components: the digital transcriptome subtraction pipeline for both the Illumina and SOLiD sequencing platforms written in R, a suite of R based statistical tools for assessing the statistical significance of viral genomes hit, a high-performance database for storing alignment results and sequence annotations, and a web application, called Xeno, for the visualization (using GBrowse), query and analysis of aligned reads. The digital transcriptome subtraction was implemented to subtract in silico known human sequences from cancer transcriptome datasets leaving candidate non-human sequences for further analysis. Reads from each sample within each cancer type are first filtered according to their mapping quality metrics and then the coverage across known viral sequences and their gene coding regions is calculated. Viruses that show hits are ranked according to the degree of coverage across their nucleotide sequences.

We demonstrate the effectiveness of our pipeline by applying it to a total of 56 transcriptome samples from different cancer types sequenced by the OICR's Cancer Genomics Group. Our results indicate the presence of a number of significant hits across both samples and viruses. We further discuss the biological significance of our results in terms of the cancer types and coverage across relevant viral gene coding regions. The new pipeline will be released as freely available software under the GNU General Public License.

1165W

Whole genome sequencing of tumor-normal pairs from 5 African Americans with late stage or metastatic prostate cancer. *N.J. Cardin[1], R. Kazma[1], J.A. Mefford[1], T.J. Hoffmann[1], A. Levin[2], D. Chitale[2], B. Rybicki[2], J.S. Witte[1].* 1) Epidemiology and Biostatistics, University California San Francisco, San Francisco, CA., California; 2) Henry Ford Hospitals, Detroit, MI.

Prostate cancer is a common but complex disease, with 186,000 new cases and 28,600 deaths in 2008 [1]. African-American men have the highest incidence and mortality of this disease worldwide. Deciphering the genomic basis for this high disease burden, and why some tumors progress rapidly while others remain relatively latent, are critical to understanding the biology and appropriate therapy for this disease. Previous work has detected numerous somatic mutations in prostate tumors. This includes the recent sequencing of seven prostate tumor-normal pairs in European-American men which found that genomic rearrangements may be related to aberrations in chromatin or transcription patterns [2].

Here we present results from sequencing five paired prostate tumor-normal samples from African-American men with metastatic or very late stage disease. Preliminary data show ~20,000 somatic SNPs and indels per sample, with ~2,000 in or near genes. Of these close to 10 per sample are non-synonymous, including some frame shifts, insertions and nonsense mutations. We also find approximately 50 somatic SNPs and indels per sample in or near known cancer associated genes. Interestingly, we observe a ~4 fold enrichment of these in genes that contained non-synonymous mutations in [2].

We also find multiple large scale somatic copy number changes, including events affecting whole chromosome arms. In particular, the loss of chromosome arm 8p, which harbors known cancer suppressor genes. We also observe a large deletion on chromosome 17 between 5.6Mb and 8Mb; a region which harbors TP53, a major cancer suppressor gene. In addition to these copy number changes we observe multiple rearrangements, including balanced rearrangements both within and across chromosomes.

These genome-wide re-sequencing data allow detection at greater resolution of a range of mutations, while confirming previous reports of numerous loss of function and large scale genomic changes in prostate cancer samples.

[1] Ahmedin J., et. al. “Cancer Statistics, 2008”. CA Cancer J Clin 58 (2): 71-96 (March 2008)

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1166W

Amplified Haplotype Calling in Next Generation Tumor Sequence Data. *N. Dewal[1], Y. Hu[2], M. Freedman[3,4], T. LaFramboise[5], I. Pe'er[2].* 1) Department of Biomedical Informatics, Columbia Univ, New York, NY; 2) Department of Computer Science, Columbia University, New York, NY 10027, USA; 3) Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA 02115, USA; 4) Medical and Population Genetics Program, The Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA; 5) Department of Genetics, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA.

During tumor initiation and progression, cancer cells acquire a selective advantage, allowing them to out-compete their normal counterparts. Identification of the genetic changes that underlie these tumor acquired traits can provide deeper insights into the biology of tumorigenesis. Regions of copy number alterations and germline DNA variants are some of the elements subject to selection during tumor evolution. Integrated examination of inherited variation and somatic alterations holds the potential to reveal specific nucleotide alleles that a tumor “prefers” to have amplified. Next-generation sequencing of tumor and matched normal tissues provides a high-resolution platform to identify and analyze such somatic amplicons. Within an amplicon, examination of informative (e.g. heterozygous) sites deviating from a 1:1 ratio may suggest selection of that allele. A naïve approach examines the reads for each heterozygous site in isolation; however, this ignores available valuable linkage information across sites. We therefore propose a novel Hidden Markov Model-based method - Haplotype Amplification in Tumor Sequences (HATS) - that analyzes tumor and normal sequence data, along with training data for phasing purposes, to infer amplified alleles and haplotypes in regions of copy number gain. Our method is designed to handle rare variants, somatic mutations, and biases in read data. We assess the performance of HATS using simulated amplified regions generated from varying copy number and coverage levels, followed by amplicons in real data. We demonstrate that HATS infers the amplified alleles more accurately than does the naïve approach, especially at low to intermediate coverage levels and in real tumor data containing stromal contamination.

1167W

GST an emerging threat to north Indian population for head and neck squamous cell carcinoma: A meta analysis of case control studies. *S. Gupta[1], A. Srivastava[2], M.C. Pant[3], B. Mittal[2], P. Seth[1].* 1) Bioinformatics, Biotech Park, Lucknow, India; 2) Department of Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow,Uttar Pradesh, India; 3) Department of Radiotherapy, C. S. M. Medical University, Shahmina Road, Lucknow, Uttar Pradesh, India.

BACKGROUND: Genetic polymorphisms in Glutathione S-transferase (GSTT1null, GSTM1null, and GSTP1 Ile105Val) results in the toxification and detoxification of (pre)-carcinogens, which plays an important role in progression of not only head and neck squamous cell carcinoma (HNSCC) but also in second primary malignancy after index HNSCC and to individual susceptibility to HNSCC. GSTP1 is markedly over-expressed in case of smoking related HNSCC as compared to other cancers. STUDY DESIGN: The purpose of the present study was to determine the outcome of the metabolic GST genes polymorphism and interaction with environmental factors on susceptibility to the risk of HNSCC. SEARCH CRITERIA: Electronic searches for comprehensive literature PubMed, Springer, EBSCO and iLibrary were undertaken up to April 2011. References lists of the selected papers were also screened to collect missing potential articles. SELECTION CRITERIA:Case-control studies were selected to examine the association of HNSCC with GSTT1, GSTM1null and polymorphism in GSTP1Ile105Val gene. The genotype distribution of these polymorphisms were described in details and the status were determined by using molecular methods of PCR or PCR-Restriction Fragment Length Polymorphism (PCR/PCR-RFLP). Moreover, the results were expressed as odds ratio (OR) and corresponding 95% confidence interval (95% CI), were included. DATA COLLECTION AND ANALYSIS: Total 110 studies regarding GSTT1null, GSTM1null and GSTP1with respect to HNSCC were identified after careful review, irrelevant 89 papers were excluded and finally 12 concerning GSTT1null, 11 concerning GSTM1null and 9 concerning GSTP1 were included in the anaysis. To estimate more precise consequence of the association, a meta-analysis has been performed for 24 appropriate case-control studies including 6709 cases and 7302 controls. Crude odds ratios (ORs) with 95% CI were used to assess the strength of the association. RESULTS: Our result suggests that GSTM1null (OR: 1.046; 95% CI: 0.942-1.161, P=0.402) and GSTT1null (OR: 1.011; 95% CI: 0.885-1.155, P=0.872) are associated with an increase in risk of HNSCC patient where as GSTP1 Ile105Val polymorphism (OR: 0.786; 95% CI = 0.590-1.047, P=0.072) was not associated with altered susceptibility to HNSCC. Whereas, In Indian population, we found that there is a significant association of GSTP1Ile105Val polymorphism with HNSCC due to higher content of Benzo[a]pyrenediol consumption through chemicals.

1168W

Meta-analysis of genome-wide mRNA expression profiles with computational analysis identifies miR-x and miR-y associated with recurrence of colorectal cancer. *E. Hsi[1,2,3], T. Kuo[2,4], I. Yang[2,4], P. Tsai[2,4], S. Juo[2,3,4].* 1) Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 3) Cancer Center, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 4) Department of Medical Genetics, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.

Colorectal cancer (CRC) is one of the leading malignant cancers with the rapid increase in incidence and mortality in Han Chinese. The recurrences of CRC are sometimes unavoidable and half of them will recur in the first year. MicroRNAs can act as biomarkers to predict early recurrence of CRC, but identifying them from over 1000 microRNAs is challenging and time-consuming. In this study, we systematically searched and extracted four studies (GSE12032, GSE17538, GSE4526 and GSE17181) for mRNA expression data of CRC from gene expression omnibus (GEO). We identified miR-x and miR-y using the IMRE method with miRbase that includes 568,071 miRNA-target connections between 711 miRNAs and 20,884 gene targets. We also performed meta-analysis and showed that the two miRs are highly significant based on the fisher p-value combination (p=9.14×10-9 for miR-x and p=1.14×10-6 for miR-y). Further more, we used our human CRC samples (46 CRC patients with early recurrence and 38 patients without early recurrence) to validate the result (p=0.0007 for miR-x and p=0.0033 for miR-y). Our empirical results showed that the two miRs are significantly downregulated in the recurrent CRC patients. This study indicated that the miR-x and miR-y could be an important tumor suppressor of CRC and potential targets for treatment.

1169W

Identification and differentiation between driver and passenger mutation applying clustering algorithm on next gen sequencing data. *S. Karmakar[1], A. Ghosh[1], A. Basu[2].* 1) Indian Statistical Institute, Kolkata, India; 2) National Institute of BioMedical Genomics, Kalyani, India.

Identification of driver mutations in cancer cells is a challenging endeavor. We hypothesize that these driver mutations arise early in the original cancer cells providing it a selective advantage to form distinct clones. Next generation sequencing data provides an opportunity to de-construct the allele frequency spectrum for all somatic mutations in a particular tumor. Partitioning the allele frequency spectrum in distinct clusters hence can provide an idea of the number of clones present in the tumor cell mass. We here simulate data that mimics the output of tumor sequence data as generated on the 454 platform (Roche Sequencing) and applied AIC and BIC model selection and clustering algorithms methods to develop a model delineating driver and passenger mutations.

1170W

Dealing with Sample Aneuploidy and Mosaicsm Using the ASCAT Algorithm on Different SNP Array Platforms. *R. Keshavan, S. Verma, S. Shams.* BioDiscovery Inc, El Segundo, CA.

Cancer samples pose a challenging problem for analysis using microarray technology. The basic Array CGH (aCGH) approach can confidently detect changes in copy number when a sample is measured against a normal control. However, the necessary normalization pre-processing step will essentially wash away the information associated with hyperdiploid samples. This can be an issue especially with cancer samples. Additionally, mosaic samples, due to different mixture of cells in the sample, can also severely complicate the analysis and interoperation of samples with microarray technology. One of the recently proposed approaches to simultaneously deal with these issues is called the Allele Specific Copy Number Analysis of Tumors (ASCAT) algorithm[1]. The approach takes advantage of the SNP information from SNP microarrays to combine the change in total signal strength (log-R value) with the B-Allele Frequency (BAF) to create an optimization function that has local minimas at values corresponding to possible sample ploidy and percent normal contamination. Here we demonstrate an efficient implementation of the ASCAT algorithm on processing cancer samples generated by The Cancer Genome Atlas (TCGA) project. Using Affymetrix and Illumina SNP array data, results are compared between the different platforms as well as with and without application of the ASCAT algorithm. Characterization of a percentage of the cells as normal contaminants within the samples using the ASCAT algorithm reveals many more gains and losses in the aberration profile of the tumors than in samples processed without ASCAT. ASCAT also identified that a large percentage of samples contain high ploidy numbers. With the sample ploidy and extent of normal contamination identified using ASCAT, clearer and more accurate calls can be made for cancer samples.

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1171W

The European Genome-phenome Archive (EGA). *I. Lappalainen, J. Almeida-King, V. Kumanduri, P. Marin-Garcia, P. Flicek.* European Bioinformatics Institute, Cambridge, United Kingdom.

The European Genome-phenome Archive (EGA), a service of the European Bioinformatics Institute (EBI), provides a permanent archive for all types of potentially identifiable genetic and phenotypic data generated in the course of biomedical or genetics research. The EGA contains data collected from individuals when the applicable consent agreements prevent open, public data distribution. As of 2011, data from more than 60 studies and 100 000 samples have been submitted to the EGA and are available for researchers who have applied for and been granted access to them. These studies include raw DNA sequence data from re-sequencing or transcriptomics projects as well as array-based genotyping experiments. The EGA also stores the submitted phenotype data associated with the samples. Data access decisions are made by a data access-granting organisation (DAO) and not directly by the EGA project. The DAO is typically part of the same organisation that approved and monitored the initial study. The EGA website includes information about the relevant DAO for each submitted dataset and instructions for interested researchers to apply for access to the data. Once DAO authorization has been granted, the EGA provides secure tools for downloading and decrypting the authorized files and general support for questions related to the data content. The EGA ensures high quality data and provides additional useful resources for our users in many different ways. All public components of the submitted study are integrated into the central EBI search tools. The EGA and NCBI's Database of Genotype and Phenotype (dbGaP) provide a shared catalogue of studies that have been submitted to either archive, which allows easy access to related studies and contact information on access control. All submitted data is required to pass basic quality and consistency analysis, and, we are able to phase submitted data or impute unobserved genotypes using public reference resources such as the 1000 Genomes project. Future developments will include the ability to view aligned sequence data and called variants through genome browsers or other visualisation tools supporting appropriate security methods. The EGA website is available at http://www.ebi.ac.uk/ega/.

1172W

Genome-wide association study of classical Hodgkin Lymphoma and EBV status-defined subgroups. *J. McKay[1], U. Urayama[1], R. Jarrett[2], H. Hjalgrim[3], K. Ekström Smedby[4], M. Melbye[3], A. Diepstra[5], A. van den Berg[5], M. Lathrop[6,7], P. Brennan[1], IARC HL GWAS.* 1) International Agency for Research on Cancer, Lyon, France; 2) MRC University of Glasgow Centre for Virus Research, Garscube Estate, University of Glasgow, Glasgow, United Kingdom; 3) Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark; 4) Department of Medicine Solna, Clinical Epidemiology Unit, Karolinska Institute and Karolinska University Hospital, Stockholm, Sweden; 5) Department of Pathology and Medical Biology, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands; 6) Centre National de Génotypage, Institut Génomique, Commissariat à l'énergie Atomique, Evry, France; 7) Fondation Jean Dausset-CEPH, Paris, France.

Accumulating evidence suggests that risk factors for classical Hodgkin lymphoma (cHL) differ by tumor Epstein-Barr virus (EBV) status. We conducted a genome-wide association study (GWAS) of 1,200 cases and 6,417 controls to identify common genetic variants associated with cHL with an evaluation by tumor EBV status. Two novel loci in the major histocompatibility complex (MHC) region, one residing adjacent to MICB (rs2248462, OR= 0.61, P=1.3x10-13) and the other at HLA-DRA (rs2395185, OR=0.56, P= 8.3x10-25), were associated with cHL irrespective of EBV status. Consistent with previous reports, EBV-positive cHL associations were observed within the class I region (rs2734986, HLA-A, OR=2.45, P=1.2x10-15; rs6904029, HCG9, OR=0.46, P=5.5x10-10), and an EBV-negative cHL association was observed within the class II region (rs6903608, HLA-DRA, OR=2.08, P= 6.1x10-31). Further heterogeneity was observed within EBV-negative cHL, with the rs6903608 association confined to nodular sclerosing cHL (Phet= 0.002). Evidence for a predominately EBV-negative cHL association was found for rs20541 (IL13, 5q31) (OR=1.53, P=5.4x10-9), a variant previously linked to psoriasis and asthma. However, the evidence for replication was less clear. Notably, one additional psoriasis-associated variant, rs27524 (ERAP1), showed evidence of an association in the GWAS (OR=1.21, P= 1.5x10-4) and replication series (P=0.028). Overall, these results provide strong evidence that EBV status is an etiologically important classification of cHL, but suggest that some components of the pathological process are common to both EBV-positive and -negative cases.

1173W

Visualizing and Exploring Genomic Alterations in The Cancer Genome Workbench. *C. Yan[1], R. Finney[1], M. Edmonson[2], N. Cu[2], Y. Hu[2], D. Meerzaman[2], K. Buetow[1,2].* 1) NCI/CBIIT, Rockville, MD; 2) NCI/LPG, Bethesda, MD.

The Cancer Genome Workbench (CGWB, http://cgwb.nci.nih.gov) provides a variety of tools with enhanced functionalities and unique features for visualizing and analyzing genomic data. CGWB consists of extended version of UCSC browser, Bambino, PathOlogist, Heatmap viewer and Protein structure viewer. Genomic data includes copy number alteration, somatic mutation, mRNA and miRNA expression, methylation, and nextgen sequencing data. Key features and their uses are highlighted below. First, integrated display of those genomic data at the individual gene level can be used to evaluate the significance of regulatory processes such as methylation and microRNA expression in controlling gene expression. The protein structure viewer illustrates the impact of mutations on protein function in the context of 3D structure models and LogR.E-value/SIFT scores for detecting deleterious mutations. Second, the heatmap viewer offers interactive displays of gene expression and copy number changes in parallel along with clinical features. The data can be sorted and clustered on a set of genes in a pathway to identify the distinct copy number and expression patterns. Third, PathOlogist transforms a large set of gene expression data into quantitative descriptors of pathway-level behavior. It provides straightforward means to identify the functional processes, rather than individual molecules, which are altered in disease. Fourth, Bambino allows users to examine the nextgen sequencing data at base level and to identify SNPs/indels from nextgen sequence mapping files in SAM/BAM format. Bambino can be invoked from browser view of sequence coverage track which is useful for examining sequencing quality, RNASeq expression and structure alteration in whole genome sequencing. Lastly, CGWB is hosting data from the following projects: The Cancer Genome Atlas (TCGA), Therapeutically Applicable Research to Generate Effective Treatments (TARGET), the Sanger Institute's Catalog of Somatic Mutations in Cancer (COSMIC), and the NCI60 cell lines. Much of the data in CGWB is available on caGrid. With comprehensive genomic alteration data from large numbers of tumor samples and cell lines, CGWB will help researchers gain new insight into cancer biology.

1174W

A chemical genetics screen identifies a novel drug that targets steroid biogenesis and receptor signaling leading to growth inhibition of pediatric malignant astrocytoma cell lines. *N. Ajeawung[1], D. Poirier[2], D. Kamnasaran[1,3].* 1) Pediatrics Research Unit - Centre de recherche du CHUL-CHUQ; 2) Laboratory of Medicinal Chemistry, Oncology and Molecular Endocrinology - Centre de recherche du CHUL-CHUQ, Québec, Canada; 3) Department of Pediatrics, Laval University, Québec, Canada.

Background: Brain tumors are among the leading cause of cancer-related deaths in children, with least 60% manifested as astrocytomas. Malignant astrocytomas represent 8-12% of all pediatric supratentorial brain tumors, with an overall median survival of 11-14 months. While those that arise in the brainstem represent an additional 10-20%, with a 10-year overall median survival of <10%. Despite current therapies, challenges still exist in the treatment of pediatric malignant astrocytomas, leading to the need to explore new therapies. Since a wide range of genes involved in steroid biogenesis and signaling are expressed in pediatric malignant astrocytomas, our objective was to investigate whether novel classes of drugs that target these gene products can be effective in inhibiting growth. **Methods and Results:** We screened using a candidate chemical structure approach, a library of 400 drugs which can potentially inhibit steroid biogenesis and cell signaling. By using a panel of human pediatric malignant glioma cell lines established from surgical specimens, we discovered a potent drug that inhibits androsterone (male sex pheromone) biogenesis and with the ability to significantly reduce the viability of pediatric malignant astrocytomas in a dose dependent manner. Cells treated with this drug responded by undergoing apoptosis, cell cycle regulatory, and invasive changes. Furthermore, significant inhibition of transformation was noted. Cells also become increasingly radiosensitive upon drug treatment. Most remarkable, the toxicity on human astrocytes (control) was minimal. **Conclusion:** We have discovered a novel drug from a chemical genetic screen which can significantly inhibit the growth of pediatric malignant astrocytomas, with minimal toxicity on non-transformed human astrocytes.

1175W

Germline deletion in the *INI1/SMARCB1* gene in a child with ependymoma and in his father with multiple schwannomatosis. *JG. Pappas[1], E. Reich[1], JC. Allen[2], J. Wisoff[3], I. Mikolaenko[4], LM. Messiaen[5].* 1) Dept Pediatrics,Human Gen, New York Univ, Sch Med, New York, NY; 2) Dept Pediatrics, Div Ped Hematology/Oncology, New York Univ Langone Med Ctr, New York, NY; 3) Dept Neurosurgery, New York Univ Sch Med, New York, NY; 4) Dept Pathology, New York Univ Sch Med, New York, NY; 5) Dept Genetics, Univ Alabama at Birmingham, Birmingham, AL.

We present a 6 year boy (case 1) with brain ependymoma and his 40 year father (case 2) with multiple schwannomatosis. Case 1 presented at 6 years with behavior changes (aggression), neck spasms and fatigue. He had history of mild global developmental delay, functioning about a year and a half below age level. Clinically he had macrocephaly and no dysmorphic features or other apparent malformations. Brain CT and MRI revealed 4-5 cm lobulated, heterogeneously enhancing mass arising contiguous to the right frontal horn of the lateral ventricle, extending into the third ventricle. A gross total resection was accomplished and there was no evidence of CNS metastases. The pathology revealed glial neoplasm with findings consistent with ependymoma. Involved field radiation therapy was administered thereafter and the patient remains in continuous remission for more than 3 years. Case 1 had a 13 month old brother with no medical problems. Case 2 had three palpable schwannomas on his upper extremities, one small cafe au lait macule and he had schwannomas excised in the past. He was diagnosed with depression. He had multiple blood relatives with multiple schwannomatosis, central nervous system tumors, sarcoma, breast cancer and depression. Both cases carry a novel heterozygous in frame deletion of 6 amino acids in exon 1 of the *INI1/SMARCB1* gene: c.57_74del; p.Glu20_Phe25del in the blood. The mutation was absent in the blood of the mother and the brother of case 1. Germline and somatic mutations in the *INI1/SMARCB1* have been associated with multiple schwannomatosis (Hulsebos TJ et al, 2007; Rousseau G at al 2011) and rhabdoid tumors (Eaton KW et al, 2011; Pfister S et al, 2009). There is no report in the medical literature of a case with ependymoma and germline mutation in the *INI1/SMARCB1*. The BAF47 immunostain was performed on the brain tumor tissue form case 1 and revealed extensive loss of INI-1 staining in many tumor nuclei. Whole genome chromosome microarray copy number analysis (1.8 million SNP/CN targets, Affymetrix, Inc.) in the blood of case 1 revealed no clinically significant copy number variations. Sequencing and deletion / duplication testing of the *TP53* gene in case 1 revealed no mutations. Our cases further confirm the association of mutations in the *INI1/SMARCB1* gene with schwannomatosis and expand the phenotype to include ependymomas.

1176W

Association of Estrogen Receptor-) Gene Polymorphisms with Breast Cancer Risk in Iran. *S. Abbasi.* Tehran University of Medical Sciences, Tehran, Iran.

Background: Receptor-mediated estrogen activation participates in the development and progression of breast cancer. Evidence suggests that alterations in estrogen signaling pathways, including estrogen receptor-((ER-( ) and -) (ER-)) occur during breast cancer development. ER-(gene polymorphism has been found to be associated with breast cancer and clinical features of the disease in Caucasians and we expected ER-)) also might have such influence. Epidemiologic studies have revealed that age-incidence patterns of breast cancer in Middle East differ from those in Caucasians. Genomic data for ER-) is therefore of value in the clinical setting for that ethnic group and we have investigated whether polymorphisms in the ER-) gene are associated with breast cancer risk among Iranian women.

Methods: A case-control study was conducted to establish a database of ER-) polymorphisms in Iranian women population for the first time and to evaluate ER-) polymorphism as an indicator of clinical outcome. Two selected coding regions in ER-) gene (exons 3 and 7) were scanned in Iranian women with breast cancer referred to Imam Khomeini Hospital Complex clinical breast cancer group (150 patients) and in healthy individuals (147 healthy control individuals). PCR single-strand conformation polymorphism technology was performed.

Results: A site of silent single nucleotide polymorphism (SNP) on exon 7 was found, but, any variation on exon 3 was found. The frequency of allele 1 in codon 392 (CTC → CTG was found only in breast cancer patients (5.7%) (χ^2=17.122, P=001). The allele 1 in codon 392 (C1176G) was found has a direct association with the occurrence of lymph node metastasis.

Conclusions: These data suggest that ER-) polymorphisms in exon 47 codon 392 is correlated with various aspects of breast cancer in Iran. ER-) genotype, as determined during presurgical evaluation, might represent a surrogate marker for predicting breast cancer lymph node metastasis.

Keywords: breast cancer, estrogen receptor-) , polymorphism, LN metastases, SSCP-PCR.

1177W

Survival analysis for genetic variants associated with colorectal cancer susceptibility. *A. Abulí[1,2], X. Bessa[2], R. Jover[3], C. Ruiz-Ponte[4], C. Fernández-Rozadilla[4], J. Muñoz[1], A. Carracedo[4], A. Castells[1], M. Andreu[2], S. Castellvi-Bel[1], Gastrointestinal Oncology Group of the Spanish Gastroenterological Association.* 1) Gastroenterology Department, Hospital Clinic-IDIBAPS-CIBERehd-CEK, Barcelona, Catalonia, Spain; 2) Gastroenterology Department, Parc de Salut Mar-IMIM-UPF, Barcelona, Catalonia, Spain; 3) Department of Gastroenterology, Hospital General d'Alacant, Alicante, Spain; 4) FPGMX, Genomics Medicine Group, Hospital Clinico-CIBERER-USC, Santiago de Compostela, Galicia, Spain.

Colorectal cancer (CRC) represents in Spain the most frequently detected neoplasm in men and women and the second cancer in mortality. Environmental factors have an important role in cancer development although genetic components seem to have major relevance. Recently, new common low-penetrance single nucleotide polymorphisms (SNP) for CRC have been identified by case-control genome-wide association studies (GWAS) permitting to point out so far 16 loci for CRC genetic susceptibility in chromosomal regions 1q41, 3q26.2, 8q23.3, 8q24.21, 10p14, 11q23.11, 12q13.3, 14q22.2, 15q13.3, 16q22.1, 18q21.1, 19q13, 20p12.3 and 20q13.33. However, the prognosis significance of the SNPs on disease-free and overall survival remains controversial. Our aim was to evaluate the prognostic and predictive value of the reported SNPs for CRC genetic susceptibility in order to identify cases at a higher risk of recurrence that may benefit from more intensive treatment and follow-up surveillance. We included 1,229 CRC cases recruited by the EPICOLON consortium, a Spanish epidemiologic, prospective, multicentre and population-based study. Present results are referred to the first 10 evaluated SNPs (8q23.3, 8q24.21, 10p14, 11q23.11, 14q22.2, 15q13.3, 16q22.1, 18q21.1, 19q13, and 20p12.3). Genotyping was performed in TaqMan and Sequenom genotyping platforms. Evaluated clinical and tumor variables included age, gender, carcinoembryonic antigen (CEA), stage, location, microsatellite instability, differentiation, histology, chemotherapy treatment, recurrence and death. Median follow-up of the whole series was 40 months (range, 1 -72). Statistical analysis was performed with the SPSS package. The primary outcomes were overall survival (OS) and disease free survival (DFS). Probability curves were calculated according to the Kaplan-Meier method and compared using the log-rank test. A multivariate analysis of hazard risk including all 16 SNPs, adjusted for TNM stage of disease, age and gender, is being performed using Cox proportional hazards regression in a stepwise manner. As expected, tumor stage was strongly associated with OS and DFS. So far, although not achieving statistical significance, three SNPs could be related either to OS (10p14, p=0.04; 16q22.1, p=0.06; 19q13, p=0.05), or DFS (16q22.1, p= 0.06). In conclusion, some CRC genetic susceptibility variants may influence OS and DFS and have prognostic value.

1178W

Detection of BRCA1 and BRCA2 mutations by High-Resolution Melting curve analysis in breast cancer patients from Thailand and Pakistan. *J. Ahmad[1,6], S. Daud[2], S. Sangrajrang[3], F. Le Calvez-Kelm[6], N. Kakar[1], C. Voegele[6], O. Sinilnikova[4], S. Tavtigian[5], F. Lesueur[6].* 1) Department of Biotechnology , BUITEMS, Quetta, Balochistan, Pakistan; 2) National Centre of Excellence in Molecular Biology, University of the Punajab, Lahore, Pakistan; 3) National Cancer Institute, Bangkok, Thailand; 4) Centre Léon Bérard, Lyon, France; 5) Huntsman Cancer Institute, School of Medicine, University of Utah, Salt Lake City, USA; 6) International Agency for Research on Cancer, Lyon, France.

The spectrum of BRCA1 and BRCA2 mutations has been characterized in different populations worldwide with significant variation of the relative contribution of these genes to breast cancer between the populations. However, the contribution of mutations in these two genes to breast cancer patients in Pakistani and Thai populations remains relatively unexplored apart from a few genetics studies. Hence, we aimed to perform comprehensive BRCA mutation screening to characterize the frequency and spectrum of mutations within these two genes in a group of high-risk familial or early onset cases, which forms the largest group of breast cancer patients in these two Asian populations. One hundred and forty three Thai cases and 85 Pakistani cases were enrolled in the study. The entire coding sequence of both genes was scanned for mutation using High Resolution Melting curve analysis (HRM) followed by DNA sequencing. Two known BRCA1 mutations (p.E1250X and p.G1770fs) and two BRCA1 mutations that are not reported in the BIC database (p.S423fs and p.S1057fs) were observed. The nonsense mutation p.E1250X was identified in two unrelated Thai cases. The frameshift mutation p.S423fs was identified in a Thai case whereas the two other frameshift mutations were identified in two Pakistani cases. In the case of BRCA2, three 3 frameshift mutations (p.E1577fr, p.R1704fr and p.S2559fr) and one nonsense mutation (p.Q1107X) were identified. The nonsense mutation was identified in a Pakistani case and was already reported in BIC database, whereas the three frameshift mutations are novel. p.E1577fr was identified in a Pakistani case, p.R1704fr in two Thai cases, and p.S2559fr in one Thai case. In addition to the known or novel pathogenic mutations described here, we report one known inframe deletion in BRCA1 (p.K1110del), two evolutionarily unlikely missense substitutions (p.G1788C in BRCT domain of BRCA1 and p.G2901D in DNA binding domain of BRCA2) that are predicted to affect protein function, as well as a number of likely to be neutral missense variants, silent variants and intronic variants in both genes. In summary, our findings show that BRCA1 and BRCA2 account for a substantial proportion of hereditary breast cancer and early-onset breast cancer cases in Thailand and in Pakistan and suggest that genetic testing for predisposing BRCA germline mutations may be justified for high-risk women from these two countries.

1179W

PTEN exon four and five mutation analysis of Iranian sporadic Breast cancer patients. *M. Akouchekian[1], S. Henati[2], M. Akbari Azirani[3].* 1) Department of genetics & molecular biology, Tehran University of Medical Science, Tehran, Iran; 2) Departments of Oncology, Isfahan University of Medical Science, Isfahan, Iran; 3) KNTU University of Technology, Tehran, Iran.

BACKGROUND: Allelic loss of human chromosome 10 short arm which contains three tumor suppressor genes (TSG) , is one of the common genetic events in cancer. These TSGs on human 10q are ERCC6, PTEN and DMBT1. PTEN gene is reported as one of the most frequently mutated tumor suppressor gene in human cancer. The PTEN gene maps to 10q23.3 and encodes a 403 amino acid dual specificity phosphatase (protein tyrosine phosphatase). It was shown to play an important role in human malignancy. Previously studies were reported somatic PTEN mutations and deletion in sporadic breast, prostate, kidney and brain cancer (Tate et al, 2007 & Yoshimoto et al. 2007). The tumor suppressor role for PTEN was further supported by the discovery that the autosomal dominant multineoplasia syndrome, Cowden disease, is associated with germ-line PTEN mutations. Family members with Cowden disease, both male and female, have an increased incidence of breast cancer. Increasing data have implicated PTEN loss in breast carcinogenesis. While loss of heterozygosity (LOH) of the PTEN locus is frequently reported in sporadic breast carcinoma. The aim of the present study is to determine mutations in PTEN gene in Iranian sporadic Breast cancer patients by direct sequencing of the exons.

METHODS: Patients with sporadic breast cancer diagnosed at the Isfahan's Sayedalshohada Hospital in year 2011 were ascertained for this study. Genomic DNA was isolated from peripheral blood lymphocytes and the entire PTEN coding region of the exons four and five were screened for mutations by PCR and direct sequencing analysis.

RESULTS: Complete sequence analysis of exon four and five demonstrated two heterozygous G to A transversions of the patient's PTEN gene. This mutation was absent in DNA from 10 unrelated healthy individuals as the control group in this study.

CONCLUSIONS: PTEN alterations were detected in fifteen of twenty Iranian Breast cancer patients. We investigated this two LOH variation in our controls and no alterations were seen in the exon four and five of our control groups. Our finding show that sporadic breast cancer seems to be associated with LOH at the PTEN locus, partially involving the mutant allele, further supports the role of PTEN insufficiency for increased proliferation of breast carcinomatos cells.

1180W

BRCA2:E3002K, a new recurring mutation in the founder French Canadian population of Quebec. *S.L. Arcand[1], S. Côté[2,3], S. Nolet[4,5], P. Ghadirian[6], W.D. Foulkes[1,7,8], M.D. Tischkowitz[7,8], A.-M. Mes-Masson[9,10], D. Provencher[9,11], P.N. Tonin[1,12,13].* 1) The Research Institute of the McGill University Health Centre, Montreal, Canada; 2) Service de médecine génique, Département de médecine, Centre hospitalier de l'Université de Montréal, Montreal, Canada; 3) Département des sciences biomédicales, Université de Montréal, Montreal, Canada; 4) Département de pathologie, Centre Hospitalier de l'Université de Montréal, Montreal, Canada; 5) Département de Pathologie et Biologie Cellulaire, Université de Montréal, Montreal, Canada; 6) Epidemiology Research Unit, Centre de recherche du Centre hospitalier de l'Université de Montréal (CRCHUM), Montreal, Canada; 7) Program in Cancer Genetics, Departments of Oncology and Human Genetics, McGill University, Montreal, Canada; 8) Lady Davis Institute, Segal Cancer Centre, Jewish General Hospital, Montreal, Canada; 9) Centre de recherche du Centre hospitalier de l'Université de Montréal (CRCHUM), Institut du cancer de Montréal, Montreal, Canada; 10) Département de médecine, Université de Montréal, Montreal, Canada; 11) Division of Gynecologic Oncology, Université de Montréal, Montreal, Canada; 12) Department of Medicine, McGill University, Montreal, Canada; 13) Department of Human Genetics, McGill University, Montreal, Canada.

Hereditary breast cancer (HBC) and/or ovarian cancer (HBOC) families of French Canadian (FC) descent in Quebec harbor recurring mutations in BRCA1 and BRCA2 cancer susceptibility genes due to common founders. Although most mutations identified are predicted to cause truncation of the encoded protein, and thus are classified as pathogenic mutations, missense mutations are often classified as variants of unknown significance (VUS). The occurrence and segregation of VUS and cancer in families often has been used to establish pathogenicity in the absence of a functional assay for these genes. Recently, we reported the BRCA2:E3002K VUS in two of 82 HBC FC families with three or more confirmed cases of invasive breast cancer under the age of 66, although it was not reported in 54 HBOC families with a case of invasive ovarian cancer and two or more cases of ovarian cancer or breast cancer under age 66. A very young age of diagnosis (<40 years) of breast cancer occurred in both VUS-positive families. Since this report, six HBC/HBOC FC families have been identified in hereditary cancer clinics in Montreal. All but two of the VUS-positive families harboured a very young age at diagnosis case of breast cancer (<40 years). To further characterize the contribution of this VUS in FC cancer families, we have investigated 52 HBC/HBOC FC families previous shown not to harbour the most common BRCA1/2 mutations reported in the FC population. Thus far no new mutation-positive families were identified. This variant has also been reported in cancer families from other populations and has recently been reclassified as likely pathogenic by commercial sequencers. The very young age at diagnosis of breast cancer in our VUS families would support this reclassification. The history of the FC population of Quebec, ascertainment of the families from a common geographic area (within or near the Island of Montreal) and occurrence of the VUS in HBC families suggest that BRCA2:E300K carriers share common ancestors and that this mutation could be added to the roster of pathogenic BRCA1/2 variants screened for in this population.

1181W

Activation of p53 as a possible therapeutic strategy for treatment of cancer. *A. Avan[1,2], M. Maftouh[2], A. Avan[3, 4], R. Mirhafez[5], H. Fiuji[6].* 1) Clinical Genetics Department, VUmc, Amsterdam, Netherlands; 2) Department of Medical Oncology, VU Medical Cancer Center Amsterdam, VUmc, PO Box 7057, 1007 MB, Amsterdam, The Netherlands; 3) Clinic of Wilson's disease, Imam Reza Hospital, Tabriz University of Medical Sciences, Tabriz, Iran; 4) Department of Emergency, Dr. Chamran Hospital, Ferdows, Birjand Medical University of Medical Sciences, Iran; 5) Department of Modern Sciences and Technologies, Faculty of Medicine, Mashhad University of Medical Science, Mashhad, Iran; 6) Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran.

P53 is a tumor suppressor gene that plays a central role in the protection of cell against cancer. Cancer cells contain two types of mutations: mutations that cause uncontrolled growth and increase in number of cells, and other mutations that block the normal defenses of cells against unnatural growth. Mutation in p53 is in the second category. Interestingly, mutations in p53 gene can lead to about half of the human cancer. Most of these mutations are no synonymous mutations. Over 90% of mutations are located in the DNA-Binding Domain (DBD) of p53. Understanding the molecular mechanisms of p53 allow us to modulate these pathways and also it would also help us to find a way in therapeutic reactivation of p53 favors apoptotic cell death in tumor cells. Activity of p53 is tightly controlled by physiological conditions. On the other hand, cellular stress signals are needed for activation and accumulation of p53 through interaction with various proteins and also posttranslational. For instance, Phosphorylation in serine and threonine residues that are located in NTD, carboxy-terminal domain, and DBD of p53. Furthermore, the half of p53 is regulated by ubiquitin modification on lysine residues in the carboxy-terminal region and degradation by proteasome pathway. Consequently, targeting p53 facilitates cell-cycle arrest and DNA repair mechanisms. For example, phosphorylation of human p53 on serine 46 has been contributed to the activation of apoptotic target genes, and mutation of this phosphorylation site reduces the ability of p53 to induce cell death but not proliferative arrest. Therapeutic strategies that result in the activation of p53 would benefit from an ability to modulate or the differential responses to p53 activation. One of the possible mechanisms for activation of p53 is stress to cancer cells, such as oncogene activation, hypoxia and loss of normal environment might result in a differential and sensitivity to undergo apoptosis compared with normal tissue.

1182W

Genetic Sequence Variants (GSV) and Head and Neck Cancer (HNC) Outcome: a Genome-Wide Association Study. *A.K. Azad[1], I. Bairati[4], L. Cheng[2], D. Waggott[3], E. Samson[4], D. Cheng[1], M. Mirshams[1], S. Savas[1], J. Waldron[5], C. Wang[6], D. Goldstein[7], P. Boutros[3], G. Liu[1,8], F. Meyer[4], W. Xu[2].* 1) Applied Molecular Oncology, Princess Margaret Hospital, Toronto, Ontario, Canada; 2) Biostatistical Unit, Princess Margaret Hospital, Toronto, Ontario, Canada; 3) Informatics and Bio-computing Platform, Ontario Institute for Cancer Research, Toronto, Ontario, Canada; 4) Centre de Recherche en Cancerologie de l'Universite Laval, l'Universite Laval, Quebec City, Quebec, Canada; 5) Department of Radiation oncology, Princess Margaret Hospital, Toronto, Ontario, Canada; 6) Département de Radio Oncologie, Centre Hospitalier Universitaire de Québec, Quebec City, Quebec, Canada; 7) Department of Otolaryngology, Princess Margaret Hospital, Toronto, Ontario, Canada; 8) Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, USA.

Background: Carcinomas of the oral cavity, pharynx and larynx are referred to as head and neck cancers; together they account for 2-3% of all newly diagnosed cancers in North America. Between 40-50% of HNC are early diagnosed at stages I-II. The 5-year and 10-year relative survival rates are 61% and 50%, respectively. Germline GSV has become increasingly studied and showed as potential prognostic or predictive markers of outcome in a variety of cancers. Identifying these GSV may therefore increase the accuracy and validity of outcome prediction models in HNC patients. **Methods:** We conducted a genome-wide scan in 531 Stage I-II radiation-treated HNC patients (originally recruited for an alpha-tocopherol/beta-carotene placebo-controlled secondary prevention study) using Illumina HumanHap 610K BeadChips platform. Sample quality filtering was applied to control the missing rate, Hardy-Weinberg equilibrium, population stratification, and heterozygousity rate that resulted in a total of 541,903 GSV. We generated multivariate models adjusted for the potential clinical factors and principal components using the genome-wide genotype data to detect association with clinical outcome of overall survival (OS). Follow-up time was calculated from the time of randomization until the date of last visit, the date of death or December 31, 2006. Survival rates were estimated by using the Kaplan-Meier estimator. Cox proportional hazards models were used to test for associations between the GSV and OS. Each GSV was modeled using an additive, dominant and recessive model of genetic inheritance effect. **Results:** In all, 330 (62%) patients had Stage I and 201 (38%) patients had Stage II disease. The most frequent tumor location was the larynx 443 (83%) and the remaining 88 (17%) were in the oral cavity and pharynx. If we only considered patients who had not experienced the outcome, the median follow-up time for overall survival (OS) was 9.21 years. Five-year OS was 77% (95% CI: 0.73-0.80); 312 patients were alive at December, 2006 the last date of follow-up for survival. Two OS associated GSV were genome-wide significant: *TGM2*:c.1099+651C>T: (aHR 2.00 (1.56-2.57); p=1.3E-8) and *CACNA1S*:c.258+5737A>G: (aHR 6.41(3.39-12.14); p=5.1E-8). **Conclusions:** Our study suggests that GSV in *TGM2* and *CACNA1S* may contribute to HNC outcome. Further validation of our findings in independent populations is ongoing.

1183W

Somatic mutations in esophageal squamous cell carcinoma revealed by exome sequencing. *J. Bai[1], Q. Wang[2], W. Chen[1], H. Cai[2], C. Zeng[1].* 1) The laboratory of disease genomics and individualized medicine, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, China; 2) School of Oncology, Peking University, Beijing, China.

Esophageal squamous cell carcinoma (ESCC) is the major histological type of esophageal cancer. To find key somatic mutations during carcinogenesis of ESCC, we conducted exome sequencing of tumor tissues and matched blood samples from 9 patients of Chinese Han descent. Sequencing was performed on Illumina GAII by paired-end protocols. Reads that passed the pre-processing filters were used to do alignment and mutation calling. The average non-redundancy coverage of the targeted region was 31×, and ~76.5% was at least covered by 10 distinct reads. Mutation analysis was conducted only in regions with their sequencing coverage no less than 10× in both tumor and blood samples. A variation was identified as a somatic mutation if it existed in at least 20% of distinct reads from the tumor but the reads in matched blood sample were wild type. All somatic mutations identified by exome sequencing were validated by genotyping in SEQUENOM MassARRAY® or by Sanger sequencing.

In total we identified 85 somatic mutations in 80 genes among nine patients. Only two genes, TP53 and DMD, showed to have mutations in more than one sample. DMD, the largest gene in the human genome, was excluded for further analysis since numerous mutations in this gene were shown as non-cancer mutations in our in-house database. In consistence with our previous result that TP53 had the most frequent molecular alterations in ESCCs, mutated TP53 was seen in five of the nine tumors (55.6%). Most of the remaining 78 genes were also involved in TP53-related processes as shown in the protein-protein interaction analysis. Therefore we removed these genes in the analysis in attempt to locate mutations involved in other pathways. Among the remaining 5 genes, two of them, SMARCD3 and TSPYL2, participate in chromatin remodeling, an enzyme-assisted nucleosome movement involved in transcriptional activation or repression. These results imply that the malfunction of chromatin remodeling caused by somatic mutations may also contribute to tumorigenesis of ESCC. Verification of mutations in these two genes in more ESCC samples is in the process.

1184W

Identification of men with a genetic predisposition to prostate cancer: targeted screening of *BRCA1/2* mutation carriers and controls. The IMPACT study. *E.K Bancroft[1,2], E. Castro[2], E.C. Page[2], R.A. Eeles[2,1], IMPACT Study Steering Committee and Collaborators.* 1) Cancer Gen Unit, Royal Marsden Hosp, Sutton, United Kingdom; 2) Oncogenetics Unit, Institute of Cancer Research, Sutton, United Kingdom.

Introduction: IMPACT is a multi-national targeted prostate cancer screening study of men with a known germline mutation which is thought to predispose to the disease. The study is recruiting male *BRCA1/2* mutation carriers and a control group, who have tested negative for a mutation present in their family, from 43 centres in 14 countries. Method: Eligible men aged 40-69 years are offered annual serum PSA testing for five years. The PSA threshold used to determine prostate biopsy is >3ng/ml. All men are offered a biopsy irrespective of PSA level after 5 years of screening. A subset of men are also invited to take part in a Quality of Life sub-study. Results: To date 1200/1700 men have been recruited, 86 have undergone biopsy and 37 men have been diagnosed with prostate cancer (22 *BRCA2* carriers, 9 *BRCA1* carriers and 6 controls). 60% of cases diagnosed in *BRCA1/2* carriers were Gleason score ⁄ 7 and T2 or higher (in the intermediate/high risk group for immediate radical treatment and not active surveillance, according to NICE guidelines) while in the general population (ERSPC study), 72% had a Gleason ≤6 and 61% were T1 (low risk group, suitable for active surveillance). The estimated positive predictive value for PSA screening in BRCA carriers is 44% vs 24% in the general population (ERSPC study). 160 men have joined the Quality of Life study and baseline health-related quality of life (HRQOL) and psychosocial measures have not identified any increased levels of distress in this sub-group. Conclusions: Our preliminary results show that annual PSA screening using a cut-off of 3.0ng/ml detects aggressive prostate cancer in *BRCA* carriers. The higher risk of PrCa observed in *BRCA1* and *BRCA2* carriers will need to be confirmed with further data. Early data indicate that a larger proportion of gene carriers have developed clinically significant prostate cancers (those that would need radical treatment on UK treatment guidelines) compared with the control group.

1185W

Somatic mutations in CCKBR alter receptor activity and promote tumor progression. *T. Barber[1], M. Lajiness[1], I. Wulur[1], B. Feng[3], M. Swearingen[1], M. Uhlik[1], K. Kinzler[2], V. Velculescu[2], T. Sjoblom[4], S. Markowitz[5], S. Powell[6], B. Vogelstein[2], M. Willard[1].* 1) Departments of Translational Science and Cancer Angiogenesis, Lilly Research Laboratories, 355 East Merrill Street, Indianapolis, IN, 46285, USA; 2) Ludwig Center for Cancer Genetics and Therapeutics, and Howard Hughes Medical Institute at Johns Hopkins Kimmel Cancer Center, Baltimore, MD, 21231, USA; 3) Center for Molecular Recognition, Columbia University, College of Physicians and Surgeons, New York, NY, 10032, USA; 4) Department of Immunology, Genetics and Pathology, Uppsala University, SE-751 85 Uppsala, Sweden; 5) Department of Medicine and Comprehensive Cancer Center, Case Western Reserve University School of Medicine and Case Medical Center, Cleveland, OH, 44106, USA; 6) Division of Gastroenterology/Hepatology, University of Virginia Health System, Charlottesville, VA, 22908, USA.

The roles of cholecystokinin B receptor (CCKBR) in numerous physiological processes in the gastrointestinal tract and central nervous system are well-documented. There has been some evidence that CCKBR alterations play a role in cancers, but the functional significance of these alterations for tumorigenesis is unknown. We have identified six mutations in CCKBR among a panel of 140 colorectal cancers and 44 gastric cancers. We demonstrate that these mutations increase receptor activity, activate multiple downstream signaling pathways, increase cell migration and promote angiogenesis. Our findings suggest that somatic mutations in CCKBR promote tumorigenesis through deregulated receptor activity and highlight the importance of evaluating CCKBR inhibitors to block both the normal and mutant forms of the receptor.

1186W

Landscape of Somatic Structural Alterations in Chronic Lymphocytic Leukemia (CLL) Detected by Whole Genome Sequencing. *L. Bassaganyas[1], J.C. Tubio[1], G. Escaramis[1], C. Tornador[1], S. Bea[2], X.S. Puente[3], D. Gonzalez-Knowles[1], R. Guigo[1], I. Gut[4], C. Lopez-Otin[3], E. Campo[2], X. Estivill[1] on behalf of the CLL Genome Project Consortium.* 1) Genes and Disease, Center Genomic Regulationn (CRG), Barcelona, Catalonia, Catalonia, Spain; 2) Hospital Clinico, Barcelona, Catalonia, Spain; 3) University of Oviedo, Oviedo, Asturias, Spain; 4) Centro Nacional de Analisis Genomicos, CNAG, Barcelona, Spain.

Cancer is driven by somatically genetic mutations that can be acquired gradually overtime or by single catastrophic events. Chronic lymphocytic leukemia (CLL) is one of the most frequent cancers in western countries, representing 35% of all cases of leukemia. Different genetic alterations have been identified in CLL, including chromosomal aberrations detected by cytogenetic analysis, FISH and aCGH. The limitations of these techniques do not allow the identification of the full set of somatic structural variation at molecular level. Our understanding of oncogenesis has benefited greatly from next-generation sequencing technology (WGS). Massively sequencing, in combination with computational approaches, offers the potential to carry out genome-wide screening of all sort of chromosomal rearrangements. Using the toolkit PeSVFisher that includes several algorithms to identify structural variations in genomic sequencing, we have analyzed WGS to annotate the entire complement of genomic rearrangements in 10 patients with CLL. By identifying read pairs that did not map correctly with respect to each other on the reference genome, we have catalogued 9,994 potential somatic rearrangements. Then, a bioinformatic tool called FinalCountDown allowed us to filter 1,989 moderate and 64 high-confidence potential somatic events, after removing those rearrangements with breakpoints involving complex regions of the genome (i.e., segmental duplications, simple tandem repeats, and low-divergent transposable elements). 53% of these high-confidence somatic rearrangements were confirmed in silico by the presence of split-reads in the predicted breakpoints. Moreover, we combined these results with the depth of coverage data of WGS in order to obtain a comprehensive catalogue of copy number variation. The analysis of these 10 CLL cases by WGS represents the first step in a comprehensive characterization of the genomic alterations at the structural level, coupled with functional analysis, of CLL in the framework of the CLL Genome Project, to decode the genetic changes that are responsible for the development and progression of CLL.

1187W

Detection of somatic mutations in hepatocellular carcinoma: a prerequiste for individualized immonotherapy. *P. Bauer[1], C. Schroeder[1], S. Junker[1], M. Loeffler[2], S. Stevanovic[2], H.G. Rammensee[2], S. Nadalin[3], A. Koenigsrainer[3], C. Hann von Weyhern[4], O. Riess[1].* 1) Dept of Medical Genetics, Univ Tuebingen, Tuebingen, Germany; 2) Department of Immunology, Institute for Cell Biology, Univ Tuebingen, Tuebingen, Germany; 3) Dept. for Surgery, Univ Tuebingen, Tübingen, Germany; 4) Department of Pathology, Univ Tuebingen, Tuebingen, Germany.

Hepatocellular carcinoma is the fifth most common cancer in the world [1]. Over 500.000 patients are diagnosed hepatocellular carcinoma per year, the total number in Europe and the USA is rising over the last decades. The project IndividualLiver has the aim to establish individualized immunotherapy for hepatocellular carcinomas and is supported by the BMBF. Prerequisite for a successful immunotherapy is the detection of somatic mutations in genes presented on the cell surface. In this context, we sequenced a total of 11 patients, diseased with hepatocellular carcinoma by means of next-generation sequencing. After surgery, two liver specimen were taken from each patient - one tumor sample and one normal liver tissue. Both samples were conditioned and sequenced using a targeted whole-exome resequencing approach (NimbleGen SeqCap V2, Illumina 2x76PE sequencing). We established a bioinformatic pipeline for detection of new somatic mutations within tumor tissue. In average, we found 582 (± 161) new somatic, coding sequence changes per sample pair. The average on-target-ratio was 0.48 (± 0.05), overall coverage at a depth of at least 10x reached 0.84 (± 0.1). The overall processing-time can be estimated to be 3-4 weeks. Though further validation of our results is under way including conventional variant sequencing and data comparison with proteome analysis sets of the respective patients, plausibility and filter criteria suggest a high quality of our data. Using this approach, we are able to detect new somatic sequence changes within tumor tissue that ultimately give rise to a patient specific tumor-directed peptide vaccine.

1188W

Identification of ethnic specific gene expression differences in breast cancer and normal breast tissue. *L.L. Baumbach-Reardon[1], C. Gomez[1], J. Yan[2], T. Halsey[2], K. Ellison[2], M.E. Ahearn[1], M. Jorda[1], M. Peagram[1].* 1) University of Miami Medical School, Miami, FL; 2) Almac Diagnostics, Durham, N.C.

Disparities in breast cancer (BC) stage of presentation and survival are well documented in patients of different ethnicities. These differences are undoubtedly a result of a combination of factors: socio-economic, lifestyle, tumor characteristics and inherent factors, such as genetic composition. Our group is focused on the genetic contributions to these disparities, to increase understanding of underlying biology, leading ultimately to individualized, ethnic-specific diagnostic and therapeutic approaches. We report results of a recently completed study focused on gene expression profiling in a multi-ethnic cohort of triple negative breast cancer patients. We analyzed BC and self-matched normal tissue samples from 10 African-American (AA), 10 Hispanic (His), and 10 non-Hispanic white (Caucasian) patients from south Florida. Study samples were cut from FFPE (Formalin Fixed Paraffin-embedded tissue) blocks marked by pathology as normal vs. tumor tissue, and sent to Almac Diagnostics for RNA isolation, cDNA preparation, and hybridization to a breast cancer enriched gene expression array (Breast Cancer DSA Research Tool). From the BC DSA data, a two-way ANOVA (disease state and ethnicity) was used to identify transcripts with a p-value less than 0.01. Data QC showed that samples clustered well with respect to ethnicity and adjacent normal vs. tumor tissue. We have identified ethnic-specific expression patterns in the matched normal and tumor samples. Initial pathway analysis indicates a number of genes in DNA repair are differentially expressed across ethnicities. In a set of ten DNA repair/cell cycle genes, the direction of change (increased or decreased expression) was the same for all three ethnic groups; however, the level of change differed greatly between ethnic groups. Fold changes ranged from -6.54 to +5.53, with all being >2 fold change in at least one ethnic group. Additional pathway analysis and validation of results are ongoing. We are completing a parallel analysis of normal tissue samples (reduction mammoplasty) from AA and Cau non-cancer patients to investigate potential normal differences. Combined analysis of tumor and normal breast expression (transcriptome) data will help to better understand the possible significance of gene expression differences in breast tissue between ethnic groups. These studies have important implications for addressing BC ethnic disparities, as well as tailored approaches to prediction, prevention and treatment.

1189W

Arsenic-related genomic and epigenomic alterations in lung squamous cell carcinomas. *D. Becker-Santos[1], V. Martinez[2], E. Vucic[1], S. Lam[1], W. Lam[1].* 1) Department of Integrative Oncology, British Columbia Cancer Research Centre, Vancouver, BC, Canada; 2) Department of Molecular and Cell Biology, University of Chile, Santiago, Chile.

Background: Arsenic is a well-established skin, bladder, and lung carcinogen. It is estimated that over 100 million people worldwide are exposed to toxic levels of arsenic, mainly through drinking water. Lung cancer is the main cause of deaths due to arsenic exposure, with this metalloid acting as the major etiological agent in cancers that occur in never smokers. Relative to other subtypes of lung cancer, lung squamous cell carcinomas (SqCCs) occur at higher rates following arsenic exposure. The mechanisms by which arsenic causes cancer is still under investigation, and it has been proposed that both genetic and epigenetic processes may be involved. In this study, a rare panel of lung tumours from a population with chronic arsenic exposure, including SqCC tumours from patients with no smoking history, was analyzed to identify arsenic-associated copy-number alterations (CNAs) and DNA methylation changes. Methods: A total of fifty-two cases of lung SqCC were analyzed by whole-genome tiling-set array comparative genomic hybridisation. Twenty-two specimens were collected from arsenic-exposed patients from Northern Chile (10 corresponding to never smokers and 12 to smokers). A panel of thirty cases was obtained for comparison from North American smokers without arsenic exposure. In addition, 22 blood samples from healthy individuals from Northern Chile were examined to identify naturally occurring germline DNA copy-number variations (CNVs) that could be excluded from analysis. DNA methylation analysis was performed using Illumina's Infinium Human Methylation 450K array. Results: We identified several CNAs and DNA methylation changes associated with arsenic exposure. These alterations were not attributable to either CNVs or smoking status. The most recurrent events represented DNA losses at chromosome bands 1q21.1, 7p22.3, 9q12, and 19q13.31. The only arsenic-associated DNA gain occurred at 19q13.33, which contains genes previously recognized as oncogenes. Conclusions: Our study has provided insights into the molecular mechanisms of arsenic-induced lung neoplasia. The distinct and recurrent arsenic-associated genetic and epigenetic alterations suggest that this group of tumours may represent a separate disease subclass.

1190W

Caprin1 is Frequently Overexpressed in Human Lymphomas. *S. Berker Karauzum[1], Z. Cetin[1], B. Akkaya[2], A. Timuragaoglu[3], IH. Ozbudak[2], H. Bagci[1].* 1) Department of Med Biol & Genetics, Akdeniz University, Medical Faculty, Antalya, Turkey; 2) Department of Pathology, Akdeniz University, Medical Faculty, Antalya, Turkey; 3) Department of Hematology,Akdeniz University, Medical Faculty, Antalya, Turkey.

Caprin1 endoded by Cytoplasmic Activation/Proliferation-Associated Protein-1 gene located in 11p13 chromosome region. Previous studies using cultivated normal and cancer cell lines indicated that Caprin1 is associated with cell proliferation in various types of cell lineages, including hematopoietic and epithelial cells. We hypothesised that Caprin1 might be overexpressed in some of these tumor cells and that this overexpression could be associated with the clinicopathological parameters in some types of the tumors. Immunohistochemistry was used to carry out expresssion profiling of 110 normal human tissue sections in tissue microarrays and 113 human cancer tissue sections in multiple tumor tissue micoarrays. Additional tests using tissue microarrays for lymphomas also utilized immunohistochemistry and included 16 cases with Hodgkins disease, 98 cases with Non-Hodgkins B cell lymphoma, and 5 cases with Non-Hodgkins peripheral T cell lymphoma. The expression of Caprin1 was observed in different types of tumors derived from different tissues including, esophageal, stomach, small intestinal, colon, rectal, kidney, gall bladder, prostate, breast, ovarian, endometrium, uterine, oronasopharyngeal, lung, liver and lymphoid tissue. Caprin1 overexpression was observed in 4 cases with Hodgkins disease (25%) and in 26 cases with Non-Hodgkins B cell lymphoma (26.5%). Caprin 1 expression was not,however, detected in any Non-Hodgkins T cell lymphoma cases. The higher frequency of Caprin1 overexpression in high grade diffuse large B cell lymphomas than the low grade non-Hodgkin B cell lymphomas indicates that Caprin1 expression levels might be correlated with the cellular proliferation potential of the lymphoma cells. In this study, we revealed the expression profile of Caprin1 in normal human and tumor tissues for the first time. Caprin1 overexpression is likely associated with the development of lymphoid and epithelial tumours derived from different organs and tissues. Further studies on the clinicopathologic importance of Caprin1 overexpression in different types of lymphoma and solid tumours are needed.

1191W

Rapid identification of cancer mutations by high throughput sequencing on the MiSeq system. *J. Betley, G. Smith, K. Hall, MiSeq program team.* Illumina Cambridge Ltd, Saffron Walden, United Kingdom.

Next generation sequencing has enabled the routine sequencing of whole human genomes at a scale of many thousands per year. One exciting application of this technology has been the complete sequencing of the cancer genomes and the identification of and cataloguing of somatic mutations in a wide variety of cancer types. We have developed a new sequencing system, called Miseq, that generates over 1Gb of data from 2x150 base reads in just over a single day. Miseq integrates all the steps of the Illumina sequencing process from cluster generation, to paired end sequencing reads, into a fully automated `hands-off' workflow that involves 20 minutes of set up time, and includes the steps of primary and secondary data analysis, including variant calling and de novo assembly. A primary application of this new system is the characterisation of variation within individual exons of clinical cancer samples. Starting with FFPE-extracted DNA, we demonstrate that it is possible to amplify and sequence a highly multiplexed set of exons within a panel of cancer samples. We identify single nucleotide variation with a limit of detection less than 1%. The rapid turnaround of the system, coupled with the simple sample preparation and operation of the instrument, makes possible the deployment of Next Generation sequencing in a clinical research setting for cancer.

1192W

Post-genome wide association studies in colorectal cancer. *M. Biancolella[1], C. Yan[1], G. Mendoza-Fandino[1,3], M.J Hitchler[4], G.A Coetzee[1,2], G. Casey[1].* 1) Department of Preventive Medicine, University of Southern California, Los Angeles, California, USA; 2) Department of Urology, Norris Cancer Center, University of Southern California, Los Angeles, California, USA; 3) Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA 90033, USA; 4) Radiation Oncology Department Kaiser Permanente 4950 Sunset Boulevard Los Angeles, CA 90027.

Several genome-wide association studies (GWAS) of colorectal cancer (CRC) have been published and have reported a number of significant associations. However, only limited characterization of the biological/functional role of these associations in cancer pathogenesis has been conducted. Meeting this challenge is not simple, since none of the associations identified to date target coding exons of genes, but instead map to non-protein coding regions near genes or within gene-poor regions. A working hypothesis is that risk allele is in linkage disequilibrium (LD) with an adjacent SNP that maps within a regulatory region and affects gene expression elsewhere. To address this we have measured the presence of regulatory elements by assessing histone modifications across the CRC GWAS regions using chromatin immunoprecipitation-sequencing (ChIP-seq) methods. ChIP-seq profiles for H3K4me1, H3K4me3, H3K27me3, H3K36me3, H3K9/14ac as well as RNAPII were generated in two colon cancer cell lines (HCT116 and SW480). Several chromatin regions were identified within susceptibility LD blocks that suggest the presence of putative enhancers. The potential regulatory activities of 11 of these regions were determined by an enhancer transfection assay using 1.5-2kb DNA fragments centered on the ChIP-seq peaks of active chromatin marks. Five of the 11 regions tested showed intrinsic enhancer activity (one region at 18q21; one region at 11q23; one region at 15q13; two region at 9p24). The peak identified at 11q23 showed pronounced unidirectional enhancer activity. Within this 1.5-kb DNA fragment, the risk alleles of two SNPs strongly correlated with the risk variant rs3802842. A 30% reduction in enhancer activity was seen when one of these SNPs was mutagenized. Studies are now in progress to determine the transcription factor binding sites that may be affected by this SNP. Studies are also in progress to correlate genotype of rs3802842 and gene expression of genes mapping within 500kb of the risk SNP using normal colon tissues from >300 subjects to identify candidate genes that may be regulated by this regulatory element.

1193W

Developing breast cancer among men and possibility of exposure to endocrine- disrupting chemicals in Iran: A case report. *F. Biramijamal[1], A. Hossein-Nezhad[2], H. Jalaikhoo[3,4], F. Ramezani[4], S.S. Mirgheydari[4], S. Klahroodi[3,4], P. Hemmati[4].* 1) National Institute of Genetic Engineering and Biotechnology- Medical Genetics, Tehran, Iran; 2) Endocrinology and Metabolism Research Center, Tehran, Iran; 3) Artesh 501 Hospital ,Tehran, Iran; 4) Clinic of Oncology, Tehran, Iran.

It is described that interaction between heavy metals such as cadmium (Cd) with estrogen receptor can be increased breast cancer risk, because the heavy metals can act as estrogen mimics. It is shown that urinary cadmium (CdU) is increased among workers in battery plants. It is induced by occupational exposure to Cd. In this study, we are present a case report of breast cancer in a man who worked in battery plants. He diagnosed with invasive ductal carcinoma grade II, and axillaries lymph-nodes showing metastatic mammary carcinoma. At the time of resection (surgery), he was 52 years old. The result of pathology is shown that the tumor surrounded by a chronic inflammation infiltrate and dissociated by bundles of connective tissue. The tumor borders are infiltrative. Stromal fibro-sclerosis and chronic inflammatory reaction is ostensible. All examined lymph-nodes are involved by tumor. Skin and surgical margins are free of tumor. Estrogen receptor (ER) and Progesterone receptor (PR) status were positive and c-erB-2 statue was negative in the tumor tissue. Also, bone Scan of patient was normal. ER and PR status has been described as an indicator of endocrine responsiveness and as a prognostic factor for recurrence too. The some of researchers found no association between these receptors and histopathological status of tumor and metastases among women patients. However, The ER and PR positive status in a man patient may be reflected to exposure to endocrine- disrupting chemicals such as Cd. Also, It is described that c-erB-2 positive rate is not common in ER^+/PR^+ tumors among women patients, and similarity, we found the c-erB-2 negative in our case with ER^+/PR^+. In addition, grade I tumors were more common in receptor positive tumors while grade II and III tumors were more common in receptor negative tumors among women patients. However, in our case, we found ER+/PR+ in grade II tumor. So, we suggest using the risk assessment kits for identification and measurement of environmental risk factors especially in battery industry. This protocol can be helped to reduce the incidence of breast cancer in Iran.

1194W

Genome-wide SNP array analyses of benign, low malignant potential and low grade serous epithelial ovarian tumours identify chromosomal anomalies, including a novel fusion gene. *A.H. Birch[1], S.L. Arcand[2], K.K. Oros[3], K. Rahimi[4], A.K. Watters[5], D. Provencher[6,7], C.M. Greenwood[3,8,9], A.M. Mes-Masson[6,10], P.N. Tonin[1,2,11].* 1) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 2) The Research Institute of the McGill University Health Centre, Montreal, Canada; 3) Lady Davis Research Institute, Jewish General Hospital, Montreal, Canada; 4) Department of Pathology, Centre Hospitalier de l'Université de Montréal (CHUM), Montreal, Canada; 5) Department of Pathology, McGill University and McGill University Health Centre (MUHC), Montreal, Canada; 6) Centre de recherche du Centre hospitalier de l'Université de Montréal (CRCHUM), Institut du cancer de Montréal, Montreal, Canada; 7) Division of Gynecologic Oncology, Université de Montréal, Montreal, Canada; 8) Department of Oncology, McGill University, Montreal, Canada; 9) Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, Canada; 10) Department of Medicine, Université de Montréal, Montreal, Canada; 11) Department of Medicine, McGill University, Montreal, Canada.

Epithelial ovarian cancer (EOC) is the most lethal gynaecological malignancy, and will result in the deaths of approximately 1750 Canadian women this year. Over 50% of all EOC tumours are of the serous histopathological subtype. Accumulating histological and genetic evidence has led to the recent proposal that low grade serous ovarian carcinomas (LGSOC) likely have a different molecular etiology than their high grade (HGSOC) counterparts. While HGSOCs grow aggressively and often exhibit extensive aneuploidy and *TP53* mutations, LGSOCs are more indolent, harbour mutations in *KRAS* or *BRAF*, and display low levels of chromosomal instability. With the advent of whole-genome SNP arrays, it is possible to interrogate the entire cancer genome to detect chromosomal aberrations. Most genome-wide SNP studies of serous ovarian tumours have focused on HGSOCs. Less is known of the genomic events occurring in benign (BOV) and low malignant potential (LMP) tumours, which are proposed precursors of LGSOCs. We have applied the Illumina BeadChip 610-Quad SNP array to genotype over 600,000 markers in 32 serous BOVs, 59 serous LMPs, and 12 LGSOCs. Mutation testing of *KRAS, BRAF* and *TP53* was also carried out, and copy number aberrations were inferred using Genome Studio and the GenoCNA algorithm. Increasing levels of chromosomal aberrations were seen between BOVs, LMPs and LGSOCs. Few BOVs showed evidence of allelic imbalance, whereas 34 of 59 LMPs displayed allelic imbalance and/or intrachromosomal gains and losses. LGSOCs displayed a wide spectrum of aberrations, ranging from aberrant copy numbers of whole chromosomes to multiple intrachromosomal gains and losses. Complex patterns of intrachromosomal breaks were associated with *TP53* mutations, in both LMPs and LGSOCs. A homozygous deletion located on chromosome 6 in an LMP sample resulted in the creation of the *GOPC-ROS1* fusion gene, a rare variant previously reported as oncogenic in a glioblastoma cell line and two cholangiocarcinoma samples. To the best of our knowledge, this is the first fusion gene observed in an EOC context. Whole-genome SNP arrays are useful tools for studying tumour-associated genomic events. Ideally, specific chromosomal signatures may be connected to pathological outcomes, and SNP arrays may be employed as a diagnostic tool to parse histologically similar tumours.

1195W

Analysis of expression profile of VEGF pro and antiangiogenic isoforms in head and neck cancer squamous cells carcinoma. *P.M. Biselli-Chicote[1], G.H Marucci[1], R. Castro[1], J.V. Maniglia[2], D.S. Neto[3], E.C. Pavarino[1,4], E.M. Goloni-Bertollo[1,4].* 1) Genetics and Molecular Biology Research Unit - UPGEM, Medical School of Sao Jose do Rio Preto - FAMERP, Sao Jose do Rio Preto, Sao Paulo, Brazil; 2) Department of Othorrinolaringology and Head and Neck Surgery, Medical School of Sao Jose do Rio Preto - FAMERP, Sao Paulo, Brazil; 3) Patology Service, Hospital de Base, Sao Jose do Rio Preto - FAMERP, Sao Paulo, Brazil; 4) Department of Molecular Biology, Medical School of Sao Jose do Rio Preto - FAMERP, Sao Paulo, Brazil.

Head and neck cancer is a broad term that encompasses paranasal sinuses, nasal cavity, oral cavity, pharynx and larynx epithelial tumors. About 40% of head and neck cancers occur in the oral cavity, 25% in the larynx, and 15% in the pharynx. The vast majority of epithelial tumors are classified as head and neck squamous cell carcinoma. Tumor growth and progression depend on angiogenesis that is a process of new blood vessels formation from a preexisting vascular endothelium. The newly formed blood vessels provide nutrients and oxygen to the tumor, increasing its growth. Therefore, angiogenesis plays a key role in cancer progression and occurrence of metastasis.Vascular endothelial growth factor (VEGF) is a mitogen of endothelial cells and its overexpression is associated whit tumor growth and metastasis. However, the selection of splicing alternative site at the 3' region of the messenger RNA results in expression of isoforms with a different C-terminal region that may have inhibitory effects in angiogenesis and they are downregulated in tumors. Protein VEGF165 is a proangiogenic factor and plays important role in the migration, proliferation and cell survival. On the other hand, altered C-terminal region of VEGF165b (antiangiogenic isoform) allows the inhibition of endothelial proliferation, migration and vasodilatation induced by VEGF165, and inhibition of physiological angiogenesis and tumor growth. We analyzed the expression of VEGF165 e VEGF165b isoforms in 20 samples of head and neck squamous cells carcinoma and adjacent normal tissue. Total RNA was obtained and the correspondent cDNA was synthesized. Specific primers and probes for each isoform were used to expression analyses by Quantitative Real Time PCR. As result no differences was observed in the expression of both genes in tumor compared to normal samples. We hope that with increasing the sample size, our results may serve as a basis for further studies regarding the splicing mechanism of VEGF gene in the head and neck cancer, aiming to identify new therapeutic targets and diagnostics.

1196W

Tylosis with Oesophageal Cancer segregates with RHBDF2 mutations leading to dysregulation of downstream EGF and EphrinB3 signalling. *D.C. Blaydon[1], S.L. Etheridge[1], J.M Risk[2], H-C. Hennies[3], H.P. Stevens[4], J.K. Field[5], A. Ellis[5], I.M. Leigh[6], A.P. South[6], D.P. Kelsell[1].* 1) Centre For Cutaneous Research, Barts and the London, London, United Kingdom; 2) Department of Molecular & Clinical Cancer Medicine, Institute of Translational Medicine, Liverpool, United Kingdom; 3) Division of Dermatogenetics, Cologne Center for Genomics, University of Cologne, Cologne, Germany; 4) Barnet Hospital, Barnet, United Kingdom; 5) The University of Liverpool Cancer Research Centre, Liverpool, United Kingdom; 6) Centre for Oncology and Molecular Medicine, Ninewells Hospital and Medical School, Dundee, United Kingdom.

Oesophageal cancer (predominantly squamous oesophageal cancer) is the seventh leading cause of cancer related deaths worldwide. There is a familial form of susceptibility to oesophageal cancer called “Tylosis” where patients have a very high risk of developing squamous cell cancer of the oesophagus (up to 95% by age 65) - we have now identified the single genetic cause of this condition. Mutations in the inactive rhomboid protease RHBDF2 predispose individuals to Tylosis with Oesophageal Cancer (TOC), an autosomal dominant syndrome with palmoplantar keratoderma, oral precursor lesions and a high lifetime risk of oesophageal cancer. Two mutations identified in 3 families from the UK, US and Germany, c.557T>C (p. Ile186Thr) and c.566C>T (p. Pro189Leu), affect residues translated from a highly conserved region of exon 6. Rhomboid proteins are typically serine proteases that cleave type-1 membrane proteins in the transmembrane region. RHBDF2 lacks protease activity but is predicted to inhibit the active rhomboid protease RHBDL2. Substrates of RHBDL2 include EGF and EphrinB3, proteins important in cell proliferation, adhesion and migration. Dysregulation of EGFR and Ephrin signalling has been implicated in many cancers and EGFR inhibitors have demonstrated clinical efficacy. Immunohistochemical staining of normal skin showed RHBDF2 predominantly at the plasma membrane whereas in tylotic skin, the distribution appeared more cytoplasmic, suggesting altered trafficking or processing. Dysregulation was also observed in the distribution of RHBDL2 and EphrinB3 in tylotic skin. Recombinant EGF had little effect on the growth of immortalised patient keratinocytes (n=2) compared with controls (n=2). In addition, tylosis keratinocytes exhibited increased migration compared with controls, particularly in the absence of EGF ($p<0.005$ in each case). This suggests a key role for RHBDF2 in regulating EGF signalling in both cutaneous and oral keratinocytes. The link with RHBDF2 mutations and oesophageal cancer could lead to new tools for early diagnosis and small molecule RHBDL2 inhibitor development for treatment of oesophageal disease and other epithelial cancers including lung and ovarian cancer.

1197W

Identification of fusion transcripts in ovarian cancer by next generation RNA-sequencing. *M. Bonin[1], J. Hoffmann[1], H. Neubauer[3], M. Walter[1], A. Staebler[2], F. Wagner[3, 4], O. Riess[1], D. Wallwiener[3], H.G. Rammensee[4], T. Fehm[3].* 1) Medical Genetics Dept., Institute of Human Genetics, Tuebingen, BW, Germany; 2) Institute for Pathology, Eberhard-Karls-University Tuebingen, Liebermeisterstr. 8, 72076 Tuebingen, Germany; 3) Department of Obstetrics and Gynecology, Eberhard-Karls-University Tuebingen, Calwerstr. 7, 72076 Tuebingen, Germany; 4) Institute of Immunology , Eberhard-Karls-University Tuebingen, Calwerstr. 7, 72076 Tuebingen, Germany.

Ovarian cancer is one of the most malignant tumors in women and the leading cause of death from gynecological cancer in the Western civilization. The standard therapy consists of aggressive cytoreductive surgery followed by platinum-based chemotherapy. In addition to the advanced stage of the disease at the time of diagnosis in most instances, the intrinsic or acquired platinum resistance in the majority of patients leads to its high mortality rate. To gain new insights in resistance mechanisms, mutation load and modified pathways, we performed RNA-seq methods using Illumina next generation sequencing technologies. Chromosomal Translocations and fusion transcripts have been a class of mutations in solid tumors. The development of RNA-sequencing methods provides an opportunity for deep characterization of cancer cell transcriptomes and the discovery of fusion transcripts arising from genomic rearrangements. Six cryopreserved tissues samples from each platinum resistant and platinum sensitive ovarian carcinomas were selected. Tissue sections (10μm) were prepared, stained with hematoxilin/eosin and characterized by a pathologist. Only samples with serous ovarian cancers containing at least 80% malignant tissue were processed further. From consecutive sections RNA was extracted and analysed by using 76nt paired-end reads with Illumina technologies. We identify 18 novel fusion transcripts in the 12 ovarian cancer samples. Supported by the Genomatix® algorithm, we were able to validate 15/18 (83%) fusion transcripts by qRT-PCR and Sanger-Sequencing. Most fusion transcripts were associated with Copy number transitions and were partially common in highly amplified DNA regions. Around 40% of the fusion transcripts partners have either been previously detected in oncogenic gene fusions, or reported by pathway analysis to be oncogenic. The results indicating that RNA-sequencing opens a great opportunity for discovering novel fusion transcripts in ovarian cancer and we are now able to analyze the impact of this alteration for the growth and resistance mechanisms of cancer cells.

1198W

BRCAPRO vs Manchester Scoring System - Experience of Genetic Health Queensland, Australia. *M.J. Boon[1], R.D. Susman[1], J. Bjorkman[2].* 1) Genetic Health Queensland, Brisbane, Queensland, Australia; 2) Pathology Queensland, Brisbane, Queensland, Australia.

Genetic Health Queensland (GHQ) is the sole provider of publically funded clinical genetic services to the 4.5 million residents of the state of Queensland, Australia. As approximately 30% of referrals relate to familial cancer, significant numbers of genetic tests for cancer predisposition syndromes are performed. Due to limitations in funding, criteria exist so that testing is only offered in cases with a considerable likelihood of identifying a pathogenic mutation.

GHQ has primarily used the computer software BRCAPRO to determine eligibility for *BRCA1* and *BRCA2* gene testing, along with clinical judgement in unique cases. Genetic testing is offered when the likelihood of identifying a mutation is 20% or above. Due to ongoing issues with the departmental use of BRCAPRO, the Manchester Scoring System (incorporating the more recent pathology adjustment) was considered as an alternative risk prediction model. Prior to implementing this new method, an audit was performed to review the current mutation detection rates using BRCAPRO and predict the sensitivity and cost-effectiveness of the alternate model. Benefits and limitations of each method were also considered.

Between the period of January 2010 and January 2011, Manchester and BRCAPRO scores were calculated for all patients seen by GHQ with a personal history of breast and/or ovarian cancer. *BRCA1* and *BRCA2* gene testing was subsequently offered to all individuals who had a BRCAPRO score of above 20% or were deemed appropriate by clinical judgement. The resultant number of mutations identified was used to calculate the mutation detection rate of BRCAPRO within our population and predict the approximate detection rate if the Manchester Scoring System had been used. Within the limits of the audit, a comparison was made between the mutations detected and/or potentially missed by each method.

The mutation detection rate using the Manchester Scoring System was predicted to be comparable or better than BRCAPRO, with similar numbers of tests being indicated by both methods. For this reason, the Manchester Scoring System (with the threshold of a combined score of 19 and above) was adopted by Genetic Health Queensland in 2011. Preliminary data from the first four months of implementation will be presented and evaluated against predicted outcomes.

1199W

What management for the asymptomatic men carriers of BRCA1 and BRCA2 mutation? Inquest to the French oncogenetics centres. *I. Brachot-Simeonova[1], G. Morin[2], C. Gillaux[1], B. Demeer[2], F. Amram[2], J. Gondry[1], M. Mathieu[2], R. Fauvet[1].* 1) Gynecology and Obstetric, Amiens University Hospital, Amiens, France; 2) Clinical Genetics and Oncogenetics, Amiens University Hospital, Amiens, France.

Objective: To define in the absence of guidelines, the management in France of asymptomatic men bearing a mutation of BRCA1-2 gene. Material and method: This multicentre study is a descriptive survey of practice. A questionnaire was addressed to the professionals working in the 90 French oncogenetics centres. Results: We obtained the answers of 46 practitioners working in 58 centres. 100% of the responders offered this screening to determine the risk of transmission to the descent and 86% to offer a personal follow-up. This follow-up concerned for 94% the prostate cancer, for 68% the breast cancer, for 49% the pancreatic cancer, and for 12% the melanoma. The screening of the prostate cancer was proposed mainly to the men bearing a BRCA2 mutation and from the age of 40 years. It was based on the clinical examination and the prostate-specific antigen. The screening of breast cancer was offered to the patient bearing a BRCA2 mutation. It was proposed by self-palpation and/or medical clinical examination and started between the age of 30 and 50 years. Imagery was only realized in case of symptoms. The screening of the pancreatic cancer was offered after the age of 40 years and by computed tomography and/or pancreatic MRI. It was mainly proposed in case of familial history of pancreatic cancer. The general practitioner was considered to be the best to perform all these screenings. Conclusion: These experts' opinions can help to establish recommendations for the management of the asymptomatic men carriers of BRCA1-2 mutation.

1200W

Single Molecule Targeted Resequencing to Individualize Cancer Therapy. *A.M.K. Brown[1], K. Ng[1], L. Timms[1], R. De Borja[1], T. Zhang[2], S. Kamel-Reid[2], J. Dancey[1], L.L. Siu[2], B. Tran[2], P. Shaw[2], S. Ghal[2], V. Ferretti[1], S. Watt[1], N. Onetto[1], T. Hudson[1], J.D. McPherson[1,3].* 1) Ontario Institute for Cancer Research , Toronto, ON, Canada; 2) Princess Marget Hospital, University Health Network, Toronto, ON, Canada; 3) University of Toronto, Toronto, ON, Canada.

Next generation sequencing technology is revolutionizing cancer research with unprecedented amounts of genome-wide data being generated for all tumor types. This is leading to a greater recognition of many of the molecular events associated with the initiation, progression and potential treatment of cancer. It is clear that cancer is a complex disease with most tumors being as unique as the individuals they arise in. A personalized medicine approach that includes the characterization of each tumor to identify key dysregulated pathways will better inform prognosis and enable targeting of therapies to improve outcomes. In order for this individualized approach to be integrated in the clinic the turn-around time, from tumor resection or biopsy to informed reporting to the clinician, needs to be short and the sensitivity and specificity needs to be high in order to accurately identify somatic variants in heterogeneous tumor samples with non-optimal cellularity. Using the PacBioRS platform, we have developed methods for the rapid characterization of a validated set of known oncogenes to uncover potential actionable mutations that can guide therapy. DNA derived from fresh frozen tissue, FFPE and blood has been tested and no appreciable difference in quality or quantity has been observed. Additionally, protocols for whole genome amplified material have also been optimized. We continue to develop library preparation methods and have increased yield over five-fold since the onset of development to over 100 MB of mapped sequence per SMRT cell in two 40 minute sequencing runs. Currently we are recruiting 1-2 patients per week with advanced metastatic cancers who are potential candidates for early phase clinical trials of targeted agents. Initially we focused on 19 oncogenes that overlap the Oncocarta V.1 panel (Sequenom, Inc.) to allow for cross technology comparisons. However, to fully take advantage of the sequencing yield of the PacBioRS, we are expanding to a larger list of ~200 genes. This clinical resequencing is being done in collaboration with a diagnostic CAP/CLIA molecular diagnostic laboratory to provide validation of all results prior to utilization. Logistics of sample handling, validation of this pipeline and reporting mechanisms are all being evaluated and optimized in a clinical trial setting for eventual routine inclusion into the clinical environment.

1201W

The MLH1 c.1-93 G>A SNP is associated with MLH1 promoter methylation and molecular features of serrated pathway colorectal cancer. *D.D. Buchanan[1], M.D. Walsh[1,2], M. Clendenning[1], R.J. Walters[1], B. Nagler[1], S. Pearson[1], D. McKeone[1], E. Pavluk[1], C. Rosty[3], J.L. Hopper[4], M.A. Jenkins[4], D.R. English[4], J.P. Young[1,2].* 1) Familial Cancer Lab, Queensland Institute of Medical Research, Herston, QLD, 4006, Australia; 2) University of Queensland School of Medicine, Herston QLD 4006, Australia; 3) University of Queensland Centre for Clinical Research, Royal Brisbane and Women's Hospital, Herston, QLD 4029, Australia; 4) University of Melbourne, Centre for MEGA Epidemiology, School of Population Health, Melbourne, VIC 3053, Australia.

The c.1-93 G>A SNP (rs1800734) within the MLH1 gene promoter has previously shown an association with MSI-H colorectal cancer (CRC). The majority of MSI-H CRCs is sporadic, resulting from methylation of the MLH1 gene promoter methylation with the remaining proportion of MSI-H CRCs seen in Lynch syndrome, resulting from a mutation in one of the mismatch repair genes (MMR). The aim of this study was to determine the association between the MLH1 c.1-93 G>A SNP and MSI-H CRC resulting from MLH1 promoter methylation. Five hundred and ninety three (n=593) individuals with CRC from the population-based Melbourne Collaborative Cohort Study were characterized for MSI, methylation of the MLH1 promoter and the CpG island methylator phenotype (CIMP) markers RUNX3, CACNA1G, SOCS1, NEUROG1 and IGF2 using MethyLight, the BRAF p.V600E somatic mutation, somatic mutations in codons 12 and 13 of the KRAS gene and loss of MMR proteins (MLH1, MSH2, MSH6 and PMS2) by immunohistochemical staining. DNA isolated from FFPE tissue from the normal colon mucosa was used to genotype for the rs1800734 SNP using a Taqman allelic discrimination assay. In this case-only analysis, carriers of the variant A allele were significantly older age at presentation of CRC (G= 66.4yrs ± 7.9yrs versus A=68.1yrs ± 7.1yrs, p=0.006). MSI-H CRCs were significantly more common in individuals carrying at least one copy of the variant allele (16%) when compared to individuals with the wildtype genotype (8%, p=0.003, OR=2.2, 95%CI=1.3-3.7). CRCs with methylation of the MLH1 gene promoter were increased two-fold in carriers of the GA or AA genotypes (15.4%) when compared to carriers of the GG genotype (8.6%, p=0.01, OR=1.9, 95%CI= 1.2-3.2), displaying an increase in the frequency of MLH1 methylation tumours with increasing variant allele dose (GG (8.6%) < AG (13.8%) < AA (27.6%)). CRCs with a BRAF p.V600E mutation (p=0.04, OR=1.4, 95%CI= 1.1-2.0) or CIMP (≥ 3 positive markers; p=0.007, OR=1.7, 95%CI=1.2-2.4) were associated with carrying at least one copy of the variant allele. No association was observed for gender, tumour site or mutations in the KRAS gene. In patients with CRC, the variant allele of the MLH1 c.1-93 G>A promoter SNP is significantly more common in CRC with methylation of the MLH1 promoter, CIMP and the BRAF p.V600E mutation suggesting that MLH1 promoter methylation and the methylator phenotype of serrated pathway CRC underlies the previously reported association with MSI-H CRC.

1202W

Improved tumour profiling using next generation sequencing approaches. *D. Burgess[1], I. Tomlinson[2], M. Middleton[2], B. Hassan[2], A. Schuh[2], D. Green[1], T. Albert[1], X. Zhang[1], J. Taylor[2].* 1) Research & Development, Roche NimbleGen, Madison, WI; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, UK.

Next generation sequencing methods have fueled research into the genetic basis of cancer, in particular the somatic mutations in tumours which may influence natural history, tendency to metastasize, response to treatment and potential for adverse events. However, tumour profiling in clinical practice has not kept pace with these developments and remains largely limited to a few selected genetic markers which are known to influence response to therapy, such as EGFR and KRAS to guide treatment with erlotinib and gefitinib in non small cell lung cancer and BRAF for BRAF inhibitors in melanoma.

Although whole genome sequencing is becoming cheaper, it is not yet cost effective in a clinical setting and the bioinformatics pipelines required to manage the wealth of data created on an individual patient basis must also be developed. Robust targeted resequencing methods are therefore critical to realise the prospect of using NGS for molecular profiling of tumours in both research and clinical settings. To address this, we developed a solution capture protocol that is robust, rapid and has high capture efficiency. The technique was evaluated on a panel of colorectal cancer samples that was well characterized by other molecular techniques and was associated with clinical outcome data. We then developed a capture design for the routine screening of a set of high-value cancer gene targets and evaluated this design against a panel of different cancer types, including colorectal, melanoma and sarcoma. The results of this evaluation study, including a comparison with other solution capture protocols will be described.

1203W

Genome-wide DNA methylation pattern analysis of childhood leukemia. *S. Busche[1,2], C. Richer[3], B. Ge[2], J. Healy[3], T. Kwan[1,2], S.H. Chen[2], D. Sinnett[3,4], T. Pastinen[1,2].* 1) Human Genetics, McGill University, Montreal, Quebec, Canada; 2) McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, Canada; 3) CHU Ste-Justine Research Center, Montreal, Quebec, Canada; 4) Department of Pediatrics, Universite de Montreal, Montreal, Quebec, Canada.

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer, and is a leading cause of cancer-related death in children. The most prevalent is the B-cell progenitor subtype (pre-B ALL; ~80%), and cytogenetic analysis has helped further classification into three subgroups based on chromosome number, recurrent genetic translocations, or lack of any known genetic changes (NP). In addition to genetic alterations, cancer cells display a deeply distorted epigenome. DNA methylation is a frequent epigenetic modification occurring almost exclusively in the context of cytosine methylation in CpG dinucleotides. An in-depth understanding of genome-wide methylation patterns, and their correlation/association with available gene expression and SNP genotyping data from the same samples has the potential for identifying novel biomarkers. We collected bone marrow and/or peripheral blood from pediatric pre-B ALL patients of French-Canadian origin at diagnosis (day 0, untreated leukemia) and remission (matched-normal material). The tumor samples display high leukemic blast rates, and represent the three subgroups. Methylation profiling was carried out using the Illumina Infinium HumanMethylation450 BeadChip, which interrogates ~450,000 CpG-sites across the whole genome. Principal component analysis reveals the tumor subgroups as a major determinant of genome-wide methylation differences among pre B-ALL samples (accounting for 22% of variance). Furthermore, we identified significant methylation level alterations of multiple CpG-sites associated with *IGF2BP*, a gene whose expression level was shown to be perturbed in B-ALL. In subsequent approaches, RNA-sequencing derived gene expression levels will be correlated with promoter methylation levels. Regulatory marker methylation is expected to negatively correlate with the corresponding gene expression levels. SNP genotyping will identify genetic variants which might be a source of DNA methylation variation and hence gene regulation. Overall, we hope that our findings will provide the basis for the development of more efficient therapeutic approaches.

1204W

High resolution melting (HRM) analysis of BRAF V600E mutation in sporadic colon cancer. *T. Cacev, S. Bulat, S. Kapitanovic.* Division of Molecular Medicine, Rudjer Boskovic Institute, Zagreb, Croatia.

Colorectal cancer arises as a consequence of continuous mutation accumulation in number of oncogenes and tumour suppressor genes. Approximately 50% of colorectal tumours have an aberrant MAPK signalling. Although mutations of genes involved in MAPK signalling pathway are known for sometime, their importance has recently been brought to scientific attention as a consequence of break-troughs in personalized medicine and modern antitumour therapy. Conventional methods of mutation detection are prone to generating false positive or negative results; therefore, in recent years a method of choice for mutation screening is the high resolution melting fluorescent detection of heteroduplexes (HRM). In this study HRM method was introduced in order to detect the most common BRAF gene mutation, V600E in sporadic colon cancer tumours. A total of 120 tumour samples were analyzed and in 6 (5%) BRAF V600E mutation was detected and confirmed by DNA sequencing. Finally, HRM method has proved itself to be the simplest and faster alternative to conventional methods of mutation detection and can be used for molecular profiling of other tumours where BRAF mutations have an important role.

1205W

Evidence for a strong genetic contribution to lethal prostate cancer. *L. Cannon-Albright[1,2,3], C. Teerlink[1], N. Agarwal[2], R. Stephenson[2].* 1) University of Utah School of Medicine, Salt Lake City, UT; 2) Huntsman Cancer Institute, Salt Lake City, UT; 3) George E. Wahlen Department of Veterans Affairs Medical Center, Salt Lake City, UT.

Decades of investigation into the genetic causes of prostate cancer and prostate cancer aggressiveness has yet to clearly identify genes or variants which explain much more than a small amount of risk for prostate cancer among a small population of men. Even less progress has been made in understanding why 30% of all patients with localized prostate cancer eventually develop recurrent, and subsequently fatal, prostate cancer. Here we investigate familial clustering for the distinct subset of prostate cancer cases whose disease recurs, those cases who go on to die from their prostate cancer. Using the Utah Population Database, a computerized genealogy of Utah combined with Utah death certificates from 1904, we analyzed the familial clustering of prostate cancer cases. We utilized only those 1.2 M individuals in the genealogy who had data for both parents, all 4 grandparents and 6 of 8 great grandparents. We identified a total of 17,207 men with a diagnosis of prostate cancer, of which 4,388 also had a death certificate including prostate cancer as a primary or contributing cause of death. It has been well demonstrated that prostate cancer shows significant excess familial clustering. Analysis of the subset of prostate cancer cases dying from prostate cancer shows a similar result. We tested the hypothesis that the subset of men dying from prostate cancer clustered even more closely than all prostate cancer cases. We performed the Genealogical Index of Familiality (GIF) test for excess relatedness on the cases dying from prostate cancer. We compared the observed relatedness of this distinct set of cases to the expected relatedness among all prostate cancer cases. Significant excess relatedness for the men dying from prostate cancer was observed beyond second cousin relationships; even when close relationships were ignored, the significant excess relatedness was observed. These data provide strong support for a genetic contribution to lethal prostate cancer and identifies a set of high-risk pedigrees who should be studied to identify the responsible genes.

1206W

Quantitative analysis of the expression of anti- and pro- angiogenic isoforms of VEGF in breast cancer by qPCR. *R. CASTRO[1], P.M. Biselli-Chicote[1], V.R. Regiani[2], D.S. Neto[3], N.A.B. Junior[4], J.L.E. Francisco[4], J.A. Thome[5], E.C. Pavarino[1], D.A.P.C. Zuccari[2], E.M. Goloni-Bertollo[1].* 1) Research Unit in Genetics and Molecular Biology, Faculty of Medicine of Sao Jose do Rio Preto, Sao Paulo, BR; 2) Center for Studies and Research on Cancer Faculty of Medicine of Sao Jose do Rio Preto, Sao Paulo, BR; 3) Pathology Service, Hospital de Base, São Jose do Rio Preto, Sao Paulo, BR; 4) Department of Gynecology and Obstetrics, Hospital de Base, Sao Jose do Rio Preto, Sao Paulo, BR;; 5) Institute of Anatomical Pathology and Cytopathology, Sao Jose do Rio Preto, Sao Paulo, BR.

Cancer is the second leading cause of death in the world after cardiovascular diseases and female breast cancer is the most frenquently diagnosed cancer and the leading cause of cancer death in females worldwide, accounting for 23% of the total new cancer cases and 14% of the total cancer deaths in 2008. About half breast cancer cases and 60% of the deaths are estimated to occur in economically developing countries. Angiogenesis plays a critical role in local growth of solid tumors and subsequently in the process of distant spread. Numerous studies have demonstrated the importance of angiogenesis in cancer. Nevertheless, 3' alternative splice site selection in exon 8 of VEGF gene results in a sister family of isoforms, VEGFxxxb, which are anti-angiogenic and downregulated in tumor tissues. We quantitatively analyzed the expression of pro-angiogenic and anti-angiogenic VEGF isoforms in breast carcinoma and adjacent normal tissue samples. For that purpose, total RNA from 15 tumor samples and their respective margins were obtained and synthesized cDNA from. We designed and synthesized primers and specific probes for each isoform, which were used for the analyses of expression by real time PCR. So far, we have not observed different expression between the anti- and pro- angiogenic isoforms in the tumor samples compared to normal tissue. These results evidence that the selection of different splicing sites do not interfere in tumor angiogenesis and consequent tumor progression and metastasis. A bigger sample size might help in more advanced studies and collaborate to better development of researches on tumor angiogenesis involving VEGF gene. Studies approaching control of VEGF splicing in order to promote the selection of the distal splicing site (anti-angiogenic) instead of proximal site (pro-angiogenic) might promote an efficient therapy for breast cancer.

1207W

Identification of MMTV/HMTV retrovirus in breast tumor samples from Mexican females. *A. Cedro-Tanda, A. Córdova-Solis, A. Pedroza-Torres, M. Rives-Güendulain, M. Moreno-Arroyo, J. Tórres-López, F. Salamanca-Gómez, D. Arenas-Aranda, N. García-Hernández, X. Rivera-Gonzalez.* Instituto Mexicano de Seguridad Social, México D. F.

Breast cancer tumor is the first cause of dead in Mexican female population. Recently was probed that MMTV retrovirus its related with tumor development and progression. Te aim of this work was to identify HMTV sequences integrated into the breast tumor genome in Mexican females. We study 80-paired tumors (stage II and III) and non-affected tissue. DNA and RNA were obtained. Nested PCR was performed to amplify Env gene fragment (660pb and 220pb) to verify retrovirus presence in all samples, Env gene contained in pBR233 was used as positive control and a housekeeping gene GADPH (500pb was used as a reaction control. Also qPCR was used to double-check the presence of the retrovirus with Taqman probes for Env gene. Total RNA was treated with DNase to use in qRT-PCR that was performed with Taqman probes to evaluated Env and Gag genes expression. Splinkerrette Method was performed to determine insertion sites of the MMTV/HMTV retrovirus in positive samples to contain the provirus. We successfully transformed the pBR322plasmid and determined that it contained Env gene. MMTV infection was determined in 30% of the tumor samples and in none of the non-affected breast tissue. This data was double-checked and confirmed by Taqman PCR. Expression of Env and Gag genes were determined in positive tumors by using TaqMan probes. All amplified fragments were sequenced from positive samples to determine their identity. In positive infected tumors, we developed Splinkerrette method to determine MMTV/HMTV retrovirus insertions, we found several samples containing expressed viruses and with insertions along the genome. We are working in order to determine the possible participation of MMTV/HMTV retrovirus in to the tumor progression and development; we will look forward in to the target genes to evaluate expression and function alterations originated from the mutagenesis insertion.

1208W

Mutations in the Sonic Hedgehog Pathway in Congenital Acute Lymphoblastic Leukemia Cells. *V. Chang[1], K. Sakamoto[1], S. Nelson[2].* 1) UCLA, Department of Pediatrics, Division of Hematology-Oncology. 10833 Le Conte Ave. MDCC A-312, Los Angeles, CA; 2) UCLA, Department of Human Genetics, Pathology and Laboratory Medicine, and Psychiatry, David Geffen School of Medicine. 695 Charles Young Drive S. Gonda 5554, Los Angeles, CA.

Background: Congenital acute lymphocytic leukemia (cALL) is diagnosed in the first month of life and is commonly associated with MLL translocations. cALL has a much worse prognosis compared to older pediatric patients with ALL. This suggests that cALL is a biologically different disease, and thus may result from a distinct set of mutations. The aim of our study was to conduct genomic analysis of paired primary tumor and normal samples from cALL patients and two MLL-positive ALL cell lines (RS411 and HB1119). Methods: Genomic DNA was enriched for coding exons using Sure Select Human All Exon or Human All Exon 50Mb kits (Agilent, Santa Clara, California). The primary sample was sequenced on the Illumina GAIIx, and the two cell lines were sequenced on the HiSeq 2000 (Illumina, San Diego, California). Reads were aligned to the human reference genome using Novoalign (www.novocraft.com). The Genome Analysis Toolkit was used for recalibration of base quality, variant calling, filtration and evaluation. GeneChip Human Genome U133 Plus 2 (Affymetrix, Santa Clara, California) was used to obtain gene expression for all samples and normal lymphocytes. Cel files were normalized and analyzed with dChip to identify differentially expressed genes, using a fold change analysis of the samples compared to normal lymphocytes. Results: We generated 72 million reads of tumor and normal sequences in the primary cALL sample, with oversampling greater than 80% of the bases at 20x. There was a novel, somatic, heterozygous nonsynonymous missense mutation in sonic hedgehog (SHH), which was validated with Sanger sequencing. We generated 312 million reads for RS411, with 91% of bases covered at 20x or greater, and 349 million reads for HB1119 with 92% of bases covered at 20x of greater. There were novel, heterozygous, nonsense mutations in Patch, which is a SHH receptor, in both cell lines. Expression data showed upregulation of SHH downstream transcription factor GLI3 at nearly 8-fold for HB1119 and 12-fold for RS411. Conclusion: This primary cALL sample and two ALL cell lines harbor novel, nonsynonymous mutations in SHH and Patch, with an upregulation of GLI-3 in both cell lines. Therefore, the SHH pathway may be involved in the pathogenesis of cALL.

1209W

***ITPR3* gene haplotype is associated with cervical cancer risk.** *T. Chang[1], Y. Yang[2,3], Y. Lee[1,4,5], T. Chen[2], S. Chang[1], W. Lin[1].* 1) Medical Research Department, Mackay Memorial Hospital, Taipei, Taiwan; 2) Department of Gynecology and Obstetrics, Mackay Memorial Hospital, Taipei, Taiwan; 3) Department of Gynecology and Obstetrics, Taipei Medical University, Taipei, Taiwan; 4) Pediatrics, Mackay Memorial Hospital, Taipei, Taiwan; 5) Pediatrics, Taipei Medical University, Taipei, Taiwan.

Cervical cancer is strongly associated with infection by oncogenic forms of human papillomavirus (HPV). However, HPV infection alone is not sufficient for progression to cervical cancer. It is now recognized that host immunogenetic background play an important role in the control of HPV infection and the development of cervical cancer. Inositol 1,4,5-trisphosphate receptor type 3 (ITPR3) is an intracellular Ca^{2+} release channel that mediates Ca^{2+} mobilization in response to the binding of a second messenger, inositol 1,4,5-trisphosphate (IP3). It has been reported that ITPR3 plays an important role in the regulation of apoptotic signaling in T lymphocytes. The aim of this study is to investigate the association between *ITPR3* gene single nucleotide polymorphisms (SNPs) and the risk of cervical cancer in the Taiwanese population. We genotyped 2 SNPs (rs3748079 A/G and rs2229634 C/T) in 272 cervical squamous cell carcinoma (CSCC) patients and 921 age/sex matched healthy controls by using the Pre-Developed TaqMan Allelic Discrimination Assay. The presence and genotypes of HPV in CSCC patients were determined by PCR. No statistically significant differences of genotype, allele, and carrier frequencies of these *ITPR3* SNPs were found among controls, all women with CSCC, and those with HPV-16 positive CSCC. Intriguingly, we found significant associations of haplotype AT between all women with CSCC and controls (OR = 3.34, 95% CI 1.87-5.97, $P_c = 6.4 \times 10^{-5}$) or between women with HPV-16 positive CSCC and controls (OR = 4.27, 95% CI 2.24-8.13, $P_c = 6.9 \times 10^{-6}$). Our results suggest that AT haplotype in the *ITPR3* gene may confer susceptibility to CSCC in the Taiwanese population.

1210W

Viability Screens with Pooled shRNA Libraries Identify Novel and Synthetically Lethal Anti-Cancer Targets. *A. Chenchik[1], D. Tedesco[1], K. Bonneau[1], M. Makhanov[1], C.G. Frangou[2].* 1) Cellecta, Inc., Mountain View, CA; 2) Fred Hutchinson Cancer Research Center, Seattle, WA.

Genes modulating proliferation and survival in oncogenic cells have been identified using pooled lentiviral-based libraries expressing many thousands of shRNAs. A viability assay with leukemic and prostate adenocarcinoma cell lines transduced with these shRNA libraries targeting thousands of genes identified a few hundred essential genes for each panel of cells. Subsequent validation using single shRNA-expressing constructs showed that about 80% of the shRNAs identified in the complex library screen did in fact lead to cell death when transduced in cells. Analysis of the identified essential genes for known biological interactions revealed several non-random clusters of interacting proteins that provide some insight into signaling pathways and protein networks specific to these cancers. Also, we have recently adapted the approach to combinatorially screen shRNA sequences targeting hundreds of genes to discover additive and synergistic combinations that generate a synthetic-lethal phenotype. Analysis of the lethal combinations indicates redundant, complementary, and compensatory responses in cancer cells. This presentation will provide an overview of the screening platform, our approach to shRNA library design, and results from the screens for cancer cell-specific lethal and synthetic-lethal genes.

1211W

A rare intronic *CHEK2* sequence variant in a child with ependymoma and glioblastoma alters substrate recognition in the NMD pathway. *H. Cheung[1], D. Wheeler[2], G. Tomlinson[3], L. Strong[4], R. Gibbs[2], S. Plon[1].* 1) Pediatrics, Baylor College Med, Houston, TX; 2) Human Genome Sequencing Center, Houston, TX, USA; 3) University of Texas Health Science Center San Antonio, San Antonio, TX, USA; 4) MD Anderson Cancer Center, Houston, TX, USA.

Both inherited and somatic mutations in DNA repair genes have been associated with multiple types of childhood cancer. Reduced DNA repair capacity can accelerate genomic instability and tumor development. We analyzed constitutional DNA from probands in kindreds with unusual patterns of childhood cancer for coding mutations in a panel of 45 cancer associated genes with an emphasis on DNA damage response and repair genes. We are now using this dataset to find sequence variants that would affect RNA expression patterns while meeting at least two of the following criteria: not present in 1000 Genomes data, having a minor allele frequency (MAF) of <0.01, and within 100 bp of an annotated alternatively spliced region. Using a custom splicing array, we measured RNA expression patterns and levels from patient-derived, non-tumor lymphoblastoid (LCL) cells from both patients and controls and analyzed for aberrant events. One heterozygous sequence variant in *CHEK2* altered nonsense-mediated decay (NMD) patterns in these transcripts as validated by RT-PCR and inhibition of NMD by cycloheximide. This is a rare intronic variant rs56109140 with MAF of 0.008 and results in increased NMD. We are now confirming this splicing phenotype in heterozygous LCL lines from the 1000 Genomes collection; if confirmed, it will be important to determine the impact of this *CHEK2* variant on cancer risk. Supported by Thrasher Foundation to HCC and 1R01CA138836 to SEP.

1212W

A Comprehensive Resequence-Analysis of 166kb *KITLG* Region in Familial Testicular Germ Cell Tumors. *C. Chung[1], M. Yeager[3], J. Boland[3], M. Greene[2], S. Chanock[1], C. Kratz[2].* 1) LTG/DCEG, NCI/NIH, Bethesda, MD; 2) Clinical Genetics Branch/DCEG, NCI/NIH, Bethesda, MD; 3) Core Genotyping Facility/DCEG, Advanced Technology Program, SAIC-Frederick Inc., NCI-Frederick, Frederick, MD.

Among several loci identified through genome-wide association studies, the *KITLG* region on chromosome 12q22 has shown the strongest risk estimate for testicular germ cell tumor (TGCT). Mutations in the KIT/KITLG system, involving the ligand for the membrane receptor tyrosine kinase, c-KIT, have been implicated in both testicular cancer development and infertility, both of which are established TGCT risk factors. We have created a comprehensive catalogue of variants within this region to lay the foundation for further investigation into the underlying functional mechanisms of this association. Our study sample set included a total of 117 individuals from the National Cancer Institute's Familial Testicular Cancer Study (FTCS): 10 parent-son trios, of which 6 trios contained 2 familial TGCT cases, 51 familial TGCT cases and 36 sporadic bilateral cases. We resequenced a 166kbps region (chromosome 12: 87371132-87537273) around *KITLG*, based on linkage disequilibrium patterns and tag analysis using HapMap III CEU and the 1000 genome CEU low-coverage pilot data (July 2010 release). No recombination hotspot was observed within a 1Mb region centered on *KITLG*. We have further extended catalogue of common and rare *KITLG* variants using deep coverage in next-generation sequencing. These study results will facilitate further functional elucidation of TGCT susceptibility loci within this genomic region.

1213W

Evaluation of RAD51C as a new breast cancer susceptibility gene in the Belgian/Dutch population. *K. Claes[1], M. Van Bockstal[1], S. De Brouwer[1], N. Swietek[1], K. Storm[2], J. Van den Ende[2], S. Willocx[2], E.B. Gomez-Garcia[6], B. Blaumeiser[2], K. Leunen[3], C.J. Van Asperen[4], J. Wijnen[4], E. Legius[5], G. Michils[5], G. Matthijs[5], M.J. Blok[6], A. De Paepe[1], B. Poppe[1], K. De Leeneer[1].* 1) Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; 2) Center for Medical Genetics, University Hospital of Antwerp, Antwerp, Belgium; 3) Department of Gynaecological Oncology, Catholic University of Leuven, Leuven, Belgium; 4) Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands; 5) Department of Human Genetics, Catholic University of Leuven, Leuven, Belgium; 6) Department of Clinical Genetics, Maastricht University Medical, Maastricht, Netherlands.

Recently, germline mutations in RAD51C were found to be associated with an increased risk for breast and ovarian cancer. Meindl et al. (2010) detected six monoallelic pathogenic mutations in RAD51C by screening 1.100 unrelated German women with gynaecologic malignancies (breast and/or ovarian tumors). Strikingly, all six deleterious mutations were exclusively found within 480 BRCA1/2 negative breast and ovarian cancer families and not in breast cancer only families. With this study we aim to determine the prevalence of germline RAD51C mutations in Belgian/Dutch breast and ovarian cancer families, previously found to be negative for BRCA1&2 mutations. We performed mutational analysis in 482 index patients. Mutation detection was performed with High resolution melting (HRM), followed by Sanger sequencing of the aberrant melting curves. No unequivocal deleterious RAD51C mutation was identified in our study population. In total our mutation analysis revealed 11 unique sequence variations of which 3 are novel. The most interesting is a novel 3'UTR variant: c.*131 A>G, identified in an ovarian cancer patient, diagnosed at the age of 60 and a positive family history of breast and colon cancer. In silico miRNA binding seed predictions (PITA Scan) revealed the creation of a miR-126 binding site. Functional assays are currently ongoing to find out if this variant alters RAD51C expression mediated by miRNAs. Two other novel variants (RAD51C c.-36 A>G (5'UTR) and c.572-17 G>T (intronic)) were detected in our study population, none of them are predicted in silico to affect splicing. Since the initial publication, we and others were unable to confirm a major role for RAD51C germline mutations in breast and ovarian cancer families. Since the investigated cohort is of similar size and geographically close to the patients investigated in the initial report, we conclude that the prevalence of RAD51C mutations may be lower than initially expected. However, further investigations in populations of various ethnic origins are required before RAD51C can be excluded as a major breast and ovarian cancer susceptibility gene.

1214W

Mutation deep within an intron of *MSH2* causes Lynch syndrome. *M. Clendenning[1], D.D. Buchanan[1], M.D. Walsh[1], B. Nagler[1], C. Rosty[1], B. Thompson[2], A.B. Spurdle[2], J.L. Hopper[3], M.A. Jenkins[3], J.P. Young[1].* 1) Familial Cancer Laboratory, Queensland Institute of Medical Research, Herston, QLD, AUSTRALIA; 2) Molecular Cancer Epidemiology, Queensland Institute of Medical Research, Herston, QLD, AUSTRALIA; 3) Centre for Molecular, Environmental, Genetic and Analytical Epidemiology, Melbourne School of Population Health, The University of Melbourne, Parkville, VIC, AUSTRALIA.

Background Lynch syndrome, a heritable form of cancer predisposition, is caused by germline mutations within genes of the DNA mismatch repair family, and can be rapidly identified in young onset cancer patients through the detection of loss of expression of at least one of these genes in tumour samples. To date, such causative mutations have only been identified within exonic and splice site regions. Though this approach has been successful in the majority of families, a considerable number remain in which no mutation has been found. Here, we address mutation detection in such a family. **Methods** An alternative mutation discovery procedure involving haplotype analysis of the locus containing the gene lost in the tumour and delineation of segregating haplotypes, followed by an investigation of splicing aberrations was utilised to uncover a cryptic splice site which lays outside the genomic regions routinely examined for mutations. **Results** We show that an intronic mutation 478bp upstream of exon 2 in the *MSH2* gene causes Lynch syndrome through the creation of a novel splice donor site with subsequent pseudoexon activation, thus highlighting the need for more extensive sequencing approaches.

1215W

Association of polymorphisms in antioxidant genes with chronic myeloid leukemia. *E. Cordova[1], E. Crespo-Solis[2], M. Ayala-Sanchez[3], C. Yerena de Vega[4], M. Lopez-Hernandez[4], C. Sanchez-Guerrero[4], I. Chavez-Perez[4], A. Martínez-Tovar[5], A. Aguayo[2], M. Morales-Marin[1], A. Martinez-Hernandez[1], L. Orozco[1,6].* 1) Investigacion, Inst Natl de Medicina Genomica, Mexico D.F., Mexico; 2) Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico; 3) Hospital de Especialidades del Centro Médico Nacional La Raza, Mexico; 4) Centro Médico Nacional 20 de Noviembre, ISSSTE, Mexico; 5) Hospital General de México, SS, Mexico; 6) Posgrado en Ciencias Genómicas, UACM, Mexico.

Chronic myeloid leukemia (CML) is the most common form of leukemia in adults, with an annual incidence of 1 to 1.3 cases for each 100,000 individuals. CML is characterized by the presence of the translocation t(9;22)(q34;q11), which generate the fusion gene BCR-ABL. The causes of CML development are barely known and only ionizing radiation has been associated with CML risk. Oxidative stress has been recognized as an important factor in cancer development. In fact, polymorphisms occurring in genes that encode for antioxidant enzymes such as GSTP, NQO1 and HMOX1 have been associated with an increased risk to develop lung, bladder and liver cancer. However, the association between variants in antioxidant genes and CML risk has been poorly studied. Hence, in this study we determined whether polymorphisms occurring in five antioxidant genes were associated with the risk to develop CML in Mexican population. Genomic DNA was obtained from 250 individuals diagnosed with CML and 400 healthy blood bank donors. Cases and controls signed an informed consent and were matched for gender, age and ethnic origin. Allelic discrimination of single nucleotide polymorphisms (SNPs) located on GSTPI (A1404G), GCLM (-588CT), GCLC (-129CT) and NQ01 (C609T) genes was carried out by using the 5' exonuclease reaction (TaqMan). Genotyping of HMOX1 (GC)n repeats was performed by DNA fragment-length analysis. Genotype frequencies were in Hardy-Weinberg equilibrium in cases and controls. Allelic frequencies of SNPs were similar to those reported in other populations. Genotype and allele frequencies of all SNPs studied did not show significant differences between cases and controls. Regarding HMOX1 (GC)n repeats we found 27 different alleles (10-39), showing significant differences with other populations. In Mexican population the most common HMOX1 (GC)n repeat alleles were 22 and 29 repeats and the frequency of the short alleles (10-25) was significantly lower than those reported elsewhere. Interestingly, when HMOX1 (GC)n short allele frequencies were compared between CML patients and controls, a significant association was observed (OR=1.497, CI[1.108-2.022], p=0.008). In conclusion, although more studies are needed with leukemic populations and other ethnic groups, we found a preliminary evidence of association between CML susceptibility and HMOX1 (GC)n repeat polymorphism.

1216W

BRCA1 and BRCA2 diagnosis: feasibility of multiplex amplification coupled to next-generation sequencing. *F. Coulet, F. Pires, M. Eyries, C. Colas, F. Soubrier.* Oncogénétique moléculaire, GH Pitié-Salpêtrière-APHP, PARIS, France.

Screening of the two large BRCA1 and BRCA2 genes are time-consuming with traditional technologies of direct sequencing or even with prescreening heteroduplexes methods. Next generation sequencing has proven to be highly efficient for research purposes, in particular to detect mutations in genetic diseases and in tumoral genomes. In this study we tested the possibility to use multiplex PCR coupled to next-generation sequencing in routine diagnosis of breast cancer predisposition. We tested a Multiplex Amplification (5 multiplex PCR reactions generating 94 amplicons of Specific Targets (BRCA1 and BRCA2) for Resequencing (MASTR by Multiplicom). Seven patients with 32 different known variants scattered over amplicons of BRCA1 and BRCA2 genes were analyzed. The 32 variants were previously detected by High Resolution Melting. All gene variants tested correspond to 7 pathogenic mutations (2 missense, 2 nonsense, and 3 frameshift), 1 unclassified variant and 24 polymorphisms. A 2-step PCR protocol allows incorporation of 7 optimized 454 MIDs (molecular barcodes) in each amplified product to unambiguously link each read to its parent sample. A small DNA amount is necessary (250 ng) for the complete screening of each sample. The 454 Titanium DNA sequencing technology on the genome GS Junior instrument (Roche Diagnostics) utilizes adapter-ligated DNA, emulsion PCR and pyrosequencing system. A single sequencing run containing the 7 biological samples was performed and we obtained 45766 reads (corresponding to a mean of 70X coverage per amplicon). All samples of known genotypes were properly clustered by the Amplicon Variant Analyzer (AVA) software from Roche Diagnostics. The 32 different nucleotide variants were identified, and the multiple polymorphisms are well genotyped (a total of 126 heterozygous or homozygous variations were identified). Fourteen previously unknown polymorphisms were identified because of the extended amplicons sequence as compared to primers previously used for HRM screening. Accurate and rapid variant detection could be additionally done by SeqNext software (JSI Medical Systems) allowing homopolymers to be specifically analyzed. Further experiments need to be done to confirm the high sensitivity and specificity obtained in this pilot experiment. Combination of MASTR assays with massive parallel sequencing technology allows high fidelity, high throughput and cost effective sequencing also for diagnostic purposes.

1217W

Association between CCRN4L gene and Non-Small-Cell-Lung Cancer (NSCLC) in Brazilian patients. *P.G. Couto[1], L. Bastos-Rodrigues[1], A. Vilhena[2], N.F. Amaral[2], L. De Marco[1].* 1) Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; 2) Hospital Julia Kubitschek, Belo Horizonte, Brazil.

The circadian rhythms comprise daily oscillations in various biological processes that are regulated by an endogenous clock. Disruption of these rhythms have been associated with cancer in humans and the understanding of these rhythms in cellular growth control, tumor suppression and cancer treatment can reveal how clock genes and clock-controlled genes are regulated in healthy humans. One of these rhythmic genes, Nocturnin (CCRN4L), encodes a deadenylase-a-ribonuclease that specifically removes the poly (A) tails from mRNAs. We genotyped three tag SNPs (rs938836, rs17050680, rs3805213) in the CCRN4L gene in 72 patients with Non-Small-Cell-Lung Cancer (NSCLC) and 67 individuals controls to investigate the association between the CCRN4L gene and NSCLC. One of the three tested markers demonstrated significant association with genotype and allele frequency in lung cancer (rs3805213; p=4.4 e-3 and p=5.7 e-2, respectively). We found a statistically significant association between tag SNP rs3805213 and lung cancer. The results of our study suggest that polymorphisms in the CCRN4L gene can represent a risk factor in the occurrence of NSCLC in Brazilian patients.

1218W

Cancer Risk-Associated Variants Disrupt FoxA1 Genomic Activity. *R. Cowper, X. Zhang, J. Wright, M. Cole, J. Eeckhoute, J.H. Moore, M. Lupien.* Dartmouth Medical School, Lebanon, NH, USA.

Genome-wide association studies (GWASs) have identified thousands of single nucleotide polymorphisms (SNPs) associated with human traits and diseases. But because the vast majority of these statistical associations map to the noncoding regions of the genome their causative mechanisms remain elusive. Employing a new methodology combining cistromics, epigenomics and genotype imputation we annotate the noncoding regions of the genome in breast and prostate cancer cells and identify the functional nature of SNPs associated with breast and prostate cancer risk. Through the holistic analysis of the genomic distribution of risk-associated SNPs we reveal that risk-associated variomes target the cistromes of transcription factors in a cancer type-specific manner and that the genomic activity of the pioneer factor FoxA1 is a key target in both breast and prostate cancer. Finally we computationally predict and experimentally validate the disruption of FoxA1 recruitment for a set of risk-associated SNPs and assess the subsequent alteration of target gene expression and cell proliferation.

1219W

Genetic Polymorphisms in NAT1, NAT2, GSTM1, GSTP1 and GSTT1 and Susceptibility for Colorectal Cancer in a Filipino Population. *E. Cutiongco[1], V. Bañez[2], M. Roxas[3], C. Padilla[1], C. Ngelangel[4], R. Santos-Cortez[5], C. Silao[1], F. Rocamora[1], O. Florendo[2], C. Sison[2], A. Roxas[3], F. Doble[6], E. Nuqui[7] for The Philippine Cancer Genetics Study Group.* 1) Inst Human Genetics, Natl Inst Health, University of the Philippines, Manila; 2) Section of Gastroenterology, Department of Medicine, Philippine General Hospital, Philippines; 3) Department of Surgery, Philippine General Hospital, Philippines; 4) Inst of Clinical Epidemiology, Natl Inst Health, University of the Philippines Manila; 5) Phil National Ear Inst, Natl Inst Health, University of the Philippines, Manila; 6) Department of Surgery, East Avenue Medical Center, Philippines; 7) Department of Pathology, College of Medicine, University of the Philippines, Manila.

BACKGROUND: Colorectal cancer is one of the leading cancers of the world. Based on incidence, it ranks fourth in men and third in women, with over one million new cases worldwide. The majority (> 90%) of cancers of the colon and rectum are adenocarcinomas. In the Philippines, cancers of the colon and rectum ranked 6th overall, 4th among males and 4th among females. Polymorphisms in metabolic genes which alter rates of bioactivation and detoxification have been shown to modulate susceptibility to colorectal cancer. METHODS: A total of 276 colorectal cancer cases and 224 controls from the Filipino population were genotyped for selected polymorphisms in GSTM1, GSTP1, GSTT1, and NAT2. Medical and diet histories, occupational exposure and demographic data were also collected for all subjects. Odds ratios for colorectal cancer status were then obtained. RESULTS: Univariate logistic regression of non-genetic factors identified exposure to UV (sunlight) (OR 1.99, 95% CI: 1.16-3.39) and wood dust (OR 2.66, 95% CI: 1.21-5.83) and moldy food consumption (OR 1.61, 95% CI:1.11-2.35) as risk factors; while the NAT2*6B allele (recessive model OR 1.51, 95% CI :1.06-2.16; dominant model OR 1.87, 95% CI: 1.05-3.33) and homozygous genotype (OR 2.19, 95% CI: 1.19-4.03) were found to be significant among the genetic factors. After multivariate logistic regression of both environmental and genetic factors, only UV radiation exposure (OR 1.21, 95% CI: 1.21-3.58) remained to be significantly associated with increasing colorectal cancer risk in the study population. CONCLUSION: The NAT2*6B allele and genotype were found to significantly increase colorectal cancer risk among Filipinos.

1220W

5-Fluorouracil induce the expression of TLR4 on HCT116 colorectal cancer cell line expressing different variants of TLR4. *h. davoodi[1], S.R. Hashemi[2], S. Heng Fong[3].* 1) Golestan University of Medical Sciences, Gorgan, Iran , Gorgan, gorgan, Iran; 2) Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran; 3) Department of Pathology, Faculty of Medicine and Health Science, University Putra Malaysia, Malaysia.

Toll-like receptors (TLRs) are a class of proteins that play a key role in the innate immune system. TLR4, which is considered one of the most important TLRs, recognizes LPS of Gram-negative bacteria. Two cosegregating single nucleotide polymorphisms (SNPs) of the human TLR4 gene, namely Asp299Gly (D299G) and Thr399Ile (T399I), have been correlated with hyporesponsiveness to inhaled lipopolysaccharide (LPS). 5-Fluorouracil (5-FU) is widely used for the treatment of patients with advanced colon cancers. Resistance to 5-FU is one of the most prominent obstacles to successful chemotherapy. The propose of this study was to investigate the effect of 5-FU on HCT116 colorectal cancer cell line with different variants of TLR4. HCT116 colorectal cancer cell line was transfected with Flag-CMV1-TLR4 wild-type (WT) and Flag-CMV1-TLR4 mutants, D299G and T399I expression plasmids. Transfected- HCT116 cells were treated with different concentration of 5-FU. The expression of TLR4 on 5-FU-treated HCT116 cells was analysed by FACS and the cytotoxic effect of 5-FU on transfected HCT116 cells was assessed by MTT assay. 5-FU significantly increased the expression of TLR4 protein on HCT116 cell line as analysed by FACS. 5-FU- Induced TLR4 expression in wild-type cells was higher than the other cells ($P<0.05$). Comparison of the cytotoxic effect of 5-FU showed that the highest IC50 value was for D299G variant of TLR4 at 48 hours treatment. As a conclusion 5-FU induce the expression of TLR4 both in cells with wild-type and mutant TLR4. Colorectal cancer cells with D229G TLR4 variant are more resistance to the drug compared to Wild type.

1221W

The role of genetic ancestry in breast cancer predisposition and tumor presentation in Puerto Rican Hispanic. *J. Dutil[1], L. Fejerman[2], E. Ziv[2], S. Huntsman[2], N. Arroyo[1], J. Matta[1], J. Gonzalez-Bosquet[3].* 1) Ponce School of Medicine & Health Sciences, Ponce, PR; 2) University of California in San Francisco, San Francisco, CA; 3) H. Lee Moffitt Cancer Center & Research Institute, Tampa, Fl.

Introduction: The incidence, clinical presentation, and mortality of breast cancer vary among different ethnic subgroups. Despite a lower incidence of breast cancer in US Hispanics, Hispanic women are more likely to die of breast cancer than non-hispanic white women diagnosed at similar age and stage. In the Hispanic population of Puerto Rico, the contribution of genetic ancestral factors in determining breast cancer risk and its clinical presentation is unknown. Methods: The study population consists of 227 breast cancer cases and 364 controls recruited from private practices of surgeons and oncologists from Puerto Rico. A panel of 106 ancestry informative markers was genotyped using the iPLEX Sequenom technology. Individual genetic ancestry was estimated using a maximum likelihood approach, which infers ancestry of each individual as a function of the probability of the genotype at each locus based on ancestral allele frequencies. The distribution of genetic ancestry was compared between cases and controls, and between different tumor subtypes. Results: The distribution of genetic ancestry among the 591 study subjects varied from 0 to 100% African, 0 to 100% European, and 0 to 38% Native American. There was no significant difference in the average European ($p=0.6$), African ($p=0.862$) and Native American ($p=0.2$) ancestry proportions between breast cancer and controls. Interestingly, we observed a 8.3% increase in average African ancestry proportion in women with triple negative tumors (estrogen receptor (ER) -negative, progesterone receptor-negative, and human epidermal growth factor receptor 2 (HER2) -negative tumors) when compared to tumors retaining at least one of the hormonal receptor ($p=0.04$). Conclusions: Global genetic ancestry does not seem to contribute significantly to the risk of breast cancer in Puerto Rican women. However, we present evidence that ancestry may contribute to the breast tumor hormonal receptor status in this population.

1222W

Identification and validation of genetic variants that influence breast cancer prognosis. *D. Eccles[1], I. Politopoulos[1], W. Tapper[1], S. Gerty[1], J.J. Liu[2], F. Couch[3], D. Easton[4], N. Rahman[5], S. Ennis[1], A. Collins[1], POSH study investigators.* 1) Faculty of Medicine, University of Medicine, Southampton, United Kingdom; 2) Genome Institute of SIngapore; 3) Mayo Clinic; 4) University of Cambridge; 5) Institute of Cancer Research.

Aim: to identify common genetic variants associated with an increased likelihood of developing metastatic breast cancer after diagnosis using a GWAS approach in a clinically well characterised patient cohort. Background: Breast cancer prognosis is predicted using tumour characteristics. Rare and common genetic variants clearly influence the tumour phenotype (e.g. BRCA1 carriers typically develop triple negative breast cancers, different variants predispose to ER +ve or ER-ve breast cancer). It is also likely that genetically determined variations lead to variable efficiency between individuals for example in immune surveillance and drug metabolism thereby influencing prognosis by altering risk of metastases or response to adjuvant drug therapy. Method: 870 breast cancer cases diagnosed before 41 years of age and participating in the POSH study (Eccles et al, BMC Cancer 2008) were genotyped as part of three separate GWAS to identify risk SNPs. The raw data from the three studies were merged and SNPs successfully typed across all three studies were included in a Kaplan Meier survival analysis at stage 1. Six additional sub-group analyses using KM survival analysis were conducted in order to try and identify SNPs with differing mechanisms of action (ER negative, ER positive, HER2 negative, HER2 positive, anthracycline and/or anti-oestrogen treated). SNPs to take forward for validation were selected based on low p-values, MAF > 5%, supporting evidence from nearby SNPs with differing MAFs and SNPs in or close to genes with potential functional significance. Results: A total of 497332 SNPs were tested, 832 cases passed all QC procedures for the stage 1 analysis. 260 SNPs were identified in one or more of the sub group analyses with nominal genome wide significance. 160 were picked for replication in stage 2. Two SNPs (located on chromosomes 5 and 15) showed evidence of replication at stage 2 with p-values from a pooled analysis of stage 1 and 2 data exceeding genome wide significance ($P \leq 10\text{-}38$). In addition, the SNP on chromosome 5 retained genome wide significance ($P \leq 10\text{-}13$) in ER-ve and anthracycline treated subsets despite the smaller sample sizes. Conclusions: we have found preliminary evidence for an effect of common genetic variants on breast cancer prognosis at two genetic loci. Additional multivariate analyses and further genotyping in well phenotyped clinical series will be needed to replicate these findings.

1223W

Overview of the Clinical ELLIPSE (ELucidating Loci Involved in Prostate cancer SuscEptibility) Consortium (CEC). *R. Eeles[1], C. Goh[1], Z. Kote-Jarai[1], P. Kraft[2], D. Easton[3], B. Henderson[4], F. Schumacher[4].* 1) The Institute of Cancer Research, United Kingdom; 2) Harvard University, USA; 3) The University of Cambridge, United Kingdom; 4) University of Southern California, USA.

Background: The inherited link of prostate cancer (PrCa) has led to large scale Genome Wide Association Studies (GWASs), which aim to identify susceptibility loci associated with PrCa. Currently, these Single Nucleotide Polymorphisms (SNPs) have only been evaluated against overall PrCa risk and several disease parameters. The challenge now is to translate these genetic risk algorithms into the clinical setting. This has led to NIH (National Institute of Health) funded post-GWAS initiatives in PrCa and the establishment of the Clinical ELLIPSE Consortium (CEC) to harmonise genetic, clinical and epidemiological data from existing consortia around the world. This will provide the necessary guidance in developing useful and powerful risk prediction models for PrCa risk, including screening outcome and treatment effects. These risk prediction models may have an immediate public health impact by providing clinicians with the necessary tools to improve clinical decision making. **Methods:** Several projects are currently underway including the analysis of Active Surveillance outcomes against these SNPs, and the analysis of the PRACTICAL (PRostate cancer AssoCiation group To Investigate Cancer Associated aLterations in the genome) consortium cohort, looking at the association of these SNPs with disease parameters. Collaborations have been established with ERSPC (European Randomized Study of Screening for Prostate Cancer), STAMPEDE (Systemic Therapy in Advanced or Metastatic Prostate cancer: Evaluation of Drug Efficacy) and RAPPER (radiogenomic) consortia, to analyse these SNPs' role in screening and treatment outcomes. **Results:** An inventory collected across the consortium showed that there are 47215 cases and 110811 controls with clinical data. There were also large numbers of tumour (10654 samples) and blood (31700 samples) available. These figures are very encouraging and discussions are currently underway to access and combine data across the groups for analyses. **Conclusion:** The CEC provides the necessary framework needed to foster successful collaborations and begin the transition from `bench-side' research to `bed-side' care. **Acknowledgments:** This project is supported by The NIH grant: No. 1 U19 CA 148537-01 and Cancer Research UK.

1224W

Genome-wide association study identifies germline susceptibility loci in *ETV6-RUNX1*-rearranged childhood acute lymphoblastic leukemia. *E. Ellinghaus[1,17], M. Stanulla[2,17], G. Richter[1], D. Ellinghaus[1], G. te Kronnie[3], G. Cario[2], G. Cazzaniga[4], M. Horstmann[5], R. Panzer Gruemayer[6], H. Cave[7], J. Trka[8], O. Cinek[9], A. Teigler-Schlegel[10], A. ElSharawy[1], R. Haesler[1], A. Nebel[1], B. Meissner[2], T. Bartram[2], F. Lescai[11], C. Franceschi[12], M. Giordan[3], P. Nuernberg[13], B. Heinzow[14], M. Zimmermann[15], S. Schreiber[1,16,17], M. Schrappe[2,17], A. Franke[1,17].* 1) Institution of Clinical Molecular Biology, Christian-Albrechts-University Kiel, Kiel, Schleswig-Holstein, Germany; 2) Department of Pediatrics, University Hospital Schleswig-Holstein, Kiel, Germany, on behalf of the German Berlin-Frankfurt-Münster study Group for Treatment of Childhood ALL; 3) Department of Pediatrics, Laboratory of Pediatric Hematology/Oncology, University of Padua, Italy; 4) M. Tettamanti Research Center, Children's Hospital, University of Milan-Bicocca, Monza, Italy; 5) Clinic of Pediatric Hematology and Oncology, University Medical Center, and Research Center Children's Cancer Center, Hamburg, Germany; 6) St. Anna Children's Hospital and Children's Cancer Research Institute, Vienna, Austria; 7) Department of Genetics, Hospital Robert Debre, Paris, France; 8) Department of Pediatric Hematology/Oncology, 2nd Faculty of Medicine, Charles University Prague, Czech Republic; 9) Department of Pediatrics, 2nd Faculty of Medicine, Charles University Prague, Czech Republic; 10) Oncogenetic Laboratory, Department of Pediatric Hematology and Oncology, Justus-Liebig-University, Giessen, Germany; 11) Division of Research Strategy, University College London, UK; 12) Department of Experimental Pathology, University of Bologna, Italy; 13) Cologne Center for Genomics, University of Cologne, Germany; 14) State Social Services Agency S-H, Kiel, Germany and Univ. of Notre Dame, Sydney Medical School, Australia; 15) Pediatric Hematology and Oncology, Hannover Medical School, Hannover, Germany; 16) Department of General Internal Medicine, University Hospital S-H, CAU, Kiel, Germany; 17) These authors contributed equally to this work.

Acute lymphoblastic leukemia (ALL) is characterized by the malignant clonal proliferation of lymphoid cells that are blocked at an early stage of differentiation. More than 60%; of patients diagnosed with ALL are children below the age of 15 years. The annual incidence rates of childhood ALL vary worldwide between one and four new cases per 100,000 children younger than 15 years, with a peak incidence at about two to five years of age. The two major genetic subtypes of childhood ALL are characterized by either hyperdiploidy (>50 chromosomes per leukemic cell) or the chromosomal translocation t(12;21)(p13;q22) leading to an *ETV6-RUNX1* (*TEL-AML1*) gene fusion, which account for approximately 25% and 22% of the entire childhood ALL population, respectively. We performed a genome-wide association study of 355,750 SNPs in 474 healthy controls and 419 *ETV6-RUNX1*-positive ALL cases. The eight most strongly associated SNPs were followed-up in 951 *ETV6-RUNX1*-positive ALL cases and 3,061 healthy controls from Germany/Austria and Italy, respectively. We identified a novel, genome-wide significant risk locus at 3q28 (P_{CMH}=8.94×10^{-9}, OR=0.65). The separate analysis of the combined German/Austrian sample only, revealed additional genome-wide significant associations at 11q11 (P=9.14×10^{-11}, OR=0.69) and 8p21.3 (P=6.12×10^{-9}, OR=1.36). These associations and another association at 11p11.2 (P=4.95×10^{-7}, OR=0.72) remained significant in the German/Austrian replication panel after Bonferroni correction for multiple testing. Our findings demonstrate that germline genetic variation can specifically contribute to the risk of ALL subtypes - in this case *ETV6-RUNX1*-positive childhood ALL. The genes identified as risk loci emphasize the role of the *TP53* gene family and the importance of proteins regulating cellular processes including cell growth, differentiation, mitotic cycle and oncogenic transformation in connection with tumorigenesis.

1225W

***ELF3* is a putative oncogene frequently gained and hypomethylated in the adenocarcinoma subtype of non-small cell lung cancer.** *K.S. Enfield[1], R. Chari[2], D. Becker-Santos[1], S. Lam[1], W.L. Lam[1].* 1) Integrative Oncology, British Columbia Cancer Research Centre, Vancouver, British Columbia, Canada; 2) Harvard Medical School, Department of Genetics, Boston, MA, USA.

Lung cancer remains the cause of the most cancer-related deaths each year, with a 5 year survival rate of less than 15%. The predominant type of lung cancer is non-small cell lung cancer (NSCLC) and the majority of these cases consist of the adenocarcinoma (AC) subtype. Oncogenes such as *EGFR* are well defined drugable targets of AC, but *EGFR* mutations are only found in approximately 20% of cases. Additional oncogenes clearly exist to drive this subtype. Genes activated by multiple mechanisms such as copy number gain and hypomethylation are more likely to be drivers of oncogenesis. Identification of such genes will lead to the discovery of novel therapeutic targets to improve NSCLC patient prognosis. Multidimensional genome-wide analysis was conducted on a panel of AC tumors with matched adjacent non-malignant tissues. *ELF3* was identified as frequently gained, hypomethylated and overexpressed. Copy number analysis was performed on 84 AC pairs using the SNP 6.0 platform, methylation analysis was performed on 60/84AC pairs using the Illumina HM27 Methylation platform, and expression analysis was performed on 83/84 pairs using the Illumina expression microarray. The role of ELF3 in cell proliferation and the ability to form colonies in soft agar was assayed using stable *ELF3* mRNA knockdown in AC cell lines with high *ELF3* expression. Knockdown was verified by qRT-PCR. Subcellular localization of ELF3 was determined by western blot on nuclear and cytoplasmic fractions and confirmed with immunofluorescence (IF). *ELF3* was found to be frequently gained (41/84), hypomethylated ($p<0.05$), and at least 2-fold overexpressed (60/83). Interestingly ELF3 is normally highly expressed in the fetal lung and minimally expressed in the normal adult lung. Knockdown of *ELF3* led to significantly reduced cell proliferation, and colony formation assays produced fewer colonies of smaller size. ELF3 is a member of the ETS family of transcription factors, so we sought to examine the subcellular localization of the protein. Western blots and IF revealed ELF3 to be predominantly located in the nucleus, indicating ELF3 behaves through its transcription factor activity. *ELF3* is a gene frequently disrupted by genetic and epigenetic mechanisms, which result in its increased expression in AC. ELF3 plays roles in cell proliferation as well as contact-independent growth. The fact that ELF3 expression is normally restricted to fetal lung development implicates *ELF3* as a fetal oncogene in AC.

1226W

The Lymphoid Cancer Families Study - A resource for identification of genetic factors contributing to lymphoma, lymphoid leukemia, and myeloma: Phase 1 Recruitment. *A. English[1], A. Brooks-Wilson[1], J. Connors[2].* 1) Genome Sciences Centre, British Columbia Cancer Agency, Vancouver, Bristish Columbia, Canada; 2) Medical Oncology, British Columbia Cancer Agency, Vancouver, Bristish Columbia, Canada.

Introduction: Although most lymphoid cancers are sporadic, the occurrence of multiple cases within the same family may indicate the existence of genetic susceptibility factors common to these disorders. By creating a collection of DNA samples with medical, family history and lifestyle information from consenting subjects and family members, we hope to identify genetic factors involved in susceptibility to lymphoid cancer.

Materials & Methods: Probands with a family history of lymphoid cancer were identified from a database begun in 1981 by the Lymphoma Tumour Group at the BC Cancer Agency, and by ascertainment of probands through local hematologists. Eligible subjects are mailed an introductory package including an explanatory letter, information and consent form, and family history questionnaire (FHQ). A pedigree is constructed from completed FHQ and a phone interview is conducted to obtain more detailed information about cancer diagnoses, treatment histories, and vital statistics of family members. Living affected and unaffected family members of participating subjects are invited to join the study. Participation consists of providing a DNA sample, permission to access medical records and stored tissue samples if applicable, and completing an epidemiological/lifestyle questionnaire. Pedigrees are then assessed to determine suitability for genetic mapping studies.

Results: To date 152 families have been ascertained. Introductory packages have been mailed to 495 eligible subjects; 282 individuals from 78 families have consented and are at various stages of participation. DNA samples have been collected from 168 participants including 22 saliva samples and 146 blood samples. Epidemiological/lifestyle questionnaires have been completed by 123 participants.

Conclusions: We have established a DNA sample and medical history collection of families with multiple cases of lymphoid cancer. Recruitment is in progress. This collection, likely applied in collaboration with other researchers in the field, will help to identify genetic risk factors for lymphoid cancers.

1227W

Initiative in profiling Filipino colorectal cancer patients based on clinical and molecular genetics criteria. *M. Enriquez[1,2], M. Nuylan[1], J. Pilante[3], L. Cabral[1], E. Cortes[4], E. Bondoc[5], F. Natividad[1], SLMC Colorectal Cancer Study Group.* 1) Research and Biotechnology, St. Luke's Medical Center, Quezon City, 1102 Philippines; 2) Physics Department, CENSER, College of Science, De La Salle University, Taft Avenue 2401 Manila, Philippines; 3) Department of Biological Sciences, College of Science, De La Salle University, Dasmarinas, Cavite; 4) Department of Surgery, SLMC, Quezon City, 1102 Philippines; 5) Institute of Digestive Diseases, SLMC, Quezon City, 1102 Philippines.

Objective: The study was done to (1) determine the presence of KRAS and P53 gene mutations in tumor samples from Filipino patients with sporadic colorectal cancer; (2) determine the degree of heterogeneity of mutation at various stages of cancer, and (3) correlate gene mutations with tumor grade, tumor stage, and site of tumor. Methodology: Paired normal and tumor frozen tissues from 35 Filipinos who underwent surgical resection for colorectal cancer (CRC) in a tertiary hospital in Metro Manila from 2000-2004 were included in the study. The sample population is made up of 19 males and 16 females with ages ranging from 34-77 years and a mean age of 60; 17 (49%) were <60 years old and 18 (51%) were > 60 years old. Seventeen (49%) samples were from distal colon and 26 (74%) were moderately differentiated. From each sample, DNA was extracted and using primers specific for exons 1, 2, 3, 4a and 4b of KRAS gene and exons 5, 6, 7 and 8 of P53 gene. These targeted regions were amplified by PCR. Results: Eighteen samples (51%) carried 20 mutations in the KRAS gene, 11 (55%) were silent type and 9 (45%) were considered pathogenic. Eight (89%) tumors with pathogenic mutations were in stage 3. Among the pathogenic mutations, 6 occurred in exon 1 and 3 in exon 2. For mutations in exon 1 only 1 was in the hot spot codon 13; 4 were in codon 19 and 1 in codon 11. On the other hand, seventeen samples carried eighteen P53 mutations and 16 (89%) were in exon 6; and two were in exon 5. Twelve (75%) samples were in stages 2 and 3. Four pathologically labelled ”normal” tissues had P53 mutation in exon 6. Interestingly, two stage 1 tumor samples carried P53 mutation. Five (42%) of the 17 samples had KRAS-P53 tandem mutations and all were in stage 3. Conclusion: Data presented support the concept of heterogeneity of mutation in cancer evolution and poses questions on the role of these two genes in tumor initiation and progression. This study is among the very few molecular genetics investigation in colorectal cancer among Filipinos.

1228W

Tdp1 and PARP-1 are synthetic lethal targets for rhabdomyosarcoma. *H.K. Fam[1], M. Chowdhury[1], S. Bajaj[2], N. Osborne[1], K. Choi[1], C. Walton[1], G. Sun[1], M. Bond[1], T. Triche[2], C. Pallen[1], C.F. Boerkoel[1].* 1) University of British Columbia, Vancouver, BC, Canada; 2) Children's Hospital Los Angeles, Los Angeles, CA.

Background: In mammalian cells, many nuclear proteins are poly ADP-ribosylated in response to DNA strand breaks by the poly ADP-Ribose Polymerase-1 (PARP-1) enzyme. Ribosylated proteins signal for the formation and localization of DNA repair complexes. Interestingly, many cancers are characterized by loss-of-function mutations in DNA repair proteins, and inhibition of PARP-1 has synthetic lethal effects on cancers with defects of double strand break repair. Since tyrosyl-DNA phosphodiesterase 1 (Tdp1), an enzyme that processes dirty 3' DNA termini, is in the same complex as PARP-1 and is highly expressed in rhabdomyosarcoma (RMS), we assessed PARP-1 inhibition and Tdp1 knockdown on RMS cell growth. Hypothesis: Inhibition of Tdp1 and PARP-1 is lethal or growth restrictive to RMS cells. Methods: Human dermal fibroblasts and pediatric RMS cell lines Rh30, Rh18, RD, Birch, A204, CW9019 were used to characterize expression of Tdp1 and sensitivity to Tdp1 knockdown and PARP inhibition. siRNA was used to knock down Tdp1 in RMS cells. Cell proliferation was measured using the MTT assay, apoptosis by TUNEL assay and homogeneous caspase-3/7 activation, and DNA breaks by alkaline comet assay. Mitochondrial function was analyzed with Mitosox red and mitochondrial DNA damage with qPCR of the common mitochondrial deletion. Results: Tdp1 is highly expressed in RMS cell lines compared to normal skeletal muscle and localizes to both the nucleus and mitochondria. siRNA knockdown of Tdp1 and PARP-1 inhibition are lethal to most RMS cells, and the combination of Tdp1 knockdown with PARP-1 inhibition is more lethal than either treatment alone. Both Tdp1 knockdown and inhibition of PARP-1 increase the CPT sensitivity of RMS cells and do so proportionate to the levels of mitochondrial Tdp1. Conclusions: Inhibition of Tdp1 and PARP-1 profoundly reduces RMS cell viability and proliferation. This suggests that RMS tumors have undescribed defects of DNA double strand break repair and that Tdp1 and PARP-1 are potential synthetic lethal therapeutic targets for treatment of RMS. Additionally, the finding that the CPT sensitivity of RMS cells correlates with mitochondrial Tdp1, not nuclear Tdp1, suggests that mitochondrial genotoxicity may be the molecular basis for some anticancer agents.

1229W

CD40 expression in gastric adenocarcinoma. *W. Fan[1], X. Kang[2], G. Ren[3].* 1) School of Life Science, Hebei University, Baoding, Hebei Province, China; 2) 180# Wusi Road, Baoding, Hebei 07002, China; 3) wfan6888@yahoo.com 151-7639-8202 (O).

[Abstract]: Purpose: to explore the possibility for CD40 to serve as an prognostic biomarker in human gastric adenocarcinoma. Mehtods: immunohistochemical detection of CD40 protein in dissected human gastric adenocarcinoma samples and the adjacent normal gastric tissues (i.e., the paracancergastric mucosa) from 100 patients. A forty eight month follow-up was conducted and the correlation between CD40 expression and overall survival time was evaluated. Results: significant differential expression of CD40 was found in gastric adenocarcinoma (65%) and the corresponding paracancergastric mucosa (7%) respectively (P<0.001). Expressions of CD40 protein was significantly higher in advanced cancer stages (TNM stages III & IV) and distant metastasis (71% and 80% respectively) than that in early cancer stage (TNM stages I & II) and primary tumor sites (52% and 39% respectively; P<0.05). After 48 month follow-up, the survival rate of CD40 negative patients was 65% (21/35) and that of CD40 positive patients was 6% (4/65, P<0.002). A significant correlation exists between CD40 expression and overall survival time. Conclusions: The CD40 was highly expressed in gastric adenocarcinoma; its expression on gastric cancer may play a role in distant metastasis and the advanced cancer stages, which may serve as an informative prognostic and survival predictor for patients with gastric adenocarcinoma. Key words: CD40; Gastric adenocarcinoma; Immunohistochemistry Authors: Fan, W., Kang, XJ., and GD, Ren. Address: Tumor Research Lab at School of Life Science, Hebei University, Baoding, 07002, China All co-authors have read and approval of this abstract, and there is no interest conflict.

1230W

Mutation Analysis exones 7,8&9 of PTEN Gene in Iranian Colorectal Cancer. *M. Firoozi[1], L. Alidoust Masouleh[2], M. Ghaffarpoor[2], Z. Hajebrahimi[3], M. Arabian[4], L. Najafi[3], N. Salim[5], K. Bidoki[1], M. Houshmand[2].* 1) Payam e Noor University, Tehran, Iran; 2) National Institute of Genetic Engineering and Biotechnology, Tehran, Iran; 3) Tarbiat Moddaress University, Tehran, Iran; 4) Tehran University of Medical. Tehran, Iran; 5) Shahid Beheshti University, Tehran, Iran.

Purpose: PTEN/MMAC1/TEP1 is a tumor suppressor gene encoding a dual-specificity protein phosphatase with homology to the cytoskeleton protein, chicken tenssin and bovine auxilin. PTEN acts by inhibiting the activation of Akt/Protein kinase B and is therefore involved in a major pathway controlling cell proliferation and cellular metabolism PTEN protein has been found to be important for its activity as a tumor suppressor. Recently, mutation at an (A)6 repeat of PTEN exons 7,8and 9 in colorectal cancer(CRC) patients with microsatellite instability(gene instability) have been detected.-Predicting response and limiting drug-induced toxicity for patients with CRC and gastrointestinal are also critical. Methods: Our subjective was to identify PTEN mutation in colorectal tumors and tissue samples from Iranian patients. Histopathological examinations were performed, and all tumors were confirmed as adenocarcinoma. We searched for PTEN mutations which had involved in exones 7,8 and 9. The entire coding region and flanking sites (exons 7,8 and9) PTEN gene was amplified and conformation sensitive gel electrophoresis(CSGE) and sequencing was done to identify alterations. Results: 23(76.66%) patients had adenocarcinomas and 7 (23.33%) had mucinous adenocarcinomas. patient had stage I, II, and III or IV disease with 14.76%, 44.44% and 40.8% respectively and histopathological grade I, II, and III with 66.6%, 25.9% and 7.4% respectively .The frequency of mutation in PTEN was not present in all of the samples. The presence of PTEN mutation was no a significance associated with gender, histipathological grade ,age or cancer stage in patients with adenomacarcinoma of the CRC(p>0.05).Although many study have been shown in CRC , 3 mutational hot spots . our study indicated no mutations in hot spots and more samples should be investigated in 3 hot spots and others exons and also determine expression and methylation profile of PTEN gene. We found no PTEN alterations among the colorectal cancer samples.So we recommend to investigate level of expression of PTEN gene, such as transcriptom, protein and hypermethylation of PTEN. Promoter hypermethylation of PTEN can be accrued in these samples. So we had less of level of transcriptom and protein of PTEN.

1231W

Whole-exome sequencing of 19 hereditary prostate cancer families with aggressive or early-onset disease. *L.M. FitzGerald[1], A. Kumar[2], L. McIntosh[1], E.A. Ostrander[3], E.W. Pugh[4], H. Ling[4], K.F. Doheny[4], J. Shendure[2], J.L. Stanford[1].* 1) Program in Prostate Cancer Research, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. N., PO Box 19024, Seattle, WA 98109-1024; 2) Department of Genome Sciences, University of Washington, Foege Building S-250, Box 355065, 3720 15th Ave N.E., Seattle, WA 98195-5065; 3) Comparative Genetics Section, National Human Genome Research Institute, Building 50, Room 5351, 50 South Dr, MSC 8000 Bethesda, MD 20892-8000; 4) Center for Inherited Disease Research, 333 Cassell Drive, Suite 2000, Baltimore, MD 21224.

Prostate cancer (PC) is one of the most commonly diagnosed cancers in Western societies and up to 42% of PC incidence is estimated to be due to inherited risk. Multiple common, low penetrance risk loci have been identified in recent genome-wide association studies (GWAS), but the discovery of rare, moderate-to-high penetrance mutations has proven difficult. Next-generation sequencing technology combined with a strong family-based study design, however, may facilitate discovery of rare disease mutations. Here we describe a whole-exome sequencing study of 19 hereditary prostate cancer (HPC) families with the aim of identifying such mutations. Whole-exome sequencing was performed on 80 PC cases from 19 families with ⁄3 affected relatives diagnosed with clinically aggressive and/or early onset disease. To increase the likelihood of detecting mutations that segregate with disease, the majority of affected men were ⁄2nd-degree relatives. Eleven elderly male relatives screened negative for PC were also sequenced. Sequencing was performed on the HiSeq Illumina platform; paired-end reads were aligned to the hg19 reference genome using BWA, and the programs SAMtools and GATK were used to perform local realignment and identify SNPs and indels distinct from the reference genome. SeattleSeq and ANNOVAR were used to annotate variants, which were then filtered according to their frequency in dbSNP and the 1000 Genomes Project. Variants that segregated with disease and were not observed in the unaffected relatives were prioritized according to the predicted function of the variant and of the gene in which the variant was present. In addition, data from a prior SNP genome-wide linkage scan of these families was used to prioritize variants present in regions with evidence for linkage. Data generation has been completed and analyses are underway. The results of these analyses and a discussion of our follow-up plans will be presented at the ASHG meeting.

1232W

Pooled analysis of GWA studies for survival from clear cell ovarian cancer reveals association at *RASGRF2*, *ANKS1B* and *KSR2*. *B.L. Fridley[1], R.A. Vierkant[1], Z.C. Fogarty[1], S. Armasu[1], Y. Tsai[2], H. Lin[2], H. Song[3], K. Bolton[3], J. Tyrer[3], S.J. Ramus[4], S. Johnatty[5], J.M. Cunningham[1], G. Chenevix-Trench[5], R. Sutphen[6], T. Moorman[7], J.M. Schildkraut[7], N. Wentzensen[8], S. Gayther[4], P. Pharoah[3], T.A. Sellers[2], E.L. Goode[1].* 1) Mayo Clinic College of Medicine, Rochester, MN, USA; 2) H. Lee Moffitt Cancer Center, Tampa FL, USA; 3) University of Cambridge, Strangeways Research Laboratory, Cambridge, UK; 4) University of Southern California, Los Angeles CA, USA; 5) Queensland Institute of Medical Research, Queensland, AUS; 6) University of South Florida College of Medicine, Tampa FL, USA; 7) Duke University School of Medicine, Durham NC, USA; 8) National Cancer Institute, Bethesda MD, USA.

Epithelial ovarian cancer (EOC) is the 6th most commonly diagnosed cancer among women in the world, with 5-year survival rate at 45%. To date, genome-wide association (GWA) studies of EOC outcomes, including overall survival (OS), have or restricted analysis to the most-common histological subtypes of high-grade serous or included all EOC cases, with limited success. Lack of findings could be attributed in part to genetic heterogeneity of effects by histological subtype. For example, women with ovarian clear cell carcinoma (OCCC) have slightly worse OS than women with serous ovarian cancer, as OCCC seems to be more aggressive. Therefore, to identify loci associated with OS, we completed a pooled analysis of 272 OCCC cases (81 deaths) from eight sites which were genotyped on the Illumina 610-quad array as part of three EOC GWA studies. Imputation of untyped markers was computed using MACH and the HapMap CEU genotypes and poorly-imputed markers (r-square < 0.30) were removed. Association analyses was completed for 2,504,879 SNPs using log-additive Cox proportional hazards models adjusted for study site, age at diagnosis and population stratification. Association analysis of OS from OCCC revealed associations at three loci: rs26419 in *RASGRF*2 (minor allele frequency (MAF) = 0.02, HR = 33, p=6.15E-07), rs10492271 in *ANKS1B* (MAF = 0.16, HR = 0.17, p=9.43E-07) and rs10850832 in *KSR2* (MAF = 0.17, HR = 2.50, p = 1.14E-06). However, none of these associations reached genome-wide statistical significance. RasGRF2 is known to interact with KSR2, a kinase suppressor of ras 2 and inhibits MEKK3-activated MAP kinase and the NF-kappa) pathway, and has an indirect interaction with TNF and KRAS. *ANKS1B* encodes EB-1 which is expressed in the ovary, where overexpression can down-regulate APP processing. Additional analyses are on-going to better understand these associations, including analyses by study site, to adjust for additional clinical covariates such as stage and grade, and to examine time to recurrence. In conclusion, these results provide new candidate genes related to OS from OCCC, demonstrating that analysis by histological subtype may better inform detection of variants associated with inter-individual differences in EOC survival.

1233W

Comprehensive analysis of somatic mutations in twenty-seven liver cancers by whole genome sequencing. *A. Fujimoto[1,2], T. Tsunoda[1,2], Y. Totoki[3], T. Abe[1], K.A. Boroevich[1], M. Nagasaki[2,4], M. Kubo[1], S. Miyano[2,4], N. Kamatani[1], Y. Nakamura[1,4], T. Shibata[3], H. Nakagawa[1].* 1) Ctr Genomic Med, Riken, Yokohama, Japan; 2) Data Analysis Fusion Team, Computational Science Research Program, RIKEN, Tokyo, Japan; 3) Division of Cancer Genomics, National Cancer Center Research Institute, Chuo-ku, Tokyo, Japan; 4) Human Genome Center, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo, Japan.

We report the whole-genome sequences of twenty-seven viral or non-viral liver cancers using massively parallel sequencing. We identified 1,000 to 20,000 single nucleotide mutations (SNVs), 40 to 800 short insertions and deletions (indels) and 0 to 60 intra- and inter-chromosomal rearrangements per sample. The pattern of mutations was significantly different among nucleotide sites and a higher mutation rate from C to T in CpG sites was observed. Dozens of genes with recurrent coding or splice-site mutations were also identified. Gene set enrichment analysis revealed that indels and nonsynonymous SNVs were significantly enriched in chromatin remodeling and cell adhesion genes after adjustments for multiple testing. Also, we detected the integration sites of the hepatitis B virus (HBV) genome into cancer genomes in the base resolution by using a paired-end method. Our results represent a comprehensive pattern of somatic mutations in liver cancer and will provide valuable insights for the mechanism of liver carcinogenesis and future molecular therapy.

1234W

Novel methods to integrate genotype and gene expression data reveal pathways genetically associated with ER breast cancer subtypes. *T. Furey[1], Q. Xinog[1], M. Weiser[1], E. Hauser[2], C. Perou[1], S. Mukherjee[3].* 1) Department of Genetics, Carolina Center for Genome Sciences, Lineberger Comprehensive Cancer Center, UNC-Chapel Hill, Chapel Hill, NC; 2) Center for Human Genetics, Duke University, Durham, NC; 3) Department of Statistical Science, Duke University, Durham, NC.

Recent GWAS studies have identified genetic variants associated with different cancers. In these studies, only genotype information is considered. To better understand potential genetic effects, we developed two complementary methods that simultaneously analyze whole genome genotype and mRNA expression to identify molecular pathways associated with cancer or a cancer subtype. Simulation studies support the ability of these methods to identify genetically and transcriptionally linked loci. We applied both methods to a small set of ER+ and ER- breast cancer tumor samples. The first method, Gene Set Association Analysis (GSAA), independently calculates gene-based scores reflecting their association to samples segregated into two classes, such as by ER status, using genotype and expression data separately. These are then combined into a single estimate of each gene's relatedness to class distinction. Using a pre-defined collection of pathway gene sets, a Kolmogorov-Smirnov (KS) statistic along with the combined gene scores then identifies pathways differentially regulated or genetically distinct between classes of samples. A second method places increased focus on genetic contributions to phenotypic differences. For each variant, an association score is calculated as follows: $Score(X_j|S_i) = As(C|S_i) + As(C|E(X_j)) * As(E(X_j)|S_i)$, where S_i is a particular variant, X_j is a gene in close proximity to S_i, $E(X_j)$ is the expression of that gene, and C is a phenotypic class. Each As(A|B) term indicates a measure of association between A and B. The first term, $As(C|S_i)$, reflects the simple association of SNP S_i to phenotype class. The second term, $As(C|E(X_j)) * As(E(X_j)|S_i)$, is product of the association of the expression of gene X_j with phenotype class and the association of SNP S_i with the expression of gene X_j (i.e. an eQTL measure). The overall score quantifies the relatedness of gene X_j to phenotype based on genetic associations of S_i directly to the phenotype and indirectly through the expression of gene X_j. Similar to above, gene scores are then used to identify pathways associated with phenotype class. When applied to a set of ~100 breast cancer samples stratified by estrogen receptor (ER) status, we found both methods indicate that HER2 and P53 pathways are upregulated and genetically associated with ER+ samples, while pathways related to cell cycle control including the BRCA/ATR pathway and pyruvate metabolism are linked to ER- samples.

1235W

Unprogrammed presentation number

1236W

Genetic and functional evidence of the presence of a tumor suppressor gene in 3p12-cen region in a model of epithelial ovarian cancer. *K. Gambaro[1], P.M. Wojnarowicz[1], M. de Ladurantaye[2], K. Leclerc Désaulniers[2], V. Barrès[2], J-S. Ripeau[4,5], S.L. Arcand[4], J. Lavoie[3], D.M. Provencher[2,6], A-M. Mes-Masson[2,8], M. Chevrette[4,5], P.N. Tonin[1,4,7].* 1) Human Genetics, McGill, Montreal, QC, Canada; 2) Centre de recherche du Centre hospitalier de l'Université de Montréal / Institut du cancer de Montréal, Canada; 3) Department of Pathology, McGill University, Montreal, Canada; 4) The Research Institute of the McGill University Health Centre, Montreal, Canada; 5) Department of Surgery, Urology Division, McGill University, Montreal, Canada; 6) Division de gynécologie oncologique, Université de Montréal, Canada; 7) Department of Medicine, McGill University, Montreal, Canada; 8) Department of Medicine, Université de Montréal, Canada.

Ovarian cancer (OC) is the fifth leading cause of death by cancer in women in western countries. The disease is largely asymptomatic with most patients diagnosed at an advanced stage often resulting in a poor outcome. Little is known of the etiology and mechanisms of tumorigenicity for this disease. Numerous studies have reported recurrent abnormalities of the chromosome 3p arm in epithelial OC (EOC) suggesting the presence of tumor suppressor genes (TSG). We have proposed that the 3p12-pcen region harbours TSG(s) based on loss of heterozygosity (LOH) studies of OC and suppression of tumorigenicity of a 3p haploinsufficient OV90 EOC cell line, which occurred as result of the transfer of chromosome 3 fragments. To identify 3p12-pcen TSG candidates, we performed a functional complementation assay based on the microcell mediated chromosome transfer of a derivative chromosome 3 defined by the 3p12-q11 region into OV-90. Of the 4 microcell hybrid clones generated hybrid 3-1 was unable to form colonies in soft agarose, and exhibited a significantly delayed tumor forming capacity in immunocompromised mice. Genome-wide SNP genotyping analysis revealed that hybrid 3-1 retained the largest amount of transferred DNA with a unique fragment containing 2 hypothetical genes and VGLL3. Comparative expression microarray analysis of OV-90, hybrid 3-1, malignant ovarian tumor samples (N=99) and normal ovarian samples not only supports VGLL3 as a top TSG candidate but also shows that a reprogramming of networks relevant to EOC had occured in the hybrid. Ectopically overexpressing VGLL3 in OV90 failed to yield stable transfectant clones. Stables clones expressing moderately increased levels of VGLL3 transcript resulted in the accumulation of cytoplasmic vacuoles. These clones exhibited tumour latency in SCID mice. Overexpression of VGLL3 using a lentivirus tetracylcine-inducible vector system resulted in the development of a similar cytoplasmic phenotype. Our results taken together suggest that VGLL3 expression plays a role in EOC tumorigenicity.

1237W

Evolutionary dynamics of blood cancer revealed by deep sequencing. *H. Gao[1,2], C. Wang[1,2], C. Bustamante[3], M. Mindrinos[1,2], D. Miklos[4], R. Davis[1,2], W. Xiao[1,2].* 1) Biochemistry, Stanford Univ, Stanford, CA; 2) Stanford Genome Technology Center, Stanford Univ, Stanford, CA; 3) Department of Genetics, Stanford Univ, Stanford, CA; 4) Department of Immunology, Stanford Univ, Stanford, CA.

In patients with chronic lymphocytic leukemia (CLL), the immune repertoires of B cells in the blood typically experience a rapid expansion of cancer clones, leading to substantial shrinkage of diversity. This implies that cancer clones go through distinct evolutionary processes from the B cell clones in healthy individuals. To understand the underlying disease mechanism, we applied 454 sequencing technology to sequence the immunoglobulin heavy chain variable region (VDJ) gene segments of B lymphocytes from 30 samples of 6 CLL patients collected at diagnostics, and after transplantation, at 56 days, 180 days, 365 days, and 550 days. Based on this rich data set, we modeled the re-generation of immune repertoire of cancer patients after bone marrow transplantation as a linear birth process with immigration. We performed extensive simulations to demonstrate this stochastic model reflects the evolution of immune repertoire. Then we applied the Ewens sampling test derived from this stochastic process to distinguish the immune repertoires of the cancer patients from the controls and to monitor their recovery or relapse accurately. Moreover, we estimated the immigration rate of donor's B cell clones entering the circulation system and the average time to reconstruct the normal immune repertoire after transplantation, both of which are of clinical importance. We anticipate that our stochastic model will be useful in accurate diagnostics of blood cancer and prognosis after transplantation.

1238W

Association of polymorphic variants of Vascular Endothelial Growth Factor (VEGF) gene in relation to risk and androgen therapy response in Prostate cancer patients of North India. *G.P. George, R.D. Mittal.* Urology, Sanjay Gandhi Post Graduate Institute of Med. Sc., Lucknow, Uttar Pradesh, India.

Prostate cancer (PCa) represents a global public health problem. In comparison to the Western countries the incidence rate of PCa in India is low. Simultaneously, Hormone Refractory Prostate Cancer (HRPC) is also a leading cause of aging-related cancer death in men. VEGF is expressed at higher levels in prostate cancer cells in the absence of androgens and might therefore be involved in the onset of androgen refractory prostate cancer. We analyzed —7C>T; —1001G>C; —2578C>A; —2549 Ins/Del SNPs in VEGF, in a population based case-control study of 215 PCa cases and 220 healthy controls of North India. The study protocol was approved by Ethical Committee of the Institute. Clinical outcomes were obtained for 58 patients on androgen-deprivation therapy (ADT). Haplotype frequencies were estimated using the expectation-maximization algorithm using the Arlequin program, version 2.0. All statistical analyses were performed with the SPSS ver. 13.0 (SPSS Inc, Chicago, USA) statistical software package. The mean age (years±SD) for PCa patients and controls was not significantly different (p, 0.203). Overall analysis showed significant association in VEGF —7C>T heterozygous genotype (CT) (p=<0.001; OR=3.14), variant genotype (AA) of —2578C>A was at 2.9 fold risk in patients (p= 0.002) and the heterozygous genotype of VEGF —2549 Ins/Del (I/D) posed a 2.2 fold risk in individuals developing PCa (p=<0.001). Haplotypes T/G/C/I and T/G/C/D showed 3.7 and 2.5 fold increased risk with PCa (p=<0.001 and p= 0.021 respectively). The HR for advancement of PCa to HRPC showed that heterozygous genotype (I/D) of —2549 Ins/Del revealed a protective trend for the patients developing PCa (p=0.051; OR=0.28). The findings suggest inheritance of VEGF variants as a predictor of PCa progression risk among North Indian men, but still require validation in larger observational studies to validate the potential effect of these SNPs in PCa patients on ADT.

1239W

Molecular characterization of pediatric high grade astrocytomas reveals mTOR pathway dysregulation in pediatric anaplastic astrocytomas. *N. Gerges[1], T. Haque[1], A. Nantel[2], N. Jabado[1].* 1) McGill University, Montreal, Quebec, Canada; 2) Biotechnology Research Institute, Montreal, Canada.

Brain tumors are the leading cause of cancer-related mortality in children. Pediatric high grade astrocytomas, including grade IV (glioblastoma, pGBM) and grade III (anaplastic astrocytomas, pAA), are rare but devastating brain tumors accounting for 15% of all brain tumor cases in children. Grade III and IV pediatric astrocytomas are similarly treated and exhibit the poorest overall prognosis in pediatric oncology as 90% will die within 3 years of diagnosis. To identify differences based on tumor grade and age, we investigated high grade pediatric astrocytomas using transcriptome profiling. Our results show independent segregation of pAA from pGBM highlighting distinct molecular characteristics between these subgroups. The 660 differentially expressed genes between grade III and IV pediatric astrocytomas were further investigated using the Ingenuity Pathway Analysis (IPA) software to achieve comprehensive analysis of biological functions. IPA identified a significant dysregulation of the mTOR pathway (p-value = 4.65x10-4) that differentiated, among others, both subgroups. Indeed, 13 genes involved in the mTOR pathway were found to be differentially regulated between pAA and pGBM. PRKCB, a major member of the mTOR pathway involved in apoptosis and transcriptional regulation, was found to be upregulated in pAA compared to pGBM with a fold change of 2.723 that was further validated using immunohistochemical staining on pAA and pGBM primary tumors. This is the first report of its kind in the literature showing differential regulation of pAA and pGBM within pediatric high grade astrocytomas and identifying upregulation in pAA of members of the mTOR pathway including PRKCB. We are further investigating the functional significance of the dysregulation of mTOR pathway in pAA compared to pGBM. These results shed light on a pathway that may be amenable to therapy as drugs targeting it, including rapamycin, are already in the clinical setting and have been used in clinical trials in children including those with neurofibromatosis. They also further emphasize the need for better molecular classification of tumors for optimal therapeutic results in patients who currently have limited options for clinical trials and dismal outcome using current targeted therapies that are conceived without improved knowledge of the inherent biology of the tumor.

1240W

PIK3CA , BRAF,KRAS&PTEN mutational alteration and association with clinicopathological features in Iranian Colorectal Cancer Patients. *M. Ghaffarpour[1,4], S. Sobhani[2], M. Firoozi[2], E. Mohammadi Pargoo[2], S. pirmoradi[2], Z. Mostakhdemin Hosseini[3], F. Kamali[3], F. Fereidooni[3], M. Houshman[1,4], Sobhani Firoozi.* 1) Medical Genetics Department,National Institute for Genetic Engineering and Biotechnology, Tehran, Iran; 2) 2Department of Biology, Faculty of Sciences, Islamic Azad University, Science & Research Campus, Tehran; 3) Iranian National Tumour Bank, The Cancer Institute of Iran, Imam Hospitals CompleX, Tehran, Iran; 4) 4Medical Genetics Department,,Special Medical Center, Tehran, Iran.

Colorectal cancer (CRC) is the third most common cancer amongst Males and the fourth amongst females in Iranian cancer patients. The prevalence of PIK3CA,BRAF ,KRAS and PTEN mutations in CRC has been reported to vary between 13.6% to 32%,5%to22%,20% to 50% and1% and 29% respectively. The aim of this study was to identify PIK3CA gene mutations in exons 9 and 20 ,BRAF gene mutations in exon 11,15 , KRAS gene mutations in exon 1and PTEN mutations in exon7,8&9 among Iranian CRC patients, and to consider whether they are associated with the clinicopathological parameters like age, sex, familial history, site of primary and histology. Materials and Methods: Patients and tissue specimens:Tissue samples were obtained from 61 consecutive patients with CRC (26 men and 36 women; age range, 28-88 years; with median age of 57.8 years).Histopathological examinations were performed, and all tumors were confirmed as adenocarcinoma Mutational analysis: We searched for PIK3CA mutations in exons 9 and 20.PIK3CA exon 9 includes codons 542 and 545, PIK3CA exon 20, BRAF mutations in exons 11 and 15,KRAS mutations in codond12 &13(exon 1) and PTEN mutations in exons 7,8&9 where the large majority of mutations occur in these genes .The PI3KCA , BRAF, KRAS and PTEN mutational analyses were made by means of PCR sequencing. Results and conclusion: 53 (85.5%) patients had adenocarcinomas and 9 (14.51%) had mucinous adenocarcinomas. patients had stage I, II and III or IV disease with16.%, 43%and 41.% respectively and histological grade I, II and III with 55.74%,39.36% and 4.9%. respectively. Among the 62 samples analyzed (51were primary tumors and 11were metastases). The frequency of mutations in PIK3CA were present in 4.33% (3 of62) of the samples (4.33%mutation in exon 9(c.1633G>A E545K and no mutation in exon 20). The frequency of mutation in BRAF were present in 2 of 62 of the samples (3.22% mutation in exon 15; V600E and IVS14-4C>T). Mutation in PTEN was not present in the colon cancer patients (0 of 30). The presence of PIK3CA , BRAF and PTEN mutations were not a significance associated with gender, histological grade, age, or cancer stage in patients with adenocarcinoma of the CRC(P>0.05).20.3% of patients in KRAS gene. About 60% of KRAS-mutations were indicated in rectum cancer patients and 41.7% in colon cancer patients. In addition our result indicated that KRAS mutations in codons 12, 13 were less common among Iranian colorectal cancer patients.

1241W

Fine-Scale mapping of the 11q13 breast cancer susceptibility locus. *M. Ghoussaini[1], S. Ahmed[1], K. Michailidou[2], K. Gregory[1], E. Dicks[2], K. Driver[1], M. Maranian[1], C. Turnbull[3], N. Rahman[3], P.D. Pharoah[1,2], D.F. Easton[1,2], A.M Dunning[1].* 1) Department of Oncology, University of Cambridge, Cambridge CB1 8RN, United Kingdom; 2) CR-UK Genetic Epidemiology Unit, Public Health and Primary care, University of Cambridge, Cambridge, United Kingdom; 3) Section of Cancer Genetics, Institute of Cancer Research, Sutton, Surrey, UK.

We previously identified a variant at 11q13 (rs614367) associated with breast cancer risk through a genome-wide association study. This variant lies in an LD block of ~700 Kb. Using data from the 1000 Genomes Project, we catalogued ~1600 common variants (Minor Allele Frequency >0.05) in this region, of which 140 have a detectable correlation with rs614367 and were thus considered to be potentially causative. 52 tag SNPs were genotyped in ~13,000 cases and controls from the East Anglian SEARCH breast cancer case-control study, using FluidigmTM technology. We also used genotyping data from 145 SNPs at this locus from two UK population based studies in ~ 3600 cases and ~5200 controls. Genotypes of all other known variants in the locus were imputed using known genotypes in combination with data from the 1000 Genomes Project. Based on the combined data from all studies, 29 variants were convincingly associated with Breast Cancer (P-values 10-8 to 10-19, odds ratios 1.14 to 1.29). Assuming a single causative variant, all but 5 variants could be excluded based on a likelihood of <1:100 compared with the most significant SNP. These 5 variants lie in a 48 Kb LD block upstream of CCND1, FGF3, FGF4 and FGF19 genes. The gene(s) directly under the control of this locus remains to be determined.

1242W

SLC39A2 and FSIP1 Polymorphisms as Potential Modifiers of Arsenic-Related Bladder Cancer. *D. Gilbert-Diamond[1], A. Andrew[1], H. Nelson[2], Z. Li[1], T. Punshon[1], A. Schned[1], C. Marsit[1], S. Morris[4], J.H. Moore[1], A. Tyler[1], M. Guerinot[1], K. Kelsey[3], M. Karagas[1].* 1) Dartmouth Medical School, Lebanon, NH, USA; 2) University of Minnesota, Minneapolis, MN, USA; 3) Brown University, Providence, RI, USA; 4) University of Missouri-Columbia, Columbia MO, USA.

Arsenic is a carcinogen that contaminates drinking water worldwide. Over 10% of the unregulated drinking wells in the U.S state of New Hampshire have arsenic concentrations above the current U.S. maximum contaminant limit of 10 μg/L. Accumulating evidence suggests that both level of exposure and genetic factors may influence one's risk of arsenic-induced malignancies. We sought to identify potential interactions between single nucleotide polymorphisms (SNPs) and elevated arsenic exposure in relation to bladder cancer in a US population with low to moderate drinking water levels of arsenic. We first screened a subset of bladder cancer cases using a panel of approximately 10,000 non-synonymous SNPs. We then genotyped top ranking hits on the SNP array (Fisher exact test P < 0.0001) in our population-based case-control study (n=832 cases and 1191 controls). SNPs in the fibrous sheath interacting protein 1 (FSIP1) gene (rs10152640) and the solute carrier family 39, member 2 (SLC39A2) in the ZIP gene family of metal transporters (rs2234636) were identified as potential hits in the initial scan and validated in the full case-control study. The adjusted odds ratio (OR) for the FSIP1 polymorphism was 2.57 (95% confidence interval (CI) 1.13, 5.85) for heterozygote variants and 12.20 (95% CI 2.51, 59.30) for homozygote variants compared to homozygote wild types in the high arsenic group, and unrelated in the low arsenic group (p for interaction = 0.002). For the SLC39A2 polymorphism, the adjusted ORs were 2.96 (95% CI 1.23, 7.15) and 2.91 (95% CI 0.99, 8.52) for heterozygote and homozygote variants respectively, and close to one in the low arsenic group (p for interaction = 0.03). Our findings suggest novel variants that may modify the risk of arsenic-associated bladder cancer.

1243W

Cancer genes are differentially expressed with age in normal tissues. *D. Glass[1], A. Viñuela[1], L. Parts[2], D. Knowles[3, 7], A. Nica[4,2], J. Nisbet[2], A. Barrett[5], M. Sekowska[2], M. Travers[5], S. Potter[2], E. Grundberg[2, 1], K. Small[1, 2], A. Hedman[5], J. Tzenova Bell[5, 1], G. Surdulescu[1], F.O. Nestle[6], P. di Meglio[6], R. Durbin[2], K. Ahmadi[1], J. Winn[7], D. Hochhauser[8], V. Bataille[1], P. Deloukas[2], M.I. McCarthy[5], T.D. Spector[1], E.T. Dermitzakis[4], The Muther consortium.* 1) King's College London, London, United Kingdom; 2) Wellcome Trust Sanger Institute, Hinxton, United Kingdom; 3) Department of Engineering, University of Cambridge, Cambridge, United Kingdom; 4) Department of Genetic Medicine and Development, University of Geneva, Geneva, Switzerland; 5) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 6) St John's Institute of Dermatology, King's College London, London, United Kingdom; 7) Microsoft Research, Cambridge, United Kingdom; 8) University College London Cancer Institute.

BACKGROUND: Age is the strongest risk factor for cancer. The relationship between the ageing process and cancer development is not fully understood; this study investigates changes in gene expression in skin and fat with age which may help to further elucidate the mechanisms involved. METHODS: 855 photo-protected abdominal skin samples were taken from female Caucasian twins aged 40 - 85 years. Abdominal fat and lymphoblastoid cell lines (LCLs) were sampled from the same individuals. RNA was extracted from all samples and hybridised with the Illumina Human Sentrix 12 micro-array. A linear mixed model was used to explain gene expression variability with age and confounding factors; (Significance determined from permutations using false discovery rate <0.05). RESULTS: There was a difference in the number of genes differentially expressed with age between the 3 tissues with the skin showing the greatest number of genes differentially expressed with age (n~2000) compared to fat (n~600). No genes were differentially expressed with age in LCLs. 100 common genes were differentially expressed with age in both skin and fat tissues. Multiple genetic pathways implicated in cancer development were significantly differentially expressed with age. These include genes in well known pathways such as MEK1, ERK1/2, EGFR, WNT and Frizzled. These results may shed some light into the complex interactions between ageing and cancer.

1244W

Clinical implications of a prostate cancer risk SNP profile in an Active Surveillance cohort. *C. Goh[1], E. Saunders[1], N. Mahmud[1], M. Tymrakiewicz[1], D. Leongamornlert[1], T. Dadaev[1], E. Castro[1], D. Olmos[1], K. Thomas[1,2], R. Woode-Amissah[1,2], M. Guy[1], K. Govindasami[1], L. O'Brien[1], A. Hall[1,2], R. Wilkinson[1], A.A. Al Olama[3], D. Easton[3], Z. Kote-Jarai[1], C. Parker[1,2], R. Eeles[1,2].* 1) The Institute of Cancer Research, United Kingdom; 2) The Royal Marsden Hospital, Sutton, United Kingdom; 3) The University of Cambridge, United Kingdom.

Background: Genome-wide association studies (GWAS) have identified 39 susceptibility loci associated with prostate cancer (PCa) so far. Currently, these have only been evaluated against overall PCa risk and several clinical disease parameters. The challenge now is to translate the current PCa risk algorithms to the clinical setting. We explore the potential prognostic role that these risk SNPs might play in patients undergoing Active Surveillance (AS) for prostate cancer. **Methods:** The AS cohorts have low-risk PCa with Gleason scores ≤3+4, T1/T2 stage tumours and percentage positive biopsy cores <50%. These patients are offered regular PSA measurements and repeat prostate biopsies (18-24 monthly). DNA from eligible patients were genotyped using the Sequenom MassARRAY iPLEX platform and Taqman assays. The cumulative risk scores for each patient were calculated by summing risk alleles for each loci using the weighted effect as estimated in previous studies. These risk scores were assessed against defined adverse outcomes in AS including, adverse histology on repeat biopsy, time to treatment, and a PSA doubling time of <2 years (PSADT), to determine their prognostic value. **Results:** 391 patients were eligible for analyses. Median age was 66.4 years (range 50-79.4), with a mean PSA of 6.8 (range 0.19-36). At the time of analysis, 35.8% of patients have undergone treatment and 35.7% have histological upgrade on repeat biopsies. On univariate analyses, there was no correlation between the calculated genetic risk scores and upgrade on repeat biopsy, or time to treatment (p=0.573 and p=0.825, respectively). When analysing differences between the higher and lower genetic risk groups (defined as the top 25% and lowest 25% of the genetic risk distribution), there was again no correlation (p=0.850, 95%CI= 0.509-1.839; and p=0.906, 95%CI=0.530-1.570, respectively). For PSADT, there was also no correlation with the genetic risk scores (p=0.317). Subgroup analyses were conducted using risk scores generated from loci thought to be associated with aggressiveness (e.g. SNPs in 8q24 region), which again showed no correlation. **Conclusion:** This study does not support the use of the current PCa risk scores to predict outcomes in an AS cohort. Further analyses to investigate risk algorithms pertaining to treatment outcomes are needed. To our knowledge, this is the first study analysing the potential prognostic significance of GWAS risk SNPs in a PCa treatment cohort.

1245W

Familial aggregation of acute myeloid leukemia and myelodysplastic syndromes in young, but not in adult patients. *L.R. Goldin[1], S.Y. Kristinsson[2], X. Liang[1], A.R. Derolf[2], O. Landgren[3], M. Bjorkholm[2].* 1) Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, MD; 2) Department of Medicine, Division of Hematology, Karolinska University Hospital Solna and Karolinska Institutet, Stockholm, Sweden; 3) Medical Oncology Branch, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland.

Apart from rare pedigrees with multiple cases of acute myeloid leukemia (AML), there is limited data on familial aggregation of AML and myelodysplastic syndrome (MDS) in the population. In a comprehensive population-based study, we estimated risk of AML, MDS, other hematological malignancies and solid tumors among relatives of AML and MDS patients. Swedish population-based registry data were used to evaluate outcomes in 24,573 first-degree relatives of 6,962 AML and 1,388 MDS patients compared with 106,224 first-degree relatives of matched controls. Using a marginal survival model, we calculated relative risks (RR) and 95% confidence intervals (CI) as measures of familial aggregation. AML, MDS, and myeloproliferative neoplasms as a group did not aggregate significantly in relatives of patients with AML, MDS or in the combined group. The risks for any hematopoietic or solid tumor were significantly but modestly increased among relatives of AML patients. In contrast, we found a significantly increased risk (RR=7.53; CI: 1.25-45.13) of AML/MDS and a 3.01-fold RR (CI: 1.09-8.31) for all myeloid malignancies combined among relatives of patients diagnosed under age 21 years. The lack of familial aggregation of AML or MDS is striking and in sharp contrast to findings in patients with other myelo- and lymphoproliferative disorders. However, relatives of young AML patients do seem to be at increased risk of AML/MDS, other hematopoetic and solid tumors suggesting that germ line genes play a stronger role in these patients. The increased risk of any hematopoetic or solid tumor suggests that genes for malignancy in general and/or other environmental factors may be shared in these families.

1246W

Genetic variations in BRAF, CDKN2A, NRAS, and PTEN and the risk of melanoma in melanoma-prone families. *A.M. Goldstein[1], R.M. Pfeiffer[1], D. Maeder[1,2], L. Burdett[1,2], M. Yeager[1,2], S.J. Chanock[1], M.A. Tucker[1], X.R. Yang[1].* 1) Division of Cancer Epidemiology and Genetics, National Cancer Institute (NCI), National Institutes of Health, DHHS, Bethesda, MD, USA; 2) Core Genotyping Facility, NCI-Frederick, SAIC-Frederick, Inc., Frederick, MD, USA.

Cutaneous melanoma (CM) is a type of skin cancer with a complex etiology. CDKN2A is the major high-risk susceptibility gene for CM identified to date. In addition, somatic mutations occur frequently in CM tumors. Overall, the genes most frequently somatically mutated in CM are BRAF, CDKN2A, NRAS, and PTEN. To examine whether germline copy number variations (CNVs) in these genes might contribute to the disease phenotype in high-risk CM-prone families, we conducted array-based comparative genomic hybridization (aCGH) studies using the Nimblegen whole-genome tiling (720k exon-focused) CGH array on 153 melanoma patients and 82 unaffected family member/spouse controls from 48 American melanoma-prone families with and without germline CDKN2A mutations. Potential disease-related CNVs were validated using qPCR. In addition, we evaluated evidence for associations between CM and tagging SNPs in BRAF, CDKN2A, and PTEN in 53 families (23 families segregating CDKN2A mutations and 30 families without CDKN2A mutations). For the association analysis, we had genotype data on 64 SNPs for 183 CM patients and 379 unaffected family members/spouse controls using an Illumina iSelect Infinium assay. Conditional logistic regression models were used to estimate the trend p-value for the association between CM and each SNP, using codominant coding for genotypes with the homozygote of the common allele as the reference group. All analyses were adjusted for age, sex, and CDKN2A mutation status. No disease-related CNVs in BRAF, CDKN2A, NRAS, or PTEN were consistently observed in CM cases. Association analyses showed no significant associations with BRAF or CDKN2A. A significant association was observed between rs1234221 in PTEN and CM risk (p-trend=0.00055; OR= 0.59, 95% CI=0.35-0.99 for CA vs CC [ref]; OR=0.11, 95% CI=0.03-0.38 for AA vs CC [ref]). The association remained significant after Bonferroni correction (p-trend=0.035). The effect also appeared stronger in families without CDKN2A mutations versus those with CDKN2A mutations. In summary, we observed no disease-related germline CNVs for the most frequently somatically mutated genes for melanoma in 48 CM-prone families. SNP evaluation of BRAF, CDKN2A, and PTEN showed one SNP tagged for PTEN to be significantly associated with CM risk. Our study is limited by the small sample size and should be considered exploratory. Additional CM-prone families are needed to further evaluate the relationship between rs1234221 and CM risk.

1247W

CYP3A4*1B POLYMORPHISM IS NOT ASSOCIATED WITH PROSTATE CANCER IN MEXICAN POPULATION. *L. GOMEZ-FLORES-RAMOS[1], E. VAQUEZ-VALLS[1], B.M. TORRES-MENDOZA[2], M.P. GALLEGOS-ARREOLA[2].* 1) UIMEC, UMAE de Especialidades, Centro Médico Nacional de Occidente, Instituto Mexicano del Seguro Social; Belisario Dominguez 1000, CP 44340, Guadalajara, México; 2) Centro de Investigacion Biomedica de Occidente, Centro Médico Nacional de Occidente, Instituto Mexicano del Seguro Social; Sierra Mojada 800, CP 44340, Guadalajara, México Occidente,.

Prostate cancer (PCa) is the second most common malignancy in mexican men over 50 years, its etiology is multifactorial, and genetic factors are important in its development. The evidence suggests that steroid hormones and genes that regulate them could influence prostate carcinogenesis. Thus, the CYP3A4 enzime, which facilitates the deactivation of testosterone as a result of NADPH-dependent monooxidative reactions, has been considered. The CYP3A4*1B (-290A> G) polymorphism, has been associated with susceptibility to PCa in various populations, so the aim of our study was to determine the association of the CYP3A4*1B polymorphism in Mexican population. The frequency of the CYP3A4*1B allele was determinate in 257 samples of genomic DNA from general population individuals and 44 patients with PCa. The wild homocygote (AA) genotype was the most common among both groups, occurring in 84% (37/44) of patients and in 79.8% (205/257) of the control group, followed by the heterocigote genotype (AG) with 16% (7/44) in the PCa group and 19.5% (50/257) in controls, the mutant homocigote (GG) genotype was not observed in the patients, and was present at 1% (2 / 257) in the control group, non significant differences were found [p>0.05; OR 0.71, 95% CI (0.28 to 1.73)]. Our results suggest that the CYP3A4*1B polymorphism is not associated with prostate cancer in Mexican population.

1248W

Confronting the Problem of Genetic Heterogeneity in Cancer Tissues: Investigating the Role of the Androgen Receptor Gene in Breast Cancer Ontogeny and Treatment. *B. Gottlieb[1,2,3], C. Alvarado[1], C. Wang[5], B. Gharizadeh[5], F. Babrzadeh[5], B. Richards[1], M. Basik[2,4], L.K. Beitel[1,2,3,4], M. Trifiro[1,2,3,4].* 1) Dept Cell Genetics, Lady Davis Institute for Med ical Research, Montreal, PQ, Canada; 2) Segal Cancer Centre, Jewish General Hospital, Montreal, PQ, Canada; 3) Dept of Human Genetics, McGill University, Montreal, PQ, Canada; 4) Dept of Medicine, McGill University, Montreal, PQ, Canada; 5) Stanford Genome Technology Center, Stanford University, Palo Alto, CA.

The extensive genetic heterogeneity discovered within cancer tissues, has raised many questions with regards to cancer ontogeny. It has been suggested that understanding the origins of genetic heterogeneity could be critical to identifying genetic markers of cancer ontogeny and developing prevention and treatment regimens. A major problem preventing any assessment of the significance of genetic heterogeneity in cancer tissues has been the inability to measure the relative frequency of the different genetic variants. We have examined somatic genetic heterogeneity within individual breast tumors by “over-sequencing” the X-linked androgen receptor gene (AR), up to 37,000 times, to quantify AR CAG repeat length variation, a functional polymorphism associated with breast cancer risk. All samples of laser capture micro-dissected cancer and non-cancer tumor tissues, as well as from matching blood leukocytes, had up to 30 AR CAG repeat length variants, instead of just two. Normal breast tissues had a majority of ARs containing zero CAG repeats, while cancer tissues had a reduced number of ARs with zero CAG repeats, and a majority of ARs with CAG repeat lengths in the normal tissue range (18-25). Blood leukocytes had many fewer AR variants, few, if any, with zero repeats, and a majority with normal length CAG repeats. These results support the observation that shorter AR CAG repeat lengths may be protective against breast cancer, and the hypothesis that selection as a result of particular hormonal microenvironments, of pre-existing minority cell populations with longer AR CAG repeat length variants, may contribute to breast carcinogenesis. One might also consider that tumor genomic heterogeneity introduces significantly greater complexity for `targeted' therapeutic approaches. However, if longer CAG repeats drive breast cancer cell growth and shorter repeats inhibit growth, and if our heterogeneity hypothesis is valid, our work may suggest creative approaches to treatment of breast cancer. One would be by identifying and then selectively eliminating cells containing longer AR CAG 1 repeat variants, and thus enhancing the relative concentration of cells with short CAG repeats. A more challenging approach, that involves targeting host factors, would be the identification and modulation of the micro-environmental conditions that result in the selection of longer AR CAG 1 repeat variants. Thus, important therapeutic implications may arise from these observations.

1249W

Replication of genome-wide discovered breast cancer risk loci in the Cypriot population. *A. Hadjisavvas, M. Loizidou, I. Neophytou, K. Kyriacou.* The Cyprus Institute of Neurology & Genetics, Department of Electron Microscopy/Molecular Pathology, Nicosia, Cyprus.

Genome-wide association studies (GWAS) have identified several loci as being associated with breast cancer in mostly European populations. It is important to evaluate whether these variants confer risk across different populations and also to assess the magnitude of risk conferred. The aim of this study was to evaluate previously GWAS-identified breast cancer risk variants in the Cypriot population. To evaluate whether the GWAS derived risk variants contribute to breast cancer in the Cypriot population, we genotyped them in 1,109 Cypriot female breast cancer patients and 1,177 healthy female controls. Overall, our results show modest concordance for breast cancer GWAS-discovered alleles and their effect sizes in the Cypriot population. The effects sizes of GWAS-discovered SNPs need to be verified separately in different populations.

1250W

Association of 8q24.21 locus with the risk of colorectal cancer: A systematic review and meta-analysis. *B.S. Haerian[1], M.S. Haerian[2]*. 1) Deapartment of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; 2) Research Center for Gastroenterology and Liver Disease, Shaheed Beheshti University, M.C., Tehran, Iran.

Objective: Recent genome-wide association studies of colorectal cancer (CRC) have identified rs6983267 and rs10505477 polymorphisms as key loci in the 8q24 region to be associated with CRC. In the present study, we performed a meta-analysis to determine whether these loci are risk factors for susceptibility to CRC. Materials and Methods: We meta-analyzed the 22 included studies (47,003 cases and 45,754 controls) that evaluated the role of rs6983267 and rs10505477 polymorphisms with CRC risk under alternative genetic models. Results: Meta-analysis of the pooled data showed allelic and genotypic association of the rs6983267 polymorphism with CRC risk in the Asian, European, and Americans with European ancestry. Sub-analysis of the American studies showed negative results in the studies with non-identified ethnicity of the subjects. Meta-analysis of included studies of rs10505477 polymorphism identified allelic and genotypic association with CRC risk in the American subjects. Further meta-analysis of the American studies demonstrated positive results in the studies with non-identified ethnicity of the samples. Conclusion: Our data suggested that the rs6983267 polymorphism is a risk factor for CRC in the Asians, European, and American with the European ancestry populations.

1251W

Analysis of the 16p11.2 region in congenital primary hypothyroidism and atypical Septo-Optic Dysplasia. *M. Dasouki[1], G. Lushington[2], K. Hovanes[3].* 1) Dept Pediatrics, Univ Kansas Med Ctr, Kansas City, KS; 2) Molecular Graphics and Modeling Laboratory, Univ of Kansas, Lawrence, KS; 3) Combimatrix Diagnostics. Irvine, CA.

The chromosome 16p11.2 region was recently found to be a hot spot for copy number abnormalities and had been associated with autism +/- congenital anomalies (29.5-30.1 Mb) and severe early childhood obesity (28.7-29 Mb). Two genes within this region, *SEZ6L2* and *SH2B1*, are considered to be strong candidates for the autism spectrum disorder and obesity phenotypes respectively. Otherwise, there is no strong genotype-phenotype correlation. In a 2 year old Caucasian girl with severe congenital hypothyroidism, growth hormone deficiency, bilateral optic nerve hypoplasia with retinal drusen and facial dysmorphism, a *de novo* chr.16p11.2 deletion [del(16)(p11.2p11.2)(RP11-301D18-).arr 16p11.2(29,366,195-30,302,387)x1] was identified. Septo-optic dysplasia (SOD) is a highly heterogeneous condition comprising variable phenotypes including midline and forebrain abnormalities, optic nerve and pituitary hypoplasia. Mutations in the transcription factors *HESX1, SOX2, SOX3* and *OTX2* had been implicated in its etiology. While several genes within the deleted 16p11.2 region are expressed in the pituitary and thyroid glands, two (*CD2BP2* & *YPEL3*) were found to interact with known SOD related genes [*CD2BP2-NR5A1-SOX2* & *YPEL3-ONECUT1-HESX1*]. Additionally, *CD2BP2* & *YPEL3* showed interaction loops with *NKX2-1, NKX2-5, HHX* and *TSHR*, genes known to be important for thyroid gland development and morphogenesis. These findings suggest a possible important role for these two genes in the pituitary and thyroid development. It is also possible that additional mutations within the intact alleles in the 16p11.2 region as well as other related genes may have contributed to this unique phenotype.

1252W

Cytogenetic and phenotypic findings in Tunisian children with Fanconi Anemia. *A. AMOURI[1,2], F. TALMOUDI[1,2], S. ABDELHAK[2], O. KILANI[1], I. CHEMKHI[1,2], H. GUERMANI[1,2], S. HENTATI[1], N. ABIDLI[1], O. MESSAOUD[2], M. BEN REKAYA[2], W. AYED[1,2], THE TUNISIAN FANCONI ANEMIA STUDY GROUP.* 1) CYTOGENETIC LABORATORY, PASTEUR INSTITUTE OF TUNIS, TUNISIA; 2) MOLECULAR INVESTIGATION OF GENETIC ORPHAN DISEASES RESEARCH UNIT (MIGOD) UR 26/04, PASTEUR INSTITUTE OF TUNIS, TUNISIA.

Background:Fanconi anemia (FA) is a rare syndrome characterized by bone marrow failure, malformations and cancer predisposition. Hypersensitivity to alkylating agents such as diepoxybutane (DEB) or mitomycin C (MMC) is the gold standard test for the diagnosis of FA. Methods:Chromosomal breakage investigation using MMC induction was carried out in 176 pediatric aplastic anemia (AA) patients suspected with FA. After cytogenetic confirmation, some of the FA-siblings were also investigated to exclude disease especially when donors for bone marrow transplantation are sought. Results:Chromosomal breakage evaluation showed 32 patients with FA among 176 paediatric AA patients (18.18%). FA-patients exhibited a high frequency of chromosome fragility compared with the controls.The rate of consanguinity was 94.11% in FA-patients. Among 21 sibs of 11 FA patients, we found 4 (19.4%) affected. Conclusion:This study is the first in Tunisia to analyses the cytogenetic and phenotypic findings in children with FA. FA seems to be frequent in Tunisia because of the frequent consanguineous marriages. So, this study calls for an urgent nation-wide screening. Results of this study indicate also that is extremely important to rule out FA in all FA-siblings.

1253W

Intrachromosomal events and presence of complexities at the breakpoints are common features in *MECP2* duplication rearrangements. *C.M.B. Carvalho[1], M. Ramocki[2, 3], D. Pehlivan[1], S.W. Cheung[1], L.M. Franco[1], M. Shinawi[4], J.W. Belmont[5], H.Y. Zoghbi[1, 2, 3], P.J. Hastings[1], J.R. Lupski[1, 2, 3].* 1) Dept Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Children's Hospital, Houston, TX; 4) Division of Genetics and Genomic Medicine, Department of Pediatrics, Washington University School of Medicine, St Louis; 5) Division of Cardiology, Department of Pediatrics, Baylor College of Medicine, Houston, TX.

Copy number gain in Xq28 including *MECP2* is the most commonly identified subtelomeric alteration in patients with developmental delay and associated clinical findings. *MECP2* duplications are nonrecurrent, that is each patient presents a unique rearrangement with variable size and gene content. To date, we collected a cohort of 44 patients with *MECP2* duplications through a collaboration established among our research laboratory, Baylor Diagnostic Laboratory (MGL) and the Blue Bird Circle Rett Center clinic at Texas Children's Hospital specifically for boys (and rare girls) with *MECP2* duplication syndrome. Previous partial analysis derived from high-resolution comparative genomic hybridization array assays (aCGH) revealed occurrence of complex rearrangements within our patient cohort, i.e. triplications or stretches of normal copy number interspersed with duplications, in approximately 27% of cases. Here we further studied 18 patients carrying duplications including *MECP2* for whom we were able to accomplish DNA sequencing for each one of the rearrangement breakpoint junctions. Surprisingly, DNA sequencing unveiled the presence of complexities in up to 50% of the rearrangements, most of which could not be anticipated by analysis of aCGH results alone. All of those complex alterations have at least one breakpoint within or flanking the low copy repeats (LCRs) that map nearby the *MECP2* gene, supporting and expanding our previous hypothesis that such LCRs stimulate rearrangements in the *MECP2* locus. In addition, we used SNP array (Illumina Human Omni1-Quad) to assess the status of the SNPs present within the altered genomic region and observed absence of heterozygosity (AOH) in almost all cases strongly suggesting that the *MECP2* duplication is mainly an intrachromosomal event. In summary, our data contributes to further understand the genomic alterations present in the *MECP2* duplication patient cohort and support the contention that the genomic architecture plays an important role for nonrecurrent rearrangement formation.

1254W

Significance of small CNVs detected below the clinical cytogenetic laboratory threshold using Affymetrix 2.7M SNP array. *Y. Qiao[1,2], C. Tyson[3], M. Hrynchak[3], E. Lopez-Rangel[2], J. Hildebrand[2], S. Martell[1], C. Fawcett[3], K. Calli[1,2], J. Holden[4], S. Lewis[2], E. Rajcan-Separovic[1].* 1) Dept Pathology (Cytogenetics), Universty of British Columbia (UBC) and Child and Family Res Inst (CFRI), Vancouver, Canada; 2) Dept. Medical Genetics, UBC and CFRI, Vancouver, Canada; 3) Cytogenetics Lab, Royal Columbian Hospital, New Westminster, Canada; 4) Depts Psychiatry & Physiology, Queen's University, Kingston, Ontario, Canada.

Whole genome screening array technology is now an established tool for detecting copy number variants (CNVs) in neurodevelopmental conditions such as autism spectrum disorders (ASDs) and intellectual disability (ID). It is expected that higher resolution arrays detect CNVs more accurately and allow identification of smaller CNVs. However, validation of smaller CNVs requires molecular methods and their clinical relevance is sometimes ambiguous, thus a cut-off of 200kb for deletion (del) and 500kb for duplication (dup) is frequently used in the clinical setting. Our work aims to determine the criteria for reliably detecting smaller CNVs and establishing their clinical relevance. We used the Affymetrix Cyto 2.7M Array first to validate 39 CNVs (31 Kb - 5.4 Mb) previously detected and confirmed by lower resolution arrays in 30 subjects with ASD and/or ID. All 39 CNVs were confirmed by the 2.7M array and in addition 10 previously undetected and unique CNVs, 100-500 Kb in size, were selected for validation using qPCR. Four of the 10 CNVs (40%) were confirmed (3 were maternal and 1 of unknown origin). We noted that array experiments with a high waviness-segment-count >10 tended to have CNVs that could not be confirmed, and we used this experience to select unique CNVs below the clinical size cut-off from 39 new subjects with a normal clinical array report. Eleven small unique CNVs (49~480 Kb) containing genes of potential brain function were assessed using qPCR. Eight (70%) were confirmed, including seven familial CNVs and one de novo dup (140 Kb) which encompasses intron 2 of KCNH7, a gene which regulates neurotransmitter release and neuronal excitability. A maternally transmitted 130 Kb dup covering exons 64-79 of the DMD gene was found in a 3 year old boy manifesting autism and neuromotor delay. Whilst his features were not strongly suggestive of DMD (with normal CPK testing) he did manifest delayed gross motor skills including crawling (at 14 months.), walking (at 20 months) and difficulty climbing stairs independently at 3 years. In light of the CNV finding, repeat CPK and neuromotor testing is continuing. In conclusion, the use of array quality control criteria such as waviness-segment-count helped decrease the number of false negative small CNVs from 60% to 30%. The small CNVs confirmed by qPCR and below the clinical cut-off tend to be of familial origin, and in most, but not all cases would be classified as variants of unknown significance.

1255W

A Genetic Study to Understand The Enigma of Holoprosencephaly. *M.I.K. Shehab[1], M. Zaki[2], H. Bassiouny[2], H. Kayed[1], M. Eid[1].* 1) Human Cytogenetics, National Research Center, Cairo, Giza, Egypt; 2) Clinical Genetics, National Research Center, Cairo, Giza, Egypt.

Holoprosencephaly (HPE) is a genetically heterogeneous disorder that affects the midline development of the forebrain and midface in humans. Classic HPE encompasses a continuum of brain malformations including (in order of decreasing severity): alobar, semilobar, lobar, and middle interhemispheric variant (MIHV) type HPE. These subtypes are defined primarily by the degree and region of neocortical nonseparation. Clinical suspicion of HPE is typically based upon compatible craniofacial findings, the presence of developmental delay or seizures, or specific endocrinological abnormalities, and is then followed up by confirmation with brain imaging. The aim of the work is to better understand and elucidate all of the clinical findings of this enigmatic syndrome in Egyptian patients. The study included HPE patients recruited during 2007-2011 from the Pediatric Neurology Clinics and Clinical Genetics Department, National Research Center, Egypt. Their age ranged from few minutes to 9.9 years (mean age is 3.3 ± 1.8 years). Patients were clinically examined to detect malformations and anomalies. A three generation pedigree was constructed with special emphasis on positive consanguinity and similarly affected family members. In addition, magnetic resonance imaging of the brain (MRI), electroencephalogram (EEG) recording, karyotyping and FISH were done for all patients. Furthermore, endocrinal functions, fundus examination and echocardiography were evaluated when indicated. A total of 25 HPE patients were studied; 15 males and 10 females. Brain imaging revealed, alobar in 6 patients (24%), semilobar in 15 patients (60%), lobar in 2 patients (8%) and MIH in 2 patients (8%). Positive consanguinity was present in 10 cases (40 %) while similarly affected family members were detected in 8 cases (32 %). All the living patients presented with delayed milestones. Poor temperature regulation was present in 12 patients (48%). Clinically, microcephaly (>-3 SD) was detected in 20 patients (80%). Chromosomal aberrations were detected in 7 (28%) of the patients . As a step towards elucidating the spectrum of the HPE, facial dysmorphism, cerebral signs and karyotype findings were highlighted.

1256W

Interstitial deletion of chromosome band 2p21 with eye anomalies. *G. LEFORT[1,2], J. PUECHBERTY[1,2], A. SCHNEIDER[1,2], M. TOURNAIRE[1,2], M. GIRARD[1,2], P. SARDA[2], P. BLANCHET[2], L. PINSON[2], D. GENEVIEVE[2], S. TAVIAUX[1,2], E. HAQUET[2], F. PELLESTOR[1,2], C. COUBES[2].* 1) Chromosomal genetics, Hôpital ADV, CHRU Montpellier, MONTPELLIER, Languedoc, France; 2) Medical genetics, Hôpital ADV, CHRU Montpellier, MONTPELLIER, Languedoc, France.

We report a patient with multiple congenital anomalies including bilateral eye anomalies with a 2p21 deletion diagnosed by microarray analysis. The patient was the third child of unrelated parents with an unremarkable family history. Pregnancy was uneventful. At birth, the male child was eutrophic and presented multiple congenital anomalies including lumbar lipoma and myelomeningocele, congenital dislocation of the right hip, right cryptorchidism and complex bilateral ocular malformation. The eye malformation concerned the anterior and posterior chambers of the eye and was interpreted as scleroderma of the cornea associated with chorioretinal atrophy. GH deficit was discovered at the age of 7 years and the patient received hormonal therapy. The patient was addressed to our Department of Medical Genetics at 17 years for genetic evaluation. At this time, the patient presented dysmorphic features (triangular face, prominent nasal bridge, dysplastic ears, microretrognathia, dental anomalies) and nail hypoplasia. The patient is blind but has no mental retardation. Course was marked by severe scoliosis which underwent surgical correction, and by paraplegia which was a sequella of surgery for lumbar lipoma. Standard cytogenetic studies showed a normal karyotype. Molecular biology analyses of the FOXC1, PITX2, and PAX6 genes found no mutations. Microarray analysis (SNP6.0 Affymetrix) revealed a “de novo” 1.46 Mb deletion of the 2p21 band. To date, few pure 2p21 deletions have been reported in the literature and recorded in genetic databases. The phenotypic expression of 2p21 deletion in our patient concerns mainly the ocular system and a few dysmorphic features. Among the 11 genes identified in the 2p21 band, there is no candidate gene for the eye anomalies. However the SIX3 gene, located approximately 375 kb proximal to the boundary of the deleted region, has been reported to participate in murine eye development. We postulate that a dysregulation of the SIX3 gene due to the adjacent deletion might be the cause of the eye anomalies in our patient.

1257W

CDH9 deletion in a patient with profound intellectual disability and autism spectrum disorder. *C. Soler-Alfonso[1], C.P. Schaaf[2], V.R Sutton[2], J. Wiszniewska[2], s.H. Lee Kang[2].* 1) Division of Medical Genetics, University of Texas at Houston, Houston, TX; 2) Department of Molecualr and Human Genetics. baylor College of Medicine, Houston, TX.

This report describes a female patient with a 5.830 Mb deletion at chromosome 5p14.1-p13.3. She presented for evaluation of profound intellectual disability and autistic behaviors. Array comparative genome hybridization revealed the aforementioned copy number variant, deleting only one annotated gene, CDH9. This gene encodes a type II classical cadherin from the cadherin superfamily, integral membrane proteins that mediate calcium-dependent interactions and play an important role in neuronal cell adhesion. In addition, common genetic variants in 5p14.1 have been associated with risk for autism spectrum disorders by genome wide association studies. Here, we report the first patient with CDH9 deletion, and present a detailed clinical and behavioral characterization. This reports strengths the proposed role of cadherin 9 in the etiology of autism spectrum disorders and intellectual disability.

1258W

Nanoscopic analysis of DNA segregation and chromatin accessibility during metaphase. *W.A. Khan[1, 2, 7], R.A. Chisholm[3], S.M. Taddayon[1, 4], A. Subasinghe[1, 5], J. Samarabandu[1, 5], L.J. Johnston[3], P.R. Norton[1, 4], P.K. Rogan[1, 6, 7], J.H.M. Knoll[1, 2, 7].* 1) University of Western Ontario, London, Ontario, Canada; 2) Pathology; 3) Steacie Institute for Molecular Sciences, National Research Council, Ottawa, Ontario Canada; 4) Chemistry; 5) Electrical & Computer Engineering; 6) Biochemistry & Computer Science; 7) Cytognomix Inc.

We have previously shown differences in accessibility of short fluorescence in situ hybridization DNA probes (FISH) to some targets on homologous metaphase chromosomes. To understand these differences, the topography of metaphase chromosomes visualized with atomic force microscopy (AFM) was correlated with the organization of integral chromosomal proteins by immuno-FISH. Initially, centromeric DNA of the alpha-satellite family was examined on chromosome 17 at nanoscale resolution. AFM revealed either two (bimodal) or three (trimodal) major ridge structures along the lateral axis of the centromere. Bimodal ridges demonstrated a wider average lateral extension (0.71 μm) relative to trimodal ridges (0.56 μm). This is indicative of sister chromatids at different segregation stages prior to splitting to their spindle poles. Upon correlating centromere 17 FISH with chromosome topography, alpha-satellite DNA fluorescence was coincident with the centromere ridge in only 23% of the chromosomes, and did not co-localize in the remaining chromosomes (n=22). Integrated intensities quantified by gradient vector flow analysis showed up to 34% difference between homologs, presumably due to polymorphisms in alphoid DNA length or differences in probe accessibility. Immunolocalization of a constitutive centromeric protein (CENP-B) at the base of the kinetochore coincided with the centromere ridge topography. Immuno-FISH studies showed that in ~ 50% of the cells, the alpha-satellite DNA was not coincident with the distribution of CENP-B protein. This suggests that the timing of centromere DNA segregation is not tightly coordinated with structural changes in the kinetochore attachment sites. Variability in chromosome topography was also studied at non-centromeric sequences along the length of the chromosome arms by correlated AFM and FISH. A short sequence-defined low-copy FISH probe spanning 3.4Kb, and detecting 3 separate homologous targets was analyzed. The targets were embedded in a segmental duplicon block in 16p12.1 with its paralogous targets 1.39Mb and 3.58Mb telomeric in 16p13.1. The context of this probe differed among homologs, as it hybridized to both ridge-like (n =11) and groove-like (n = 9) structural targets at similar frequencies. In summary, differences in metaphase hybridization patterns observed with short FISH probes (1.5-5Kb) may be related to the chromatin compaction state or the distribution of underlying epigenetic marks in mitosis.

1259W

A short stature girl with a complex chromosomes X and Y translocation. FISH and array-CGH studies. *V. Català[1,2], K. Torabi[1], E. Masip[1], T. Escabias[1], A. Serés-Santamaria[1], E. Cuatrecasas[1].* 1) Prenatal Genetics, Barcelona, Catalonia, Spain; 2) Unitat de Biologia Cel·lular i Genètica Mèdica. Facultat de Medecina. Universitat Autònoma de Barcelona. Spain.

The cytogenetic prenatal diagnosis in a twin pregnancy of two female fetuses, one of the karyotypes showed a translocation between chromosome X and chromosome Y [46,X,der (X) t(X,Y)(p22.33;q11.2)] and the other showed a normal female karyotype. The parents' karyotypes were normal, implying that the reorganization was de novo. At the time of birth, the karyotype of the twins to determine who was the translocation carrier was carried out. At the age of 10 years, the girl with the translocation has short stature, and the endocrinologist recommended a study of the SHOX gene given karyotype. The study for the SHOX gene by FISH (Cytocell) resulted in a deletion on the translocated X chromosome. Spanish Health administration for the use of Growth Hormone accept the treatment when the patient has an altered gene SHOX. Since in our laboratory was setting up the array-CGH technique, applied microarray platform Constitutional Focus BACS (BlueGnome, Cambridge, UK) and the result obtained was that the translocation involved the majority of the long arm of chromosome Y Including the AZF region genes. The follow up of the patient by the geneticist and the endocrine is very important because premature ovarian failure was descrived in adult patiens with similar translocations.

1260W

X-linked congenital hypertrichosis syndrome is associated with interchromosomal insertions mediated by a human-specific palindrome near SOX3. *X. Zhang[1], H. Zhu[1,2], D. Shang[1], M. Sun[1], S. Choi[3], P. Patel[3], T. Jing[2].* 1) Dept Med Gen, Peking Union Med College, Beijing, China; 2) Lanzhou University School of Basic Medical Sciences, Lanzhou, China; 3) Institute for Genetic Medicine, University of South California, Los Angeles, CA 90033, USA.

X-linked congenital generalized hypertrichosis (CGH), an extremely rare condition characterized by universal overgrowth of terminal hair, was first mapped to chromosome Xq24-q27.1 in a Mexican family. However, the underlying genetic defect remains unknown. We ascertained a large Chinese family with an X-linked congenital hypertrichosis syndrome combining CGH, scoliosis and spina bifida, and mapped the disease locus to a 5.6-Mb critical region within the interval defined by the previously reported Mexican family. Through a combination of high resolution copy number variation (CNV) scan and targeted genomic sequencing, we identified an interchromosomal insertion at Xq27.1 of a 125,577-bp intragenic fragment of COL23A1 on 5q35.3, with one X breakpoint within and the other very close to a human-specific short palindromic sequence located 82-kb downstream of SOX3. In the Mexican family, we found an interchromosomal insertion at the same Xq27.1 site of a 300,036-bp genomic fragment on 4q31.2, encompassing PRMT10 and TMEM184C and involving parts of ARHGAP10 and EDNRA. Noticeably, both the two X breakpoints were within the short palindrome. The two palindrome-mediated insertions fully segregate with the CGH phenotype in each of the families, and the CNV gains of the respective autosomal genomic segments are not present in the public database and were not found in 1274 control individuals. Analysis of control individuals revealed deletions ranging from 173-bp to 9,104-bp at the site of the insertions with no phenotypic consequence. Taken together, our results strongly support the pathogenicity of the identified insertions and establish X-linked congenital hypertrichosis syndrome as a genomic disorder.

1261W

Apparent monosomy 21- revision of the diagnosis Case report. *K. Adamová[1], M. Godava[1], M. Holzerová[2], M. Jarošová[2], V. Koudeláková[3], J. Šantavý[1].* 1) Department of Medical Genetics, University Hospital and Palacky University, Olomouc, Czech Republic; 2) Department of Haematology, University Hospital and Palacky University Olomouc, Czech Republic; 3) Laboratory of Experimental Medicine, Palacky University Olomouc, Czech Republic.

We report a case of a mentally and physically afflicted woman (33) with complete monosomy 21 (45,XX,-21) diagnosed by cytogenetic analysis 30 years ago. Patient presentes with severe mental retardation, cerebellar ataxia, convulsions, incontinence both fecal and urinary and facial stigmatization. As the complete monosomy 21 is rare and suggested to be incompatible with life other techniques were currently used to exclude potential partial monosomy and/or imperceptible structural rearrangement. G- banded karyotype showed repeatedly 45,XX,-21 whereas array utilising Cytogenetics Whole-Geonome 2,7M Array (Affymetrix) detected a large deletion of the proximal part of 21q and a small deletion of the terminal part of 6q. FISH performed both with centromeric and paint probes (CEP 13/21 Kreatech, WCP 6,21 Cambio) showed moreover a loss of the centromeric part of chromosome 21 along with translocation of the distal part of 21q onto the deleted chromosome 6. Revised karyotype of the patient is thus 45,XX,der(6)t(6;21)(q26;q21.3),-21.

1262W

Spectrum of chromosome abnormalities in spontaneous abortions: a chromosome CGH study of consecutive products of conceptions. *L. Apel-Sarid, K. Schlade-Bartusiak, B. Lomax, P. Eydoux.* Pathology, Children's & Women's Hosp, Vancouver, BC, Canada.

Chromosome abnormalities are a major cause of loss of pregnancy in the first trimester of pregnancy. The spectrum of chromosome abnormalities has been well characterized using karyotyping, but the failure rate of cell culture is high. Chromosome Comparative Genomic Hybridization (cCGH) in association with flow analysis allows characterization of the chromosome complement of products of conception without cell culture. We report on our 10-year experience of cCGH performed on 2233 products of conception. In our center, cCGH is performed for the following reasons: 1) Failed karyotype (56.2% of cases); 2) Material not available for karyotyping (11.2%); 3) Possible maternal contamination (Normal female karyotype obtained from chorion: 30.1%); 4) Other indications, including characterization of a chromosome abnormality detected on the karyotype (2.5%). CGH failed in 188 cases (8.4%). Chromosome abnormalities were detected in 796 cases (35.6%). Autosomal trisomy was the most frequent abnormality (509 cases, 63.9%). All chromosomes except 1 and 19 were represented; the most frequent abnormalities were trisomy 22 (11.6%), trisomy 16 (9.2%), trisomy 21 (7.7%) and trisomy 15 (5.7%). Double trisomies were seen in 7.7% of cases and triple trisomies in 1.1% of cases. Sex chromosome abnormalities were present in 74 cases, including 70 cases of monosomy X (8.8%). Monosomy 21 was present in 1.8% of cases and monosomy 9 was seen once. Polyploidy was detected by flow analysis in 6.2% of cases. Structural rearrangements were detected in 9.5% of cases. Four cases of isochromosome 8q were identified: this is a novel de novo recurrent rearrangement in products of conception. These frequencies are significantly different from those obtained after karyotyping in our laboratory or as reported in the literature. This difference is likely to be due to a lower success rate in culturing tissues from products of conception with specific abnormalities. In association with karyotyping results, this study allows better characterization of the overall spectrum of chromosome abnormalities in products of conception.

1263W

Micronuclei in bone marrow cells of mice exposed to chloroquine, in vivo. *I. Aranha.* Univ. do Estado do Rio de Janeiro, Rio de Janeiro Brazil.

Chloroquine is a drug that was initially used for the treatment of malaria but has also been successfully used for the treatment of arthritis and lupus eritematosus. In the present study, the bone marrow cells of mice were used to assess the effect of chloroquine, in vivo using the micronucleus assay. Six animals were injected intraperitoneally with the drug at a concentration of 25% LD_{50}. Chloroquine was injected during 5 consecutive days. On the sixth day the animals were sacrificed by cervical dislocation, femurs were removed, the bone marrows collected and smears were made for slides preparation. After 24 hours the cells were stained with Giemsa Gurr (2%) and analyzed under optical microscope. As positive control, 6 animals received cyclophosphamide once (50 mg/mL)and 6 animals not exposed to any drug served as negative control for the experiment. In the test group 12080 cells were observed, 93 with micronuclei. In the positive control group 12535 cells were analyzed and 173 showed micronuclei. In the negative control group 12697 cells were studied and 9 had micronuclei. The chi-square test for independence showed that chloroquine was the responsible for the micronuclei observed.

1264W

44,X,t(4;12)(q12;q13),der(13;14)(q10;q10).An unusual case in a girl with Turner syndrome and two diferent translocations. *G. Arteaga Ontiveros[1], C. Uria Gomez[1, 2], R. Garcia Cavazos[3].* 1) Genecon Diagnostico Laboratorio, Toluca, Mexico; 2) Facultad de Medicina UAEM Toluca, Mexico; 3) Unidad de Genetica Clinica y Perinatal, Mexico D.F.

The ocurrence of X chromosome monosomy and a Robertsonian translocation is a rare event. But the additional presence of a balanced reciprocal translocation is even more. To our knowledge, there are some reports of patients with Turner syndrome combined with a 13;14 translocation, but no one with an additional balanced reciprocal translocation. A 15 years old girl was referred to our laboratory for cytogenetic analysis because she presented phenotypic characteristics of Turner syndrome. Chromosomal analysis in peripheral blood lymphocytes with GTG bands showed 44,X,t(4;12)(q12;q13,der(13;14)(q10;q10) in all the metaphases. Parental karyotype were 46,XY and 45,XX,der(13;14)(q10;q10). Therefore, Robertsonian translocation in the proband is of maternal origin and balanced reciprocal translocation is de novo. The mother reports that she had two spontaneous abortions and have no more children. At the time of the study, the mother was pregnant. Prenatal diagnosis was offered but she refused to do. Studies in oocytes suggest Robertsonian translocation carriers have a higher risk of generating aneuploid oocytes. Probably in our case, the monosomy X was a consequence of interchromosomal effect, because the Robertsonian translocation could influence non-disjunction. The presence of balanced reciprocal translocation (4;12) could be attributed to the same effect.

1265W

Molecular and clinical characterizations of patients with tetrasomy and pentasomy 15q11q13. *L. Bao[1,2], J. Yang[2], Y. Yang[3], Y. Huang[2], H. Li[2].* 1) Divison Human Gen, Cincinnati Child Hosp Med Ctr, Cincinnati, OH; 2) 2Center for Clinical Molecular Medicine, Children's Hospital of Chongqing Medical University, Chongqing, China; 3) Shanghai Children's Hospital, Shanghai Jiaotong University Affiliated Children's Hospital, Shanghai, China.

Chromosome 15q11q13 contains a group of imprinted genes that play an important role in development. Deletions of paternal and maternal 15q11q13 segment are associated with Prader-Willi syndrome and Angelman syndrome, respectively. Although duplications of 15q11q13, particularly maternally transmitted have been reported in patients with developmental delays and autism spectrum disorders, there have been limited studies on tetrasomy for 15q11q13 and fewer on pentasomy for 15q11q13. Moreover,the relationship between genomic dosage of 15q11q13 and clinical phenotypes remains to be further defined. We identified two pediatric patients with development delays and mental disabilities. Conventional chromosome banding analysis revealed each had a large derivative chromosome 15, one with a karyotype of 47,XY,+del(15)(q14) and the other 47,XY,+del(15)(q21q26). High resolution whole genome SNP microarray analysis (Affymetrix Cytogenetics Whole-Genome 2.7 M Array) and FISH analysis revealed that both derivative chromosomes 15 are the products of duplication or triplication a 12-Mb genomic segment involving 15q11q13. The affected segment overlaps with the PWS/AS critical region on 15q11q13. The 47,XY,+del(15)(q14) was revised to 47,XY,+iso(15)(q13) and contains two extra copies or tetrasomy for 15q11q13, and the other derivative chromosome 15 was derived from triplication of 15q11q13 resulting three extra copies or pentasomy for 15q11q13 (revised karyotype 47,XY,+trp(15)(q11q13)). Gene expression and methylation study correlated maternal origin of the derivative chromosomes. Moreover, it was found that patients with penasomy 15q11q13 showed more severe mental impairments and speech delays than the one with tetrasomy 15q11q13. Our study underscores the importance of microarray analysis in characterizing chromosomal abnormalities and shed some light on molecular genetics of 15q11q13 aberrations and 15q11q13 dosage effects on clinical features.

1266W

Microarray characterization of a partial trisomy 1q23-qter in a premature liveborn male with multiple congenital anomalies. *K. Button, X. Li, J. Kobori.* Genetics, Kaiser Permanente, San Jose, CA.

Partial trisomy 1q is associated with multiple congenital anomalies and is rarely seen in liveborn. We report a case with the largest documented trisomy 1q in a liveborn premature male. A 29 Y old G2P1 female was referred for amniocentesis due to ultrasound anomalies discovered at 18w5d. Ultrasound findings included large left hydrothorax, right displacement of the heart, single umbilical artery, a unilateral left sided cleft lip and palate, nuchal thickening of 7-9mm, and bilateral clenched hands. Follow up ultrasound at 19w2d also showed a small right pleural effusion. The fetal echocardiogram at 19w4d showed no major structural heart defects. The heart is displaced towards the right due to a large left pleural effusion. At 20w2d the patient was notified of the chromosome abnormality and decided to continue the pregnancy. Ultrasound at 21w5d showed increased amniotic fluid, frank hydrops, generalized edema and ascites, and marked increase in the size of the left pleural effusion. Ultrasound at 26w5d showed polyhydramnios, worsening hydrops, massive left hydrothorax with severe mediastinal shift, hypoplastic lungs, severe ascites and skin thickening, ventriculomegaly, and a head circumference consistent with 39w gestation age due to soft tissue edema. At 29w0d the patient went into spontaneous labor. Male infant of 1620 grams was born breech with a weak heart rate of <60/min which dropped to 10-20/min within 5 minutes, and was absent by 7 minutes at time of death. Cause of death was respiratory and cardiac failure due to severe hydrops and pulmonary hypoplasia. The infant was described as mottled, blue, limp, apneic, with notable edema/hydrops of head and body. Autopsy was declined but the following dysmorphic features were noted: midface hypoplasia, microphthalmia, low-set poorly formed ears, cleft lip, and camptodactyly. Karyotype on the amniotic fluid was 46,XY,der(4)t(1;4)(q23;q35). Array CGH (Agilent/ISCA 8x60K) revealed a terminal duplication of 85.83 Mb from band 1q23.3-qter at genomic coordinates chr1:161,347,151-247,179,432 and a terminal deletion of 4.42 Mb from band 4q35.1-qter at genomic coordinates chr4:186,610,285-191,028,016. The patient's father has a balanced t(1;4)(q23;q35). The clinical features reported for patients with 1q duplications overlap with the current case except for the findings of hydrothorax and pleural effusion. Our patient also had some features previously described in association with 4q35 deletions.

1267W

X Chromosome structural variants in patients with intellectual impairment and developmental delay: summary of 2200 cases. *T.-J. Chen[1,2], Y. Wang[2], M. Li[1,3], M. Phelan[1].* 1) Hayward Genetics Center and Dept. of Pediatrics, Tulane University School of Medicine, New Orleans, LA; 2) Dept. of Pathology, College of Medicine, University of South Alabama, Mobile, AL; 3) Dept. of Molecular and Human Genetics, Baylor College of Medical, Houston, TX.

Structural aberrations of the X chromosome are major etiological factors for X-linked mental retardation (XLMR) and developmental delay (DD). We report results of aCGH studies performed as part of the clinical assessment of 2200 patients with unexplained intellectual impairment (II) or DD with or without dysmorphic features. Clinical information and aCGH results of the 2200 patients were collected from two clinical laboratories at Tulane University School of Medicine and University of South Alabama. aCGH studies were performed using the standard protocol recommended by the manufacturer on the Agilent 44K platform. We identified 55 cases (2.5%) of X chromosome abnormalities from the 2200 patients. There were 5 numerical X chromosome abnormalities (5/55, 9.09%), 2 X chromosome mosaics (2/55, 3.64%), 3 translocations (3/55, 5.45%), 27 duplications (27/55, 49.09%), and 18 deletions (18/55, 32.73%). The median size of the duplications was 407 kb (310 kb~9.355Mb), and the median size of the deletions was 1.43 Mb (346 kb~51.363Mb). The most recurrent aberrations were Xp22.31 with 5 cases of deletions and 7 cases of duplications, followed by Xq28 with 5 cases of deletion and 4 cases of duplication. All 5 deletions at Xp22.31 were males with clinical features of DD, II, ichthyosis, and behavior problems, while the 7 duplications at Xp22.31 included 4 females and 3 males. All 5 deletions at Xq28 were females with the deletion size ranging from 340 kb to 3.21 Mb. Three of these 5 deletions resulted in loss of the MeCP2 gene. The 4 cases of duplications at Xq28 included 2 males and 2 females. The duplication size ranged from 313 kb to 669 kb. MeCP2 duplication was identified in two cases. The clinical features of patients with Xq28 abnormalities were widely variable, even among the patients with deletions or duplications of MeCP2. In our previous report, the detection rate by aCGH for all structural chromosome abnormalities of the autosomes and sex chromosomes was about 11-14%. The current study demonstrates that approximately 20% of aCGH positive cases are due to X chromosome numerical and structural aberrations. Identification of these deletions and duplications will facilitate the discovery of XLMR genes, aid in characterizing the associated phenotypes and assist in defining the gene functions of X-linked genes.

1268W

A Fugitive Occupational Hazard related to Diamond Workers detected by Cytogenetic Alterations. *S. Chettiar[1], J. Dattani[2], D. Jhala[2].* 1) Depaartment of Biotechnology, Shree Ramkrishna Institute of Comp. Edu. App. Sci., Surat, India; 2) Department of Zoology, School of Sciences, Gujarat University, Ahmedabad, India.

Increasing economy and international trade policy of Government of India has resulted in 300% raise in diamond trade consequently hailing more than 1.5 million of worker in Surat itself engaged in diamond cutting and polishing. The introduction of new high speed grinding tools with a polishing surface of microdiamonds cemented in very fine cobalt powder, resulted in dusts of cobalt silicatic complex formed by the grinding and polishing which enter the physiology of the workers engaged in. Thus occupationally exposed to cobalt and other heavy metals individuals are reported with multiple symptomatic cobalt pneumopathy. Current exercise has been undertaken to evaluate the genotoxic effect of the diamond cutting and polishing process in diamond workers of Surat city. A cross sectional study was undertaken incorporating 14 individuals of diamond occupation with equitant age and sex matched cases controls. Individuals engaged in the occupation for more than five years were included in the study after availing written consent from the subjects. Venous blood (2ml) was collected and subjected to culture by standard Perioheral Blood Lymphocyte Culture (PBLC) and endpoints like chromosomal aberration and micronucleus were scored. Subjects exposed to cobalt and other heavy metals by procedural diamond cutting and polishing showed significant (P=0.001) increased aberration by elevated frequency of chromatid break and gaps. Incidence of micronculie was also enhanced in the individuals exposed as compared to control. Heavy metal exposure definitely brings about genotoxic effect in the individuals, thus curative and precautionary measures should be implemented in the work environment of diamond cutting and polishing industries.

1269W

IsoUPD as a mechanism of fetal rescue detected by Oligo-SNP array. *N. Christacos, S. Schonberg, Z. Dai, D. Boles, J. Kelly, M.L. Solvak, K. Burks, T. Simanivanh, P. Mowrey.* Dept Cytogenetics, Quest Diagnostics, Chantilly, VA.

We report the cytogenetic and microarray results of a 6 year old female with known mosaicism for a marker chromosome and clinical features that included macrocephaly, developmental delay and self injurious behavior. Conventional cytogenetic analysis confirmed the presence of a small marker (ring) chromosome. High resolution microarray analysis using an oligo-SNP array (Affymetrix 6.0) determined the ring chromosome to be derived from chromosome 7. Specifically, the ring was observed as a gain of 18.7 Mb at bands 7p11.2 to 7q11.23. Furthermore, there appeared to be an internal duplication within the ring (7) encompassing 2.8 Mb at nucleotide position 70,428,242 to 73,208,896 including bands 7q11.22 and 7q11.23. Such duplications within ring chromosomes have been reported previously and are believed to occur in processes closely related to those that give rise to the more commonly noted inverted duplications with terminal deletions. In addition, homozygosity for nearly all SNP markers on chromosome 7 was observed, corresponding to uniparental disomy or isoUPD7. Only the region encompassed by the ring appeared to retain heterozygosity. The genomic alterations identified are expected to cause phenotypic and/or developmental abnormalities. Duplication of proximal chromosome 7 by the presence of a ring chromosome is rare and as such, a clearly recognized syndrome has not emerged. Reported patients have in common speech and language delay, developmental delay and mild dysmorphic features. UPD for only a few chromosomes has been shown to have a phenotypic effect. The effects of the UPD7 are dependent on parental origin of imprinted regions. Maternal UPD7 is associated with Russell-Silver syndrome. Paternal UPD7 has not been associated with any consistent clinical findings. We hypothesize a mechanism of fetal rescue by early mitotic nondisjunction of the one normal copy of chromosome 7 inherited from one parent serving to rescue the lethal deletion represented by the r(7) inherited from the other parent. This is a unique case with a rare r(7) associated with complete isoUPD7. To our knowledge, cases with this pattern have not been reported. This case also illustrates how oligo-SNP array testing can be informative in demonstrating unanticipated levels of complexity and should be considered for patients with small ring chromosomes associated with abnormal clinical manifestations.

1270W

Molecular cytogenetic prenatal diagnosis of a fetus with a mosaic supernumerary neocentromeric derivative chromosome 13. *K. Chun[1,2], M. Hryshko[3], D. Konkin[3], S. Bal[3], D. Bernier[3], M. Tomiuk[3], S. Burnett[4,5], P. Frosk[4,5], B.N. Chodirker[4,5], A.J. Dawson[3,4,5].* 1) Molecular Genetics Laboratory, North York General Hospital, Toronto, Canada; 2) Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada; 3) Cytogenetics Laboratory, Diagnostic Services of Manitoba, Winnipeg, Canada; 4) Section of Genetics and Metabolism, Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Canada; 5) Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, Canada.

Neocentromeres are defined as a group of mitotically stable human derivative centromeres without alpha satellite DNA that are able to form a primary constriction and participate in kinetochore formation. Neocentromeres provide stability to rearranged chromosome fragments that would otherwise be acentric and rapidly lost. A 33-year-old G1P0 woman of English/Irish ancestry was referred to prenatal genetics following a screen positive maternal serum screen with a 1:129 risk of Down syndrome. Family and pregnancy histories were unremarkable. The patient's partner is 33 years old and of Scottish/English ancestry. There is no known consanguinity. The karyotype of the fetus was found to be: 47,XX,+mar[3]/46,XX[36]. Interestingly, one of these three colonies appeared itself to be a mosaic colony. FISH analysis with the whole chromosome paint (wcp) for chromosome 13 confirmed that the marker was of chromosome 13 origin. Based on the G-banded pattern, this supernumerary derivative chromosome 13 was interpreted as an inversion duplication of segments 13q21 through 13qter, with a neocentromere present at 13q21, in ~8% of colonies examined. A repeat amniocentesis showed the same karyotype in ~11% of colonies examined. Parental blood karyotypes were normal. QF-PCR performed on blood samples from both parents and the second amniotic fluid sample showed evidence of a second maternal allele at markers D13S258 (13q21) and D13S628 (13q31-q32). This suggests that the fetus may have originated as a trisomy 13, with attempted rescue resulting in a supernumerary neo(13), similar to the supernumerary der(15) that is sometimes associated with UPD15. The fetus was not UPD13, however, as evidenced by the inheritance of paternal alleles at D13S258 (13q21) and D13S628 (13q31-q32). After an induced abortion, the fetus was examined and noted to display mild dysmorphism with round facies, hypertelorism, down-slanting palpebral fissures and a bifid nasal bridge. Gross anatomy was unremarkable. Full autopsy was consented to and results are pending. A fetal blood sample at delivery showed mosaicism for the supernumerary neo(13) in 2 of 30 (~7%) cells examined. There is only one report of prenatal detection of a neo(13)(qter->q21:) (Mascarenhas et al., 2008). We report the second prenatally detected case of a mosaic neo(13), but the first case where the origin of a low level mosaic prenatal neo(13) was confirmed by QF-PCR.

1271W

Intragenic and sub-megabase deletions detected on whole genome screens in patients with developmental anomalies reveal loci potentially linked to novel disorders. *J. Compton, L. Schmidt, D.M. Riethmaier, A. Fuller, B. Boggs, G. Richard, J.M. Meck, S. Aradhya.* GeneDx, Gaithersburg, MD.

Chromosomal abnormalities account for a large proportion of the causes of developmental retardation in the pediatric population. Mendelian disorders are often clinically diagnosed and confirmed by sequencing of single genes rather than by molecular cytogenetic analysis. Whole-genome screens, such as high-resolution array CGH, are typically reserved for those patients with syndromic presentations and for whom a diagnosis is elusive. We have used whole genome array CGH in over 10,000 patients and identified pathogenic changes in 18%. Among these causative events, we have found several deletions that each affected only one gene associated with a Mendelian disorder. These genes included MECP2, NSD1, CREBBP, ROR2, and more than 25 others. Most of these were de novo events. We have also identified deletions less than 1 Mb that were de novo and affected less than five genes, one or more of which may contribute to the pathogenesis of hitherto unknown clinical syndromes. Our data highlight the importance of surveying at the individual gene level in whole genome screens for causes of developmental anomalies and point to loci potentially underlying novel genomic disorders.

1272W

Conventional and molecular cytogenetic characterization of a complex rearrangement involving 9p in an infant with craniofacial dysmorphism and cardiac anomalies. *D.L. Di Bartolo[1], M. El-Naggar[3], R. Owen[3], T. Sahoo[3], F. Gilbert[2], V. Pulijaal[1], S. Mathew[1].* 1) Department of Pathology and Laboratory Medicine, Weill Cornell Medical College/ NewYork Presbyterian Hospital, New York, NY; 2) Department of Pediatrics, Division of Human Genetics, Weill Cornell Medical College/ NewYork Presbyterian Hospital, New York, NY; 3) Quest Diagnostics Nichols Institute, San Juan Capistrano, CA.

We present clinical, conventional cytogenetic, fluorescence in situ hybridization (FISH), array comparative genomic hybridization (aCGH), and single nucleotide polymorphism array (SNP array) data on a one-month-old female infant with a terminal deletion and an inverted duplication of the short arm of chromosome 9. Although numerous cases with either partial trisomy or partial monosomy of 9p have been reported, only four involved both duplication and deletion of 9p. Chromosome analysis of our patient identified add(9)(p23); FISH analysis established the add(9)(p23) as deriving from chromosome 9 and detected a terminal 9p deletion. Array CGH analysis, using more than 3,200 BAC markers genome-wide, further characterized the abnormality as a terminal deletion of about 11 Mb, from 9p23 to 9pter, and a duplication of about 27 Mb, from 9p23 to 9p13.1. The ISCN designation is as follows: 46,XX,der(9)del(9)(p23)dup(9)(p23p13.1).ish add(9)(p23)(wcp9+),del(9)(pter)(305J7T7-). arr 9pterp23(CTD-217316-->CTD-2601L6)x1,9p23p13.1(CTD-2320C9-->RP11-293C6)x3 SNP array, using the Affymetrix 6.0 platform, revealed no normal copy number segment between the deleted and duplicated segments, suggesting U-type reunion between sister chromatids, following a double strand break in both chromatids, as the underlying mechanism. Clinical features included craniofacial dysmorphism: wide open anterior fontanelle and sagittal suture, prominent nose, flat nasal bridge, long grooved philtrum, thin lips, and extra skin at the back of the neck. The patient also exhibited low muscle tone and a single transverse palmar crease on the left hand. An echocardiogram revealed a perimembranous ventricular septal defect, and a secundum atrial septal defect. Normal female external genitalia was present. In short, abnormalities common to both 9p deletion syndrome (trigonocephaly, flat nasal bridge, and long philtrum) and 9p duplication syndrome (hypotonia, bulbous nose, single transverse palmar crease, and cardiac anomalies) were observed. It is possible that cryptic terminal deletions in previously reported cases of 9p duplication may have gone undetected by conventional cytogenetic analysis. Characterization of 9p rearrangements, through the use of aCGH/ SNP array, is critical to provide an accurate genotype-phenotype correlation, to establish 9p deletion-duplication syndrome as a distinct clinical entity, and to define critical regions for the identification of candidate genes.

1273W

Submicroscopic genomic alterations investigated by array-CGH in Finnish and Brazilian patients with Müllerian Aplasia (MA). *E.L. Freitas[1], M. Sandbacka[2,3], H. Laivuori[3,4], M. Halttunen[5], P.A. Otto[1], K. Aittomäki[2,6], C. Rosenberg[1].* 1) Department of Genetics and Evolutionary Biology, Institute of Bioscience, University of São Paulo, São Paulo, Brazil; 2) Folkhälsan Institute of Genetics, Helsinki, Finland; 3) Haartman Institute, Medical Genetics, University of Helsinki, Finland; 4) Research Programs Unit, Women's Health, University of Helsinki; 5) Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Finland; 6) Department of Clinical Genetics, Helsinki University Central Hospital, Finland.

Developmental defects of the Müllerian ducts result in abnormalities of the female genital tract ranging from upper vaginal atresia to total absence of Fallopian tubes, uterus and upper vagina, while ovaries and secondary sexual characteristics are normal. Patients often exhibit additional renal and vertebral defects. In most cases of MA the etiology is unknown. The vast majority of patients with MA have a normal female karyotype. Previously, our group investigated by 1 Mb BAC array CGH syndromic MA patients and found chromosomal imbalances affecting 1q21.1, 17q12, 22q11.21 and Xq21.31 chromosome regions (Cheroky et al., 2008). Recently Nik-Zainal and collaborators (2011) found similar results in patients with isolated and syndromic MA, in addition to describe a recurrent microdeletion on 16p11.2 region. In order to verify whether smaller genomic alterations contributes to the etiology of MA, we investigated by high-resolution array-CGH 30 Finnish and 15 Brazilian MA patients. The Brazilian ones were previously screened and presented negative results on 1 Mb BAC array. For this study, we used a CGH microarray platform from Agilent Technologies containing 180.000 oligonucleotides. Among the Brazilian MA patients only described polymorphic copy number variations of DNA segments (i.e., without a major pathogenic effect) were detected. A possible explanation is that patients with chromosome alterations had already been ascertained by 1 Mb array and, therefore, were not included in the present study. Among the Finnish MA patients we detected two different deletions not commonly found in control populations, respectively at 16p11.2 and 17q12. Deletions in these regions have been previously related to the etiology of MA. The 17q12 deletion contains the LHX1 and HNF1B genes, both related to malformations of the female genital tract. The 16p11.2 deletion was found in 3/63 MA patients studied by Nik-Zainal et al. (2011), and our data supports the finding that this copy number change contributes significantly to the pathogenesis of MA. In conclusion, two out of 30 Finnish and none of the 15 Brazilian MA patients were found to carry deletions not found in control persons. The role of copy number variations need further study in MA.

1274W

Cytogenetic studies in primary amenorrhea cases. *E. Ghadirkhomi, H. Khani, S. Jafarzadeh.* Department of Cytogenetic, Genetic laboratory of ACECR (Jahad daneshgahi), Tabriz, East Azarbaijan,Iran.

Amenorrhea, is the absence of a menstrual period in a woman of reproductive age. It is a symptom with many potential causes such as an abnormality in the hypothalamic-pituitary-ovarian axis, anatomical abnormalities of the genital tract, or functional causes.In this study we aimed to investigate chromosomal abnormalities in patients present with primary amenorrhea employing GTG banding. Chromosomal analysis was carried out in 134 cases that were referred to our laboratory from 2006 to 2010. The karyotype results revealed 77.6% (n=104) with normal chromosome composition and 22.38% (n=30) showed chromosomal abnormalities. Among the patients with abnormal chromosome constituents 53.54% exhibit numerical aberration and 46.66% showed structural abnormalities.Chromosomal abnormalities among the patients can be classifie into five main types with or without mosaicism: 1-The most frequent karyotype was X chromosome aneuploidies (n=14) that include Turner syndrome 45,X (n=6),mosaic Turner 45,X/46,XX(n=7) ,and 48,XXXX(n=1). 2-Male karyotype 46, XY was present in 4 patients. 3-Structural anomalies of the X chromosome were detected in 10 cases .Four patients were found to have isochromosome of long arm of X chromosome [46X,i(Xq)],one patient has isochromosome of short arm of X chromosome [46X,i(Xp)],5 patient has partial deletion of X chromosome . 4-Mosaicism of male chromosome constitution and X chromosome aneuploidy was present in one cases (45XO/46XY). 5-Mosaicism of X chromosome aneuploidy and structural anomalies of X chromosome was found in one case 45,X/46,i(Xq)/47,X i(Xq)i(Xq). The present study has emphasized that karyotyping is one of the fundamental investigations in the evaluation of primary amenorrhea.

1275W

Duplication Of Chromosome 12 Due to Familial Balanced Robertsonian Translocation Case Report. *s.s.h. hammad, s.i.h helal, n.a. abdelmeguid, a.k.k kamel.* human cytogenetics, national research center, cairo, Egypt.

ABSTRACT Trisomy of the short arm of chromosome 12 is a rare chromosomal anomaly with an estimated incidence of 0.2:10,000 births and in the majority of reported cases the trisomy 12p results from malsegregation of a balanced parental translocation. Approximately 40 patients have been reported and the majority of reported cases resulted from malsegregation of a balanced paternal translocation, We report a case of a (45; XY) der (12) (qter•p12.3:: 12.3•pter). p13) and rob (13; 14) (q10; q10) by direct FISH. The mother is a carrier of a balanced robertsonian translocation 45, XX; t (13; 14).The father has a normal karyotyping. and in this case the clinical findings also reflect monosomy of the other chromosome involved in the original translocation. Keywards: Trisomy 12, Children with Special Needs.

1276W

Co-occurrence of 22q12.1-12.3 deletion encompassing the neurofibromatosis type 2 (NF2) locus in conjunction with 16p13.11 deletion in an infant with multiple congenital anomalies. *A.M. Jay[3,4], S. Ebrahim[1,2], D. Stockton[3,4], V. Misra[3,4], R. Awwad[3,4], M. Hankerd[1], A.N. Mohamed[1,2].* 1) Detroit Medical Center Univ. Lab. Cytogenetics; 2) Dep of Pathology, Wayne State Univ; 3) Children's Hospital of Michigan; 4) Department of Pediatric, Wayne State Univ, Detroit MI., USA.

We report on the cytogenetic and molecular characterization of two deletions involving chromosomes 22q12.1-q12.3 and 16p13.1 regions in a 3-days-old infant. The infant presented with ambigious genitalia and multiple congenital anomalies. Dysmorphic features included hypertelorism, prominent ear tags, micrognathia, flattened nasal bridge, and high arching palate, as well as duodenal and choanal atresia, intestinal malrotation and Pierre Robin sequence. The infant was born at 38 weeks of gestation to a 31 year G5P4 mother who had a previous infant born with congenital diaphragmatic hernia who died at 5 weeks of age. The mother also had one miscarriage which occurred at 11 weeks into the pregnancy. The current pregnancy was remarkable for severe polyhydramnios diagnosed by prenatal ultrasonography. After delivery via C-section, the patient's apgars were 8,9 and birth weight was 2.270 kg. A head ultrasound identified mild hydrocephalus with dilation of both lateral ventricles. At day two of life the infant had bilious emesis and had not passed meconium. Chromosome analysis revealed a female karyotype with a partial deletion of chromosome 22q. Chromosomal Microarray revealed that the deletion is ~ 8.9 Mb at 22q12.1-q12.3 region including the NF2 gene locus. In addition, a second deletion of ~1.24 Mb was found at 16p13.11 region. Parental testing is being pursued which may contribute further in elucidating the etiology of this patient's aberrations and prognosis. To our knowledge deletions of chromosome 22q12.1q12.3 encompassing the NF2 locus has been reported in two individuals with developmental delays and other congenital abnormalities, and has been implicated in the development of neurofibromatosis type II, a syndrome characterized by multiple tumors on the cranial, auditory and spinal nerves, and by other lesions of the brain. On the other hand, deletions of 16p13.1 region has been reported as a region of recurrent microdeletion, which may contribute to a specific clinical phenotype of epilepsy, significant learning difficulties and distinct facial dysmorphism.

1277W

MTHFR Gene polymorphism in mothers having Down syndrome babies. *A. Kaur, A. Kaur.* Human Genetics, Guru Nanak Dev University, Amritsar,143005, Punjab, India.

Down syndrome (DS) is most common chromosomal aneuploidy and a cause of human intellectual disability, with an incidence of 1-600/1-1000 live births. The origin of the extra chromosome is maternal in 95% of cases and is due to failure of normal chromosomal segregation/non-disjunction during meiosis. Folic acid has an important role in the process of genetic material distribution during cell division, because of its importance to cellular methylation reaction. The most common missense mutation is C-T substitution at the nucleotide 677 in coding region of MTHFR gene converting an alanine to valine residue in the gene product and creating a new Hinf I restriction site resulting in the MTHFR 677 C-T polymorphism and reduction in enzyme activity which may lead to a decrease in the levels of S-adenosylemethionine, inhibition of methyltransferase and subsequent DNA hypomethylation. Cytogenetic investigations of Down syndrome children is being carried out in our department. This study was undertaken with the aim of finding out the prevalence of the MTHFR C677T polymorphism in mothers having Down syndrome children in our region. Study was done on 41 blood samples from mothers of cytogenetically confirmed Down syndrome children and 31 control women with healthy children. Polymorphism was analysed by PCR-RFLP method and statistical analysis was done. The genotypic analysis showed that the frequency distribution of CC genotype was 24.4% while that of CT genotype was 75.6% in cases. Among controls, genotype frequency was observed to be 61.3% for CC, 35.5% for CT and 3.23% for TT. Among cases, allele frequency for 677C and 677T was found to be 0.62 and 0.38, respectively while in controls it was 0.8 and 0.21 respectively. Though chi square revealed statistically significant difference of the genotype frequencies between cases and controls ($\chi 2=12.17$); the odds ratio OR:0.204, 95% CI: 0.95-56 p>0.05) showed no association between cases and controls. The study is being carried out on larger sample.

1278W

Cytogenetic screening for female infertility: A Tunisian report. *O. KILANI[1], W. HAMMAMI[1,2], M. OUNI[1,2], W. AYED[1], A. FADHLAOUI[3], M. MEFTEH[3], F. ZHIOUA[3], S. HENTATI[1], N. ABIDLI[1], S. ABDELHAK[2], A. AMOURI[1,2].* 1) CYTOGENETIC LABORATORY, PASTEUR INSTITUTE OF TUNIS, TUNIS, Tunisia; 2) MOLECULAR INVESTIGATION OF GENETIC ORPHAN DISEASES RESERCH UNIT, IPT, TUNISIA; 3) GYNOCOLOGY OF AZIZA OTHMANA HOSPITAL, TUNIS, TUNISIA.

Infertility is an important health problem affecting 10-15% of couples. Of all couples classified as infertile, female infertility accounts for about 40-50% while 10-30% either is attributed to both male and female infertility or is unexplained. Some major causes underlying female infertility are polycystic ovary syndrome (PCOS), premature ovarian failure (POF) and recurrent pregnancy loss (RPL). The objective of the present study is to investigate the contribution of chromosomal abnormalities in infertile women. This study included 175 infertile women aged from 20 to 45 years who were referred to for routine cytogenetic analysis between January 2002 and January 2011. Chromosomal studies using peripheral blood and RHG-banding technique were performed on all patients. In some cases, FISH analysis using X centromere, Y centromere and SRY probes confirmed the cytogenetic findings. Cytogenetic study revealed chromosomal abnormality in 11 infertile women (6.28%). Structural chromosome abnormalities involving autosomes (45.45%) and sex chromosomes (54,54%) were detected. The results of this study are consistent with those reported in the literature which is associated with a greater prevalence of chromosomal abnormalities in infertile women compared with the general population. These findings show the importance of consider the cytogenetic study in the initial diagnosis protocol of infertile couples.

1279W

Validation studies of a disease-oriented whole-genome scanning as a diagnostic test for genetic and structural variations. *T. Kubota[1], S. Sakazume[2], T. Nagai[2], M. Kamiyama[3], K. Ichikawa[3], H. Sato[4], S. Saito[3].* 1) Fac Med, Epigenetic Med, Univ Yamanashi, Chuo, Yamanashi, Japan; 2) Dept Pediatrics, Dokkyo Univ Koshigaya Hosp, Koshigaya, Saitama, Japan; 3) Riken Genesis Co Ltd, Riken Yokohama Inst, Yokohama, Kanagawa, Japan; 4) DNA Chip Research Inc, Yokohama, Kanagawa, Japan.

Thanks to advance of cytogenetic techniques with high resolutional DNA chip devices, a number of new genetic and structural variations have been reported. However, these assays potentially detect non-pathogenic variations, and they usually cost high. A whole-genome scanning panel that only contains disease-associated probes has recently been developed, but we do not know the clinical usefulness by the assay using this panel. Therefore, we validated the assay on the patient samples with various karyotypes.

400 ng of DNA extracted from the peripheral blood of the patients was hybridized on the HumanCytoSNP-12 BeadChip (Illumina) using Infinium beads assay. Karyotyping of the patients had been performed using conventional methods such as G-banding and FISH.

As a result, the new assay demonstrated that (1) there was no change between the original lymphoblast (LB) sample and the advanced passage sample, (2) the previously detected deletions of the patients with Prader-Willi syndrome (PWS) (n=1) and Williams syndrome (WS) (n=2) were precisely recaptured (furthermore, no single nucleotide difference was found between the two WS patients), (3) PWS uniparental disomy (UPD) case showed long range of loss of hetrozygosity, (4) precise deleted regions were shown in two translocation cases [46,Y,der(X),t(X;Y)(p22.3;q12)] and [46,XY,der(X)t(X;15)(p21.1;q11.2),-15], and (5) origin of the marker chromosome of [47,XY,+mar[27]/46,XY,[3]mos] was detected (14q12-q23.33).

These results indicate that the disease-oriented whole-genome scanning assay can accept advanced passage LB samples and that it may be used as a diagnostic test for various deletions, UPD status and marker chromosomes in mosaic cases.

1280W

Pericentric inversion of Y chromosome in an infertile man reported as a normal variant. *E.E. Kurt[1], O. Ozer[1], E.O Ote[1], H.B. Zeyneloglu[2], F.I. Sahin[1].* 1) Medical Genetics, Baskent University Faculty of Medicine, Ankara, Turkey; 2) Baskent University Faculty of Medicine Department of Gynecology and Obstetrics, Ankara, Turkey.

Pericentric inversions of Y chromosome have been reported to have an incidence of approximately 0,6-1/1000 in the general population. According to some authors, Y chromosome inversions have been regarded as rare chromosome heteromorphisms; however others report infertility impairment in inv(Y) cases. Here we report a 26 years old infertile male patient with a 46,X,inv(Y)(p11.2;q11.2) karyotype. The result of FISH analysis performed on metaphase spreads of the patient revealed that SRY was located on Yp, excluding a paracentric inversion. Hybridization with whole chromosome X and Y probes resulted in staining of the X and derivative Y chromosome respectively, excluding a translocation. The karyotype was reported as a normal variant and resulted in a pregnancy of the female partner achieved after intracytoplasmic sperm injection, with a male fetus possessing the same karyotype. Although the chromosomal rearrangement has been reported to be normal variant, specific cases could be associated with gene deletions which could not be detected with FISH analysis.

1281W

Monosomy 3p25.3 and trisomy 1q42.13 in a boy with multiple congenital anomalies, profound growth and developmental restrictions and early demise. *C. Li[1], V. Mahajan[2], J. Wang[1], B. Paes[1].* 1) Dept Pediatrics, McMaster Children's Hosp, Hamilton, ON, Canada; 2) Dept Pathology, McMaster University Medical Center.

Both monosomy 3p25 and trisomy 1q42 have been reported in the literature as recurring chromosomal anomalies. However to our knowledge the combination of both anomalies in one patient has not been documented. We report a newborn boy of abnormal karyotype with a combination of monosomy 3p25 and trisomy 1q42. The patient was noted to have multiple congenital anomalies that included brain malformation (under developed frontal lobes with abnormal gyration patterns and mildly to moderately enlarged ventricles), ocular anomalies, tracheo-laryngomalacia, cardiovascular malformation, intestinal malrotation, hypoplastic scrotum and bilateral undescended testes, in addition to hirsutism, melanocytic nevus, dysmorphic features and biochemical anomalies. Birth weight and height were at the 3rd % and the head circumference was at the 25th %. A routine karyotype revealed 46,XY,add(3)(p25). Parental karyotyping revealed a maternal balanced translocation between chromosome 1q42 and 3p25. Array-CGH analysis of the proband showed a gain of 21.556 Mb from 1q42.13 to 1qter and a loss of 10.788Mb from 3pter to 3p25.3. The revised karyotype was 46,XY,der(3)t(-1:3)(q42.13;p25.3)mat. The patient subsequently developed profound growth and developmental restriction. A review at 7 months of age revealed severe growth restriction with weight, height and head size all very significantly below the 3rd %. He also had profound psychomotor retardation without detectable head control, gaze fixation, social smile and localization to sounds. The patient expired at 9 months of age after cardiopulmonary arrest. To our knowledge this is the first case report with this unique chromosomal imbalance. As such it may also be of value to cytogenetics databases such as the ECARUCA (European Cytogenetics Association Register of Unbalanced Chromosome Aberrations).

1282W

A Down Syndrome Patient with a de novo Recombinant Chromosome 21. *G. LULECI[1], Z. CETIN[1], S. YAKUT[1], E. MIHCI[2], A.E. MANGUOGLU[1], S. BERKER KARAUZUM[1], I. KESER[1].* 1) Department of Medical Biology, Akdeniz UNiversity Faculty of Medicine, ANTALYA, Turkey; 2) Department of Pediatric Genetics, Akdeniz University, Faculty of Medicine, ANTALYA,-Turkey.

Pure partial trisomy of chromosome 21 is a rare event and these cases are very important for karyotype-phenotype correlations in Down syndrome phenotype. We present here a Down syndrome patient with a de novo recombinant chromosome 21. According to conventional cytogenetic and FISH analysis his karyotype was designated as; 46,XY, add(21)(p13).ish dup(21)(WCP21+, RUNX1+, KCNJ6++, DSCR4++,DSCR8++, VIJ2yRM2029++). Non-continuous trisomic, tetrasomic, euploid and monosomic chromosomal segments across the recombinant chromosome 21 was detected by Oligoarray CGH analysis. STR analysis showed that this de novo recombinant chromosome 21 was maternal in origin. The dual-specificity tyrosine (Y)-phosphorylation regulated kinase 1A and Down Syndrome Critical Region 1 genes located in Down syndrome critical region supposed to be responsible for most of the clinical findings of Down syndrome. Our case is the first Down syndrome patient with a de novo recombinant chromosome 21 resulting from multiple chromosome breaks did not contain DYRK1A and DSCR1 genes.

1283W

Intrachromosomal arm duplication-deletion rearrangements. *J. Meck, S. Warren, V. Nelson, L. Schmidt, L. Matyakhina, S. Aradhya.* GeneDx, Gaithersburg, MD.

We analyzed 10,000 cases of whole-genome array CGH in individuals referred mainly for developmental disorders. Among the 18% of individuals positive for known clinically significant genomic imbalances, 46 carried coincident duplications and deletions on a single chromosome arm. Five individuals showed mosaicism suggesting a mitotic aberration. The most frequently involved chromosome arms were 8p (n=8), 5p (n=4) and 1p (n=4). The deletions ranged from 200 kb - 29 Mb, while the duplications were 290 kb - 48.5 Mb. Thirty-three (73%) cases had duplications and deletions that were immediately adjacent to each other with no intervening normal copy number region. This observation indicates that a U-type exchange mechanism is more common for complex intra-arm rearrangements than NAHR mediated by low copy repeats, although other mechanisms likely exist. While some individuals carried an interstitial duplication-deletion aberration with a terminal region of normal copy number, most (23/33 = 70%) had terminal deletions. The most frequent aberration, on 8p, was often present as the classic inverted duplication-deletion with an intervening normal copy number segment of 4-5 Mb, a constant deletion size of 7 Mb, and a variable size duplication of 3.7 - 32 Mb. On the other hand, 8p aberrations with an immediately adjacent duplication and deletion and a terminal normal copy number segment, had variable normal copy number (8-13 Mb) and deletion (1.6 - 11 Mb) sizes. One way of correcting telomere loss, the initiating event in many duplication-deletion rearrangements, is by telomere capture from another chromosome. Telomere capture was shown by FISH in four individuals and inferred in another three in whom array CGH showed a terminal duplication on a non-homologous chromosome but FISH confirmation was not available. We also found complex duplication-deletion rearrangements associated with known microdeletion/microduplication syndromes in 17 individuals (37%). These syndromes included the 1p36, 2q37, and 22q13.3 deletion syndromes, as well as Wolf Hirschhorn, Cri du Chat, Williams-Beuren, Prader Willi/Angelman, Miller-Dieker, and DiGeorge/Velocardiofacial syndromes. The involvement of low copy repeats in mediating these recurrent deletions or duplications likely holds true for the more complex duplication-deletion rearrangements at these same loci. These data highlight the complexities of intra-arm rearrangements and offer insight into their frequencies and mechanisms.

1284W

Cytogenomic characterization of a dysmorphic patient with congenital multiple anomalies presenting a normal G-banding Karyotype. *V.F.A. Meloni[1], F.B. Piazzon[4], M.F.F. Soares[2], S.S. Takeno[1], D.M. Christofolini[3], L.M. Kulikowski[4], D. Brunoni[1], M.I. Melaragno[1].* 1) Morphology and Genetics, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil; 2) Department of Imaging Diagnosis, Universidade Federal de São Paulo, São Paulo, SP, Brazil; 3) Gynecology and Obstetrics Division, ABC School of Medicine, Santo André, SP, Brazil; 4) Department of Pathology, School of Medicine, LIM 03, Universidade de São Paulo, SP, Brazil.

Array techniques are improving the diagnosis success in patients with intellectual disability, dysmorphic features and multiple congenital anomalies revealing the etiology of new microdeletion and microduplication syndromes. We describe a female patient born as the first child of a non-consanguineous young couple. First genetic evaluation at six months of age showed: weight 4370g (< 3rd centile); length 62 cm (10th centile); OFC 37cm (< -2SD); microcephaly, trigonocephaly, prominent metopic suture, bitemporal narrowness, flat occiput, synophrys, arched and sparse eyebrows, strabismus, hypertelorism, upslanted palpebral fissures, prominent eyes, midface hypoplasia, long philtrum, microstomia, three superior oral frenula, small mandible, V-shaped cleft palate, small ears, pre-auricular sinus at left, short broad neck, wide-set nipples, large haluces, dislocation of the left 2nd toe, and hypoplastic labia majora and minora. She also presented hypotonia, tricuspid valve insufficiency and right atrium enlargement, seizures, recurrent pulmonary infection, and a gastrostomy had to be maintained for feeding. At 3 years and 11 months of age the patient died of bronchopneumonia. These clinical findings strongly suggested chromosome imbalance, but the chromosome analysis by G banding was a female normal karyotype. Considering the patient's abnormal phenotype, array and MLPA techniques were used, and a large 9p deletion associated to a 20p duplication was disclosed. FISH analysis was necessary to detect the balanced translocation t(9;20)(p22.2;p12.1) in the patient's mother. Our patient had typical 9p-deletion clinical manifestations and also some features of 20p duplication. This study shows the relevance of using molecular techniques to evaluate patients with dysmorphic features and multiple anomalies suggestive of chromosome aberrations, even if their karyotypes are found to be normal, in order to make the right diagnosis, establish an accurate genotype-phenotype correlation, be able to provide a better patient management and also an appropriate genetic counseling to their families. Key words: duplication 20p; deletion 9p; array; FISH; MLPA. This work was supported by FAPESP (Brazil).

1285W

Isodicentric 15q: Report of two Colombian cases. *P. Paez[1,3], J. Acosta[1,2].* 1) INGM Universidad el Bosque, Bogota, Colombia; 2) Instituto de Ortopedia Infantil Roosevelt, Bogota, Colombia; 3) Hospital Universitario Clinica San Rafael, Bogota, Colombia.

Introduction. Abnormalities of the 15q11-q13 region are a significant cause of autism spectral disorder (ASD), accounting for approximately 1% of cases. The 13 MB region encompassed by the scope of 15q11.2-q13 deletion and duplications syndromes contains at least 30 characterized genes. We present the two first Colombian individuals whit increased dosage of distal 15q and neurodevelopmental delay and ASD.Case1. This is a two years old boy born from of non consanguineous parents with generalized developmental regression, congenital hypotonia, autism spectrum and refractory epilepsy. The pregnancy and delivery were normal. No family history. Physical examination reveals minor facial dysmorphisms, marked truncal hypotonia, horizontal nistagmus, finger contractures in both hands, failure adequately to use eye toe eye gaze, facial expression, body posture and gesture. No language. Normal brain magnetic resonance, normal base line biochemistry (glucose, lactate, pyruvate, creatinkinasa), normal amino and organic acid screening. Karyotype: 47,XY + mar. Array Comparative Genomic Hibridization (CGH): isochromosoma 15 q 11,1; 15q 13.1 (47,XY +der 15q11-15q13.1). Case 2. This is a 6 years old female, born from non consanguineous parents, the patient has severe congenital hypotonia, microcephaly, developmental delay, language absent, sterotypies, refractory epilepsy , ASD and minor facial dysmorphisms. Karyotype: 47,XX,+(mar), SKY FISH analysis report 47,XX,+der(15) and (CGH) :Idic (15)(q11). Discussion. These are the first Colombian cases reported with this cytogenetics and molecular characterization. Chromosome 15q11-13 contains a cluster of imprinted genes essential for normal mammalian neurodevelopment. Idic (15) syndrome (inverted duplication of proximal chromosome 15 or isodicentric 15 chromosome) displays distinctive clinical findings represented by early central hypotonia, developmental delay and intellectual disability, epilepsy, and autistic behavior. These phenotypic features are present in our patients. International literature suggests that patients with ASD should be routinely screened for 15q genomic imbalances. Conclusion.This report let us confirm the utility of molecular and cytogenetic analysis in the clinical approach of these patients.

1286W

Ameliorative Potential of Melatonin and Curcumin on Fluoride and Arsenic Induced Genotoxicity in Human Blood Cultures. *H.H. Pant, M.V. Rao.* Department of Zoology, School of Sciences, Ahmedabad, Gujarat, India.

Effect of fluoride and arsenic at concentrations of 34uM and 1.5uM respectively were investigated with respect to Chromosomal anamolies(CAs), sister chromatid exchanges(SCEs) ,micronuclei(MN) and comet assay in human blood cultures. Further protective role of melatonin (0.2mM) and curcumin(7.7uM) were investigated on these treated blood cultures. The data revealed fluoride and arsenic induced significant genotoxic alterations as compared to control cultures. These effects were more in combination of toxicants. Upon addition of melatonin and curcumin these effects were reduced in treated cultures evidenced by percent amelioration. This could be due to antioxidant properties of these products ie., melatonin and curcumin. Further this protective effect of these antioxidents seemed to be similar. The siginificance of these data is highlighted in this investigation.

1287W

The importance of metaphase FISH analysis in parental follow-up of microarray CNVs. *P. Papenhausen[1], J. Tepperberg[1], R. Burnside[1], I. Gadi[1], V. Jaswaney[1], J. Smith[2], R. Potluri[2], E. Keitges[3], H. Risheg[3], S. Schwartz[1].* 1) Dept Cytogenetics, Labcorp America, Res Triangle Park, NC; 2) Dept Cytogenetics, Labcorp America, Houston, TX; 3) Dept Cytogenetics, Labcorp America, Seattle, WA.

Parental studies following the detection of a CNV in microarray analysis generally consist of either region specific BAC/oligonucleotide FISH performed on interphase and metaphase preparations or Q-PCR. The former supplies genomic location as well as dosage while Q-PCR can supply accurate dosage, but not location. While terminal associated gains and losses are strongly associated with transmission from a balanced translocation/inversion carrier parent, the incidence of interstitial imbalance resulting from a balanced parental insertion is not well known. Since these alterations have a high reproductive recurrence risk, are difficult to anticipate and would not be recognized by Q-PCR, their relative incidence is important. We report five cases of balanced insertions found in BAC metaphase FISH follow-up of interstitial CNVs: 1) a maternal carrier of a 9.3 Mb intrachromosomal insertion of 19q into 19p with two children receiving the duplication recombinant , 2) a paternal carrier of a 19 Mb insertion of 8q into 17q (inverted orientation) with the derivative 17 passed to the proband, 3) a maternal carrier of a 8.8 Mb 16q segment inserted into 5p with the proband inheriting the derivative five, 4) a complex maternal carrier of three separate segments of chromosome four (two from 4q and one from 4p) contiguously inserted into 19q13.1 with her son inheriting the derivative 19 and the combined 6.25 Mb insert, and 5) a maternal carrier of a 922 Kb intrachromosomal insertion of a 3q22.3 segment into 3q28 with a 517 Kb deletion at the insertion site. Unbalanced recombination in her son resulted in duplication of the 922 Kb segment along with the directly inherited deletion. These cases provide important information concerning parental follow-up of detected interstitial CNVs. If one makes a reasonable assumption that the common microdeletion/duplication syndrome intervals with flanking segmental repeats are due to NAHR and not subject to insertions (none were found in our studies), these cases represent about 1-2% of our parental follow-up of the remaining interstitial CNVs. The absence of small insertions was notable. All of the five cases involved significant imbalance with pathogenic impact. Since these have very high associated reproductive risk, the importance of utilizing FISH rather than Q-PCR for parental follow-up studies of this alteration subclass is paramount.

1288W

15 Years Experience :Egyptian Metabolic Lab. *E.M. Fateen, A. Gouda, M. Mahmoud.* Biochemical Genetics Departmen, National Research Centre, Cairo, Egypt.

Inherited metabolic diseases are a group of genetic disorders characterized by a specific enzymatic defects leading to accumulation of metabolites and pathologic sequel. The detection of metabolic disorders is either by measuring the enzyme activities or detection of the abnormal metabolites by the different available techniques. The Biochemical Genetics department, NRC started as part of the Human Genetics department 30 years ago and became a Biochemical Genetics unit 15 years later. Staring 2003 it becomes a separate Department serving whole Egypt. This is a retrospective study for the last 15 years of our experience ,where 14220 sample has been analyzed.6320 patient had been screened by thin layer chromatography. Aryl Sulfatase enzyme activity (ASA) had been measured to 502 patients 76 (15%) showed low activity (MLD),) - glucosidase activity had been measured to 1628 patients 200 (12.3%) showed low enzyme active (Gaucher patients), sphingomylinase activity was measured to 450 patients only 16 (2.7%) showed low activity (NPD type A), chitotriosidase activity was measured to 1940 and found high in 330 patients (34%)) galactocerebrosidase activity was measured to 37 patients and all showed normal activity, hexozaminidase A&B activities was measured to 476 patients 36 (7.6%) were diagnosed as Tay Sachs and 4 Sandhoff (1.6%).1145 patients were screened for MPS by quantitative determination of the GAGS. 540 of them (47.2%) showed high GAGS. Electrophoretic separation of GAGS diagnosed 278 (51.5%) MPS patients Morquio 39(14%),Hurler 79(28%),Hunter46(16%-),Sanphilippo 43(15.5%) Maroteaux lamy71(25.5%) 13 cases of Mucolipedosis Out of 2198 patients screened for PKU by quantitative determination of phenylalanine 156 (14.2%) showed high level Out of 447 patients the level of total galactose determined was high for 34 patients (7.6%).

1289W

Phenylketonuria Scientific Review Conference: State of the Science and Future Research Needs. *M.A. Parisi[1], T.K. Urv[1], R.R. Howell[1], K.M. Camp[2], P.M. Coates[2], H.D. Hyatt-Knorr[3], L.S. Nerurkar[3], J.H. Ferguson[3], S.C. Groft[3].* 1) Intellectual and Developmental Disabilities Branch, NICHD, NIH, Bethesda, MD; 2) Office of Dietary Supplements, NIH, Bethesda, MD; 3) Office of Rare Diseases Research, NIH, Bethesda, MD.

Phenylketonuria (PKU) is an amino acidopathy and one of the most common inborn errors of metabolism, typically caused by mutations in the phenylalanine hydroxylase gene. Newborn screening for PKU continues to be a successful public health program. Early identification and initiation of treatment in infants with PKU prevents intellectual disability in children and adults, and severe symptoms of untreated PKU are rarely seen. The National Institutes of Health (NIH) last published a screening and management statement for PKU as the result of a Consensus Conference held in 2000. Although the cornerstone for treatment of PKU has been a specialized diet with protein restriction, there is now a pharmacologic intervention, tetrahydrobiopterin (BH4; Kuvan), that can allow liberalization of diet for those who respond. In addition, technological advances now allow for molecular testing that can inform genotype-phenotype predictions, as well as facilitate prenatal diagnosis and identification of carriers. The combination of new treatments, new screening technologies, and a larger body of literature on outcomes for persons with PKU warrants revisiting the 2000 guidelines to determine their current applicability. In order to address this need, an NIH Scientific Review Conference will be held in early 2012, to consider recent research findings, current treatments, the role of BH4, and future research priorities. Five working groups of topical experts (clinicians and researchers), patients and advocacy group members, and federal stakeholders have been established to answer specific questions related to PKU that will be addressed at the conference. These include: Diet Control and Management; Pharmacologic Interventions; PKU and Pregnancy; Long-Term Outcomes and Follow-up; and Molecular Testing, New Technologies, and Epidemiologic Considerations. In a parallel and concordant effort, an Evidence-based Practice Center (EPC) of the Agency for Healthcare Research and Quality (AHRQ) is conducting an evidence-based review of the comparative effectiveness of BH4 and diet in the treatment of PKU. The EPC report will be available in late 2011. Together, the PKU Scientific Conference and the Evidence-based Practice Center report will provide information on important issues, recommend future research, and provide guidance to patients, their families, and health care professionals on the current understanding of PKU.

1290W

Clinical features of L-2-Hydroxyglutaric aciduria and mutations of the L2HGDH gene in 4 Arab kindreds. *M. Ul Haque[1], M. Al-Sayed[3], E. Faqeih[6], M. Jamil[4], M. Amoudi[5], N. Kaya[5], H. Abalkhail[2], A. Al Abdullatif[2], M. Rashed[5], M. Al-Owain[3], M.A. Chishti[1], I. Peltekova[7], S.H.E. Zaidi[8].* 1) Pathology, King Saud University, Riyadh, Saudi Arabia; 2) Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 3) Department of Medical Genetics, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 4) Department of Radiology, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 5) Department of Genetics, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 6) Department of Pediatric Medicine, King Fahad Medical Citiy, Riyadh, Saudi Arabia; 7) Department of Medicine, Queen's University, Kingston, Canada; 8) Ontario Institute for Cancer Research, Toronto, Ontario, Canada.

L-2-hydroxyglutaric aciduria (MIM: 236792) is an autosomal recessive neurometabolic disorder in which affected individuals display elevated levels of L-2-hydroxyglutaric acid in urine, blood and other body fluids, central nervous system abnormalities, and elevated risk of brain tumor. In this disease, several mutations have been described in the L2HGDH gene, which encodes for the L-2-hydroxyglutarate dehydrogenase. This study describes the biochemical data, clinical presentations, and radiological findings of patients from consanguineous Arab families and genetic analysis of the L2HGDH gene. We have identified misssense mutations and a single nucleotide deletion in the L2HGDH gene of DNA isolated from the blood of the patients. The missense mutations produce amino acid changes and the single nucleotide deletion produce truncation of the L-2-hydroxyglutarate dehydrogenase protein. Recently it was reported that the single nucleotide mutation in the Italian and the Portuguese patients had a common origin, as they share an identical SNP haplotype of the disease-causing chromosome. Here we show that in Arab patients, this mutation has arisen independently as they carry a SNP haplotype that is different from the hapolotype reported for the Italian and the Portuguese patients. These suggest that mutations in the L2HGDH could arise independently in patients of different ethnic origins. This study is the first detailed description of clinical manifestations and molecular analysis of Arab patients with L-2-hydroxyglutaric aciduria. As such the present study is significant for the diagnosis and management of Arab L-2-hydroxyglutaric aciduria patients.

1291W

TIDE-BC: IMPLEMENTATION OF AN EVIDENCE-BASED PROTOCOL TO IDENTIFY TREATABLE METABOLIC DISORDERS CAUSING INTELLECTUAL DISABILITY. *C. van Karnebeek, S. Stockler.* Biochemical Diseases, Pediatrics, B C Chld & Womens Hlth Ctr, Vancouver, Canada.

Background: Intellectual disability (ID) is a debilitating condition with deficits in cognitive functioning (IQ<70) and adaptive skills, affecting 2.5% of children/adults worldwide. Currently, diagnostic yield rather than treatability directs the etiologic work-up. As causal therapy has become available for many inborn errors of metabolism (IEM) with the potential to improve health outcome, this approach might be changed. **Aims:** To collect and transform the available evidence on treatable IEM as a cause of ID into a protocol which prioritizes treatability in the diagnostic evaluation of ID patients. **Methods:** Two independent reviewers performed all systematic review steps according to Cochrane Collaboration guidelines, and translated the data to formulate an evidence-based diagnostic protocol in consensus with international experts in the field in the field. **Results & Discussion:** The review identified 75 `treatable IEMs' presenting with ID as a major feature and provides data to change the following paradigms: 1) treatable IEM are not a rare cause of ID, rather as a group these 75 may comprise a considerable proportion of ID; 2) treatable IEM are not extremely difficult to identify; 50% are identified by routinely available group tests and for the remaining disorders (requiring a `single test for single disease approach') digital tools have been developed to direct the evaluation based on additional neurologic/systemic manifestations (www.treatbleID.org). 3) Therapeutic modalities are not always expensive; aside from 1 IEM requiring substrate inhibition and 8 IEM requiring stem cell transplant) the remaining 56 therapies with proven effect were accessible and affordable (diet, co-factor/vitamin supplements). 4) The level of evidence (LoE) for these treatments is not always limited; 25% ranked at level 1 or 2. Even with a low LoE, the effect on outcome (IQ, developmental performance, behavior, epilepsy, neuro-imaging) can be considerable. **Applications:** This knowledge has been translated into a step-wise protocol prioritizing the identification of treatable ID, which integrates the use of digital tools to help the clinician. Implementation in a tertiary care centre is underway as part of our funded study ` Treatable Intellectual Disability Endeavor in BC (www.tidebc.org)', of which we will report our first experiences (yield, usability, physician satisfaction, effectiveness).

1292W

FIVE MAPLE SYRUP URINE DISEASE CASES REPORT IN A 16 YEARS PERIOD OF TIME AT THE HOSPITAL PARA EL NINO POBLANO, MEXICO. *H. Velazquez[1], J. Aparicio[2,4], M.L. Hurtado[3], I. Marroquin[3], A.G. Rojas[3], P. Concha[1], F. Gutierrez[1], R. Herrera[1], E. Huitzil[4].* 1) Neonatology; 2) Genetics; 3) cytogenetics, Hospital para el Nino Poblano, Puebla; 4) Estomatology, Benemerita Universidad Autonoma de Puebla, Mexico.

The congenital inborn errors of metabolism (IEM) were identificables diseases since 1908. Some of the most frequent IEM reported are fenilketonuria, galatosemy as albinism, cystinuria and porfiria and more rarely maple syrup urine disease reported in this study. is caused by a deficiency of the branched-chain alpha-keto acid dehydrogenase complex (BCKDC), leading to a buildup of the branched-chain amino acids (leucine, isoleucine, and valine) and their toxic by-products in the blood and urine. Case reported. 102 clinical cases with polymalformed muscle esqueletal and skull and dental dismorfies from this Hospital were already published. Only 5 patients with maple syrup urine disease have been reported in a 16 years period of time. Only the clinical evolution of one of the patients is reported in this study. Conclusions. Actually, the IEM are defined as monogenic inherited diseases or mendelian, due to a metabolic error for a protein or enzyme absence. It might be incompatible with the patient life and sometimes if the patient lives it will modify its quality of life, especially in a severe metabolic disease as it is maple syrup urine disease.

1293W

Hypertrophy of the clava, a new MRI sign in patients with PLA2G6 mutations. *A. Al Maawali[1], G. Yoon[1,2], W. Halliday[3], JTR. Clarke[1], A. Feigenbaum[1], B. Banwell[2], D. Chitayat[1], S. Blaser[4].* 1) Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; 2) Division of Neurology, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; 3) Division of Pathology, DPLM, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; 4) Division of Paediatric Neuroradiology, The Hospital for Sick Children and University of Toronto, Canada.

Background and Purpose: Heterogeneous phenotype and delayed diagnosis are common in infantile neuroaxonal dystrophy (now infantile PLAN) due to PLA2G6 gene. Onset in infantile PLAN is by 2 years with psychomotor delay. Ataxia, truncal hypotonia, optic atrophy, dementia, tetraparesis and death follow. Onset in atypical INAD (now childhood PLAN) is later with a prolonged course. We characterise clinical and MRI phenotypes in 7 children with PLA2G6 mutations. Materials and Methods: An REB approved retrospective study for MRI and medical record review identified 7 confirmed patients. Results: All 7 had novel PLA2G6 gene mutations. 6 had infantile PLAN, onset by 12 months, psychomotor delays, hypotonia, rapid progression to a non-ambulatory state by 5 years and mean age of death of 10 years. One with childhood PLAN, who presented at 3 years with abnormal behaviour and delays in fine and gross motor coordination and language, remains ambulatory at 17 years. MRI showed hypertrophied clava in 7/7 regardless of age. Previous reports, as well as brain autopsy in one of our patients, note prominent spheroid bodies in the clava nuclei. All 7 had chiasmatic atrophy and abnormal signal surrounding the peritrigonal optic radiations at any age. Vermian-cerebellar atrophy was present as early as 17 months and present in all after 21 months. All developed a bright cerebellar cortex. This finding, present as early as 17 months, was not initially present on MRI in 2 patients who were 25 and 33 months old respectively. All infantile PLAN patients had transient bright signal in nucleus accumbens. Globus pallidus iron deposition was seen in 4/7, the earliest at 29 months. Iron was inconsistently identified as magnet strength and sequence have variable magnetic susceptibility. Only 1 child developed significant supratentorial atrophy. Conclusion: Neuroimaging in children with PLA2G6 mutations may be non-specific early in the disease course, resulting in delayed diagnosis. We identify an additional early MRI finding in patients with PLA2G6 mutations, clava hypertrophy. This feature may predate vermian atrophy and cerebellar cortex signal increase and was present at any age in our patient cohort of PLA2G6 confirmed patients. The presence of clava hypertrophy may aid in earlier identification of those who would benefit from specific PLA2G6 mutations genetic testing, allowing specific genetic counselling including prognostication and prenatal testing.

1294W

Unravelling the leukodystrophies: clinical, biochemical and molecular studies of sixty Brazilian patients with genetic white matter disorders. *C. Lourenco[1], GN. Simão[2], AC. Santos[2], CAR. Funayama[1], C. Sobreira[1], W. Marques Jr[1].* 1) Neurology, Univ Sao Paulo, Ribeirao Preto, Brazil; 2) Internal Medicine, Univ Sao Paulo, Ribeirao Preto, Brazil.

Background: Leukodystrophies are a group of rare genetic diseases that affect myelin, the major constituent of brain and spinal cord white matter. An integrated description of the clinical, neuroimaging and pathophysiological features is crucial for categorizing myelin disorders. Aim: To describe the clinical/biochemical evaluation of 60 patients with a white matter disorder. Methods/patients: All patients were evaluated in neurogenetics clinics. A full medical history was taken and the following were performed: neuroimaging studies (CT, MRI with spectroscopy), ophthalmologic and auditory evaluations, neurophysiologic studies (EEG, ERG, EMG/NCV), hormone and biochemical tests, muscle biopsy with OXPHOS analysis, screening for IEMs (lysosomal studies; peroxisomal/sterol panels; cholestanol dosage; organic acids, sulfatides and aminoacid chromatography; analysis of GAGs) and, when indicated, nerve/skin biopsy for EM studies, karyotype and molecular tests. Results: In forty patients was possible to stablish a diagnosis for the leukodystrophy. X-ALD, metachromatic leukodystrophy and Krabbe disease were the commonest inborn errors of metabolism implicated in the pathophysiological of the “metabolic leukodystrophies”. Infantile Refsum disease, Canavan disease, AMACR deficiency, Niemann-Pick type C were other rare IEMs diagnosed. It was worthy of note the fact that Vanishing White Matter disease was a leading cause of leukodystrophies in our patients (eight patients). Conclusion: Almost 50% of patients with white matter abnormalities remain without a specific diagnosis even after an exhaustive investigation. Therefore a specific protocol for investigation is crucial. Treatment options are becoming a reality for some disorders , so reaching a specific diagnosis is important so that patients can be offered appropriate therapeutics.

1295W

Identification of a novel cause of autosomal dominant, adult-onset distal myopathy. *M.C. Malicdan[1], C.F. Boerkoel[1], Y. Huang[1], J. Kwan[2], C. Groden[1], W.A. Gahl[1], C. Toro[1].* 1) Undiagnosed Diseases Program, Office of Rare Disease Research, and Medical Genetics Branch, NHGRI, National Institutes of Health, Bethesda, MD; 2) National Institute of Neurological Disorders and Stroke, NHGRI, National Institutes of Health, Bethesda, MD.

Distal myopathies are genetically heterogenous diseases. Associated disease genes are known to encode proteins in the sarcomere (titin, myosin), plasma membrane (dysferlin, caveolin), or cytoskeleton (desmin, myotilin, (B-crystallin, ZASP, filamin C, and nebulin). Despite this progress, the majority of cases remain undefined and a systematic explanation for a disease mechanism is lacking. Only one cause of adult-onset distal myopathy affecting predominantly the posterior compartment has been genetically characterized. We define a second genetic cause in a two-generation family. Affected individuals have progressive weakness of distal muscles beginning late in the third decade of life without evidence of neuropathy, increased serum creatine kinase, respiratory involvement, or cardiac symptoms. MRI studies showed fatty replacement of the posterior compartments of the legs, mainly of the medial gastrocnemius. Muscle pathology showed variation of fiber size due to fiber atrophy and esoinophilic cytoplasmic inclusions that were devoid of desmin, plasma membrane proteins and oxidative activity. There were no rimmed vacuoles. Ultrastructural studies showed non-membrane bound fibrillary deposits 6-12 microns in diameter in the Z-bands. Whole exome and Sanger sequencing excluded mutations in known genes associated with distal myopathy and identified an intronic splice site mutation causing exon skipping and encoding a frameshift mutation in an unstudied enzyme. Based upon the dominant inheritance and comparable expression of the mutant and wild type mRNAs, the truncated enzyme is an antimorph or neomorph. We present spatiotemporal expression analyses and elucidate the cellular function of the enzyme and the potential disease mechanism.

1296W

MUCOPOLYSACCHARIDOSIS: OTORHINOLARINGOLOGICAL AND AUDIOLOGICAL EVALUATION AND TREATMENT. *J.C. Neto, L.S. IKARI, J.C. BERTONCELLO, M.G. ANDRADE, S.M. MARONE, B.B. TAGUCHI, J.A. FRANCISCO.* Genetics, Pontificia Universidade Catolica Faculdade de Medicina , SãoPaulo, São Paulo, Brazil - Adress: Largo de Padre Pericles, 145 cj 12 São Paulo SP 01156-040.

BACKGROUND: The mucopolysaccharidoses are lysosomal storage disorders resulting from the deficiency of a lysosomal enzyme involved in the degradation of glycosaminoglycans. Nowadays this topic has been in evidence because of the morbidity and mortality of the disease and because it causes many disorders since the mucopolysaccharidoses is a progressive multisystem disease and may lead to difficulties in diagnose. About the otorhinolaryngological disorders, these patients may present: hearing loss, otorrhea, recurrent otitis, enlarged tonsils and adenoids, recurrent sinusitis, speech disorders, snoring, apnea, mouth breathing and nasal obstruction. The study of this pathology, the clinical presentation and audiological data provides theoretical support for patients with mucopolysaccharidosis, involving multidisciplinary treatment and resulting in better quality of life for them. OBJECTIVES: Evaluate patients with MPS regarding their ERT, clinical history, audiologic data and therapy at the Clinic of Otorhinolaryngology of PUCC Hospital, during 12 months of treatment with infusion CONCLUSION: According to this study, the results concluded that patients with mucopolysaccharidosis have different changes in otorhinolaryngological physical exams and symptoms. All of them had great improvement in all clinical aspects of the reassessment after 12 months of enzyme infusion. The audiological evaluation of patients was essential for the diagnosis of hearing loss, because it allowed the improvement of the audiological quality with hearing aids. Regular monitoring of the clinical status of patients with mucopolysaccharidosis receiving enzyme infusion is needed to determine long-term benefits.

1297W

Gene expression aberrations in brains of CLN3 deficient mice suggest possible pathways in Juvenile Batten Disease. *N. Dolzhanskaya, M. Velinov.* NYS Institute for Basic Research , Staten Island, NY.

Juvenile Batten Disease (JBD) is the most common condition in the group of devastating neurodegenerative disorders referred to as Neuronal Ceroid Lipofuscinoses. JBD is typically the result of homozygocity for mutations in the gene CLN3. Better understanding of this gene's function and the consequences of its deficiency may help develop strategy for treatment of JBD that would include correcting/modifying such downstream consequences. We have studied genome-wide brain expression in a mouse model of JBD referred to as CLN3Δex7/8 mice. This model creates a close molecular approximation to the common human CLN3 mutation. To-date we have studied 6 male and 6 female brains of 5 month - old mice, homozygous for the CLN3 mutation and 12 age and gender matched littermates homozygous for the wild allele as controls. Array expression analysis was done on brain homogenates using the 44K expression array system of Agilent Inc. Analysis of the obtained results was done using GeneSpring 11.0.2 data analysis software. We have observed aberrant expression in over 300 genes, with cutoff of minimum 2 times difference with controls. Some of the expression abnormalities noted in our studies replicated those previously reported in primary neuronal cultures derived from the CLN3-/- mouse model. For instance we also observed up-regulation of genes Frg1 and Synaptoporin. Twelve coding genes in our study had more than 3 times increased expression in homozygous mutant brains compared to controls. These were as follows: Zer1, Pttg1, Phb, Ube2i, Pgpep1l, Ndufa2, Gm10240, A330084C13, Gh, Ccr6, Gdp3. Five genes had 3 or more times decreased expression compared to controls. These genes were: Hoxb 13, Hoxd8, Tlr1, Zfp264, Spint3. From the genes listed above, of most interest to us are Ndufa2 and Ccr6. Ndufa2 is an enzyme that is part of mitochondrial complex I. Homozygous mutations in this gene result in a phenotype of Leigh syndrome in humans. Abnormalities in the mitochondrial function as part of the disease mechanism in JBD were previously suggested. Ccr6 has a possible role in the T-lymphocyte entry into the CNS. Immune abnormalities as part of the disease mechanism in JBD were also previously suggested. In conclusion, our studies further confirm the evidence of several important pathways in JBD progression involving mitochondrial dysfunction, neuronal dysregulation and immune abnormalities.

1298W

Association between a new 3216-3217 delGA mutation in AGL gene and common haplotype in Tunisian population: evidence for a founder effect. *M. GRIBAA[1], A. MILI[1,2], I. BEN CHARFEDDINE[1], O. MAMAI[1], L. ADALA[1], T. BEN LAZEREG[1], A. AMARA[1], J. BOUGUILA[3], D. H'MIDA[1], K. LIMEM[2], A. SAAD[1].* 1) Human Cytogenetics, Molecular Genetics and Reproductive Biology Laboratory. Farhat Hached hospital. Sousse - Tunisia; 2) Biochemistry Department. Faculty of Medicine. Sousse - Tunisia; 3) Paediatric Department. Farhat Hached Hospital. Sousse - Tunisia.

Glycogen storage disease type III (GSD III) is an autosomal recessive disorder characterized by excessive accumulation of abnormal glycogen in the liver and muscles and caused by deficiency in the glycogen debranching enzyme, the amylo-1,6-glucosidase (AGL). In this study, we report the clinical, biochemical and genotyping features of two unrelated GSD III patients from the same region in Tunisia. Erythrocytes glycogen concentration and AGL activity were measured by colorimetric and fluorimetric methods respectively. Four CA/TG microsatellite markers flanking the AGL gene in chromosome 1, were amplified with fluoresceinated primers. The full coding exons and their relevant exon-intron boundaries of the AGL gene were directly sequenced for the patients and their parents. The two patients showed a striking increase of erythrocytes glycogen content. No AGL activity was detected in peripheral leucocytes. Sequencing of the AGL gene identified a novel 3216-3217 delGA mutation in the two patients which is predicted to a premature termination, abolishing the AGL activity. Haplotype analysis showed that the mutation was associated with a common homozygote haplotype. Our results suggested the existence of a founder effect responsible for GSD III in this region of Tunisia.

1299W

Characterization of biomarkers during investigative therapy of hydroxy-propyl-beta-cyclodextrin in Niemann-Pick Type C disease. *D.W. Killilea[1], J. Suh[1], N. Mattsson[2], H. Zetterberg[2], E. Portelius[2], K. Blennow[2], Y.H. Hung[3], A.I. Bush[3], C.A. Hastings[4].* 1) Nutrition & Metabolism Center, Children's Hospital & Research Center Oakland, Oakland, CA; 2) Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Molndal, Sweden; 3) Oxidation Biology Laboratory, Mental Health Research Institute, University of Melbourne, VIC, Australia; 4) Children's Hospital & Research Center Oakland.

Niemann-Pick Type C (NPC) is a lysosomal storage disease associated with mutations in NPC1 or NPC2 genes and characterized by progressive neurologic dysfunction. Pathological changes include accumulation of unesterified cholesterol, mineral dysregulation, oxidant stress, and neurofibrillary tangles (NFT), but characterization of these biomarkers remains incomplete. Hydroxy-propyl-beta-cyclodextrin (HPBCD) has been used in NPC animal models to reverse cholesterol trafficking defects and significantly lengthen life expectancy. We are currently treating identical twin girls with NPC with HPBCD (intravenously (IV) and intrathecally (IT)) under the FDA's Individual IND exemptions and monitoring changes in specific biomarkers. Oxysterols, beta-amyloid (A-beta) and total-Tau (T-Tau) levels, mineral levels and metabolomic compounds were assessed in plasma and cerebrospinal fluid (CSF). Plasma oxysterols were reduced during the course of the IV infusions. CSF T-Tau levels were higher in NPC patients than in normal controls, decreased following IT HPBCD, increased during a period of time without drug, and (in one twins) decreased again with drug re-initiation; this pattern suggested time-dependent changes in neuronal pathology over the course of treatment. CSF A-beta peptide levels generally decreased over the course of treatment. Initial evaluation of mineral levels in the CSF indicated that homeostasis was altered. CSF copper, iron, and zinc levels fell below the normal pediatric reference range, though were not re-established with IT HPBCD; however, changes in calcium and potassium did return to the normal range following IT drug course. Mineral dysregulation is likely to affect tau expression and promote NFT formation. NPC-dependent changes in mineral homeostasis might contribute to various pathologies in the disease, such as tau expression and NFT formation. Measurements of sulfur amino acid redox states showed decreased plasma GSH and ergothioneine levels, whereas only ergothioneine was found to be decreased in erythrocytes. The lower plasma GSH and ergothioneine content suggest that NPC is associated with impaired thiol antioxidant defense that may contribute to neurodegeneration. Monitoring of oxysterols, trace minerals, tau proteins, and ergothioneine may give us new insight into common pathways of neurodegeneration and might be useful for clinical management and development of new therapeutic strategies for NPC patients.

1300W

Abnormal Metabolomic Profiles in Pregnancies Complicated by Small-for-Gestational-Age. *M. Shinawi[1], D. Dietzen[1,2], A. Odibo[3], A. Gronowski[2], K. Moley[3].* 1) Dept Pediatrics, Division of Genetics and Genomic Medicine, Washington Univ in St. Louis, St Louis, MO; 2) Department of Pathology and Immunology, Washington Univ in St. Louis, St Louis, MO; 3) Department of Obstetrics and Gynecology, Washington Univ in St. Louis, St Louis, MO.

Background: Small for gestational age (SGA) babies are prone to increased perinatal morbidity and mortality and increased risk for hypertension and metabolic disorders in later life. The etiology is incompletely understood, and there is no clinically useful early detection test. A significant proportion of SGA infants fail to achieve their optimal and genetically determined growth potential resulting in intrauterine growth restriction (IUGR). There is accumulating evidence suggesting that the SGA may result from uteroplacental insufficiency and/or compromised placental supply of nutrients to the fetus. The aim of this study is to identify maternal metabolomic signatures that specifically characterize SGA in early pregnancy. **Methods:** We conducted a discovery-phase, nested case-control metabolic profiling study using blood samples obtained at 11-14 weeks' gestation from 31 women who subsequently delivered SGA babies and gestational age-matched 31 controls taking part in a study for adverse pregnancy outcomes. Serum samples were analyzed for acylcarnitine species and amino acids using gas chromatography-tandem mass spectrometry (LC-MS/MS). Analyte distributions in the control and SGA group were compared using the Kruskal-Wallis technique (SPSS 18.0.3). **Results:** We found 5 metabolites (cystine, hydroxyproline, ornithine, histidine, and lysine) that were significantly different ($P<0.05$) between pregnancies complicated by SGA and controls. The arginine/ornithine ratio and hydroxyproline were significantly higher ($P<0.005$ and $P < 0.026$ respectively; and $P< 0.001$ when combined) in the cases with SGA. The area under the curve (AUC) using arginine/ornithine ratio and hydroxyproline was 0.75. **Conclusions:** These specific metabolomic signatures offer insight into SGA pathogenesis and suggest a potential presymptomatic screening test for this condition.

1301W

Correlation Assessment among Clinical Phenotypes, Expression Analysis and Molecular Modeling of 14 Uncharacterized Mutations in the Human Galactose-1 phosphate Uridylyltransferase Gene. *M. Tang[1], A. Facchiano[2], R. Rachamadugu[1], F. Calderon[3], R. Mao[3], L. Milanesi[4], A. Marabotti[2,4], K. Lai[1].* 1) Diviosn of Medical Genetics, Departmet of Pediatrics, University of Utah, Salt Lake City, UT. U.S.A; 2) Laboratory of Bioinformatics and Computational Biology, Institute of Food Science - CNR, Via Roma, 64, 83100 Avellino, Italy; 3) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT 84108, U.S.A; 4) Laboratory of Bioinformatics, Institute of Biomedical Technologies - CNR, Via F.lli Cervi, 93, 20090 Segrate (MI), Italy.

Galactose-1 phosphate uridylyltransferase (GALT) catalyses the conversion of galactose-1 phosphate to UDP-galactose, a key step in the Leloir pathway of galactose metabolism. Deficiency of GALT activity in humans caused by deleterious mutations in the GALT gene can lead to a potentially lethal disease called Classic Galactosemia (CG). In this study, we selected 14 uncharacterized nucleotide sequence changes in the GALT gene associated with reduced GALT enzyme activities in patients for expression analysis and molecular modeling. When over-expressed in GALT-less *Escherichia coli* host, all but two (Y34N and H132Q) of the 14 mutant GALT proteins were expressed at level comparable to the wild-type control. Yet decreased abundance in the soluble fraction of the *E. coli* cell extracts were observed for all 14 mutant GALT enzymes, suggesting altered stability and solubility among these mutants. Interestingly, one mutation, I170T (c.509T>C), is located at the second nucleotide of exon 6 and might potentially affect splicing in human patient cells. When the mutant proteins were purified from the soluble bacterial host extracts, six mutant GALT enzymes (R201C, E200K, R233S, I278N, L289F and E291V) had detectable enzymatic activities that correlated with the clinical data. Kinetic studies showed the K*M* of these six mutants for both substrates didn't change significantly, especially for gal-1P. However, the V_{max} of these mutants decreased significantly. To further understand how these mutations affect the enzyme, we performed molecular modeling using MODELLER software, and analyzed mutants searching for variations in their structural features. Major structural effects were predicted for the Y34N, R201C, E220K mutants and some structural effects, especially at local level, were anticipated for all the others mutants but V168L, I278N and L289F. For the H132Q and P185H mutants it is possible to hypothesize a catalytic problem since these residues make an interaction with a residue in the active site. Perturbation in stability was also predicted for the majority of mutants. Overall, these studies confirm the complexity of the effect of mutations on GALT enzyme, since a combination of multiple factors, probably involving also the dynamics of the protein, is responsible for its altered enzymatic activity.

1302W

Transcobalamin deficiency ; a treatable metabolic cause of severe pancytopenia and recurrent infections. *Y.J. Trakadis[1], T. Rupar[2], S. Melancon[1], A. Alfares[1], B. Schrewe[1], D. Watkins[1], D.S. Rosenblatt[1], N. Braverman[1].* 1) Department of Medical Genetics, McGill University Health Centre and Department of Human Genetics, McGill University, Montreal, Qc; 2) Departments of Biochemistry and Paediatrics, University of Western Ontario, London, ON.

Case presentation: After an uneventful pregnancy and delivery, a baby boy born to first cousin consanguineous parents from Sri-Lanka, presented at 3 months of age with failure to thrive, generalized hypotonia and pancytopenia (Hb: 58g/L, MCV: 95.1fL). He became transfusion-dependent for both platelets and red blood cells and required nasogastric feeding. He developed recurrent opportunistic infections and chest abscesses despite intravenous antibiotics, leading to severe respiratory failure requiring intubation. He had persistent neutropenia and lymphopenia with low levels of T and B cells. A bone marrow showed dysplastic features. Cytogenetic studies, including chromosome breakage and 7q31 deletion FISH, were normal. A repeat bone marrow biopsy showed hypercellularity and this time megaloblastic features were also noted. The differential diagnosis included leukemia. A genetic-metabolic work-up showed high blood total homocysteine levels, high methylmalonic acid (MMA) levels in both blood and urine, and low blood methionine levels. The serum cobalamin level was low (112 pmol/L at 4 months of age), but the folate level was within the reference range. A diagnosis of transcobalamin (TC) deficiency was reached based on a very low uptake of [57Co]-cyanocobalamin in cultured fibroblasts in the absence of a source of exogenous TC. Molecular analysis of the TCN2 gene showed a novel homozygous mutation (c.1013_1014delinsTAA) leading to a premature stop-codon at codon 364. Treatment with hydroxycobalamin 1 mg IM QD and folate 1 mg PO QD with regular follow-up in the genetics clinic resulted in normalization of hematological, immunological, and biochemical markers and clinical status improved dramatically. No further transfusions were needed. He was progressively weaned off oxygen and restarted on oral feeds with good weight gain and improved development (currently 15 months of age). **Discussion**: TC transports vitamin B12 from blood into cells. TC deficiency is a rare disorder and has not been detected routinely where newborns are screened for MMA. A delay in diagnosis can result in life-threatening infections, anemia and neurological disease. Similar to the present patient, most mutations reported have been deletions or insertions resulting in frame shifts and predicted protein truncation. TC deficiency should be considered in any newborn suspected for leukemia. Response to treatment is excellent but the long term prognosis is not known.

1303W

Additive effect of variants in TNFRSF11A and TNFRSF11B genes in the etiopathogenesis of Paget's Disease of Bone. *F. Gianfrancesco[1], G. Morello[1], D. Rendina[2], T. Esposito[1], D. Merlotti[3], M. Di Stefano[4], S. Gallone[5], R. Nuti[3], P. Strazzullo[2], G. Isaia[4], L. Gennari[3].* 1) Inst Gen & Biophysics, National Research Council of Italy, Naples, Italy; 2) 2Department of Clinical and Experimental Medicine, Federico II University Medical School, Naples, Italy; 3) 3Department of Internal Medicine, Endocrine-Metabolic Sciences and Biochemistry, University of Siena, Italy; 4) Department of Internal Medicine, University of Turin, Italy; 5) Department of Neuroscience, University of Turin, Italy.

Paget's disease of bone (PDB) is a metabolic disorder characterized by focal abnormalities of bone remodelling. Mutations in SQSTM1 gene were identified as a common cause of the autosomal dominant mode of inheritance, but the aetiology of multifactorial inheritance remains unclear. We identified a variant (rs2073617) located in the promoter region of the gene TNFRSF11B (-223 C/T), encoding Osteoprotegerin, associated with PDB in 811 affected individuals and 367 controls ($P=1.62x10-8$, Odds Ratio=1.65, CI=1.38-1.97). T risk allele produces a binding site for the transcriptional repressor Egr1 that decrease the serum concentration of Osteoprotegerin in 56 individuals analysed (3,9 pmol/L, 4,2 pmol/L and 4,5 pmol/L in individuals with TT, CT, CC genotype, respectively). Moreover, we evaluated an additive effect between this variant and the recently identified functional variant in TNFRSF11A (V192A) encoding RANK receptor. OPG risk allele shows an OR of 2.98 while the OR for the Rank risk variant was 1.78. Additive effect between these two variant was confirmed with an OR of 4.09. In conclusion, these results provide evidence that -223 C/T variant in TNFRSF11B gene influence etiopathogenesis of multifactorial form of PDB together with variant in TNFRSF11A.

1304W

New cellular models for MPS IVA and LINCL lysosomal storage disorders using patient specific induced pluripotent stem cells. *A. Swistowski, M. Richards, M. Vellard.* Research and Development, BioMarin Pharmaceutical Inc, Novato, CA.

Mucopolysaccharidosis type IVA (MPS IVA) and Late-infantile neuronal ceroid lipofuscinosis (LINCL) are hereditary diseases of childhood. MPS IVA is caused by a deficiency of N-acetylgalactosamine-6-sulfate (GalNAc-6-sulfate) sulfatase (GALNS) an enzyme, which is needed for lysosomal degradation of keratan sulfate (KS) and chondroitin-6-sulfate (CS). GALNS deficiency has an impact on the phenotypic properties of chondrocytes in particular, resulting in the formation of cartilage that is more prone to degeneration. LINCL is a neurodegenerative disease that is caused by mutations in the gene CLN2 encoding the lysosomal protease tripeptidyl peptidase 1 (TPP1). Lack of TPP1 activity leads to accumulation of storage materials in the brain and consequently to CNS degeneration and neurological decline. Research on these diseases is significantly limited by either complete lack (CLN2) or limited availability of material from patients. Novel cellular reprogramming technologies allow derivation of induced pluripotent stem cells (iPSCs) from patient cells, which can further be differentiated into relevant cell types of interest. Generation of such cells is without doubt a great alternative for researches as it may provide an unlimited source of patient specific material and thus facilitate more accurate research on various types of diseases. We have successfully generated MPS IVA and LINCL disease specific iPSCs by transducing patient fibroblasts with retroviruses individually encoding the four human transcription factors (Oct4, Sox2, Klf4, and c-Myc). Here, we describe the process of derivation of these cells and their characterization, as well as our progress in differentiating them into chondrocytes, macrophages (MPS IVA), neurons and glia (LINCL). The purpose of our work is to generate valid cellular models for studying mechanisms of pathology and development of cell based assays for monitoring efficacy of enzyme replacement therapy in these diseases. To our knowledge these are the first iPSCs based models for MPS IVA and LINCL.

1305W

First clues of a possible inflammation process in MPS IVA (Morquio): keratan sulfate-induced macrophage activation. *Y. Zhu, M. Vellard.* BioMarin Pharmaceutical Inc., Novato, CA, USA.

Mucopolysaccharidosis IVA (MPS IVA or Morquio) is an inherited lysosomal disease that is caused by N-acetyl-galactosamine-6-sulfate sulfatase (GALNS) deficiency. Insufficient GALNS activity leads to the accumulation of keratan sulfate (KS) and chondroitin-6-sulfate (C6S) primarily in chondrocytes and cartilage. Clinical symptoms of Morquio include abnormal bone and heart development, bell-shaped chest, coarse facial features, hypermobile joints and short stature. Recently, emerging evidence suggests that inflammation might play a role in the pathophysiology of other MPS disorders. This study investigates possible inflammation processes in Morquio, particularly focusing on the inflammatory responses of macrophages in the presence of KS. Macrophage cell lines THP-1 and RAW 264.7 as well as human primary peripheral blood mononuclear cells from healthy donors were treated with various doses of KS. The release of cytokines and chemokines and time dependent activation of intracellular signaling pathways were examined. Additional cellular functions (e.g. chemotaxis, cell growth, viability) were also documented using corresponding assays. In summary, we have observed that several cytokines and chemokines were released from macrophages following KS treatment. Furthermore, an acute stimulation of macrophages with KS led to the activation of several intracellular signaling pathways. Our results indicate that KS can induce the activation of intracellular signaling pathways and the release of pro-inflammatory cytokines and chemokines in macrophages. The distinct pattern of the macrophage-derived factors in response to KS suggests the presence of a specific receptor. These data support a pro-inflammatory role of macrophages in MPS IV disease and possibility of finding specific biomarkers of inflammation in Morquio.

1306W

Genome-wide expression profiling in the Sjögren-Larsson syndrome (SLS). *J.-B. Roullet[1], S. Impey[1], Q. Yang[1], R.D. Steiner[1], W. Rizzo[2].* 1) Pediatrics, Oregon Health & Science University, Portland, OR; 2) Pediatrics, University of Nebraska Medical Center, Omaha, NE.

Background: SLS is caused by mutations in ALDH3A2 which codes for fatty aldehyde dehydrogenase (FALDH). The typical phenotype includes congenital ichthyosis, spastic diplegia or tetraplegia, and mental retardation. Seizures occur frequently and patients often present with pathognomonic white glistening dots in the retina. SLS pathophysiology remains poorly understood and there is no effective treatment. Objectives: to characterize the molecular changes caused by FALDH deficiency. Methods: Genome wide expression profiling (RNA-Seq) was performed using skin fibroblasts isolated from a patient with SLS in comparison with a skin fibroblast cell line obtained from an unaffected individual. Results: The SLS gene expression profile showed significant ($p \leq 10\text{-}5$) down-regulation of ~2,400 genes (total = 31,400 genes) including genes implicated in skin diseases (ADAMTS2, COL1A1 & COL3A1/Ehrlers-Danlos syndrome, SOCS3/atopic dermatitis, KRT10/bullous ichthyosis, SAT1/keratosis), spastic diseases (KIAA0196, ZFYVE26), psychomotor disorders/mental retardation (B4GALT1 & GBA/Gaucher disease, ATXN1/spinocerebellar ataxia, CSTB1/progressive myoclonic epilepsy, GRIK2/long-term potentiation and memory, NDN/Prader-Willi), neurogenesis (NEFM & NEFL/axonal type Charcot-Marie Tooth disease, GOLSYN/formation of presynaptic boutons, NETRIN/axon guidance), and eye diseases (ADAMTSL4/ectopia lentis, EFEMP1/Doyne-honeycomb retinal dystrophy, C1QTNF5/retinal degeneration, SLC1A47/retinal glutamate transporter, EYA2/forebrain patterning). Approximately 4000 genes were upregulated including genes implicated in the regulation of cell cycle, DNA repair, cytoskeleton integrity, endoplasmic reticulum function and intracellular protein trafficking. An increase in genes particularly relevant to SLS pathogenesis (SOX5/inhibitor of oligodendrocytes differentiation, ARHGD1B/inhibitor of actin cytoskeleton formation, KRTAP-1 & KRT19/keratin production, and PKP1/skin ectodermal structure) was noted. Conclusions: The study provides the first account of the impact of FALDH deficiency on gene expression. The data show that gene expression is severely altered in SLS and suggest potential molecular mechanisms underlying the dermatologic, neurological and retinal manifestations of the disease. Future studies with cell lines from other patients or from brain cells derived from animal models of the disease are needed to confirm and expand these findings.

1307W

Genetics variants implicated in COPII-mediated vesicle formation in chylomicron retention disease. *R. Sanchez[1], E. Levy[1,2], C. Garofalo[1], D. Sinnett[1,3].* 1) Research Center CHU Ste-Justine, University of Montreal, Montreal, Canada H3T 1C5; 2) Department of Nutrition, University of Montreal, Montreal, Canada H3C 3J7; 3) Department of Pediatrics, University of Montreal, Montreal, Canada H3C 3J7.

The COPII coat protein complex II (COPII) mediates formation of transport vesicles that bud from the ER, an essential process for traffic of secretory proteins to the Golgi organelle. Importantly, COPII vesicles play a crucial role in the transport of chylomicrons and VLDL lipoproteins in enterocytes and hepatocytes respectively. The basic functional units of the COPII complex are the proteins Sar1B, Sec23/Sec24, and Sec13/Sec31. To date, three human diseases have been found to result from disruption of COPII-related genes, chylomicron-retention disease (CRMD) (Sar1B gene), cranio-lenticulo-sutural dysplasia (CLSD) (Sec23A gene) and congenital dyserythropoietic anemia (CDAII) (Sec23B gene). Given the wide range of biochemical and clinical manifestation of CRMD, we suspect the presence of new genetics variants (SNPs) in the different genes contributing to the formation of the COPII complex. To detect SNPs that could be associated with CRMD phenotypes, we resequenced the exons of Sar1B, Sec23B, Sec24B, Sec13 and Sec31 genes. Genomic DNA from peripheral blood cells was isolated from 14 CRMD patients that have been treated for a fat malabsorption syndrome associated with diarrhea and steatorrhea. We identified 3 coding SNPs in Sar1B gene; 2 in exon 4 and one in exon 8. However none of these SNPs was polymorphic in the group of patients (n=14). Concerning the Sec genes, we found two SNPs in Sec23B gene (Ile426Val and Leu433-Pro). The allelic frequencies for these polymorphisms (Ile426Val and Leu43-3Pro) were significantly higher in patients versus control individuals. To investigate the impact of these polymorphisms in the COPII function, we performed site directed mutagenesis to create the different alleles combining these two SNPs. We transfected these plasmids in HepG2 cells, while simultaneously silencing the endogenous Sec23B gene. To characterise the role of these Sec23B variants in the function of COPII complex, we will measure enzymes (ACAT, MTP, MGAT, and DGAT) implicated in secretion of lipoproteins. In conclusion modifier genes in the ER to Golgi COPII-dependent transport might play an important role for absorption, assembly transport and metabolism of cholesterol in cells.

1308W

Evaluation of endothelial function by Endothelial Pulse Amplitude Testing in Patients with Mucopolysaccharidosis. *S. Yano, K. Moseley.* Pediatrics/Gen Div, 1G24, LAC+USC Med Ctr, USC, Los Angeles, CA.

Background: Progressive cardiovascular changes have been known as one of the major causes of death in patients with mucopolysaccharidosis (MPS). All types of MPS have been reported to present with cardiovascular manifestations. Evaluation of endothelial function (EF) by flow-mediated dilation (FMD) of the brachial artery has been studied and endothelial dysfunction (ED) characterized by decreased percent mediated dilatation was demonstrated in individuals with risk factors for atherosclerosis. Close relation of EF in coronary and brachial artery has been demonstrated. Studies to evaluate peripheral vascular EF with finger arterial pulse wave amplitude (PWA) with a finger plethysmograph (PAT) have been performed and showed that PAT hyperemia and FMD were significantly correlated. Histopathological studies of the coronary artery stenotic lesions in a patient with MPS-I showed the characteristic findings with abnormal intimal proliferations and decreased nitric oxide synthase activities in the endothelial cells that are also seen in atherosclerosis. Objectives: To evaluate coronary artery ED in MPS patients for early detection of coronary artery lesions. Material and Methods: Finger arterial PWA with a PAT device (Endo-PAT2000) was used to evaluate coronary EF. Total of 17 patients were studied: MPS-I (11), II (2), III (2), VI (1), and Mucolipidosis (1). All MPS-I and II patients have been treated with enzyme replacement therapy with laronidase and idursufase, respectively, more than 3 years. The patient with MPS-VI had bone marrow transplantation at age 3 years. Results: Ten out of 11 patients with MPS-I, and one MPS-II, one MPS-III, and one MPS-VI patients showed abnormal results. Conclusion: A high prevalence rate of ED was demonstrated in patients with MPS by the finger PAT device. Evaluation and monitoring ED in patients with MPS are important and can be life saving. It might be possible that progress of the cardiovascular lesions or improvement due to therapeutic measures could be monitored by this operator independent non-invasive method. Further studies are indicated to verify these hypotheses.

1309W

Generation of a mouse model of the attenuated type I form of sialidosis. *E. Bonten[1], G. Yogalingam[2], H. Hu[1], E. Gomero[1], A. d'Azzo[1].* 1) Deptartment of Genetics, St Jude Children's Research Hospital, Memphis, TN; 2) Dept of Chemical and Systems Biology, Dept of Micro and Immuno, Stanford U School of Med, Stanford, CA.

Lysosomal neuraminidase (NEU1) catalyzes the removal of terminal sialic acid residues from sialoglycoconjugates. The enzyme associates with the protective protein/cathepsin A (PPCA) for its efficient routing to the lysosomal compartment, as well as its intra-lysosomal catalytic activation and stability. NEU1 is linked to the neurodegenerative lysosomal storage disorder sialidosis, which is caused by structural lesions in the NEU1 gene. Different clinical phenotypes of sialidosis are distinguished according to the age of onset and severity of the symptoms; type I or normomorphic sialidosis is an attenuated, non-neuropathic form of the disease that corresponds to the cherry-red spot-myoclonus syndrome, while type II or dysmorphic sialidosis is a severe, neuropathic form of the disease. We have shown previously that the severity of the disease correlates closely with the type of gene mutation, which determines whether a patient has a complete or partial loss of functional NEU1. We have generated a faithful model of the type I form of sialidosis by cross-breeding the Neu1 null mice, a model of type II sialidosis, with a new transgenic mouse line, which ubiquitously expresses a NEU1 variant carrying the amino acid substitution V54M. This mutation was previously identified and characterized in a sialidosis patient with the attenuated form of sialidosis. Type I sialidosis mice have residual Neu1 activity in most organs and cell types. This activity may be increased by augmenting the levels of available PPCA that in this case acts as a molecular chaperone. Unlike Neu1-null mice that have severe neurologic and systemic disease, and a shortened lifespan (5-7 months), Neu1-/-/V54M mice develop normally until the age of 1 year, after which they show a slow progression of lysosomal storage in the visceral organs, predominantly in the kidneys, spleen and liver. We believe that patients with the attenuated form of sialidosis would likely benefit the most from enzyme replacement and/or gene therapy, for which this attenuated sialidosis mouse model may proof to be a valuable tool. (Supported by the American Lebanese Syrian Associated Charities of St. Jude Children's Research Hospital and NIH grant RO1-GM060950).

1310W

Genes Mediating Natural Variation in ER Stress Response. *C.Y. Chow, M.F. Wolfner, A.G. Clark.* Department of Molecular Biology and Genetics, Cornell University, Ithaca NY 14853 USA.

The endoplasmic reticulum (ER) is responsible for synthesis and maturation of many proteins essential for cellular function. ER dysfunction can have devastating consequences when misfolded proteins accumulate in the ER. The resulting “ER stress” has been associated with diseases from diabetes to neurodegeneration, as a primary cause or as a secondary exacerbating effect. Cells respond to ER stress with the “unfolded protein response” (UPR). The UPR reduces ER stress by increasing transcription of ER chaperones, attenuating translation and degrading misfolded proteins in the ER. To understand the effect of ER stress resistance on disease, it is important to determine the extent of natural variation in ER stress resistance in a population. We characterized variation in ER stress resistance in the Drosophila Genetic Reference Panel (DGRP), a collection of 192 inbred wild-derived lines whose whole genome sequences are available. We compared their relative survival on food supplemented with tunicamycin, a drug that causes ER stress by inhibiting N-glycosylation. Molecular markers of ER stress, such as Xbp1 splicing, indicate that tunicamycin induces ER stress in the fly. We observed extensive natural variation in ER stress resistance among the DGRP lines. Mortality rates, as measured by the Cox proportional hazard ratio, varied by more than 100 fold, indicating that extensive genetic variation in ER stress response is present in a single population. To understand genetic variation contributing to differential survival to ER stress, we compared gene expression between four lines that were resistant to ER stress and four that were sensitive (based on survival rates upon tunicamycin treatment). Expression measured by Agilent microarrays demonstrate that genes involved in the cellular response to ER stress, such as chaperones and genes previously implicated in human disease, showed a strong response across all eight genotypes. We found that transcriptome responses to ER stress between lines with high vs. low sensitivity to ER stress differed qualitatively and quantitatively. Many of the genes showing these sensitivity differences had not previously been implicated in ER stress. A focused study into a group of ER stress-responsive genes in all 192 lines will be presented. ER stress response is highly variable in a population and this variation likely arises from minor expression changes in genes not previously associated with ER stress.

1311W

Plasmalogen deficiency in the PEX7 hypomorphic mouse, a model for rhizomelic chondrodysplasia punctata, causes neonatal lethality associated with abnormalities in late lung maturation. *W. Cui[1], X. He[1], S. Jiralerspong[1], G. Nimmo[1], Y. Chen[1], J. Hacia[2], S. Steinberg[3], A. Moser[3], N. Braverman[1,4].* 1) Montreal Children Hospital Research Institute, Montreal, Quebec, Canada; 2) Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA, USA; 3) Kennedy Krieger Institute, Department of Neurogenetics, Baltimore, MD, USA; 4) Department of Human Genetics, McGill University, Montreal, Quebec, Canada.

The biological functions of plasmalogens (PLs), a specialized class of membrane glycerophospholipids, are largely unknown. Cell-based studies implicate roles in oxidant protection, inflammatory responses, signal transduction and cell-cell interactions. PLs are also integral components of surfactant. Inherited defects of PL synthesis cause rhizomelic chondrodysplasia punctata (RCDP), a heterogenous disorder commonly due to defects in the peroxisome transporter, PEX7. The clinical features of RCDP include growth and developmental retardation, cataracts and skeletal dysplasia. A proportion of patients die in the neonatal period, survivors suffer chronic respiratory compromise that ultimately causes death; the latter has been attributed to the effects of the skeletal dysplasia on pulmonary function. To investigate PL functions, we generated hypomorphic Pex7 mouse models that recapitulated the cataract and small size phenotype, but did not have obvious neurological deficits or chondrodysplasia punctata. These mice survived on a mixed 129/C57Bl6 background, but died within a few days of life on a C57Bl6 background. Pex7 transcript was reduced to <5%, and Pex7 protein to <12%, of wild type levels in both strains. Lung total PL levels were also equivalent in the two strains, suggesting that lethality was due to different downstream effects of PL utilization. Morphometric studies showed that death was associated with increased saccular wall tissue and decreased air spaces, indicating a delay, or arrest, in alveolarization. Using immunohistochemistry, we show that the increased interstitial tissue was mesenchymal in origin, and found increased numbers of terminal bronchiole epithelial cells (Clara cells) and alveolar type II epithelial cells, as well as increased lipid peroxidation protein adducts. Gene expression studies showed significant decreases in the transcription factor, Meox2, and further reduction in the congenic vs. the mixed background strain. We suggest that PL deficiency may affect cell signaling in late lung maturation and independently, also promote oxidant damage. Moving from Pex7 deficient mice to humans, we propose that PL deficiency contributes to early neonatal demise in RCDP as a function of lung maturation, and to the chronic lung disease in survivors, independent of the skeletal dysplasia.

1312W

Missense Mutations in the Hemochromatosis (HFE) Gene are Genetic Risk Factors for Secondary Polyosteoarthritis. *J.A. Di Battista[1], V. Martelli[1], P. Panopolis[1], J. Antoniou[2], M. Sebag[1], B. Gilfix[1], H.A. Menard[1].* 1) Medicine, McGill University, Montreal, Quebec, Canada; 2) Orthopaedic Surgery, Jewish General Hospital, Montreal, Quebec, Canada.

Rationale and Objectives: Clinical hereditary hemochromatosis type I (HH-I) is defined as the progressive accumulation of iron in organs caused by missense mutations in the HFE gene. In population studies, two prevalent mutations, the C282Y and the H63D, exhibit clinical penetrance rates varying from 1:400 (frequent) to 1:10,000 (rare) individuals with any manifestation. Rheumatic complaints of the osteoarthritis (HHOA) phenotype are the leading clinical manifestations of HH-I. Yet, nothing is known about the expression in chondrocytes of proteins regulating iron metabolism. Our study aimed at verifying their presence, at exploring variations of OA-related biomarkers in normal vs. HFE-mutation-carrying cultured human chondrocytes and, their variations in normal chondrocytes transfected with normal or mutated HFE. **Methods and Results**: Cultured chondrocytes were derived from tissues obtained at total knee replacement (TKR) of patients not carrying (OA) or carrying mutations (HHOA). Ferroportin, Divalent Metal Transporter 1, ferritin and transferrin receptor 1 were all identified by PCR in human chondrocytes. The in vivo mutated cells spontaneously expressed in vitro, significantly higher levels of MMP-1, MMP-3, MMP-13, iNOS and COX-2 (referred to as an “activation profile”) compared with cells obtained from age-matched patients with idiopathic primary OA. In vitro transfection of a construct expressing the wild type (normal) HFE gene into normal donor-derived chondrocytes had similar effects on basal or cytokine-induced MMP-13 or COX-2 expression as cells transfected with an empty vector or under mock transfection conditions. However, transfecting plasmids with HFE containing the mutations, generated through site directed mutagenesis, resulted in a 3 to 7-fold increase in MMP-1/MMP-13/COX-2/iNOS expression levels under basal and cytokine-induced conditions. Those effects were similar in the presence of normal vs. elevated iron concentrations in the culture medium. **Conclusions:** This is a first attempt to define the molecular mechanisms driving the characteristic HHOA and the role that HFE mutant genotypes may play. Our clinical observations suggest that many patients with the so-called “silent” H63D HFE mutation have early rheumatic symptoms. As such given their high frequency, that mutation while presenting a normal biochemical phenotype, is not clinically silent and may be a major, still insufficiently recognized, genetic risk factor for OA.

1313W

Case Report; Cerebrotendinous Xanthomatosis (Sterol 27-HydroxylaseDeficienc). *F. Hadipour[1], Z. Hadipour[1], P. Sarkhail[1], M. Noruzinia[1,3], Y. shafeghati[1,2].* 1) Medical Genetics, Sarem Cell Research Center & Hospital, Tehran, Iran; 2) Genetics Research Center, University of Social Welfare and Rehabilitation Science, Tehran. Iran; 3) Department of Medical Genetics, School of Medical Sciences, Tarbiat Modares University, Tehran Iran.

Abstract: Background Cerebrotendinous xanthomatosis is a rare genetic disorder of cholesterol and bile acid metabolism that results in systemic and neurologic abnormalities. The disease was first described in 1937 by Van Bogaert and has been characterized clinically, biochemically, and genetically. In 1991, mutations in the gene CYP27A1 were discovered as causative, Since then, more than 50 mutations have been implicated. The disease begins in infancy with chronic diarrhea. Cataracts become evident in childhood or adolescence, and xanthomata develop in the second and third decades of life. Significant neurologic impairment includes seizures, dementia, and extra pyramidal dysfunction and begins in the third decade of life and progresses until death. The presentation and course widely varies, and treatment can dramatically alter the natural history, especially with early initiation. Case Report We report an Iranian family with three affected child who are suffering from Cerebrotendinous Xanthomatosis. Cardinal features were: Motor dysfunction, ataxia, spastic paresis, Xanthomas of the Achilles tendon, Cataracts double. MRI of the brain show diffuse cerebral atrophy and increased signal intensity in the cerebellar white matter on T2-weighted scans. In this report, we present 3 patients with Cerebrotendinous Xanthomatosis, who confirmed by Molecular Analysis. The patients are suffering from disease resulting from a homozygous splice- mutation in intron 2 of the CYP27A1 gene. Key word; Cerebrotendinous Xanthomatosis, Motor dysfunction, CYP27A1 gene.

1314W

Innate immunity and Toll-like receptors in lysinuric protein intolerance (LPI). *J. Kurko[1], M. Tringham[1], L. Tanner[2], K. Näntö-Salonen[2], H. Niinikoski[2], M. Vähä-Mäkilä[2], O. Simell[2], J. Mykkänen[2].* 1) Dept. of Medical Biochemistry and Genetics, University of Turku, Turku, Finland; 2) Dept. of Pediatrics, Turku University Hospital and University of Turku, Turku, Finland.

Lysinuric protein intolerance (LPI; MIM222700) is a rare autosomal recessive disorder caused by a defect of cationic amino acid transport in the small intestine and kidney tubules. All Finnish patients share the same homozygous mutation c.1181-2A→T (c.859-2A>T) in the *SLC7A7* gene. Altogether 51 mutations have been found worldwide but the Finnish founder mutation has not been detected in any other population. The main symptoms of LPI consist of protein aversion after weaning, failure to thrive, muscle hypotonia, osteoporosis and hepatosplenomegaly. However, some findings vary markedly even in the same family and may include severe pulmonary and renal complications, such as alveolar proteinosis and end-stage renal disease. Some patients also suffer from normochromic anaemia with poikilocytosis and anisocytosis and unexplained immunological problems such as leukopenia, deficiencies in T and B cell functions and severe viral infections.

Immunological problems suggest a defect in innate immunity which functions through the Toll-like receptor signalling pathway. We cultured macrophages from 23 patients and 15 sex and age-matched controls and stimulated them with three PAMPs (pathogen associated molecular patterns) Pam_3CSK_4, LPS and CpG DNA which activate TLR1/2, TLR4 and TLR9 receptors, respectively. We collected plasma, culture medium and RNA samples at three time points in order to measure cytokine and chemokine levels with Luminex technology, and to perform expression profiling of TLR signalling genes with quantitative real-time PCR. Stimulation of the cells with synthetic viral CpG DNA resulted in decreased expression levels of TLR signalling genes. This refers to defects in patients' innate immunity during viral infections which may be connected to the severe immunological and pulmonary complications the patients suffer from.

1315W

Metabolic cutis laxa syndromes. *E. Morava[1], T. Gardeitchik[1], M. Mohamed[1], D. Kouwenberg[1], D. Lefeber[2], U. Kornak[4], B. Heuvel[3], R.A Wevers[3].* 1) Institute of Genetic and Metabolic Disorders and Department of Pediatrics, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands; 2) Institute of Genetic and Metabolic Disorders and Department of Neuology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands; 3) Institute of Genetic and Metabolic Disorders and Laboratory for Genetic Endocrine and Metabolic Diseases, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands; 4) Department of Human Genetics, University Hospital, Charite, Berlin, Germany.

Cutis laxa (CL) is a disorder, either acquired or inherited, in which patients have wrinkled, abundant, sagging skin with abnormal elasticity. Skin symptoms may be associated with variable systemic involvement. Recently, several new genetic defects have been discovered to cause cutis laxa. Surprisingly, a number of these syndromes are inborn errors of metabolism. These include disorders of glycosylation, like COG7-CDG and ATP6V0A2-CDG, as well as deficiencies in enzymes localized in mitochondria and involved in de novo proline synthesis (P5CS and PYCR1). Here we describe eleven children diagnosed with with metabolic cutis laxa and novel mutations and report on the discriminative metabolic and clinical features in our patients. The four children diagnosed with autosomal recessive cutis laxa type 2A (ARCL2A) due to PYCR1 mutations had a triangular face with prominent ears and progeroid appearance, abnormal wrist position and two of them showed athetoid movements. Cerebral MRI detected hypoplastic corpus callosum in three of the four patients, and two of them had increased serum alanine levels. All patients had improvement of their progerioid skin features. In four of the five so far unreported patients with (autosomal recessive cutis laxa type 2B (ARCL2B) or ATP6V0A2-CDG, in the absence of the above described abnormalities, a cobblestone like brain dysgenesis was detected, associated with glycosylation abnormalities and elevated liver enzymes. The patients with P5CS deficiency had similar features without any change of the skin symptoms with time. Discriminating between the different forms of autosomal recessive cutis laxa is challenging due to several overlapping clinical features: the severely affected skin, microcephaly, facial dysmorphisms, joint hyperlaxity, cognitive deficits, growth delay and late closing of the fontanel. We suggest to perform a brain MRI, measure serum alanine levels and liver enzymes additional to screening for glycosylation in patients with cutis laxa syndrome to obtain a timely diagnosis.

1316W

The expression pattern of genes involved in the Vitamin B_{12} metabolic pathway during mouse embryogenesis. *M.M. Moreno-Garcia[1], D.S. Rosenblatt[1], L.A. Jerome-Majewska[2].* 1) Department of Human Genetics, McGill University, Montreal, Canada; 2) Department of Pediatrics, McGill University, Montreal, Quebec, Canada.

Vitamin B_{12} (cobalamin, (Cbl)), is essential for human development and survival. A series of inborn errors of cobalamin metabolism have been identified, designated *cblA-cblG*, which affect the ability of cells to generate the two important cobalamin cofactors, methylcobalamin and adenosylcobalamin. These inborn errors result in homocystinuria and methylmalonic aciduria, either isolated or in combination. Those patients with elevation of both homocysteine and methylmalonic acid in blood and urine, have functional defects of both the cytoplasmic (methionine synthase) and the mitochondrial (methylmalonyl-CoA mutase) branches of the pathway. To examine whether genes involved in vitamin B_{12} metabolism show tissue-specific expression during mouse embryogenesis, we are characterizing their sites of expression in wild type mice at 9.5, 11.5 and 12.5 days post conception by *in situ* hybridization. We previously reported cell type and tissue specific expression of *Mmachc* and ubiquitous expression of *Mmadhc*, two genes involved in the early steps of vitamin B_{12} metabolism [Pupavac *et al*, 2011]. Here we describe expression of three genes associated with isolated methylmalonic aciduria (*Mut*, *Mmaa* and *Mmab*). We did not observe any organ, tissue or cell type specific expression for these three genes at 9.5 and 11.5 days. At 12.5, we observed cell type specific expression of *Mmab* in lung and ubiquitous expression of *Mut* and *Mmaa*. At the same time, we found higher expressions of these genes in the developing lung, heart, circulatory and nervous system. This suggests that during organogenesis these three genes may interact in only a subset of cells. In conclusion, this study presents for the first time the analysis of *Mut*, *Mmaa* and *Mmab* gene expression patterns during mouse development and demonstrates overlapping and differential *Mmab* expression, notably in the lung.

1317W

Genetic Analysis of Galactosemia genes from Indian Galactosemia Patients: Structural and Functional Implications of Mutations. *R. Singh[1], G. Kaur[2], B.R. Thapa[3], R. Prasad[1].* 1) Department of Biochemistry, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, Chndigarh, India; 2) Department of Physiology, Government Medical College and Hospital, Sector - 32, Chandigarh, India; 3) Division of Pediatric Gastroenterology, Postgraduate Institute of Medical Education and Research, Chandigarh-160012, India.

Introduction: Galactosemia is an inborn error of metabolism resulting from defective galactose metabolism. Three types of galactosemia occur according to the identity of the impaired enzyme: galactokinase (GALK), galactose-1-phosphate uridyltransferase (GALT) and UDP-galactose-4-epimerase (GALE). Disrupted metabolism leads to the accumulation of galactose and its derivatives leading to several symptoms. Objective: To determine blood GALT and GALK activity in infants with cholestasis and congenital cataracts and to establish a spectrum of mutations in GALT and GALK genes. Methods: 450 infants with cholestasis were evaluated for GALT deficiency galactosemia. Basic investigations included hemogram, liver function tests, blood culture, urine culture, urine for non-glucose reducing substances, eye evaluation, abdominal ultrasound, TORCH serology, mebrofenin scan and peroperative cholangiogram were done when indicated. Screening for GALT deficiency was done using Perkin-Elmer neonatal GALT kit. The levels of galactose-1-phosphate were also measured. 140 patients with congenital cataract were screened for GALK deficiency. Mutation analysis for most common Q188R and N314D mutations in GALT gene was performed by Restriction Fragment Length Polymorphism (RFLP). Single Stranded Conformational Polymorphism (SSCP) analysis and subsequently DNA sequencing were done for identification and characterization of unknown and novel mutations in GALT and GALK genes. Structural and functional implications of the mutations were also studied. Results: 55 (12.2%) infants were found to have reduced GALT activity with male: female: 37:18, jaundice in 55 (100%), hepatomegaly in 44 (80%), splenomegaly in 28 (51%), encephalopathy in 8 (15%), coagulopathy in 12 (22%), cataracts in 18 (32%), sepsis in 8 (15%) and failure to thrive in 30 (54%) while three patients died during the hospital course. A significant negative correlation was observed between GALT activity and galactose-1-phosphate levels. A total of 16 mutations and 4 polymorphisms were detected in GALT gene. 10 were novel mutations. N314D and Q188R mutations were detected in 37 and 3 patients respectively. Reduced blood galactokinase activity was found in 16 (11.4%) patients with congenital cataracts. 5 mutations were identified in GALK gene.4 were novel. Conclusion: N314D was the most common mutation in GALT gene from our population. 10 and 4 novel mutations were detected in GALT and GALK genes respectively.

1318W

Effects of genetic variants and nutritional factors on metabolic diseases among Chinese populations. *X. Lin.* Inst Nutritional Sci, Shanghai Inst Biological Sci, CAS, 294, Taiyuan Rd, Xuhui District Shanghai, 200031, P.R.China.

Paralleling to rapid nutrition transition in past 3 decades, the prevalence of metabolic diseases like metabolic syndrome and type 2 diabetes have been increased in an alarming rate in Chinese population. Asian people were known to have higher susceptibility of suffering type 2 diabetes than Caucasian, although limited data is available among Chinese. Therefore, we have established a population-based cohort study including 3289 Beijing and Shanghai residents aged 50-70 years. The baseline data indicated that prevalence of obesity, MetS and type 2 diabetes were significantly higher in Beijing than in Shanghai. Approximately 70% of our participants had vitamin D deficiency [25(OH)D <50 nmol/l]. Plasma 25(OH)D level was inversely associated with a higher risk of MetS and insulin resistance. Meanwhile, elevated ferritin level was associated with a high risk of diabetes independent of inflammatory markers, adipokines and MetS. Recently, we also found a unique erythrocytes fatty acids pattern and their associations with metabolic risks. In genetic association studies, common variants in CDKAL1, CDKN2A/B, IGF2BP2, SLC30A8 GCKR and KCNQ1 were found to be independently or additively associated with the risk of type 2 diabetes. However, different genetic architecture and allele frequencies between Chinese Hans and Caucasians were observed for FTO variants with obesity and type 2 diabetes. Moreover, circulating 25-hydroxyvitamin D and ferritin levels were found to be modified by genetic variation related with the genes involving vitamin D and iron regulating pathway. Currently, we are conducting a 6-year follow up study and also using genomic DNA obtained from our baseline survey for whole-genome genotyping as a part of Chinese national type 2 diabetes GWAS project. Collectively, all the efforts have been made to enhance our knowledge about major nutrition/lifestyle, genetic factors and their interaction on in development of metabolic diseases in Chinese population.

1319W

Neonatal Multiple Sulfatase deficiency with tetralogy of Fallot: Homozygosity for a Novel Mutation. *F. Al Jasmi[1], J. Hertecant[2], L. Al Gazali[1].* 1) Faculty of Medicine and Healthy Science, Al Ain, United Arab Emirates; 2) Department of Paediatrics, Tawam Hospital, Al-Ain, United Arab Emirates.

Background: Multiple Sulfatase deficiency (MSD) (OMIM 272200) is a rare autosomal recessive condition caused by mutations in the sulfatase-modifying factor-1 gene (*SUMF1*) located at 3p26. MSD is characterized by deficiencies in all 12 known sulfatases and leading to a complex clinical presentation. It may present with clinical features of any or combination of the following diseases: metachromatic leukodystrophy, mucopolysaccharidosis, X- linked ichthyosis and chondrodysplasia punctate. Methods and Results: We describe a female infant, product of a consanguineous union, who presented with tetralogy of fallot, hypoplastic lungs and recurrent chest infection, non immune hydrops, short limbs, inguinal hernia and facial dysmorphism. Urine oligo was negative but Urine MPS TLC analysis showed dermatan sulfate and heparan sulfate. Skeletal survey showed dysosotosis multiplex and short limbs. Sulfatase enzymes measurement in the proband's fibroblast showed low activities of arylsulfatase A, arylsulfatase B and iduronate 2-sulfatase which confirmed the diagnosis of MSD. *SUMF1* gene analysis revealed homozygosity for a novel mutation, c.603-2 A>G predicted to result in the loss of the normal acceptor splice site at the junction between intron 4 and exon 5. Conclusion: To our best knowledge, this is the first case report of MSD in association with tetralogy of Fallot. MSD should be suspected in patients who have clinical features typical of single sulfatase deficiencies enzymes.

1320W

A 680 kb duplication at the *FTO* locus in a subject with extreme obesity. *R.W. Davies[1], P. Lau[2], R. Dent[3], R. McPherson[2].* 1) Cardiovascular Research Methods Centre, Univ Ottawa Heart Inst, Ottawa, Canada; 2) Atherogenomics Laboratory, Univ Ottawa Heart Inst, Ottawa, Canada; 3) Weight Management Clinic, The Ottawa Hospital, Ottawa, Canada.

Background: Common intronic SNPs in the human *FTO* gene are strongly associated with body mass index. In mouse models, inactivation of the *Fto* gene results in a lean phenotype whereas over-expression of *Fto* leads to increased food intake and obesity. The latter finding suggests that copy number variants at the *FTO* locus might be associated with extremes of adiposity. **Methods:** SNP genotyping was carried out on Affymetrix 6.0 arrays in a cohort of 985 obese and 869 lean subjects drawn from the extremes of the BMI distribution (mean BMI 43.1+8.7 and 20.3+1.84 kg/m^2 for obese and lean subjects, respectively). Copy number variation was assessed using Birdseye and subsequently verified by quantitative RT-PCR. **Results:** Single marker analysis confirmed previously shown associations between intronic SNPs in *FTO* and obesity (rs9939609, OR=1.63, p=$6x10^{-13}$). In addition, a ~680 kb duplication, confirmed by real time PCR, was observed putatively between rs11859825 and rs9932411 in a 61 year old male with severe obesity of childhood onset (max BMI 53kg/m^2). The duplicated region on chromosome 16 spans from approximately 53,325,073 to 54,005,163 (build 37), including the entire GWAS risk locus and encompassing *RBL2, AKTIP, RPGRIP1L* and all but the last exon of the *FTO* gene. **Conclusion:** We have identified a novel ~680 kb duplication at the *FTO* locus in an extremely obese subject. Studies are underway to fine map the duplicated region, quantify effects on *FTO* transcript expression, and determine segregation with obesity in an extended kindred.

1321W

Association between BMI variants previously identified in adult populations in association with adolescent BMI in an ethnically-diverse cohort. *M. Graff[1, 2], K.E. North[1, 3], K.L. Mohlke[3, 4], L.A. Lange[3, 4], E.M. Lange[3, 4], C.S. Fox[5], L.A. Cupples[5, 6], K.M. Harris[2, 7], P. Gordon-Larsen[2, 8].* 1) Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; 2) Carolina Population Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; 3) Carolina Center for Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; 4) Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; 5) Framingham Heart Study, Framingham, Massachusetts, USA; 6) Department of Biostatistics, Boston University School of Public Health, Boston University Medical Campus, Boston, Massachusetts, USA; 7) Department of Sociology, University of North Carolina, Chapel Hill, North Carolina, USA; 8) Department of Nutrition University of North Carolina, Chapel Hill, North Carolina, USA.

Adolescence is a period with a high risk for weight gain, yet nearly all well established genome wide association (GWA) single nucleotide polymorphisms (SNPs) have been identified in middle aged European American adults. Using 9,350 participants of the National Longitudinal Study of Adolescent Health, or Add Health (ages 12-21 years, 52.6 %female), we selected 43 SNPs at 40 loci from previous GWA studies and assessed their association with BMI (measured in 1996) across four ancestrally diverse US subpopulations (5,531 European American, 1,904 African Americans, 1,437 Hispanics, and 478 Asians). Buccal cells from participants were extracted and genotyped using TaqMan (sample call rate: 97.5%, SNP call rate: 100%). Inverse normal transformed BMI residuals, adjusted for gender and age, were analyzed using linear regression models implemented in PLINK. Analyses were stratified by ethnicity and an inverse-variance-weighted meta-analysis was used to combine results across the 4 ethnic groups. Average BMI across all ethnic groups was 23.6±5.2 kg/m2, ranging from 22.6±5.2 kg/m2 in Asian Americans to 24.3±5.8 kg/m2 in African Americans. The effect estimates for all 43 SNPs were directionally consistent across ethnicity with previously published results. Of the 43 SNPs, 19 displayed nominally significant associations with BMI at p<0.05 in the meta-analysis (16 were nominally significant in Caucasians, 7 in African Americans, 4 in Hispanics, and 5 in Asians). Based on t-test comparisons, all but 3 of the 19 SNPs had significantly larger effect sizes (p<0.05) in our adolescent sample than published effect sizes for BMI in adults. Only FTO (rs9939609) had an I2 heterogeneity statistics across ethnicity that was significant, p=0.01. The strongest result in the meta-analysis, TMEM18 (rs6548238; p=4.9E-12), had one of the largest differences in effect size compared to published results (Willer et al 2009, stage 2 GIANT consortium), which was based on 51055 adults, with a beta of 0.74±0.11 kg/m2 in this sample versus a beta of 0.26±0.07 kg/m2 in GIANT (beta difference [SE]=0.48[0.08]; p=4.5E-10 for difference). These results show that variants found to be associated with BMI in adults are also associated with BMI in adolescents, and that some may have larger effects during this vulnerable period. R01HD057194.

1322W

Identification of novel mutations in HEXA gene in children affected with Tay-sachs disease from India. *M. Mistri*[*1], P. Tamhankar*[*2], S. Thomas[3], P. Kondurkar[2], S. Mehta[4], D. Sanghavi[2], J. Sheth[1 #].* 1) Biochemical and Molecular Genetics, FRIGE's Institute of Human Genetics, Satellite, Ahmedabad, Gujarat, India. *(joint first authors), #(Correspondence author); 2) Genetic Research centre, NIRRH, ICMR, Parel, Mumbai, India.*(joint first authors); 3) Biomedical Informatics Center, NIRRH, ICMR, Parel, Mumbai, India; 4) Usha-deep hospital, Naranpura, Ahmedabad, Gujarat, India.

Tay - Sachs disease (TSD) is an autosomal recessive storage disorder caused by impaired activity of the lysosomal enzyme hexosaminidase-A (EC 3.2.1.52) due to the mutation in HEXA gene. As per HGMD database, 134 mutations have been reported from different ethnic groups, although disease causing mutations are not known in Indian children. Hence, the present study was undertaken to identify mutations in HEXA gene in Indian Tay-sachs patients to evaluate genotype-phenotype correlation and to establish the common disease causing mutations that can be utilized for carrier screening in mass population. Five missense mutations were found in 10 patients with infantile onset Tay-Sachs disease, through bidirectional sequencing of HEXA gene using BigDye Terminator v3.1 and capillary electrophoresis was performed on automatic sequencer ABI-3130. The most common mutation detected in six individuals [60%] was c.1385A>T (p.E462V). This homozygous mutation was observed in five unrelated patients with one consanguineous and remaining non-consanguineous parentage of the same ethnic group. Four other patients were found homozygous for mutations viz. c.340G>A (p.E114K), c.964G>A (p.D322N), c.1178C>G (p.R393P) and c.508C>T (p.R170W) respectively. All the mutations identified are novel except c.508C>T (p.R170W) which has been previously identified in French-Canadian population. Protein homology modeling studies further established the effects of novel mutations occurred at highly evolutionarily conserved and functionally active domain residues in the protein leading to conformational changes in HEXA protein. The mutation c.1385A>T (p.E462V) could be a founder mutation in a single ethnic group and can be used for targeted mutation analysis in the affected individuals and mass screening at large. In addition, the study identified the mutational spectrum of Indian TSD patients, which will be helpful in offering precise genetic counseling, carrier detection followed by prenatal diagnosis.

1323W

Variants in or near PNPLA3, GCKR and PPP1R3B associate with ultrasound-defined steatosis in NHANES III. *R. Hernaez[1,2,3], J. McLean[4], M. Lazo[1,3], F.L. Brancati[1,3,5], J.N. Hirschhorn[6,7,8], T. Nguyen[6,7], I.R. Kamel[9], S. Bonekamp[9], J.M Clark[1,3,5], W.H.L. Kao[3,5], E.K. Speliotes[7,10].* 1) Medicine, Johns Hopkins School of Medicine, Baltimore, MD; 2) Medicine, Washington Hospital Center/Georgetown University Hospital, Washington, DC; 3) Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 4) NOVA Research Company, Bethesda, MD; 5) Welch Center for Prevention, Epidemiology and Clinical Research, Johns Hopkins University, Baltimore; 6) Divisions of Endocrinology and Genetics and Program in Genomics, Children's Hospital, Boston, MA; 7) Broad Institute, Cambridge, MA; 8) Department of Genetics, Harvard Medical School, Boston, MA; 9) Radiology, Johns Hopkins School of Medicine, Baltimore, MD; 10) Department of Internal Medicine, Division of Gastroenterology, and Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI.

Five genetic variants associated with computerized tomography (CT) measured hepatic steatosis in a genome-wide association meta-analysis of individuals of European ancestry. Here we aim to test whether these variants are associated with ultrasound-measured hepatic steatosis (HS) in 4,804 participants from 3 ancestries [1,825 Non Hispanic Whites (NHW); 1,442 African Americans (AA), and 1,537 Mexican Americans (MA)] in the Third National and Nutrition Examination Survey (NHANES III, Phase 2, 1991-1994). HS was defined as presence of any steatosis versus none. We examined whether HS was associated with rs738409 (PNPLA3), rs2228603 (NCAN), rs12137855 (LYPLAL1), rs780094 (GCKR), and rs4240624 (PPP1R3B) using population-specific logistic regression analysis adjusted for age, age2, gender, and alcohol consumption using an additive genetic model. We estimated overall association across the ancestries using a beta/standard error meta-analysis in METAL if there was no significant heterogeneity across ancestries. In each population, we have >80% power to detect an odd ratio of 1.15 if the allele frequency is at as high as 0.53 (PNPLA3) and an odds ratio of 2.05 if the frequency is 0.01 (GCKR). Of 4,804 participants, 57.2% were female, mean age was 43.2 years, and, the HS prevalence was 40%. The presence of HS was associated with older age, increased BMI, waist circumference, fasting glucose, triglycerides, and the presence of the metabolic syndrome. The G allele at PNPLA3 was consistently associated with HS across ancestries with an overall OR of 1.19 (p=0.0004067; p-heterogeneity=0.71) across all three ancestries. Other SNPs were not consistently associated with HS across ancestries. We observed an association between HS and the T variant in GCKR (OR=1.20, p=0.0099) and the A variant of PPP1R3B (OR=1.30, p=0.0376) in NHW. In conclusion,this is the first study to validate the association between the genetic variation in PNPLA3 (rs738409) and ultrasound defined HS in three nationally representative ancestries from the United States. The lack of consistent associations between variants in NCAN, LYPLAL1, GCKR, PPP1R3B and HS across the populations may be due to lack of power, differences in LD amongst ancestries, or true lack of association, but two of these (GCKR, PPP1R3B) are significantly associated with HS in NHW. More genetic analyses of these variants in larger populations of different ancestries are needed to distinguish amongst these possibilities.

1324W

A Genome-Wide Association Study Reveals a Quantitative Trait Locus of Resistin on RETN That Predicts Metabolic Outcomes. *C. Chung[1,2], T. Lin[3], J. Chen[4], H. Leu[4], H. Ho[5], C. Ting[5], S. Sheu[3], W. Tsai[6], J. Chen[6], W. Pan[1].* 1) Inst Biomedical Sci, Academia Sinica, Taipei, Taiwan; 2) Faculty of Public Health, Kaohsiung Medical University, Kaohsiung, Taiwan; 3) Division of Cardiology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 4) Cardiovascular Research Center, National Yang-Ming University, Taipei, Taiwan; 5) Taichung Veterans General Hospital, Taichung, Taiwan; 6) College of Medicine, National Cheng Kung University, Tainan, Taiwan.

Objective - The plasma resistin level, a potential upstream and internal facet of obesity and diabetes, has a reasonably high heritability. Whether other novel genes influence the variation in resistin level, and the roles of these genetic variants on subsequent clinical outcomes has not been thoroughly investigated. Therefore, we aimed not only to identify genetic variants modulating plasma resistin levels but also to investigate whether these variants are associated with metabolic syndrome and diabetes. Research Design and Methods - We conducted a genome-wide association study to identify quantitative trait loci associated with resistin levels by genotyping 382 young-onset hypertensive (YOH) subjects with Illumina HumanHap550 SNP chips. The culpable single-nucleotide polymorphism (SNP) variants responsible for resistin levels were then confirmed in another 559 YOH subjects, and the association of these SNP variants with the risk of metabolic syndrome (MS) and type 2 diabetes mellitus (T2DM) was examined in an independent community-based prospective cohort, the CardioVascular Disease risk FACtors Two-township Study (CVDFACTS, n= 3350). Results - The SNP (rs1423096) most significantly associated with resistin levels was located in downstream of RETN gene in the first stage ($p = 2.54\times10\text{-}20$). We replicated and confirmed the association between rs1423096 and plasma resistin levels in an additional 559 YOH subjects ($p = 1.13\times10\text{-}7$). This SNP was further associated with the risk of MS (OR = 1.577, p = 0.0432), and T2DM (OR = 2.213, p = 0.0034) in the CVDFACTS. Conclusion - The results indicate that rs1423096 seems to have a role in the determination of plasma resistin levels and seems to be associated with more deleterious metabolic syndrome and diabetes.

1325W

Genetic Risk Assessment of Type 2 Diabetes Associated Polymorphisms in African-Americans. *J. Cooke[1,3,4], M. Ng[3,4], L. Lu[6], J. Li[4], S. An[2,3,4], J. Hester[3,4,5], J. Xu[4], B. Freedman[7], C. Langefeld[6], D. Bowden[2,3,4,8], N. Palmer[2,3,4].* 1) Molecular Medicine and Translational Science, Wake Forest School of Medicine, Winston-Salem, NC; 2) Department of Biochemistry, Wake Forest University School of Medicine, Winston-Salem, North Carolina; 3) Center for Genomics and Personalized Medicine Research, Wake Forest School of Medicine, Winston-Salem, North Carolina; 4) Center for Diabetes Research, Wake Forest School of Medicine, Winston-Salem, North Carolina; 5) Program in Molecular Genetics and Genomics, Wake Forest School of Medicine, Winston-Salem, North Carolina; 6) Department of Biostatistical Sciences, Wake Forest School of Medicine, Winston-Salem, North Carolina; 7) Department of Internal Medicine - Section on Nephrology, Wake Forest School of Medicine, Winston-Salem, North Carolina; 8) Department of Internal Medicine - Section on Endocrinology, Wake Forest School of Medicine, Winston-Salem, North Carolina.

African Americans have the highest prevalence of type 2 diabetes (T2D) of any American population. Multiple single nucleotide polymorphisms (SNPs) associated with T2D susceptibility have been identified in predominantly European-derived populations. These SNPs have not been extensively investigated for individual and cumulative effects on T2D risk in African Americans. Seventeen index T2D risk variants, including rs10923931 (NOTCH2), rs7578597 (THADA), rs1801282 (PPARG), rs4607103 (ADAMTS9), rs4402960 (IGF2BP2), rs10010131 (WFS1), rs10946398 (CDKAL1), rs864745 (JAZF1), rs13266634 (SLC30A8), rs10811661 (CDKN2A/B), rs1111875 (HHEX), rs7903146 (TCF7L2), rs2237892 (KCNQ1), rs5215 (KCNJ11), rs7961581 (TSPAN8/LRG5), rs8050136 (FTO), and rs4430796 (HNF1B) were genotyped in an 2652 AA cases with T2D and 1410 non-diabetic controls from the southeastern United States. Individual SNPs and cumulative risk allele loads were assessed for association with risk for T2D. Risk allele frequencies ranged from 8.6-99.9%. Eleven SNPs had odds ratios (OR) > 1 and five from ADAMTS9, WFS1, CDKAL1, JAZF1, and TCF7L2 trended or had nominally significant evidence of T2D association (P-value < 0.05). Individuals carried between 13 and 29 risk alleles. Cumulative risk was first assessed by simple counting of the number of risk alleles and statistically evaluating differences in cumulative risk scores between cases and controls. A second analysis weighted risk scores based on European-derived effect sizes (ln[OR]) from prior publications. Association was observed between T2D and increase in risk allele load; unweighted P=0.010, OR=1.04 (95% CI 1.01-1.08), weighted P=8.10x10-5, OR=1.06 (95% CI 1.03-1.10). When the number of TCF7L2 SNP rs7903146 risk alleles was included as a covariate, risk score was no longer associated with T2D in either model; unweighted P=0.33, OR=1.02 (95% CI 0.98-1.05); weighted P=0.40, OR=1.02 (95% CI 0.99-1.06). The trend of increase in risk for T2D with increasing risk allele load is similar to observations in European-derived populations; however, these do not contribute to T2D prediction beyond that of rs7903146 in African Americans. Although differences in risk allele burden exist between cases and controls, T2D genetic risk is primarily mediated through the impact of TCF7L2 in African Americans.

1326W

MTHFR C677T and MTHFR A1298C polymorphisms in Mexican patients with Gestational Diabetes Mellitus. *I.P. Davalos[1,2], K.I. Lares-Castellanos[3], J.P. Mena-Ramírez[1,2], M. Salazar-Páramo[4,5], E.L. Chávez-González[4,5], I.M. Salazar-Dávalos[6], M.G. González-Mercado[1,2], M.A. Aceves-Aceves[6], F. Grover-Páez[3].* 1) División de Genética, CIBO, Instituto Mexicano del Seguro Social (IMSS); 2) Doctorado en Genética Humana, IGH, CUCS, Universidad de Guadalajara; 3) UMAE, Hospital de Ginecología y Obstetricia, CMNO, IMSS; 4) División de Investigación, UMAE, HE, CMNO, IMSS; 5) Doctorado en Farmacología, CUCS, Universidad de Guadalajara; 6) Facultad de Medicina, CUCS, Universidad de Guadalajara, Guadalajara, Jalisco, México.

Introduction: Gestational Diabetes Mellitus (GDM) is a common medical condition during pregnancy. The MTHFR C677T and MTHFR A1298C polymorphisms have been associated with hyperhomocysteinemia, thrombotic and cardiovascular risk, vascular disease in Diabetes Mellitus type 2, pregnant women with obstetric complications and postmenopausal women. Objective: To determine the genotype (GF) and allele frequencies (AF) of the MTHFR C677T and MTHFR A1298C polymorphisms in a group of Mexican patients with GDM. Methods: A group of 49 pregnant Mexican patients with GDM according to ADA criteria (GDM Group) and a group of 54 pregnant Mexican patients without DM (Control Group) were typed for the MTHFR C677T and MTHFR A1298C polymorphisms by the PCR/RFLP's technique. Results: For MTHFR C677T GF, %(n), were in GDM Group: CC 28%(14), CT 41%(20), TT 31%(15); in Control Group CC 30%(16), CT 63%(34), TT 7%(4). AF allele C in GDM Group 49%(48), in Control Group 61%(66), allele T in GDM Group 51%(50) and in Control Group 39%(42). For MTHFR A1298C: GF, %(n) in GDM Group AA 59%(29), AC 33%(16), CC 8%(4), in Control Group AA 61%(33), AC 37%(20), CC 2%(1) AF allele A in GDM Group 76%(74) and in Control Group 80%(86) , allele C in GDM Group 24%(24) and in Control Group: 20%(22). Conclusions: Control Group showed an homozygous deficiency for allele T, the MTHFR 677TT genotype was associated with GDM (p=0.02). The MTHFR A1298C genotype frequency and allele frequency differences between both groups were NS (p>0.05). Additionally, the MTHFR 677CT and MTHFR 677TT genotypes were associated with macrosomic products and the 1298AA genotype with easier management of glycemic control in GDM patients.

1327W

Genetic variation in UBE2E2 is associated with risk of type II diabetes in a European population. *A. Dehghan[1, 2], E.J.G. Sijbrands[3], A. Hofman[1,2], A.G. Uitterlinden[3], J.C.M. Witteman[1,2].* 1) Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands; 2) Member of Netherlands Consortium for Healthy Aging sponsored by Netherlands Genomics Initiative, Leiden, Netherlands; 3) Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands.

Genome-wide association (GWA) studies on diabetes in Asians have identified new genetic loci including *KCNQ1*, *C2CD4A/4B* and *UBE2E2* that were not previously reported in Europeans. The association of type II diabetes with *KCNQ1* and *C2CD4A/4B* has also been shown in Caucasians, however, the association with *UBE2E2* in Caucasians remains to be studied. We examined the association of genetic variants in *UBE2E2* and 60kb up/downstream of the gene with risk of type II diabetes in the Rotterdam Study, a prospective population based cohort study of individuals living in Rotterdam, the Netherlands. The population for analysis comprised of 7464 Caucasian individuals of 55 years and older, including 1411 incident cases of type II diabetes. DNA samples were genotyped using the version 3 Illumina Infinium II HumanHap550 SNP chip array and were used to impute the genotypes of nearly two million SNPs in HapMap based on the observed haplotype structure. Given that the region included 508 SNPs, the significance threshold was set at 9.8×10^{-5} (0.05/508). The three *UBE2E2* candidate SNPs that were formerly reported in a Japanese GWA study (rs6780569, rs7612463, and rs9812056) were not associated with risk of type II diabetes (smallest p > 0.12). However, we found 57 SNPs in the region of *UBE2E2* to be significantly associated with risk of type II diabetes, of which rs7648425 was the most significant one (coded allele G; hazard ratio = 1.24 [95% confidence interval = 1.13 - 1.36]; $p < 4.8 \times 10^{-6}$). This SNP is close to rs7612463 which is one of the three Japanese candidate SNPs (distance = 9.9 kb, $R^2 = 0.03$). All significant SNPs were limited to the *UBE2E2* gene region. In conclusion, genetic variants in the *UBE2E2* gene region are associated with increased risk of type II diabetes in Caucasians. Lack of replication for specific SNPs in our study may be due to the difference in LD patterns between Japanese and Caucasian populations.

1328W

Three obesity-related loci are associated with insulin resistance independently of body mass index. *T. Fall[1], J. Ärnlöv[2], C. Berne[3], E. Ingelsson[1].* 1) Dept of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; 2) Department of Public Health and Caring, Uppsala University, Sweden; 3) Department of Medical Sciences, Uppsala University, Sweden.

Decreased tissue sensitivity to insulin (i.e. insulin resistance) and pancreatic beta-cell dysfunction are the two main physiological features of type 2 diabetes (T2D), and thus, an increased understanding of the genetic underpinnings of these conditions are of great importance in predicting and preventing T2D. Despite the rapid development within the field of diabetes genetics, few genetic variants associated with insulin sensitivity have been described, potentially due to the lack of cohorts examined with gold standard methods for insulin sensitivity assessment. There is a strong link between obesity and insulin sensitivity, and obesity susceptibility loci may also affect insulin sensitivity. A cohort of 71-year old non-diabetic men (ULSAM) underwent a euglycemic hyperinsulinemic clamp (n=926). The ULSAM cohort has undergone prior genotyping on the “Metabochip”, a custom iSELECT array, which is designed to interrogate ~195,000 SNPs of interest for cardiovascular and metabolic diseases. For this study, we extracted genotypes representing the 32 loci associated with body mass index (BMI) recently reported by the GIANT consortium. In 23 of 32 SNPs, the lead SNP was available. For the remaining 9 loci, the proxy SNP with highest r^2 was selected (based on phased haplotypes from HapMap rel21). For each locus, the effect allele reported by the GIANT consortium was set to be the effect allele also in our cohort. The effect of these loci on insulin sensitivity expressed as M/I-value (insulin-mediated glucose uptake divided by steady state plasma insulin concentration) was examined using linear regression techniques. The BMI-raising alleles at three loci (*NEGR1* () -0.22±0.1, p=0.03), *MTCH2* () -0.21±0.1, p=0.03) and *SH2B1*() -0.19±0.1, p=0.05)) were associated with decreased insulin sensitivity at a nominal significance (P≤0.05) even after adjustment for BMI. Our study supports earlier reports of *SHB21* to be of potential importance in insulin sensitivity, and suggests a role of *NEGR1* and *MTCH2*. Further studies combining several samples with gold standard measurements of insulin sensitivity to maximize statistical power are needed to further determine the genetic architecture of insulin sensitivity.

1329W

Fine-scale genetic mapping reveals multiple signals of association in type 2 diabetes susceptibility loci. *T. Ferreira[1], J. Yang[2], M.E. Goddard[3], A. Mahajan[1], T.M. Teslovich[4], N.W. Rayner[1,5], B.F. Voight[6], H. Khan[7], P.M. Visscher[2], M.I. McCarthy[1,5], A.P. Morris[1] on behalf of the DIAGRAM Consortium.* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; 2) Queensland Institute of Medical Research, Brisbane, Australia; 3) Melbourne School of Land and Environment, University of Melbourne, Melbourne, Australia; 4) Center for Statistical Genetics, University of Michigan, Ann Arbor, MI, USA; 5) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK; 6) Broad Institute of Harvard and MIT, Cambridge, MA, USA; 7) Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK.

There are now more than 40 established type 2 diabetes (T2D) susceptibility loci. However, these loci often extend over hundreds of kilobases and contain many genes with plausible functional impact on the disease. In most cases, only the lead SNP is reported for each locus. However, multi-SNP analyses may provide a better understanding of the contribution of genetic variation in each locus to disease susceptibility.

We considered 20,483 T2D cases and 55,630 controls from 24 cohorts of European descent and 1,178 T2D cases and 2,472 controls from a cohort of Pakistani descent. The samples were genotyped using the “Metabochip”, a custom iSELECT array containing ~195,000 SNPs, designed to support replication and fine-scale mapping of established loci for T2D and other cardio-metabolic traits. We also considered GWAS samples imputed up to 2.5M SNPs (17,791 T2D cases and 50,337 controls from 12 cohorts of European descent). We tested for association of each SNP (MAF>1%) with T2D in each cohort and combined results via fixed-effects meta-analysis. We performed approximate conditional analysis using summary statistics from the meta-analysis and the correlation between SNPs obtained from Metabochip genotype data from a reference cohort of 1,117 T2D cases and 4,224 controls from the Warren 2 Repository and 1958 British Birth Cohort.

We identified three independent signals of association (conditional $p<5\times10^{-8}$) at the *KCNQ1* locus: rs231362 ($p=3.1\times10^{-12}$), rs74046911 ($p=6.8\times10^{-9}$) and rs163184 ($p=9.2\times10^{-9}$). We also observe two weakly correlated (CEU $r^2=0.06$) signals of association at the *ANKRD55/MEKK1* locus: rs459193 ($p=8.2\times10^{-15}$) and rs3843467 ($p=1.8\times10^{-8}$). At the *CDKN2A/B* locus, we observe two correlated signals of association (CEU $r^2=0.36$) which have been previously shown to tag three clades of haplotypes: rs10811661 ($p=4.3\times10^{-53}$) and rs10757283 ($p=7.5\times10^{-32}$). At these three loci, including all independent signals of association increases the explained sibling risk ratio from 1.003 to 1.011. These results highlight the importance of fine-mapping to fully reveal the genetic architecture of T2D associations within established loci, and thus show promise for furthering our understanding of the biological mechanisms underpinning susceptibility to the disease.

1330W

Mutational spectrum in a sample of Mexican patients with Hunter syndrome (MPSII): A preliminary report. *M. Alcantara, A. González -del Ángngel, L. Fernández-Hernández, B. García de Teresa B.* Dept Genetica, Inst Nacional Pediatria, Mexico City, Mexico.

INTRODUCTION: Over 300 pathogenic mutations have been described in IDS gene leading Hunter syndrome (MPSII). The mutational spectrum of Mexican MPSII patients isn't well known. OBJECTIVE: Identification of IDS mutations in a sample of Mexican patients with a clinical and enzymatic diagnosis of MPSII. MATERIAL AND METHODS: DNA samples from 7 male non-related MPSII patients (2 familial and 5 sporadic) and their mothers were analyzed by a PCR-RFLP assay to identify the IDS/IDS2 inversion and by full sequencing of IDS gene. RESULTS: Six different mutations were ascertained: p.His335Tyr, p.Asp45His, c.508-1G>C, the IDS/IDS2 inversion, a complete deletion of IDS that extends to the IDS2 and FMR2 genes and one complex IDS rearrangement not yet full characterized involving a deletion of exons 4 to 7. Carrier status for p.His335Tyr and the IDS complex rearrangement was confirmed in two pregnant mothers and prenatal diagnosis (PD) was offered in both. CONCLUSIONS: A genotype-phenotype correlation still can't be attempted. However it can be noted that the 3 patients with large rearrangements show classic phenotypes. Large IDS rearrangements are observed in the 10% MPSII cases, yet here they represent 42% (3/7), this last might be due to the small size of our sample. Large deletions that eliminate IDS/IDS2 and FMR-2 genes lead to a modified phenotype featuring seizures and ptosis; however these clinical features were absent in our patient. The p.His335Tyr mutation is still not reported. Availability of molecular diagnosis for MPSII in Mexico opens the possibility of PD and early ERT.

1331W

Screening for MPS VI in newborns on a high-incidence area of Northeast Brazil. *F. Bender[1,2], T. Amorim[3,4], A.X. Acosta[5,6], F. Costa-Motta[1], A. Purificacao[3], M.G. Burin[1], R. Giugliani[1,2,7,8], S. Leistner-Segal[1,2].* 1) Med Gen Service, HCPA, Porto Alegre, RS, Brazil; 2) Postgraduate Program in Medicine: Medical Sciences, UFRGS, Porto Alegre, RS, Brazil; 3) APAE, Salvador, BA, Brazil; 4) Esc Bahiana Med Saúde Pública, Salvador, BA, Brazil; 5) FIOCRUZ, Salvador, BA, Brazil; 6) Dep Pediatrics, UFBA, Salvador, BA, Brazil; 7) Dep Genetics, UFRGS, Porto Alegre, RS, Brazil; 8) INAGEMP, Porto Alegre, RS, Brazil.

Mucopolysaccharidosis VI (MPS VI, or Maroteaux-Lamy syndrome) is caused by the deficiency of the lysosomal enzyme N-acetylgalactosamine 4-sulfatase(ARSB). This deficiency causes the storage of dermatan sulphate in tissues, leading to a progressive and severe bone dysplasia and to problems in many organs and systems. MPS VI is a very rare condition, which was a relatively high incidence (13 cases identified so far) in the county of Monte Santo (50,000 inhabitants), in Bahia state, Northeast region of Brazil, where a common mutation (H178L) was found in all cases. As MPS VI could be treated with ERT and as there are indications that a better outcome may be expected in early treated cases, a newborn screening program for this condition was set up on this specific area. To the program already in place for PKU, hypothyroidism and hemoglobin disorders, screening for MPS VI was added by ARSB activity assay and by the detection of the common mutation, both performed on DBS. The standardization of the techniques of enzyme assay, DNA extraction and mutation detection were already completed and the test on newborn samples has started on January 1st, 2011. The possibility of detecting carriers will help to calculate the expected frequency of this disease in the area, and will also be a tool to target genetic counseling to the more susceptible families.

1332W

Screening for MPS IV A in dried blood spots with a practical and reliable fluorimetric method. *M.V. Camelier[1,2], G.E.S. Civallero[1], J.F Mari[1], G. Marasca[1], T.A. Vieira[1,2], M.G. Burin[1], R. Giugliani[1,2,3].* 1) Med Gen Service, HCPA, Porto Alegre, RS, Brazil; 2) Postgraduate Program in Medicine: Medical Sciences, UFRGS, Porto Alegre, RS, Brazil; 3) Dep. Genetics, UFRGS, Porto Alegre, RS, Brazil.

Mucopolysaccharidosis IV A (MPS IV A) is a rare autosomal recessive disease caused by deficiency of the lysosomal enzyme N-acetylgalactosamine-6-sulfatase(GALNS),resulting in storage of keratan-sulfate in many tissues and organs. This accumulation causes a severe skeletal dysplasia with short stature,and affects the eye, heart and other organs. Clinical trials with ERT for this disease are in progress. We describe an innovative fluorometric method for the assay of GALNS in dried blood spots (DBS). We used DBS as the enzyme source and compared it with leukocytes,having studied 25 MPS IVA patients and 54 healthy controls. We optimized the assay conditions in DBS, including incubation time and stability on different storage temperatures and along time. Results in DBS were compared to the ones obtained in leucocytes using the standard technique. The described fluorescent methodology was validated in our laboratory and the assay was found sensitive and specific,allowing reliable detection of MPS IVA patients. The use of DBS simplifies the collection and transportation,and is especially useful for testing patients from more remote areas of large countries,and when samples need to cross country borders. We believe this assay could be easily incorporated by reference laboratories and play a role in the screening for MPS IVA, contributing to the earlier detection of affected patients.

1333W

Variation in Heat-Shock 70-kDa Protein 5 (*HSPA5*) is Associated with Longitudinal Change in Body Fat. *M.H. Black[1], R.M. Watanabe[2], M. Takayanagi[1], E. Trigo[3], J. Hartiala[4], H. Allayee[4], J.M. Lawrence[1], T.A. Buchanan[3], A.H. Xiang[1].* 1) Department of Research & Evaluation, Kaiser Permanente Southern California, Pasadena, CA; 2) Department of Preventive Medicine, Division of Biostatistics, Keck School of Medicine, University of Southern California, Los Angeles, CA; 3) Department of Medicine, Division of Endocrinology, Keck School of Medicine, University of Southern California, Los Angeles, CA; 4) Institute for Genetic Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA.

ER stress in adipocytes may play a significant role in obesity. *HSPA5* is a mediator of the adaptive unfolded protein response elicited by ER stress and highly expressed in adipose tissue. *Hspa5* $^{+/+}$ mice have increased fat mass and greater insulin resistance than *Hspa5* $^{-/+}$. The contribution of *HSPA5* to changes in human body composition has not been investigated. We screened the *HSPA5* gene region in participants of BetaGene, a family-based study of obesity, insulin resistance and beta cell function in Mexican Americans. Body fat was assessed by DEXA. Insulin sensitivity (S_I), acute insulin response (AIR) and disposition index (DI) were estimated from the frequently sampled intravenous glucose tolerance test (FSIVGTT) with Minimal Model analysis. We hypothesized that variation in *HSPA5* may be associated with longitudinal changes in body fat percentage, with concomitant effects on other glucoregulatory phenotypes. We genotyped 6 SNPs in the *HSPA5* gene region, of which 3 had minor allele frequency (MAF) > 5% (MAF range: 11-26%). We tested these 3 SNPs for association with longitudinal change in phenotypes in a subset of 228 BetaGene participants (mean baseline age: 35 ± 8 years; 69% female) with follow-up phenotyping (mean length of follow-up: 4.5 ± 1.4 years). Analyses were performed assuming a dominant genetic model adjusted for baseline age and sex. All analyses were conducted using SOLAR (v 4.3.1). P-values are Bonferroni-corrected for the number of SNPs tested. The rs12009 C allele and rs391957 T allele were significantly associated with an increasing rate of change in body fat percentage (corrected p = 0.012 and 0.033, respectively). On average, carriers of the rs12009 C allele had a significantly greater increase in body fat percentage over follow-up than non-carriers (mean increase: 0.29 vs. 0.01 percent body fat per year, respectively). SNP associations with rate of change in BMI were similar in direction to those for body fat percentage. Consistent with an increase in adiposity, there was suggestive evidence for association between the rs12009 C allele and a decreasing rate of change in SI (nominal p=0.027). These data suggest that variation in *HSPA5* may contribute to increased adiposity and associated insulin resistance over time in Mexican Americans.

1334W

Novel associations for hypothyroidism include known autoimmune risk loci. *B.T. Naughton[1], J.Y. Tung[1], A.K. Kiefer[1], D.A. Hinds[1], U. Francke[1,2], J.L. Mountain[1], C.B. Do[1], N. Eriksson[1].* 1) 23andMe.com, Mountain View, CA; 2) Department of Genetics, Stanford University, Stanford, CA.

Hypothyroidism is the most common thyroid disorder, affecting about 5% of the general population. Here we present the first large genome-wide association study of hypothyroidism, in 2,564 cases and 24,448 controls from the customer base of 23andMe, Inc., a personal genetics company. We identify four genome-wide significant regions, two of which are well known to be involved with a large spectrum of autoimmune diseases: rs6679677 near PTPN22 and rs3184504 in SH2B3 (p-values 3.5E-13 and 3.0E-11, respectively). We also report associations with rs4915077 near VAV3 (p-value 8.3E-11), another gene involved in immune function, and rs965513 near FOXE1 (p-value 3.1E-14). Of these, the association with PTPN22 confirms a recent small candidate gene study, and FOXE1 was previously known to be associated with thyroid-stimulating hormone (TSH) levels. Although SH2B3 has been previously linked with a number of autoimmune diseases, this is the first report of its association with thyroid disease. The VAV3 association is novel.

1335W

Cognitive testing in patients with Fabry disease: a pilot study using a computerized, self-administered tool. *D. Elstein[1], G.P. Doniger[2], E. Simon[2], G. Altarescu[3].* 1) Gaucher Clinic, Shaare Zedek Medical Ctr, Jerusalem, Israel; 2) NeurTrax Corporation, New York, USA; 3) Genetics Institute, Shaare Zedek Medical Ctr, Jerusalem, Israel.

Background: Recently, cognitive profiles of Israeli patients with Fabry disease highlighted neuro-cognitive impairment not solely due to a chronic, pain-ridden disease. Neuro-cognitive tests are time-consuming and patients often are non-compliant. A validated standardized computerized tool for neuro-cognitive testing which is user-friendly and supplies clinically-relevant information was tested. Methods: All males and females with enzymatic and/or molecular diagnosis of Fabry disease seen in our clinic underwent assessment with the Fabry-specific Mainz Severity Score Index (MSSI) with sub-scores (neurological, renal, cardiac, and general) and a MindStreams neuro-cognitive battery (1 hour) for mild impairment evaluating memory, executive function, attention, information processing, visual spatial processing, verbal function, and motor skills; a Global Cognitive Score (GCS) was also computed. Results: Ten patients (3 males, 7 females) were tested (mean age: 41.5 (25-56) years). Males were younger, had moderate nephropathy, and none had had a cerebrovascular accident (CVA). The range of MindStreams GCS among the males was 85.6-107 points. Three females had mild to moderate neurological impairment as reflected by MSSI neurological sub-scores of 8,10, and 15 points; two females had had a CVA in the past. The range of MindStreams GCS among the fe males was 59-107.7 points. Below-average performance was prevalent, particularly in information processing and motor skills, often in a range consistent with mild impairment Average GCS in females (90.3 points) was lower than in males (98.2 points). For individual patients, performances was poorest in information processing (n=4), attention (n=2), motor skills (n=2), verbal function (n=1), and visual spatial (n=1). Conclusion: MindStreams may be useful for rapid cognitive assessment in Fabry disease. Affected domains in Fabry disease were different than in patients with other lysosomal diseases but comparable to those reported by others in Fabry disease using time-intensive batteries.

1336W

Evaluation of plasma globotriaosylsphingosine in patients with Anderson - Fabry disease in Brazil on enzyme replacement therapy with agalsidase alfa. *W. Marques Jr[1], O.M. Vieira Neto[1], M. Moysés Neto[1], I.M. Furquim[2], A.C. Paula[2], J.C. Coelho[3], C.M. Lourenco[1].* 1) Hospital das Clinicas de Ribeirao Preto - USP, Ribeirao Preto, Sao Paulo, Brazil; 2) Hospital Municipal da Criança, Guarulhos, Sao Paulo, Brazil; 3) Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Rio Grande do Sul, Brazil.

Introduction: Anderson-Fabry disease (AFD) is an inherited disorder due to an enzymatic deficiency of alpha-galactosidase activity, resulting in glycosphingolipid accumulation. As enzyme replacement therapy (ERT) involving recombinant enzyme has been introduced for this disease, finding a reliable surrogate biomarker for monitoring the therapy is very important for evaluation of benefits and better follow-up of patients on ERT. Material and Methods: We studied 06 Fabry patients four males and two females) over a period of 6 months regarding disease progression and clinical outcome under ERT. Plasma globotriaosylsphingosine (lysoGb3) levels were measured in dried blood spots before and 06 months after treatment with agalsidase alfa (Replagal®). Results: Both male and female patients had elevated levels of lysoGb3; in the males subjects, the increase was impressive before starting treatment. After 6 months, plasma lyso Gb3 were reduced in all patients; in males, plasma lyso Gb3 dropped more dramatically than in the females subjects. Pre-treatment plasma lysoGb3 concentrations of Fabry females were relatively low but remained stable or decrease after ERT. At baseline, only only two patients showed abnormal cardiac findings (mild LVH and mitral valvulopathy, respectively). Brain MRI with angiography was abnormal in two patients (multiple hypersignal loci with arterial tortuosity and periventricular white matter changes). Proteinuria and microalbuminuria were present in all patients; after 6 months under ERT, brain MRI changes remained stable and no further kidney deterioration was seen. Cardiac evaluation still showed no abnormal findings in the other patients and remained unchanged in the other two. Conclusion: Our Anderson-Fabry cohort showed reduction of plasma lysoGb3 after ERT introduction. Although it was not possible to evaluate differences in other systems because of the short period of observation, our finding confirms previous studies which show that ERT can correct plasma lysoGb3 in Fabry patients even in a short period of 6 months.

1337W

Enzymatic characterization of *ALDH7A1* (antiquitin) missense mutations associated with pyridoxine dependent epilepsy. *M.B. Coulter-Mackie[1,2], Q. Lian[1], A. Li[2], E. Struys[1,3], S. Stockler[1], P.J. Waters[1,2].* 1) Dept Pediatrics, Univ British Columbia, Vancouver, BC, Canada; 2) Dept Pathology and Laboratory Medicine, Univ British Columbia, Vancouver, BC, Canada; 3) Dept Clinical Chemistry, VU Medical Center, Amsterdam, Netherlands.

Pyridoxine dependent epilepsy (PDE) is an autosomal recessive disorder causing seizures that are uncontrollable by standard antiepileptic medications but which typically respond to pyridoxine (vitamin B6). PDE results from mutations in the *ALDH7A1* gene, which encodes alpha aminoadipic semialdehyde (AASA) dehydrogenase, also known as antiquitin (ATQ). In order to determine post-translational effects of disease-associated missense mutations on ATQ enzyme function, we established an automated assay of enzyme activity using the natural substrate, AASA.

Methods: Missense mutations in a cloned copy of human *ALDH7A1* were generated by site-directed mutagenesis and expressed in E. coli. We developed a novel source of the substrate using a cloned gene for a fungal lysine aminotransferase to produce AASA from lysine and 2-ketoglutarate. Enzymatic activity was determined using an NAD-dependent spectrophotometric kinetic assay monitored by an autoanalyzer.

Results: We have thus far studied the wild-type enzyme and three missense mutants, p.R307Q, p.E399Q and p.S430N. Levels of ATQ protein expression, visualized by SDS-PAGE, were similar for all. Assay linearity with protein and the lower limit of detection of ATQ enzymatic activity were established with the wild type. Activity for each of these three mutants was below the limit of detection (<1%).

Discussion and Conclusions: The lack of activity associated with these three mutations in our *in vitro* system is supportive of their pathogenicity *in vivo*. For the common p.E399Q mutation our findings are consistent with a previous report based on expression in CHO cells. The other two mutations have not previously been studied. We are in the process of characterizing additional mutations, some associated with variant late onset clinical phenotypes. Our method offers a simple rapid first step in evaluating the post-translational effects of missense mutations on the function of ATQ and their correlation with PDE.

Financial support for this project was provided by CURE (Citizens United for Research in Epilepsy) and the Savoy Foundation.

1338W

Two Cases of Congenital Disorders of Glycosylation Type II Without Detectable Defect in Conserved Oligomeric Golgi Subunits. *D. Handa[1], R. Gilbert[2], B. Ng[3], M. Kozenko[1], M. Ssybowska[1], H. Freeze[3], C. Li[1].* 1) McMaster University Medical Center, Hamilton, Canada; 2) Southampton General Hospital & University of Southampton, UK; 3) Sanford-Burnham Medical Research Institute, USA.

Congenital disorders of glycosylation (CDG) are autosomal recessive multi-system conditions showing defective glycosylation. To date, 29 mutated genes are known to cause CDG. The best diagnostic test for CDG is analysis of serum transferrin glycosylation status using iso-electric focusing (TIEF) or mass spectrometry. Both methods distinguish two major categories: Type I due to absence of entire glycan chains and Type II due to incomplete glycan chains. There are 18 subtypes in type I and 11 subtypes in type II disorders. For most subtypes of type II, only a few cases have been reported and the defects usually involve one of the components in the Conserved Oligomeric Golgi (COG) subunits. We present here two patients with abnormal TIEF patterns consistent with type II CDG. Patient 1 was a full term boy diagnosed with CDG type II at 6 months of age due to clinically suspected seizure, prolonged neonatal jaundice, rhizhomelic short stature, central hypotonia, failure to thrive, GI bleeding, thrombocytopaenia and nephrotic syndrome with transient, unexplained renal failure which recovered spontaneously. Patient 2 is an infant girl who also has central hypotonia, rhizhomelic short stature, failure to thrive, and a history of GI hemorrhage. In addition, she has inverted nipples, adducted thumbs, 2-3 toe syndactyly bilaterally and retinitis pigmentosa. Further testing of Patient 2 using COG-specific antibodies and brefeldin A treatment showed normal COG subunit expression and function. Patient 1 is undergoing further investigation. Whole Exome Sequencing is also underway to identify the pathological genetic changes in these patients. Prior to receiving their diagnosis of CDG type II, both patients were considered for a host of other genetic and metabolic disorders, in part due to the extremely low prevalence of CDGs, particularly CDG type II. These two cases therefore draw attention to this group of potentially under-diagnosed conditions. The fact that all COG subunits are normal in patient 2 also further highlights the genetic heterogeneity underlying this extremely rare group of disorders.

1339W

Genetic Counseling of Donor Gamete Use: The Problems Presented By Donor Anonymity. *J.L. Lauzon[1, 2], J.R. Guichon[2], I. Mitchell[2, 3].* 1) Medical Genetics, Alberta Children's Hospital, 2888 Shaganappi Trail NW Calgary, Alberta Canada T3B 6A8; 2) Faculty of Medicine, University of Calgary, 3330 Hospital Drive NW Calgary Alberta Canada T2N 4N1; 3) Department of Respirology, Alberta Children's Hospital, 2888 Shaganappi Trail NW Calgary, Alberta Canada T3B 6A8.

It is accepted practice in genetic counseling to present as an option to certain patients the use of donor gametes to conceive a child. Authors of standard genetics textbooks state that there can be “genetic indications” for using donor gametes, including when a prospective parent has an autosomal dominant, autosomal recessive or X-linked disorder, or has a heritable chromosome defect. Yet, in the United States and Canada, most gamete donations remain anonymous. Therefore, when geneticists present gamete donation as an option, they are, albeit perhaps inadvertently, presenting a procreative practice that can make it almost impossible for geneticists to obtain a complete family history of the resulting offspring. The medical consequences of anonymous gamete donation as well as a recent Canadian Court decision that banned donor anonymity will be explored with a view to suggesting richer practice guidelines.

1340W

Genetic diseases in the next edition of the International Classification of Diseases: challenges and opportunities. *S. Ayme, A. Olry, B. Bellet, A. Rath.* INSERM, SC11 /Orphanet, Paris, France.

The classification of diseases which is used worldwide and by a wide range of stakeholders is the International Classification of Diseases (ICD10). Most genetic diseases are absent in ICD10 and those with a specific code are often misclassified. As a consequence, morbidity and mortality due to genetic diseases is invisible in health information systems. To overcome this difficulty, Orphanet (www.orpha.net) has established a partnership with WHO to ensure a fair representation of rare diseases in general. Orphanet has collected all published expert classifications and established a database of phenotypes indexed with ICD10 codes, MIM codes, genes, mode of inheritance, age of onset and class of prevalence. Phenotypes are assigned to as many classification systems as necessary to represent them. The visualisation of the classification systems and of the place of each disease within the classification is available on the Orphanet website. The Orphanet nomenclature of rare diseases is a stable one, directly exploitable by information systems and freely accessible at orphadat.org. A Topic Advisory Group on rare diseases has been established to manage the revision process at WHO. The whole community of experts is involved in the validation process. Revised chapters follow a primarily clinical approach, only secondarily an aetiological one, up to the gene level. When several possible names are possible for a disease, descriptive names formed in accordance with a clinical approach are preferred. Every entity is assigned a unique identification number. Rare diseases affecting several body systems are included in every relevant chapter, as ICD11 will be poly-axial, but a main code is proposed to allow for linearisation, according to the most severe involvement and/or the specialist most likely to be relied on for disease management. In some cases, the choice is open to debate. The genetic disease community is invited to take an active part as the results will condition the visibility of all activities in the field. All the revised chapters open for comments are available on the EUCERD website at www.eucerd.eu. The alignment of the Orphanet nomenclature with OMIM poses the question of the fair representation of genetic diseases and of the genetic contribution of genomics to disease definition, in relation with the needs of the end-users.

1341W

Digi-ID: From Researcher/Resource Traceability to Participant De-identification. *M. Deschenes[1], A. Brookes[2, 7, 8], P. Burton[6, 2, 8], A. Cambon-Thomsen[3], J. Kaye[4], B.M. Knoppers[5, 1], L. Mabile[3], M. Murtagh[6, 8], A.-M. Tassé[5], G. Thorisson[2, 7], S. Wallace[6, 8], BRIF working group.* 1) P3G Consortium, Montreal, Canada; 2) Department of Genetics, University of Leicester, UK; 3) Inserm UMR 1027 and Université de Toulouse, France; 4) Oxford Department of Public Health, University of Oxford, UK; 5) Centre of Genomics and Policy, McGill University, Canada; 6) Department of Health Sciences, University of Leicester, UK; 7) Gen2Phen Project; 8) Data2Knowledge Center, University of Leicester.

Effective data sharing serves to accelerate biomedical science by optimising the availability of extant information and its use in generation scientific insights. But data sharing also raises challenges, such as how to ensure fair use of the shared information, and how to acknowledge contributions in consistent and transparent manners. It also revives existing privacy concerns. Can digital identifiers enhance global research and make it more efficient? Through Digi-ID, P3G brings together innovative projects to explore these challenges: 1)(BRIF)Bioresource Research Impact Factor (C.-Thomsen et al.)BRIF proposes solutions to the problem of the traceability and the recognition of the contribution of research resources to discovery science. It will provide a global register cataloguing unique identifiers for biobanks, databases, and repositories. Such a unique identifier would acknowledge use of the resource in publications and grants and enable such resources to establish frequency of use. 2)Using Open Researcher and Contributor ID (ORCID) developments to enhance data utility (Brookes et al.). ORCID aims at solving the name ambiguity problem in scholarly communication by creating “a central registry of unique identifiers for individual researchers and an open and transparent linking mechanism between ORCID and other current author ID schemes”. Global identification infrastructure exists for content but not for the producers of that content, creating challenges in establishing the identity of authors/contributors and reliably linking them to their published works. We foresee extensions to ORCID system use in areas such as the unambiguous tracking and accrediting of data sharing, and streamline IT solutions for ensuring control of data access. 3)DataSHIELD (Data Aggregation Through Anonymous Summary-statistics from Harmonized Individual levEL Databases) (Burton et al.)Social expectations of access, security, privacy and transparency and the consequent ethico-legal imperatives shape how we use, share and analyse data. DataSHIELD provides a key solution to new challenges by enabling pooled data analysis to be carried out across several collaborating studies as if one had full access to all of the individual level data. But, under a `parallelized' analysis these data remain completely secure on the host computers at their home bases. In effect, full individual-level meta-analysis is realised without breaching local law or research governance requirements.

1342W

Human Genome shares genes from lower plants and animals:Patenting genes is biologically undesired !! *H.K. GOSWAMI.* Genetics Department, Retired from Bhopal University, BHOPAL, Madhya Pradesh, India.

Genes are being cloned and patented which amounts to copyright of human genes, their forms and functions. Many chromosomes have several patented loci and within a decade or so, we shall have entire chromosomes patented. Hereunder this is argued, that the genes “cloned” and patented, are not exclusively human; exact copies of these genes (DNA sequences) are also present in other organisms. This has been aptly demonstrated by now, that human X and Y chromosomes and other chromosomes too, have evolved by sharing and transferring DNA sequences at various stages of evolutionary steps from diversified groups of organisms. Lately, recent data have even suggested with molecular evidences reagrding the presence of long identical DNA stretches among plant sex chromosomes and human Y chromosomes. Patent for a gene can not amount to patent of that gene present in other organisms; this needs serious scientific review. Apart from this, the patented gene is a chemical copy/pirated gene of the original gene present in a chromosome. How can a copy of the gene (man made) in the laboratory lead to patenting of an original gene present in a chromosome ? This may be a violation of legal, scientific, as well as ethical values. Technically, the genes owned in test tubes can not hold patent for the genes / chromosome domains in situ, because these are not exactly the same.

1343W

Indication of legal abortion in genetic disorders in Iran. *m. amirian[1], z. nafei[2].* 1) Yazd legal medicine organization, Yazd, Iran, yazd, Iran; 2) Yazd medical science university, Yazd, Iran.

Multiple congenital anomalies are common disorders that may be occur in fetus due to acquired, genetical or multifactorial causes.Whenever pregnant women or fetuses are seriously suffering from critical disorders it can lead to infirmity, before 16 weeks from last menstrual period, therefore the legal abortion is recommended . This subject in Iran would be done by requesting from judicial authorities.It would be accepted by three experts who are related to fetus or pregnant women disorders, then the legal abortion is issued by the legal medicine organization and eventually the legal abortion will be done by Obstetrics and gynecologist in public hospital. This case was referred to the legal medicine organization for the permission of legal abortion. Nuchal fold thickness were found by sonography and the amniocentesis were found 47xx+21 too that has shown Down syndrome in female fetus. The proband was gravid 2 and the result of non consanguineous marriage. The previous pregnancy lead to abortion . father and mother age are 36 and 27 years respectively. Bioethics is the most important concept in medical evaluation and legal aspects which is admitted in Islamic parliament of Iran. A recent research has shown that there are important conflicts in main branches of Islam that is related to the legal abortion.This subject is exist in different religions too . any disorders which can lead to infirmity or critical condition for pregnant woman is an issue related to the legal medicine organization in Iran and the legal abortion is issued.

1344W

PERSPECTIVES ON AFRICAN GENOMICS COMMOTION AND ADVANCEMENT: THE NIGERIAN CASE STUDY. *V.A. Counted[1, 2].* 1) Publication & Research, Counted for Christ International , Lagos, Nigeria; 2) Publication, Internet Missionary Journal, Berlin, Germany.

In March 2002, the Advisory Committee on Health Research of WHO issued a report on the implications of genomics in the developing countries, and the role they have to play (as was done for the Hispanics) in ameliorating a fractional imbalance while also aiming at harnessing global genomics. However, in recognizing the potential of genomics in improving health, the unprecedented advances in the science of genomics and its actions, plus its widely predicted revolutionary prevarication - as we look at these realities, how then can we present and apply this scientific knowledge to our global world? Also, while proffering solutions in some genomic problem domain via culminating a positive evolvement of human genome, where do developing countries (for example Nigeria) stand in this revolutionary emancipation? As we mull over this thought, the question of geographical negligence troubles the mind and quakes one to vomit truth. These truths will be exclusively uncovered and harkened to by applying the research questions of “What”, “Why”, and “How” - just as follows: 1. WHAT is the future or hope of maintaining a univocal or unified balance in global managerial genomics, and what should be the expected contributions of these developing countries in advancing the science of genomics? 2. Since the under-developed countries have over the years lacked trained and qualified bioethicists, scientists, geneticists and sufficient regulatory infrastructures, to deal with genomic issues, (a.) WHY is it so important for this “lack” to be met? And (b.) WHY is the discipline of genomics typical to the developed countries and then atypical to under-developed countries? Also (c.) WHY are students of these developing countries (like Nigeria) not embracing this concrete discipline? 3. HOW can we come in as Individuals, Research bodies, Scholars, Bioethicists, Scientists, Researchers, Medical Practitioners, and Geneticists, to workout this problem of parity, to give scientism hope, and rekindle the joy of many in these under-developed nations where the discipline of genomics has been condoned for hollow reasons? The researcher hopes to challenge and engage folks with these resolving questions in order to bring about a balanced polarity in global genomics healthcare through ethical, cultural, empirical, theoretical and comparable evidences.

1345W

Simons VIP Connect: The power of an online community for research recruitment. *W. Faucett[1], A. Bibb[2], E. Hanson[5], D. Ledbetter[1], C. Martin[2], A. Paal[2], T. Page[2], E. Sherr[4], J. Spiro[3], J. Tjernagel[3], W. Chung[6], Simons Variation in Individuals Project.* 1) Genomic Medicine Institute, Geisinger Health System, Danville, PA; 2) Department of Human Genetics, Emory University, Atlanta, GA; 3) Simons Foundation, New York, NY; 4) University of California at San Francisco, San Francisco, CA; 5) Harvard University, Boston, MA; 6) Columbia University, New York, NY.

Recruitment, engagement and retention of study participants are challenges for most genetic research studies, but modern methods of information provision and social networking have dramatically changed the opportunities for recruitment for rare genetic disorders. Simons VIP Connect (www.simonsvipconnect.org) was created as an online community to recruit individuals and families for the Simons Variation in Individuals Project, a study of patients with 16p11.2 deletions and duplications. The second goal of the online community is to provide support and information to families. Contact with families and the enrollment process is managed by two genetic counselors with a website for online registration and a toll-free telephone number available for interested families without internet access. The online community provides information about 16p11.2 deletions and duplications; discussion forums; literature summaries; helpful links, webinars; and an option to “ask an expert.” Participants complete a ten question survey during the registration process and can review the aggregate responses of all registered participants. Our recruitment strategies have included: directed traffic from Google ads, links from other chromosomal disorder patient advocacy websites, Facebook, referrals from molecular cytogenetics laboratories, and referrals from genetic counselors and geneticists we informed through direct mailings. The website was launched on 08/27/2010 and 162 individuals were registered as of 05/30/2011 for an average of 4 new participants per week. This includes 40 registrants from outside North America and 122 from the US and Canada (101 families). The majority of participants with deletions are between age 3 and 17 and the majority of participants with duplications are between ages 3 and 7. In the registration survey participants provided the following sources as to where they first heard about the online community: genetic counselors 28%; Facebook 23%; internet searches 23%; patient advocacy groups 10% and medical geneticist and another family were less than 10%. Of qualified families, approximately 80% agree to participate in the research study. We have found developing an online community to be an effective recruitment tool which facilitates participant engagement and is associated with high participation rate. This may be an effective tool for others to consider when recruiting and retaining study participants for rare conditions.

1346W

Stakeholders' perspectives of genetics in primary care: Will patients “slip through the cracks”? *L.E. Forrest[1], B.J. McClaren[2], S.A. Metcalfe[2], J.M. Hodgson[2], J. Emery[3].* 1) APHCRI, Australian National University, Canberra, Australian Capital Territory, Australia; 2) Genetics Education and Health Research, Murdoch Childrens Research Institute, Parkville, Victoria, Australia; 3) School of Primary, Aboriginal and Rural Health Care, University of Western Australia, Crawley, Western Australia, Australia.

Rapid developments in genetic technologies will change practice in all specialties of medicine, including primary care, due to the constant generation of new genetic/genomic information, genetic tests and screening procedures. However the ability of general practitioners (GPs) to practise genetic medicine is not only dependent on adequate education but on understanding how genetic medicine can be appropriately integrated in this healthcare setting. There is concern that consumers' genetic needs will not be met by their primary care provider. The aim is to examine how genetics is currently managed in primary care from the perspective of primary care providers, genetic health professionals and consumers, and whether changes to practice will be required in the future. A qualitative approach was taken with stakeholders including primary care representatives, genetic health professionals and health care consumers. Data were collected by semi-structured interviews addressing current practice of genetics in primary care; approaches to referrals and management; views on changes to practice and suggestions for ideal and acceptable practice; models which might deliver genetics in primary care. Thematic analysis was conducted on the transcribed interviews to identify key concepts and ideas. Fourteen interviews have been conducted to date with GPs, practice nurses, genetic counsellors, professional organisation representatives and consumers, with more planned during this year. Preliminary findings suggest that GPs willingly engage genetic health professionals for genetic support and advice as necessary. The generalist nature of general practice, a heavy workload, and time constraints were recognised by all participants as barriers to routine integration of genetics into primary care. Concern exists that patients requiring genetics care may “slip through the cracks”, especially with regard to the urgency of testing in pregnancy. Nevertheless, responses were mixed regarding whether practice will need to change to successfully integrate genetics into primary care and various models of practice were discussed.

1347W

Knowledge and attitudes toward medical genetic services and the new genetic technologies in the Cuban population. *B. Marcheco-Teruel[1], I. Rojas-Betancourt[2], E. Fuentes-Smith[1], N. González-Lucas[3], L. Martínez-Rey[2], A. Lantigua-Cruz[3], O. Mors[4].* 1) Genetic Epidemiology, National Center of Medical Genetics, Havana, La Habana, Cuba; 2) Clinical Genetics Dept, National Center of Medical Genetics, La Habana, Cuba; 3) Consultant, National Center of Medical Genetics, La Habana, Cuba; 4) Center for Psychiatric Research, Aarhus Psychiatric Hospital, Aarhus University, Denmark.

The application of knowledge gained from the characterization of the human genome holds considerable potential for the development of new health care innovations over the next years. The information generated by genomics research may have, in short and long-term, major benefits for the prevention, diagnosis and management of genetic and common diseases. In the last 8 years we have systematically explored the knowledge and attitudes toward medical genetic services and the benefits of new technologies for diagnosis and research of genetic diseases, in the Cuban population. 10630 individuals from 4874 families in contact with genetic services living in 97 out 169 Cuban municipalities were interviewed. 85% participants expressed they are satisfied with medical genetic services at the primary care level. We also explored their opinions about priorities for medical genetic services in the country: 9035 respondents considered primary and secondary prevention of genetic diseases should be the main priority; 75.4% considered that genetic researches should be also a priority in order to increase the benefits of medical genetics for public health; the need of offering medical and social support to families and individuals with genetic disorders and disabilities they may cause, was recommended as four priority by 7377 individuals; and education in medical genetics for health professionals and the broader population was the fifth. In Cuba, abortion is a legally and socially accepted procedure to end pregnancies in case of severe congenital malformations, 94.7% participants expressed that abortion should be offered in every case of severe congenital defect. 75,3% individuals were in favour of non-directiveness in genetic counselling. More specific questionnaires were conducted for complex disorders in Cuban families with Familial Early Onset Alzheimer`s Disease and with Bipolar Affective Disorder, in order to know about their attitudes and expectations toward genetic testing. We found a limited knowledge about genetics in general and the meaning of genetic risk factors for both diseases. We also observed a positive interest in presymptomatic testing including prenatal diagnosis if it would become available. The information derived from these studies is being used for developing educational strategies focused on health promotion and prevention for medical genetic services at the community level in Cuba.

1348W

The Duty to Recontact Revisited. *R. Pyeritz.* Dept Medicine, Perelman Sch Med, Univ Pennsylvania, Philadelphia, PA.

The question of whether a duty exists to recontact patients about new genetic information has been discussed for several decades, with no consensus. The emergence of new testing technologies forces reconsideration of this complex matter. A major conundrum is the uncertain meaning of the results of many genetic tests, such as gene sequencing and cytogenomic arrays. Variants of unknown significance (VUS), which comprise 5-30 percent of all results of sequencing a gene, will explode with clinical whole genome sequencing (WGS). What is the duty to keep patients informed about new interpretations of prior results? Ten issues need attention. How can new information be catalogued and make clinically relevant and available? What is the responsibility for dealing with pathologic findings not related to the indication for the original test (incidentalomes)? Who decides whether to recontact a patient—the consultant or the primary care provider? Are any data entered into a medical record (e.g., a WGS, or a direct-to-consumer genotype) obligated to re-interpretation? What are the expectations of patients? What are the implications for relatives of a re-interpretation, and who bears the responsibility for notifying? Can new interpretations be made ignoring a host of modifiers—genetic interactions, the epigenome, the microbiome, the environment, and personal behavior? Do the results of direct-to-consumer testing carry the same responsibilities if the patient wants them inserted in the record? Does an agreement to recontact maintain a physician-patient relationship that would otherwise be vacated and does failure to recontact constitute abandonment? Finally, what strategies are needed to educate all parties about these concerns. The existing policy statement of the American College of Medical Genetics, more than a decade old, is largely silent on these issues. Until science, technology and education improve our capabilities, health care providers would be wise to accept with caution an explicit duty to recontact a patient about new knowledge resulting from genetic tests. Including this perspective in the pre- and post-test counseling, even in an informed consent document, bears consideration. Similarly, the one-time consultant, even if he or she ordered a genetic test and discussed the result, might consider explicitly terminating the physician-patient relationship.

1349W

National Institutes of Health Controlled Data Access: Experience of the Genetic Association Information Network Data Access Committee. *C. Din-Lovinescu[1], E. Bookman[1], C. Baker[2], E.L. Harris[3], T. Lehner[4], C. McKeon[5], J. Moss[6], V. Starks[7], G. Godynskiy[8], S. Sherry[8], L. Rodriguez[1], E.M. Ramos[1].* 1) National Human Genome Research Institute, Bethesda, MD; 2) National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD; 3) National Institute of Dental and Craniofacial Research, Bethesda, MD; 4) National Institute of Mental Health, Rockville, MD; 5) National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD; 6) National Heart Lung and Blood Institute, Bethesda, MD; 7) National Cancer Institute, Rockville, MD; 8) National Library of Medicine, Bethesda, MD.

Genome Wide Association Studies (GWAS), a strategy for identifying genetic variants associated with health and disease, produce vast amounts of data suitable for addressing many research questions. To maximize the scientific uses of GWAS data through broad data sharing, the National Institutes of Health (NIH) developed a controlled-access data repository and data access system designed to provide GWAS data to qualified investigators for research uses consistent with the informed consent of study participants. The Genetic Association Information Network (GAIN), one of the first NIH GWAS programs, has been at the forefront of rapid and broad data sharing since its inception in 2006 and helped shape the development and design of the database of Genotypes and Phenotypes (dbGaP) and its data access system. GAIN includes GWAS of ADHD, diabetic nephropathy, psoriasis, major depression, schizophrenia, and bipolar disorder. Data Access Requests (DARs) for GAIN studies are reviewed by the GAIN Data Access Committee (DAC). The DAC grants access to controlled-access data after ensuring that investigators have adequate credentials and meet the requirements of the data access request process, including proposing research that is consistent with the data use limitations of the requested data. As of April 30, 2011, 808 DARs have been submitted, 654 (81%) of which were approved with an average submission to decision time of 12.9 days. The schizophrenia and bipolar disorder datasets are requested most frequently. 57% of submitted DARs include schizophrenia; 46% include bipolar disorder. The most common proposed research use is to study GAIN-specific phenotypes (53% of submitted requests), followed by methods development (18%) and adding controls to other studies (17%). 316 investigators have been approved to access GAIN data with 165 resulting publications. Eight investigators approved to access GAIN data (2.5%) were involved in a data management incident where the terms of the NIH Data Use Certification agreement were not followed. The majority of these incidents occurred in the early years of dbGaP and all were resolved. As the number of studies deposited in dbGaP continues to grow, a summary of how GAIN data are being accessed and used along with the experience of the GAIN DAC can serve as an example to inform the NIH and the research community about the impact of making GWAS and other large-scale genomics datasets broadly available.

1350W

Statewide Genetic Services Patient Management System in New South Wales, Australia: planning, implementation and integration. *M. Tom, A. Colley.* Clinical Genetics, Liverpool Hospital, Sydney, NSW, Australia.

Over the last year New South Wales (NSW) Health has implemented a state-wide genetic services patient management system across all Local Health Networks (LHN). This is a review of the process and integration of the database into the multiple different clinical units across the state with a view to identifying the costs, benefits, successes and barriers. Background: NSW Health is currently divided up into eighteen LHNs. To cover these areas there are six metropolitan clinical genetics services, five cancer genetics services and fourteen outreach genetic counsellors. Historically, each unit's development depended on the services of the base hospital and the needs of the local community, creating independent and unique services. In the past three different database programs were used to collect patient genetic information with data being collected and accessible only by the specific genetics unit. A proposal was put forward for a state-wide genetic database to enable easy identification of patients that have links with existing families and to prevent duplication of information, resources and testing. This database was rolled out across NSW from early 2010 to early 2011. Methods: This was a qualitative review in which questionnaires were used to find specific areas of interest. Fifteen in depth semi-structured interviews were conducted with data managers, administration and/or clinical staff from each of the cancer and clinical genetics units, outreach genetic counsellors, NSW Health and the company providing the database program and support (Kintrak). Results: The interviews identified particular areas of concern, including expectations prior to implementation, changes in work flow, time and staff costs, complexity of data entry, privacy, accuracy, affects on clinical practise and future plans. Conclusions: An effective database system is extremely useful to identify families who have attended other genetic services and assist in more accurate genetic counselling and fewer redundancies of service and testing. However, the process of integrating any new system into the complexities of clinical genetic services involves a number of adjustments and compromises which are difficult to predict in advance.

1351W

The dbGaP data repository: Who is using it for what kinds of research? *L. Walker[1,2], H. Starks[1,2,3], K. West[2,3], S. Fullerton[1,2,3].* 1) IPHG, University of Public Health Genetics, Institute for Public Health Genetics, University of Washington, Seattle, Washington, 98195, USA.Seattle, WA; 2) Center for Genomics and Healthcare Equality, University of Washington, Seattle, Washington, 98195, USA; 3) Department of Bioethics and Humanities, University of Washington, Seattle, Washington, 98195, USA.

The database for Genotypes and Phenotypes (dbGaP) was launched in December 2006 to support NIH policy that strongly promotes sharing data from genome-wide association studies. While secondary use of aggregate and individual-level data in dbGaP is encouraged (with NIH-affiliated Data Access Committee approval), there has been no systematic analysis of how this resource has been used. We manually accessed and reviewed all approved Data Access Requests (DARs) on the dbGaP website from its inception through August 10, 2010 to assess their number, type of proposed secondary use, and to characterize the institutional affiliation and location of each requestor. 2724 DARs were granted to 851 investigators in 330 institutions, located in 28 countries. Reported uses include genetic discovery (39%), methods development (26%), replication (18%), study controls (11%), investigations of population structure (1%), and quality control (1%). Requesting investigators were primarily affiliated with U.S. academic institutions (60%); other affiliations included U.S. non-profits (13%) and for-profits (8%), and non-U.S. academic (13%), non-profits (5%), and for-profits (1%). dbGaP data are being shared widely, yet the full impact on scientific advancement and public benefit is difficult to gauge because publications resulting from secondary uses are not yet identified, and DARs are not readily searchable with respect to the characteristics summarized here. To ensure broad stakeholder support for the on-going development and use of dbGaP and to advance generalizable knowledge of relevance to public health, the database should be made more accessible to primary investigators, research participants, and those responsible for certifying data for dbGaP submission.

1352W

Shwachman Diamond Syndrome diagnosis and care, comparison in six countries. *L.J. Siderius[1, 2, 3], T. Chigladze[3, 4], G. Abesadze[5], O. Kvlividze[4].* 1) Youth Health Care, Icare, Meppel, Netherlands; 2) Shwachman Diamond Syndrome represenative, SSSSH, Waddinxveen, Netherlands; 3) European Academy Pediatrics, Working Group Rare Diseases; 4) Genetic and Rare Diseases Foundation, Tbilisi, Georgia; 5) Tbilisi State Medical University, Tbilisi, Georgia.

Purpose: To access the diagnostics and care provision in for individuals with Shwachman Diamond Syndrome in Canada, Italy, Netherlands, United Kingdom, United States and Georgia. Background: Shwachman Diamond Syndrome initially described in 1964, on the basis of its features now seems to be due to a single gene defect. Canada, Italy, Netherlands, UK and US are listed in the top 20 of world's Gross Domestic product (GDP), Georgia in the lower half. We compared the healthcare systems in relation to diagnosis and care of persons with Shwachman Diamond Syndrome. Methods: A questionnaire addressing the care as perceived by individuals with Shwachman Diamond Syndrome (SDS) was send through the national patient organizations in five countries with a representing patient organization. The situation in Georgia was evaluated with information of national representatives of medical specialist, academy and patient organizations. Results: Considering the expected frequency of SDS 1: 100.000- 150.000, the highest response of persons with SDS was from Italy (7,75%) and the Netherlands (10,2 %) . Predictive health care can be coordinated on the basis of the genetic diagnosis. The Italian persons with SDS perceived significant (p= .040) more frequent coordinated care compared to other countries, and more specific to the persons in Netherlands (p=.031) and USA (p= .017). In Georgia diagnostic tests are not available for confirmation. Conclusion: Health care systems may be an underestimated factor influencing early detection and optimal care provision for persons with SDS, a rare and complex genetic disorder. The data gathered with this questionnaire may be used for the development of optimal model of management of rare diseases and extrapolation of best world practices in the countries with low GDP, and respectively, with different health cares systems, such as Georgia in particular.

1353W

Comparison of Delivery Models of Genetic Risk Information for Type 2 Diabetes. *S.B. Haga[1], W. Barry[2], R. Mills[1], J. Sullivan[3], H.F. Willard[1], L.P. Svetkey[4], G.S. Ginsburg[1].* 1) Inst Genome Sci & Policy, Duke University, Durham, NC; 2) Department of Biostatistics and Bioinformatics, Duke University Medical Center; 3) Department of Pediatrics, Div Medical Genetics, Duke University Medical Center; 4) Department of Medicine, Div Nephrology, Duke University Medical Center.

With the advent of direct-to-consumer testing, online communication has increased as a mode of delivery of genetic test results. However, the degree to which the public is able to comprehend their results without the assistance of a trained professional and the opportunity to discuss the meaning of the results is uncertain. We are conducting a randomized study to assess the impact of the method of delivery of genetic risk results for Type 2 diabetes mellitus (T2DM) on risk comprehension, risk perceptions, and health behavior. Three hundred participants recruited from the general public in Durham, NC were randomized to undergo testing provided by deCode Genetics of several polymorphisms associated with T2DM and receive their test result in-person from a genetic counselor or online through the company's website; 246 participants have completed the 1-week follow-up. Overall, 60% of individuals self-identified as White and 29% as African-American. Seventy percent of participants are female, and 65% have a Bachelor's degree or higher. A total of 38% of participants indicated that they completely trusted the genetic test result and 50% somewhat trusted the result. Overall, 73% of participants correctly reported their genetic risk for T2DM as compared to the general population (increased, decreased, or equal to). Participants who received their results in-person from the genetic counselor were significantly more likely to correctly indicate their comparative risk (85%) than those who reviewed their results on-line (61%) ($p < 0.0001$). A smaller proportion of respondents (58%) correctly reported their actual genetic risk (relative risk of developing T2DM based solely on genotype) based on their test report. Participants who received their result in-person from the genetic counselor were also more likely to correctly report their actual genetic risk (69%) as compared to those who reviewed their results on-line (48%) ($p = 0.001$). However, there was no statistically significant difference in the rate of correct response about their overall risk (lifetime risk and genetic risk) between the counseling group (65%) and online group (54%) ($p = 0.11$). In summary, participants who received their genetic risk results in-person from a genetic counselor were more likely to correctly comprehend the different risks included in their test report compared to reviewing their test result online.

1354W

Knowledge and attitudes regarding genomic medicine and research in South Florida's Hispanic and Black communities. *S. Hahn, K. Czape, A. Rupchock, M. Gavier, C. Jean, LD. Adams, J. Lee, MA. Pericak-Vance.* Hussman Institute for Human Genomics, University of Miami, Miami, FL.

While participation of minority groups in genetic and translational research is crucial, it has been historically low. Focus group research among Hispanics and Blacks in South Florida shows they may have unique attitudes and barriers to research participation. An anonymous paper survey was administered in South Florida to assess knowledge and attitudes about genetic testing and research. The survey, comprised primarily of previously published questions and available in English, Spanish, and Creole, was offered at public locations and events. Preliminary data shows a completion rate of 78% (n=242), with 51% and 27% self-identifying as Hispanic and Black, respectively. Average age was 40; 71% were female and 50% completed college. Most appreciated the concept of inheritance and the significant role of genetics in health. 77% were aware that family history influences disease risk; 80% were aware we each have genetic variations that put us at risk; and 89% knew most health problems result from a combination of genetic and other factors. Fewer knew that specific disorders have known genetic contributions such as diabetes (71%), cancer (62%), heart disease (54%), and autism (28%). Only 57% (76% of Blacks) knew sickle cell has a known genetic component. 72% answered genetic tests are used frequently to predict who will get common diseases such as heart disease and Alzheimer disease indicating misconceptions about current utility. While most were receptive to genetic testing, 48% were very or somewhat worried people will be able to learn their results. 37% thought they could be denied health insurance based on genetic information even if they didn't have the disorder. Views about genetic research were generally favorable. 78% indicated genetic research is very good and 82% would be very likely or somewhat likely to participate. While 46% said nothing would keep them from participating in genetic research, many indicated reasons that would, including worry about being harmed (20%), lack of time (19%), and worry about misuse of private information (15%). 52% answered incorrectly that all ethnic groups benefit equally from research even if a group does not participate. Overall, South Florida communities have comparable levels of knowledge of genetics to other US areas, and have a positive attitude towards genetics and genetic research. Gaps in knowledge and awareness identified may be used to improve educational initiatives, and increase minority involvement.

1355W

Recorded Interviews with Human Geneticists; an International Web-based Historical Resource. *P.S. Harper.* Institute of Medical Genetics, Cardiff University, Cardiff, United Kingdom.

A series of 80 recorded interviews with older human and medical geneticists has been undertaken by the author over the past 8 years, 62 of which have transcripts accessible on the website of the Genetics and Medicine Historical Network (www.genmedhist.org). Both laboratory and clinical workers are included in the series, which is international and principally European orientated, complementing a separate American series.(www.socgen.-ucla.edu/ hap/). These interviews form a valuable resource as part of the documentation of the history of human and medical genetics world-wide (see Harper PS, A Short History of Medical Genetics, OUP, 2008), giving information that often does not appear in written documents, in additon to providing a personal picture of the individuals involved and often of their older colleagues and teachers. There is an urgent need for extension of this work by both geneticists and historians; 10 subjects in the current series are already deceased since interview. Of particular importance will be interviews with the founders of `newer' fields such as human molecular genetics.

1356W

Managing ethical, legal, and social issues in cancer genome sequencing projects: a survey of current practices. *C. Allen[1,2], W.D. Foulkes[2].* 1) Biomedical Ethics Unit, McGill University, Montreal, Quebec, Canada; 2) Program in Cancer Genetics, Departments of Oncology and Human Genetics, McGill University, Montreal, Quebec, Canada.

Background: Large-scale whole genome sequencing (WGS) studies promise to revolutionize cancer research by identifying targets for therapy and discovering molecular biomarkers to aid in early detection, diagnosis, prognosis, and prediction of treatment response. Such projects raise a number of ethical, legal, and social (ELS) issues that must be examined. This study analyses informed consent (IC) forms from cancer genome sequencing research to determine how investigators currently engaged in such studies are managing these issues. Methods: Thirty IC forms were collected from the US, Canada, Australia, Belgium, and the Netherlands. A thematic analysis examined (1) stated purpose of sample collection, (2) scope of consent requested, (3) data sharing protocols (4) privacy protection measures, (5) described risks of participation, (6) subject re-contacting, and (7) protocol for withdrawal. Results: Seventeen of the forms collected were seeking samples for the purpose of discovering the genetic basis of a particular cancer type. Five stated the purpose of broader medical research. Three forms were seeking consent only for the study described, while six intended to use samples for related research, and sixteen for any type of future research. Three forms did not discuss data sharing, while eleven stated the intention to share data with affiliated researchers, and sixteen with the public in general, though in the latter case gaining access to sensitive data would require authorization. All but one of the forms stated that samples and data would be de-identified, though twenty-one described re-identification as a risk. Participant re-contacting practices varied but could occur to obtain additional information, to suggest additional research participation, or in five instances, to return individual research results. Twenty-nine forms discussed withdrawal, though the described consequences of this for existing samples and data varied. Conclusion: Examining IC forms from WGS research elucidates how investigators are handling some of the ELS challenges that this research poses. This information is important for ensuring that while the public benefits of research are maximized, the rights of participants are also being appropriately respected. Acknowledgements: Funding for this article was provided by the APOGEE-Net/CanGèneTest Research Network and by Susan G. Komen for the Cure.

1357W

Parents' Preferences for Return of Results in Pediatric Genomic Research. *J.G. Amatruda[1], S.I. Ziniel[2,3], E.D. Harris[1], C.M. Clinton[1], S.K. Savage[4], N.L. Huntington[2,5], R.C. Green[6,7], I.A. Holm[1,2,8].* 1) Division of Genetics and Program in Genomics, Children's Hospital Boston, Boston, MA; 2) Department of Pediatrics, Harvard Medical School, Boston, MA; 3) Clinical Research Program, Children's Hospital Boston, Boston, MA; 4) Children's Hospital Informatics Program, Children's Hospital Boston, Boston, MA; 5) Division of General Pediatrics, Children's Hospital Boston, Boston, MA; 6) Partners Center for Personalized Genetic Medicine, Boston, MA; 7) Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 8) The Manton Center for Orphan Disease Research, Children's Hospital Boston, Boston, MA.

Objectives: Current guidelines for returning individual research results (IRR) from genomic studies focus on criteria including analytic and clinical validity, actionability, and severity of the outcome. However, many argue that the personal meaning of genomic information and participant preferences should play a role in return of IRR. Children's Hospital Boston (CHB) has taken the latter approach by initiating a large pediatric genetic repository, The Gene Partnership, in which IRR are returned in accordance with participant preferences. Recognizing that there is skepticism that participants truly understand the implications of their preferences for IRR to receive, the goal of this study was to explore parents' preferences for return of IRR on themselves and their children. We hypothesized that although most parents will report a desire to receive all IRR on themselves and their children, once presented with specific types of IRR, many would choose only a subset of possible results. **Methods:** We designed a survey to assess the interest of parent in enrolling themselves and their children in a hypothetical genetic research repository, and their preferences for return of return of IRR. The survey was mailed to 6872 parents of patients at CHB. **Results:** Preliminary analysis of data on over 800 respondents showed that 83% wanted to receive IRR on themselves and 85% wanted to receive IRR on their children. When asked if they wanted to receive all IRR as opposed to choosing which IRR to receive, 77% of respondents wanted to receive all results on themselves and 83% on children. However, when later asked about specific categories of results (based on analytic validity, clinical validity, actionability, and severity of the outcome) only 54% of respondents who initially wanted all IRR, wanted to receive results in all categories on themselves; the percentage was 57% for results on their children. Further, those respondents who wished to choose which IRR to receive were significantly more likely to be concerned about the impact of receiving IRR on various aspects of their lives (e.g., worry, insurance status, job status, discrimination) ($p < 0.001$). **Conclusions:** These results suggest that although participants may not understand the implications of receiving all IRR arising from genomic research, education on the types of results that may arise will enable participants to set preferences that better reflect their desires for return of results.

1358W

"It's really good judgment on their part not to tell people the stuff they can't control": Experience of whole exome sequencing among breast cancer survivors. *B. Bernhardt[1, 2], H. Pang[2], J. Powers[3], J. Stopfer[3], S. Domchek[3], K. Nathanson[1].* 1) Dept Med Gen, Univ Pennsylvania, Philadelphia, PA; 2) Center for the Integration of Genetic Health Care Technologies, Univ Pennsylvania, Philadelphia, PA; 3) Abramson Cancer Center, Univ Pennsylvania, Philadelphia, PA.

Although whole exome sequencing (WES) provides a powerful tool for gene discovery, there is considerable debate about the obligations of clinicians and researchers to disclose incidental findings. In an ongoing study, we are offering WES for identification of novel cancer susceptibility genes to women from "high risk BRCA1/2 negative" families recruited from a research registry. As a part of the informed consent process, participants are told that a multidisciplinary expert panel will review all results and determine which are both medically actionable and significant. Participants would be given the option of choosing to learn those results only. Participants were consented for WES by telephone or at a clinic visit by a study genetic counselor, and all acceptors and decliners subsequently were offered an opportunity to participate in a telephone interview to describe motivations for participation in the WES research, assess participant understanding of WES, and to assess attitudes towards returning of results. Interviews were recorded, transcribed and coded. Of the 53 women offered WES to date, 35 consented to WES, 3 declined and 17 did not respond to the offer. From the 13 interview transcripts that have been analyzed, all women correctly understood that the study's purpose is to find "new cancer genes." All women participated for altruistic reasons or to obtain information that could provide medical benefit to themselves or family members, primarily their children. Few women identified risks to participation, but when probed, some mentioned hypothetical risks of insurance discrimination or anxiety. With the exception of one woman, all participants understood "medically actionable" as meaning some intervention could modify health outcomes. If given the option to receive results for non-actionable diseases, the majority of the women would want such results so they could keep abreast of treatment advancements, or be prepared. However, all participants accepted the limitations placed on the types of results they could receive, either because they trusted the researchers to make decisions in their best interests, or they found the research design acceptable. These preliminary findings suggest that after a 30 minute consenting process, women can understand the purpose of WES research, agree with researchers on the interpretation of medically-actionable results and accept that all findings will not be returned to them.

1359W

Citizens' values regarding expanded newborn screening and the role of parental consent: A public engagement study. *Y. Bombard[1], F.A. Miller[1], R.Z. Hayeems[1], J.C. Carroll[2], D. Avard[3], J. Allanson[4], R. Axler[1], J. Bytautas[1], P. Chakraborty[4], Y. Giguere[5], J. Little[6], B.J. Wilson[6].* 1) Department of Health Policy, Management and Evaluation, University of Toronto, Toronto, ON, Canada; 2) Department of Family and Community Medicine, Mount Sinai Hospital, University of Toronto, Canada; 3) Centre for Genomics and Policy, Department of Human Genetics, McGill University, Montreal, Canada; 4) Department of Genetics, Children's Hospital of Eastern Ontario, and Department of Pediatrics, University of Ottawa, Canada; 5) Department of Medical Biology, Centre Hospitalier Universitaire de Quebec (CHUQ), University of Laval, Canada; 6) Department of Epidemiology and Community Medicine, University of Ottawa, Canada.

Introduction: Historically, newborn screening (NBS) programs identified serious conditions where early detection and pre-symptomatic treatment were necessary to avert clinical harm. Today NBS has expanded to screen for disorders for which there is limited evidence of health benefits for infants, yet many continue to operate on a mandatory or implied-consent model. The expansion of NBS has produced policy dilemmas about the scope of NBS and the role of parental consent. Continued debate about expanded NBS has led to calls for stakeholder engagement to inform policy. We investigated citizens' values regarding the scope of NBS and the type of consent parents should provide for NBS. Methods: We conducted eight focus groups (FG; n=60), and one Citizens' Panel (CP) with a subset of the participants (n=16). Recruitment was designed to ensure socio-economic, ethnic, age and family life diversity. FG and CP approaches included an educational component, deliberative discussion and pre- and post-questionnaires. Data were analyzed with descriptive statistics and qualitative content analysis. Results: Knowledge about NBS significantly improved (mean `knowledge scores': FG: pre=6.87 (SD:2.68) vs. post=7.8(SD:2.12) $p<0.0001$ & CP: pre=7.53(SD:1.25) vs. post=8.50 (SD:0.83) $p=0.018$). The majority (>94%) supported NBS that identifies serious treatable disorders; a majority (>69%) also supported NBS for untreatable conditions. Participants valued newborns' rights to open futures and informational benefits for family, such as avoiding the diagnostic odyssey, preparation and reproductive risk information. Potential harms, such as learning false positive results, were minimized, but some identified risks from stigma and unwanted knowledge. A majority endorsed having parents either required or strongly encouraged to have their infants screened. Finally, the CP recommendation was to screen for CF (16/16 in favour) while 12 were in favour and 4 against NBS for DMD. Conclusions: Most participants anticipated benefits from early identification of treatable and untreatable disorders through NBS. Participants' general endorsement of broad-scoped panels stemmed from their value-based trade-offs that prioritized benefits (e.g., newborns' open futures and informational benefits for affected infants and family) over considered harms. Further research is warranted to better understand the sources of these value-based trade-offs and their generalizability across different populations.

1360W

Legislation on direct-to-consumer genetic testing in seven European countries. *P. Borry[1,9], R.E. van Hellemondt[2], D. Sprumont[3], C. Fittipaldi Duarte Jale[4], E. Rial-Sebbag[5], T. Matthias Spranger[6], L. Curren[7], J. Kaye[7], H. Nys[1], H. Howard[8].* 1) Biomedical Ethics and Law, University of Leuven, Leuven, Belgium; 2) Leiden University Medical Centre, Leiden, the Netherlands; 3) Institute of Health Law, University of Neuchâtel, Switzerland; 4) Portuguese Catholic University, Portugal; 5) Inserm U558, University of Toulouse III, France; 6) Institute for Public Law, University of Bonn, Germany; 7) HeLEX Centre for Health, Law and Emerging Technologies, University of Oxford, U.K; 8) Inst. of Biomedical Ethics, University of Basel, Switzerland; 9) Dept. Clinical Genetics and Medical Humanities, VU University Medical Centre Amsterdam, The Netherlands.

An increasing number of private companies are now offering direct-to-consumer (DTC) genetic testing services. The tests offered range from tests for single gene, highly penetrant disorders to susceptibility tests for genetic variants associated with common complex diseases or with particular non-health-related traits. Although a lot of attention has been devoted to the regulatory framework of DTC genetic testing services in the U.S.A., only limited information about the regulatory framework in Europe is available. We will report on the situation with regard to the national legislation on direct-to-consumer (DTC) genetic testing in seven European countries (Belgium, the Netherlands, Switzerland, Portugal, France, Germany, United Kingdom). The paper will address whether these countries have legislation that specifically address the issue of DTC genetic testing or have relevant laws that is pertinent to the regulatory control of these services in their countries. The findings show that France, Germany, Portugal, Switzerland have specific legislation that defines that genetic tests can only be carried out by a medical doctor after the provision of sufficient information concerning the nature, meaning and consequences of the genetic test and after the consent of the person concerned. In the Netherlands, some DTC genetic tests could fall under legislation that provides the Minister the right to refuse to provide a license to operate if a test is scientifically unsound, not in accordance with the professional medical practice standards or if the expected benefit is not in balance with the (potential) health risks. Belgium and the United Kingdom allow the provision of DTC genetic tests. Although relevant legislation that bind DTC companies exists at the European level (E.g. the in vitro medical diagnostic devices, consumer protection legislation, data protection legislation), the lack of a harmonized (European) approach at all levels is problematic in a context where services are offered through the internet.

1361W

PGD and PGS, between practice and ethics, what happens in Quebec? *C. Bouffard[1], S.L. Tan[2], A.K. Maglo[1], M.A. Dubois[1], R. Drouin[1].* 1) Sherbrooke University, Dept Ped, Fac Med Hlth Sci, Sherbrooke, PQ, Canada; 2) McGill University, Health Centre Royal Victoria Hospital ,687 Pine Avenue West Women's Pavilion 6th floor Montreal, Quebec 687 Pine Avenue West Women's Pavilion 6th floor Montreal, Quebec.

In August 2010, the Quebec government made a landmark decision to publicly fund in vitro fertilization and other related assisted procreation services, including preimplantation genetic diagnosis (PGD) and but not preimplantation genetic screening (PGS). According to the new Quebec regulations, PGD will be funded publicly if performed by a licensed hospital for the purpose of detecting specific chromosomal or single-gene defects. Under these conditions, Quebec's policy will likely impact the provision of assisted reproductive technologies in the rest of Canada. However, despite the political will, the legal battles between federal and provincial governments and the needs of patients, the infrastructures are not ready to absorb the demand. Under these conditions, a little over a year after the announcement of the supervision of PGD and PGS by the Quebec government, what is the situation? Has the system kept its promises? What are the opinions of practitioners? Can we consider that the conditions of delivery of reprogenetic services are ethical? Methodology - Qualitative Research Design: Phase I) Online questionnaires for 10 obstetricians working in fertility clinics and laboratories, 10 geneticists engaging in activities related to PGD and PGS, and 05 genetic counselors working within a Quebec context. Data analysis: Quantitative and qualitative analyses (open and closed questions). Result: The preliminary results presented here are part of a vaster research on the conditions of delivery of PGD in Canada, but they apply only to Quebec. Several reasons are such that waiting lists are growing and services are being delivered in dribs and drabs. It seems that the current conditions of delivery of reprogenetic services covered by the Quebec government and the desire to keep these within the public system, promote the expansion of private enterprise, reprogenetic tourism and the exile of specialists. Conclusion: The current conditions of delivery of reprogenetic services in Quebec can bring about ethical issues as to accessibility and equity which mainly affect people who cannot afford to offer themselves these services through the private system, as well as issues related to the underutilization of expertise in place.

1362W

Parental Perspectives on Pediatric Genetic Research. *T. Brazg[1], H.K. Tabor[1,2], J. Crouch[1], S.M. Fullerton[3], B.S. Wilfond[1, 2].* 1) Center for Pediatric Bioethics, Seattle Children's Research Institute, Seattle, WA; 2) Department of Pediatrics, University of Washington, Seattle, WA; 3) Department of Bioethics and Humanities, University of Washington, Seattle, WA.

Increasingly, parents of children with both Mendelian and complex diseases, as well as parents of healthy children, are being asked to enroll their offspring in genetic research studies. Limited data exist about how parents decide whether or not to have their children participate in these kinds of studies, or their perspectives on the possibility of receiving individual or aggregate research results. We interviewed 23 parents whose children with autism or diabetes were enrolled in one of three genetic research studies. These interviews were conducted as part of a larger study on genotype-driven research recruitment. The interviews were transcribed and analyzed using thematic qualitative analyses. The specific research question addressed in this analysis was what are parents' perspectives on research participation and return of research results for genetic studies in which their children are enrolled? Parents discussed a wide range of people for whom their child's participation in genetic research could have implications. They made reference to the impact on themselves as parents and care-takers, their family more broadly, their affected and unaffected children, grandchildren, future generations, others with the disease and society at large. The most frequently cited concepts that influenced parents' decisions to enroll their children in genetic studies were: time and convenience; “helping” themselves, their children, or others; the relevance of the research to their family; and weighing burdens and benefits of participation. When asked about the possibility for the return of research results, parents explained that receiving both individual and aggregate results would be an incentive for further research participation. They also described potential positive and negative psychological impacts of receiving genetic research results, ranging from providing an explanation for their child's disease to possible guilt and anxiety. Parents of children in autism research also cited reproductive planning - for themselves or in the future for their unaffected children - as a specific potential benefit of receiving their children's individual genetic research results. By better understanding how parents make decisions about enrolling their children in genetic research and how they feel about the possibility of receiving individual genetic research results, we can develop the most effective and appropriate ways to design pediatric genetic research.

1363W

ELSI challenges encountered when integrating population-based and clinical biobanks into a national biobank infrastructure: The case of Biobank Norway. *I. Budin-Ljøsne[1], B. Solberg[2], J.R. Harris[1].* 1) The Norwegian Institute of Public Health, Oslo, Norway; 2) Norwegian University of Science and Technology, Trondheim, Norway.

Norway hosts a rich collection of population-based biobanks, clinical biobanks and national health registries. Biobank Norway is a recently funded infrastructure project that aims to integrate these biobanks into a national platform in order to enhance the use of these data and biological samples in genomic and health research and innovation. In addition to building interoperability at a national level, Biobank Norway will be optimally coordinated with international biobanking initiatives. Establishing a platform that spans clinical and population-based biobanks requires streamlined and secure procedures for the handling and sharing of data and biospecimens. Furthermore, consideration of diverse stakeholders in the development of policies regarding a host of issues is essential for maximizing the success and sustainability of biobanks. Most of the ethical, legal and social implications (ELSI) that are encountered in the development of a national biobank platform are universal, though some are country-specific depending on national ethical-legal frameworks. This presentation describes the most challenging ELSI issues confronting Biobank Norway and discusses approaches being used to address these issues in Norway and internationally. Examples of these ELSI challenges include the ELSI implications associated with the “blurring” of traditional boundaries between research biobanks and disease biobanks, between clinic and research and between predictive and diagnostic testing. Further issues include determining which human biological samples and associated data can be stored in a national biobank infrastructure and can be shared internationally knowing that informed consent may not have been collected or is restricted, implementing procedures to guarantee the respect for privacy and confidentiality of stored data as well as the development of policies related to the return of results and incidental findings.

1364W

The role of the human growth receptor exon 3 polymorphism (hGHRd3) in maternal glycemia and neonate size. *E.S. Ingersoll[1], H. Weis[1], C.M. Ackerman[1], L.P. Lowe[2], M.G. Hayes[1], B.E. Metzger[1], W.L. Lowe[1], M. Urbanek[1], HAPO Study Cooperative Research Group.* 1) Division of Endocrinology, Metabolism, and Molecular Medicine, Northwestern University, Feinberg School of Medicine, Chicago, IL; 2) Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL.

Background: The GH/IGF-1 axis plays a role in both longitudinal bone growth and glucose homeostasis. A common polymorphism in the human growth hormone receptor (hGHR) is a complete deletion of exon 3. The truncated receptor (hGHRd3) shows greater sensitivity to growth hormone therapy in patients and this may be due to increased downstream signaling rather than increased binding affinity for GH. The hGHRd3 allele has also been associated with short stature and protection against type 2 diabetes and insulin resistance. Our objective was to investigate the contribution of the hGHRd3 allele to neonatal size and glycemia. **Methods:** We genotyped 3300 European ancestry mothers and neonates (2056 mothers, 1238 neonates) and 1594 Mexican-American mothers and neonates (795 mothers, 788 neonates) from Bellflower, CA from the Hyperglycemia and Adverse Pregnancy Outcome Study cohort. We used a previously established multiplex PCR method. The hGHRd3 (minor) allele frequency observed for this study was 0.279 and 0.248 for European ancestry and Mexican-Americans respectively and genotypes were in Hardy Weinberg equilibrium. We analyzed association of several neonatal size and metabolic parameters (birth weight, length, head circumference, sum of skin folds, cord glucose and C-peptide, 2 hour glucose) and maternal height with hGHR allele using a previously established model, fully adjusted for confounding variables. **Results:** There was a negative association between the GHRd3 allele and birth length in mothers and babies. This effect was significant (-0.12cm per d3 allele, CI:-0.24cm, -0.003; p=<0.05) for the effect of mother's genotype on birth length and approached significance for the effect of neonatal genotype on birth length. None of the other outcomes showed significant association with genotype, including maternal height which can impact fetal growth. **Discussion:** This study is the largest evaluation of the impact of the hGHRd3 polymorphism on fetal growth to date and confirms previous reports that hGRd3 allele is associated with shorter birth length, although this effect is modest. The role of interaction of maternal and neonatal genotype in this association will require further study. There was no evidence for an appreciable effect of hGHRd3 on other common measures of birth size or newborn glycemic control.

1365W

Chromosomal prenatal diagnosis: Study of 250 cases. *C. Albu[1,2], D. Albu[1,2], E. Severin[1], M. Dumitrescu[2,3].* 1) University of Medicine and Pharmacy Carol Davila, Bucharest, Romania; 2) Alco San Clinic, Bucharest, Romania; 3) Marius Nasta Hospital, Bucharest, Romania.

Antenatal cytogenetic diagnosis is an accepted component of prenatal care for women at increased risk for chromosomally abnormal offspring. Objective: to investigate the frequency of the different types of chromosomal abnormalities in order to highlight the importance of prenatal cytogenetic test. Patients and Methods:This study included 250 prenatal karyotypes carried out in the last year. The age of the caucasian pregnant women was between 24 and 46 years, caucasian womens. The study included karyotypes performed by cultivation of amniocytes and trophoblastic cells obtained by amniocentesis and chorionic villus sampling. Results: 92,78% from a total of the karyotypes were normal and 4,1% presented a chromosomal abnormalities. Sex chromosome aberrations were present in 8,49% of the abnormal karyotypes. Autosomal aneuploidies represented the highest percent of the chromosomal abnormalities, respectively 50,99%. Translocations were the second most frequent, with a total of 14,32%. Lower rates were found in inversions (2,66%), duplications (5,20%), deletions (3,49%) and marker chromosomes (2,65%). Conclusions: The necessity of the prenatal detection of fetal chromosomal abnormalities to all pregnancies and especially for the risk categories.

1366W

Prenatal molecular diagnosis of Down syndrome: a comparison between molecular and traditional karyotyping. *D. Chu[1], J. Liou[2].* 1) Graduate Inst Med Biotech, Chang Gung Univ, Dept Med Biotech, Tao-Yuan, Taiwan; 2) Department of OB/GYN, Chang Gung Memorial Hospital, Taipei , Taiwan.

Trisomy 21, a.k.a. Down syndrome, is the most commonly seen chromosome aneuploidy in live births. The incidence is estimated to be around 1 in 900 to 1 in 1000 worldwide. Currently, multiple maternal serum markers screening test (MMST) in the second trimester plus karyotyping as the confirmation test are still standard procedures to identify fetal Down syndrome. However, due to the limitations associated with these methods, molecular approaches using chromosome 21-specific short Tandem repeats (STR) markers are suggested to be a rapid and accurate alternative for this purpose. In this study, we performed our previously established STR analysis on 602 amniotic fluid samples which were collected for traditional karyotyping due to increased risk for trisomy 21 based on MMST positive results or advanced maternal age. Data indicated that 2 of the 602 were positive for trisomy 21 and the rest 600 cases were negative. These results were consistent with those of traditional karyotyping. It is concluded that prenatal molecular diagnosis of chromosome aneuploidy is a rapid yet reliable alternative of karyotying. It is also practical for clinical application.

1367W

RAPID PRENATAL DIAGNOSIS OF COMMON CHROMOSOME ANEUPLOIDIES BY QF-PCR: EAST-SLOVENIAN EXPERIENCES. *a. erjavec skerget[1], s. stangler herodez[1], a. zagorac[1], b. zagradisnik[1], n. kokalj vokac[1,2].* 1) Laboratory of Medical Genetics, University Clinical Centre Maribor, Maribor, maribor, Slovenia; 2) Medical Faculty Maribor, Department of Molecular Biology, SI - 2000 Maribor.

INTRODUCTION Quantitative-fluorescence polymerase chain reaction (QF-PCR) was used to detect common foetal aneuploidies in the Slovenian population. The results were obtained within 48 - 72 hours after sample collection in order to alleviate parental anxiety in cases with only raised risk of trisomy (maternal age) and in cases with high risk for foetal aneuploidy (positive serum screening tests, nuchal translucency measurements, ultrasound abnormalities or others). METHODS The QF-PCR tests were performed on 265 prenatal samples (73.3 % amniotic fluids, 26.7% chorionic villus) which were analysed blind, without knowledge of the results obtained using conventional cytogenetic analysis. DNA from prenatal samples were analysed using on in-house-developed method with 20 micro-satellite markers located on the chromosomes 13, 18, 21, X and Y. The QF-PCR results of 61.8% samples were compared with their respective karyotypes. RESULTS 262 samples were successfully analysed. In 7.2% of cases a numerical chromosome abnormalities with QF-PCR were detected: 18 autosomal trisomies and 1 triploidy. Results of QF-PCR and karyotyping were compared in 162 cases. In a group with raised risk of fetal trisomy the specificity and sensitivity of QF-PCR method was 100%. Among cases with high risk for fetal aneuploidy sensitivity was 100% but specificity was lower, 91% to 95%, depending up on referral reason. CONCLUSIONS Our results demonstrates that QF-PCR is a reliable and rapid method for detection of common numerical chromosome disorders. In a group of older woman with raised risk for foetal aneuploidy the results of QF-PCR can be used alone without karyotyping. In cases with higher risk, especially those with pathological ultrasound, analysis only with QF-PCR method isn't enough. However, karyotyping is still required for all prenatal samples in Slovenia.

1368W

A comparison of emerging technologies for aneuploidy detection in spontaneous pregnancy loss. *C.N. Paxton[1], S. South[1,2,3], A. Brothman[1,2], K. Geiersbach[1,3].* 1) ARUP Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, UT, USA; 2) Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, UT, USA; 3) Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT, USA.

Background: An estimated 15% of clinically recognized pregnancies are spontaneously lost in the first trimester, and of these, up to 50% are chromosomally abnormal. Genetic testing permits identification of the cause of pregnancy loss and facilitates genetic counseling and future pregnancy planning. Cytogenetic cell culture is the historic standard method for analyzing products of conception (POCs), but due to the poor viability of many of such samples, the failure rate is approximately 10-20%. Several molecular methods have recently been introduced for detection of genomic abnormalities in POCs. We have compared three hybridization based technologies for accuracy, ease of use, and analytic sensitivity.

Methods: All samples were previously characterized by cytogenetics or oligonucleotide array platform. The samples included 65 POCs with abnormal karyotype and 5 POCs without karyotype due to growth failure in culture. DNA was extracted from backup flasks or directly from residual cleaned villi or fetal somatic tissue. Testing was performed according to manufacturer instructions using the Affymetrix SNP6.0 array, Perkin Elmer Karyolite BACs-on-Beads (BoBs), and NanoString Karyotype CodeSet.

Results: Results were 100% concordant between methods for SNP6.0 array and BoBs, and 90% concordant for Nanostring. All 5 culture failure samples yielded results by at least one method, with 100% concordance between methods. 5 samples with an abnormal karyotype showed normal female results with 100% concordance between methods, consistent with maternal cell contamination (MCC) of the backup flask used for DNA extraction in these cases. 3 samples showed evidence of placental mosaicism. As expected, BoBs and Nanostring did not detect polyploidy and neither method reliably detected segmental aneusomies.

Conclusions: The Affymetrix SNP6.0 method had the highest analytic sensitivity and was superior to karyotype for characterizing segmental aneusomies. MCC in cultured cells and placental mosaicism in uncultured cells can contribute to discrepant results by any of the three methods tested. Novel lower resolution methods (PE BoBs and Nanostring) show an overall lower analytic sensitivity but are attractive solutions for lower cost and secondary testing on samples for which a karyotype cannot be obtained.

1369W

Rapid prenatal diagnosis of common chromosome aneuploidies using quantitative fluorescent (QF)-PCR: 10 years experience in a center from the Republic of Macedonia. *D. Plaseska-Karanfilska, S. Madjunkova, I. Maleva, S. Kiprijanovska.* Research Center for Genetic Engineering and Biotechnology “Georgi D. Efremov”, Macedonian Academy of Sciences and Arts, Skopje, Macedonia.

The quantitative fluorescent (QF) PCR of selected small tandem repeat (STR) markers enables rapid and accurate prenatal diagnosis of the aneuploidies of chromosomes 21, 18, 13, X and Y. Here, we present our results of the use of QF-PCR for prenatal detection of common chromosomal aneuploidies in 2200 pregnancies at risk performed in a period of 10 years. It was also used in the prenatal cases for monogenic diseases to control for maternal contamination of the fetal material. The prenatal diagnosis was performed on genomic DNA isolated from fetal cells collected by amniocentesis or chorionic villus samples. All samples were analyzed by at least four STR markers on each of chromosomes 21, 18 and 13. In addition, amelogenin, TAF9, SRY and STR markers on X chromosome were used for the determination of the gender and sex chromosome aneuploidies. Maternal blood samples were analyzed in all blood contaminated amniotic samples and in most chorionic villi samples. In most instances the QF-PCR analysis was performed as a stand-alone test. No discordant results were obtained when cytogenetic analysis was performed in addition to QF-PCR. In one case the discordant sex between ultrasonography and QF-PCR was due to sex reversal subsequently confirmed by cytogenetic analysis. Forty seven (2.1%) samples could not be analyzed due to maternal contamination of fetal material. We have detected 46 trisomy 21 fetuses, 18 trisomy 18, five trisomy 13, five monosomy X, four XXY, two triploidy, one XYY, one triple X and one double trisomy 21 and XXY fetus. Polymorphic duplications involving STR markers D13S631, D21S1441, D18S978 or D18S535 were detected in seven fetuses; in all fetuses the duplications were inherited from one of the parents. The parental origin of the aneuploidy was determined in 64 cases; trisomy 21 (n=39), trisomy 18 (n=13), trisomy 13 (n=3), monosomy X (n=5) and XXY syndrome (n=4). The origin was maternal in the majority of the autosomal trisomies (all except one trisomy 21) and paternal in most of the sex chromosome aneuploidies (four of the five monosomies X and three of the four XXY syndrome cases). Triple X syndrome was detected in one woman with a fetus with trisomy 18. In conclusion, the QF-PCR method is an efficient, rapid and reliable method for prenatal diagnosis of the most common chromosome aneuploidies. In addition, it can provide information about the origin of the aneuploidy and maternal contamination of the fetal material.

1370W

Bac on Beads technology a rapid and reliable screening for Chromosomal Aneuploidies: One year experience. *G. Queipo[1,2], J. Duran[2], Z. Najera[2], N. Najera[2], R. García- Cavazos[3].* 1) Human Genetics, Hospital General de Mexico, Mexico City, Mexico; 2) NanoLab, Next Generation Diagnosis Mexico City, Mexico; 3) Perinatal Genetics Medicine, Mexico City, Mexico.

There have been significant advances in antenatal screening for fetal aneuploidy. Prenatal screening is a world wide tool used to detect those high risk aneuploidies pregnancies in the first o second gestational trimester with non invasive technique as ultrasound and biomarkers. However invasive studies are necessary in positive cases. Chromosome abnormalities are a well-established cause of pregnancy loss and birth defects. The most common are autosomal aneuploidy (~75%), followed by polyploidy (~13%), sex chromosome abnormalities (~8%) and structural imbalance (~4%). Trisomy of chromosome 21, 13, or 18 as well as sex chromosome aneuploidy account for 60-80% of abnormal fetal karyotypes detected in cultured amniotic fluid cells. For non-mosaic standard trisomy, cultured karyotype analysis has been considered a reliable detector of fetal abnormality. Nevertheless, the sensitivity of karyotyping depends on the number of cells established in a particular culture, and results are usually not available for 3-4 days or more. Furthermore, it is very difficult to identify chromosome microdeletions. In addition to karyotype analysis, fluorescence in situ hybridization (FISH), is an easy-to-handle, rapid, and highly sensitive tool for genetic analysis, it has been developed in the past two decades for prenatal assay. However, that detection power depends on the probes included in the analysis. Recently molecular and genomic technology development has been improved rapid detection of chromosomal gain or loses in one assay with the CGH-array strategy. BACs-on-Beads™ based multiplex assay designed to detect the 5 most common aneuploidies and gains and losses in 9 well characterized target regions in 24 hr. Constitutional BoBs is a simple, robust assay that offers significant benefits in terms of ease of handling, minute sample volumes, reduced time-to result and improved pick-up rates for today's cytogenetics and molecular genetics. Here we present our successful experience using this strategy in prenatal analysis and discuss the possibilities in reproductive genetics diagnosis as Preimplantational (PGD), pregnancy lost analysis using Bac on beads analysis.

1371W

Female infant with t(X;Y), including *SRY*. *S. Ramanathan[1], M.D. Maxwell Lutz[2], H.S Brar[2], R.D. Clark[1].* 1) Pediatric Genetics, Loma Linda University Health Care, 2195 Club Ctr Dr, Ste A Loma Linda, CA; 2) Maternal Fetal Medicine and Genetics, 4000 14th St, Ste 502, Riverside, CA.

We report a 6-week-old female, the product of an uncomplicated pregnancy, who was diagnosed prenatally with a *de novo* sex chromosome abnormality. Amniocentesis done for advanced maternal age detected 46,X,add(X)(p11.4) on routine chromosome analysis. There was a terminal deletion of 22 megabases from Xpter→p22.11 and addition of 5 megabases from Ypter →Yp11.2 on SNP microarray analysis. The baby was inferred to have an unbalanced t(X;Y) with Yp material translocated onto the deleted Xp. The pseudoautosomal region common to both Xp and Yp was present in two copies, one from the normal X and one, presumably, from the Y chromosome on the derivative X. There were two copies of the *SHOX* gene and one copy of the *SRY* gene. The presence of the *SRY* gene was also confirmed by FISH analysis. The gene implicated in the development of gonadoblastomas, *TSPY*, localized to distal Yq, was absent. The baby was born at term by normal vaginal delivery to a 36-year-old G7P4SAb2 mother. Birth weight was 5lb 11oz and length was 18”. An echocardiogram detected a trivial muscular ventricular septal defect (VSD). A normal uterus and normal endometrium were seen on pelvic ultrasound. The ovaries were not visualized; the exam will be repeated at 6 months. At 6 weeks, the baby is healthy, with no stigmata of Turner syndrome and normal external female genitalia on physical examination. Translocations between the X and Y chromosomes are rare. When *SRY* is present, it usually results in phenotypic males or true hermaphrodites, with rare exceptions. The *SRY* gene is typically deleted in females with X/Y translocations (Burnside *et al*., 2008). One possible explanation for the phenotype in our patient could be skewed X-inactivation of the derivative X-chromosome, silencing the *SRY* gene; X-inactivation studies are underway. Alternately, position effect, disrupting *SRY* gene expression due to the chromosome rearrangement could be another explanation (Sharp A *et al*., 2004). The large deletion on the X-chromosome in our patient predisposes to variable features of Turner syndrome. She is, however, expected to have normal stature and to be at negligible risk for gonadobastomas. Other than the trivial VSD, she appears to be a normal female, although the risk for gonadal dysgenesis remains. To our knowledge, this is the first report of prenatal diagnosis of t(X;Y) in a female, with the *SRY* gene being present.

1372W

Detection of identical unbalanced karyotype in two consequent fetuses due to a maternal pericentric inversion of chromosome 18. *F.I. Sahin[1], O. Ozer[1], E. Tarim[2], Z. Yilmaz[1].* 1) Department of Medical Genetics, Baskent University Faculty of Medicine, Ankara, Turkey; 2) Department of Obstetrics and Gynecology, Baskent University Faculty of Medicine, Ankara, Turkey.

Amniocentesis is the most preferred diagnostic method for detection of chromosomal abnormalities in the prenatal period. Rapid and correct diagnosis for chromosome abnormalities is important during the prenatal diagnostic process. Different diagnostic approaches could be use for prenatal diagnosis but conventional cytogenetic analysis is always accepted as the gold standard. QF PCR is a method for using rapid diagnosis of numerical chromosome abnormalities, enabling rapid diagnosis of numerical and to some extent structural abnormalities such as partial trisomy. During prenatal diagnosis, diagnostic tools need to be used in time and situation. Recombinant chromosome 18 due to familial inherited pericentric inversion chromosome 18 was detected previously in prenatal and postnatal cases. In the current case, we report a recurrent partial trisomy 18 due to a maternally inherited inversion 18 karyotype. Amniotic fluid sample of a 29 years old 17 weeks pregnant woman was sent to our department due to an increased nuchal fold and risk for trisomy 18 in second trimester maternal serum screening. QF-PCR resulted in partial trisomy 18, confirmed by culture results. Parental blood karyotypes were studied revealing a 46,XX,inv(18)(p11.31q21.1) karyotype of the mother and a normal karyotype of the father. The karyotpe of the fetus was reported as 46,XX,der(18)ish rec(18)dup(18q)inv(18)(p11.31q21.1)(18qter++)mat. The parents decided to terminate the pregnancy after genetic counseling and a new spontaneous pregnancy came up 5 months after termination revealing a karyotype similar to the first pregnancy. The mother did not give her decision on to terminate or continue the pregnancy yet. As experiencing two repetitive unbalanced fetuses was so annoying to the parents, during the genetic counseling session after the second unbalanced pregnancy, preimplantation genetic diagnosis was suggested to the parents to prevent another unbalanced fetus. Partial trisomy 18q and monosomy 18p are uncommon chromosomal abnormalities with variable phenotypic expressions. Using prenatal and preimplantation diagnostic tools to prevent liveborn unbalanced infants is important in such cases enabling to take home a healthy baby.

1373W

Limitations of quantitative fluorescent polimerase chain reaction (QF-PCR) method on detection of common chromosome aneuploidies. *Y.K. Terzi[1], O. Ozer[1], Z. Yilmaz[1], E. Tarim[2], F. Yanik[2], F.I. Sahin[1].* 1) Department of Medical Genetics, Baskent University Faculty of Medicine, Ankara, Turkey; 2) Department of Obstetrics and Gynecology, Baskent University Faculty of Medicine, Ankara, Turkey.

The quantitative fluorescent polymerase chain reaction (QF-PCR) assay is one of the rapid aneuploidy detection (RAD) tests. It allows detection of common chromosome aneuploidies prior to full karyotype analysis. Besides its' advantages, QF-PCR results have some technical limitations. Analysis of QF-PCR results is very difficult and sometimes impossible in case of maternal cell contamination, low levels of mosaicism, absence of informative marker for one chromosome, and partially trisomies or monosomies. In this study we aimed to discuss limitations of QF-PCR in our cohort. Genomic DNA was extracted from amniotic fluid or chorionic villi samples and QF-PCR was performed by a commercially available diagnostic kit including a set of STR markers for chromosomes 13, 18, 21, X and Y (Devyser, Sweden). In addition, our kit provided us to make a comparison of X chromosome copy number with an autosomal chromosome (Chromosome 7). PCR products were analyzed with ABI310 Genetic Analyzer (Applied Biosystems, Foster City, CA) and GenescanV3.7 software. In this study, a retrospective analysis was performed on 538 prenatal samples which have been analyzed with QF-PCR for chromosomes 13, 18, 21, X and Y. Five hundred and nineteen samples were reported as normal or abnormal accurately, however, results of the 19 samples were not assessed because of maternal cell contamination (2.42%). Absence of adequate copy of informative markers was detected for chromosomes 13, 21, X/Y and 7/X (1.11%). Absence of PCR products was observed in one patient (0.19%). As a conclusion, although QF-PCR is a rapid and useful diagnostic tool for prenatal diagnosis for common chromosomal aneuploidies, it is insufficient or makes it hard to evaluate in some circumstances due to its own limitations. Because of this, we believe that QF-PCR should be an adjunct to conventional karyotype analysis.

1374W

A recurrent familial L410R mutation in the TSC2 gene associated with isolated cardiac rhabdomyomas. *R. Teitelbaum[1], F. Golding[2], G. Ryan[4], D. Myles-Reid[1], R.K. Basran[5], A. Milunsky[5], D. Chitayat[1,3].* 1) The Prenatal Diagnosis & Medical Genetics Program, Mount Sinai Hospital, Toronto, ON, Canada; 2) Division of Cardiology, Hospital for Sick Children, Toronto, ON, Canada; 3) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, ON, Canada; 4) The Fetal Medicine Unit (SPP), Mount Sinai Hospital, Toronto, ON, Canada; 5) Center for Human Genetics, Department of Pediatrics, Boston University School of Medicine.

The tuberous sclerosis complex (TSC) is an autosomal dominant condition with clinical and genetic heterogeneity. Many cases are caused by de novo mutations in the TSC2 gene and the penetrance is 100%. We report a family with an L410R mutation in exon11 of the TSC2 gene with isolated cardiac rhabdomyomas. The first pregnancy of a non-consanguineous Caucasian couple initially presented with cardiac rhabdomyomas on fetal ultrasound. The pregnancy was interrupted and the autopsy showed a female fetus (46,XX) with isolated multiple rhabdomyomas. Mutation analysis of TSC1 and TSC2 showed a heterozygous L410R mutation in exon 11 of the TSC2 gene. This change had not been previously reported in the literature and its clinical significance was not known. It was predicted to be possibly damaging by Poly Phen analysis and not tolerated by SIFT analysis. Parental physical examination including Wood's lamp skin examination, brain CT scan, echocardiography and eye examination was normal. The L410R gene mutation was detected in the father. It was not detected in the paternal grandmother. The paternal grandfather was lost to follow-up. A subsequent pregnancy was again complicated with isolated fetal rhabdomyomas. The pregnancy continued and the multiple cardiac rhabdomyomas were confirmed postnatally and decreased in size. Mutation analysis detected the familial L410R TSC2 mutation in the female newborn. At the age of 3-years, no other findings associated with TSC have been identified and her development is normal. Physical examination including Wood's lamp skin examination, brain CT scan, and eye examination have been normal. This family raises the possibility that the L410R mutation in the TSC2 gene may be associated with isolated cardiac rhabdomyomas. Longer follow-up is needed to confirm this observation.

1375W

Mutations in the planar cell polarity gene CELSR1 associated with neural tube defects in humans. *R. Allache[1,2], P. De Marco[3], E. Merello[3], V. Capra[3], Z. Kibar[1,2].* 1) Pathology and Cell Biology, University of Montreal, Montreal, Quebec, Canada; 2) Department of Obstetrics and Gynecology, CHU Sainte Justine Research Center, Montreal, Quebec, Canada; 3) U.O. Neurochirurgia, Instituto G. Gaslini, Genova, Italy.

Neural tube defects (NTDs) represent a group of very common congenital malformations in humans, affecting 1-2 infants per 1000 births and represent a major cause of perinatal mortality. They are caused by a partial or complete failure of the spinal column to close during early development of the embryo. The causes of NTDs are complex involving both genetic and environmental factors. The most common forms of NTDs are anencephaly and spina bifida. Animal models have proven to be a powerful approach in dissecting the complexity underlying these traits. Studies from these models have provided strong evidence for the involvement of a signaling pathway called the non-canonical Frizzled (Fz) /Dishevelled (Dvl) or the planar cell polarity (PCP) pathway in NTDs. This pathway controls an important morphogenetic process of convergent extension (CE) which is essential during formation of the neural tube. Mutations in genes of this pathway cause NTDs in mouse models, and, importantly, recent studies have shown that mutations in two PCP genes, VANGL1 and VANGL2, were associated with human NTDs. These studies strongly implicate the Fz/Dvl pathway in the causation of NTDs. Our study aims at molecular and genetic analyses of another gene of this pathway, CELSR1, in human NTDs. The open reading frame and exon-intron junctions of CELSR1 were amplified by PCR and the products were sequenced in a large cohort of 471 non-syndromic NTD patients. We have identified 31 novel and rare missense mutations which were absent in 637 ethnically-matched controls. Ten of these mutations affect highly conserved amino acid residues. Our findings implicate CELSR1 as a risk factor for NTDs in a subset of patients and provide additional evidence for a pathogenic role of PCP signaling in these malformations.

1376W

Association of A80G polymorphism in the RFC1 gene with the risk for having spina bifida-affected offspring in Southeast Mexico and interaction with C677T-MTHFR. *L. Gonzalez-Herrera, O. Vargas-Sierra, S. Contreras-Capetillo, G. Perez-Mendoza, I. Castillo-Zapata, D. Pinto-Escalante, T. Canto-Cetina.* Dept Genetica, Univ Autonoma de Yucatan, Meridal, Yucatan, Mexico.

Spina bifida (SB) is the most prevalent neural tube defect (NTD) in Yucatan, at Southeast Mexico. Polymorphisms in folate metabolism genes have been suggested as susceptibility candidates for SB. Ingested folate is hydrolyzed into monoglutamate forms in the intestine by the reduced-folate carrier (RFC-1). RFC1 carrier transports folate across the placenta. The polymorphism A80G of the RFC-1 gene could impair folate transport from maternal blood to the fetus; and has been demonstrated as a genetic risk factor for NTD. Additionally this variant may interact with low folate status and MTHFR mutations to increase NTDs risk. This study analyzed the association of A80G polymorphism in the RFC1 gene with the risk for having spina bifida affected-offspring and its interaction with C677T-MTHFR polymorphism. A case-control association study was performed with 119 mothers and 64 fathers of children with open-dorsolumbar SB as cases. The control group was 140 mothers and 55 fathers with healthy offspring. Genotyping for A80G polymorphism in the RFC1 gene was performed by PCR-RFLP. Statistical standard X2 analysis was calculated using STATA 10.2 software. Genotype frequencies were distributed according to Hardy-Weinberg expectations ($p>0.05$). Distribution of allele G was significantly higher in cases than controls ($p= 0.04$), suggesting that the allele G is associated with the parental risk of having an SB-affected child. Comparison of genotypes GG vs AA+AG supported this significant finding: OR=1.763, IC 1.15-2.70, $p= 0.009$. Stratification by gender showed that risk was only significant in mothers (OR=1.63, IC 0.968-2.75, $p= 0.04$). The interaction of A80G-RFC1/C677T-MTHFR, did not show significant differences between case and control parents ($p>0.05$). However, stratification by gender showed differences for the genotype combinations AG/CT and GG/CT in mothers ($p=0.039$). In fathers, the genotype combination with both mutants GG/TT was significantly associated with the risk for having SB-affected offspring ($p= 0.043$). Our findings suggested the association of allele G of A80G-RFC-1 with the genetic risk for having an SB-child, which might be controlled maternally. There might be an interaction between A80G-RFC1 and C677T-MTHFR, since the genotype combinations GG/CT in mothers and GG/TT in fathers were associated with the higher risk to have a child with SB in Southeast, Mexico.

1377W

Spectrum and frequency of pathogenic chromosomal structural variants detected among foetuses with increased nuchal translucency and have a normal karyotype. *K. CHOY, H.K. WONG, W. CHONG, K.Y. KWOK, K.W. SUEN, C.C. WANG, T.K. LAU, T.Y. LEUNG.* The Chinese University of Hong Kong, Shatin, Hong Kong.

Large nuchal translucency (NT) has been known to be associated with chromosomal disorders. To determine the prevalence of submicroscopic chromosomal abnormalities of fetuses that had increased nuchal translucency and an apparently normal karyotype, DNA was extracted from a cohort of 97 stored CVS samples retrieved from a database of fetuses with increased first trimester nuchal translucency >3.5mm and normal karyotype result during first trimester screening. These samples were examined by microarray based comparative genomic hybridization (aCGH) using a 44K custom designed oligonucleotide array specifically constructed for prenatal screening with an average genome-wide resolution <100kb across the genome. Variations in copy number (CNVs) were reported after excluding known non-pathogenic variants. Nine of the 97 (9.3%) cases that were tested had abnormal aCGH results with known pathogenic micro-deletions or micro-duplications ranging from 269kb to 8 Mb size. Our data indicate that most of the pathogenic CNVs identified (7/9) were known to be associated with neurodevelopmental delay and/or mental retardations phenotype. There was also a significant association between the severity of increased NT and an abnormal aCGH result (NT>4mm; $p<0.05$). Fetuses with morphological anomalies or normal morphology respectively had an abnormal aCGH result indicating pathogenic CNVs are not restricted to fetuses that have other morphological anomalies visible by ultrasound scan. This finding indicates that the incidence of submicroscopic chromosomal abnormalities in the group of fetuses with increased NT but normal karyotypes is substantial. Chromosome microarray analysis may improve clinical recognition of chromosomal abnormalities that are currently not identified prenatally.

1378W

Diagnostic utility of array-based comparative genomic hybridization (aCGH) in a prenatal setting- our experience of 502 cases. *i. maya[1], b. Davidov[1], e. taub[1], y. Zalzstein[1], m. shohat[1,2].* 1) genetic institute, rabin medical center, petah tikva, Jordan; 2) Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Objective: Array-based comparative genomic hybridization (aCGH) is a technique for detecting submicroscopic deletions and duplications. There is limited information regarding its use in the prenatal setting. Here, we present our experience of 502 prenatal aCGHs between 2006 and 2011. Materials and Methods: We reviewed the medical files of 502 prenatal cases seen in the Recanati Genetic Institute between 2006 and 2011 on whom prenatal aCGH was carried out. Results: The indications for testing were fetal anomalies on ultrasound (U/S) (41%), advanced maternal age (AMA)(18%), family history of a disorder of unknown etiology (17%), parental concern (13%), Abnormal routine karyotype (9%) and abnormal serum biochemical screening for common fetal aneuploidies (1%). Of 45 cases with a known abnormal karyotype, 35 (80%) had a normal aCGH. This enabled us to reassure the families and the pregnancies were continued. The remaining 10 (20%) showed an abnormal aCGH, confirming the chromosomes were unbalanced, and after proper genetic counsling they were terminated. Of 457 cases with a normal karyotype, 7 had an abnormal aCGH with known clinical significant and additional 4 cases with unclear clinical significance. Overall, new clinically relevant results were detected by aCGH in 52 cases, providing additional information for prenatal genetic counseling and risk assessment (detection rate of 1: 10). Conclusion: Our results suggest that prenatal aCGH should be offered particularly in cases with abnormal U/S (detection rate was 1:52). We found the rate of detecting an abnormality by aCGH in low-risk pregnancies (normal fetal U/S) was 1:83, but larger studies will be needed to expand our knowledge and validate our conclusions.

1379W

Cockayne Syndrome and prinatal diagnosis. *B. Bozorgmehr, F. Afroozan, A. Kariminejad.* Dept Clinical Genetics, K-N Pathology & Genetics Ctr, Tehran, Iran.

Cockayne Syndrome is a rare autosomal recessive disorder characterized by profound postnatal growth deficiency with loss of adipose tissue, microcephaly, mental retardation, unsteady gait and peripheral neuropathy. We are reporting a 6-year-old girl with severe growth and developmental delay, microcephaly, mental retardation, sunken eyes and photosensitive dermatitis.Her parents are related and had a history of death of their first daughter 3 years ago.Her diagnosis confirmed by a defect in DNA repair in fibroblast followed exposure to ultraviolet light.we performed prenatal diagnosis for their next pregnancy and they have normal child now.

1380W

Prenatal Noonan and aCGH testing for increased nuchal translucency/thickness and cystic hygroma. *C. Coffeen[1], N. Nakata[1], S. Bhatt[2], V. Watiker[2].* 1) Esoterix Genetic Laboratories, LLC, Los Angeles, CA; 2) G-Path Medical Associates, Inc., Los Angeles, CA.

Increased nuchal translucency/thickness (NT) and cystic hygroma confer an increased risk for chromosome abnormalities as well as Noonan syndrome. Genetic counselors at Genzyme Genetics[SM1] offer counseling for Noonan syndrome evaluation and array CGH to patients with increased NT or cystic hygroma and normal prenatal karyotype. A retrospective analysis was performed to evaluate the utility of this testing. Patients who were referred to Genzyme Genetics for genetic counseling from April 2010 through January 2011, and had an abnormal ultrasound finding of increased NT or cystic hygroma and subsequent normal fetal karyotype were included in the review. A total of 393 patients met the inclusion criteria, 82% (322/393) had increased NT and 18% (71/393) had cystic hygroma. All patients were counseled regarding the ultrasound findings, possible impact, and were offered prenatal Noonan testing and aCGH. Fifty-seven (15%) patients elected further testing, 6 had only Noonan testing, 35 had only aCGH, and 16 had both tests. Among the 22 patients who had Noonan testing, 4 (18.2%) were found to be mutation positive. Among the 51 who had aCGH testing, 4 (7.8%) had an abnormal aCGH result. Forty of the 57 patients who underwent further testing (70%) had an ultrasound finding of increased NT; 3/40 (7.5%) were mutation positive for Noonan and 3/40 (7.5%) had abnormal aCGH results. Among the 17/57 (30%) with a cystic hygroma; 1/17 (5.9%) was mutation positive for Noonan and another one had a variant of unknown clinical significance. Overall, 14.0% (8/57) of patients who had Noonan or aCGH testing were found to be mutation positive for Noonan syndrome or had an abnormal aCGH result. These findings support the importance of offering Noonan and aCGH testing to patients with increased NT or cystic hygroma and normal fetal karyotype.

1381W

Diagnosis and clinical presentation of 20 patients referred for antenatal hyperechogenic kidneys with renal cysts and diabetes syndrome (RCAD). *K. Dahan[1], K. Lolin[2], K. Ismaili[2], L. Collard[3], B. Grisart[1], N. Godefroid[4], V. Benoit[1].* 1) Ctr Human Gen, Institut de Pathologie et de Génétique, Gosselies, Belgium; 2) Department of Pediatric Nephrology, Hôpital Universitaire des Enfants-Reine Fabiola, Université Libre de Bruxelles (ULB), Brussels, Belgium; 3) CHC, clinique de l'Espérance, CHU Sart-Tilman, rue Saint-Nicolas, 4420 Montegnée, 4000 Liège 1; 4) Department of Pediatrics, Cliniques Universitaires St-Luc, Université Catholique de Louvain, Brussels, Belgium.

Hepatocyte nuclear factor 1beta (HNF1beta) mutations have been recognized to cause renal cysts and diabetes syndrome (RCAD), also referred to as maturity-onset diabetes of the young, type 5 (MODY5). We performed mutational analyses including cytogenomic array analysis and we found HNF1B abnormalities in 20 patients, all referred for antenatal hyperechogenic kidneys with renal hypodysplasia (n =11), kidney enlargement (n=2), unilateral multicystic dysplastic kidney (MCDK; n = 3) and bilateral MCKD (n = 1). The recurrent 1.4 Mb deletion at 17q12 comprising HNF1B gene was detected in 11/20 while a single exon deletion/duplication was found in 2/20, missense changes in 5/20, nonsense change in 1/20 and a single-nucleotide deletion in one of them. Compound heterozygosity was observed in one girl who is carrier for a paternally inherited missense mutation and a de novo HNF1B deletion. HNF1B anomalies were responsible for severe oligohydramnios and termination of pregnancy in 1/20. In the remaining patients, the GFR decreased with longer follow-up and was lower in patients with solitary functioning dysplastic kidney (3/20). Extrarenal manifestations are frequent especially among the carriers of HNF1B deletion including mild facial dysmorphic features with macrocephaly in 5, jejunum atresia in one, elevated liver enzyme levels in 5, short stature in 2 while no one had presented autistic features. Additionally, regular ultrasound examination had revealed a Wilms tumor in an asymptomatic girl (2 years-old), raising the question on hypothetical relationship between HNF1B germline mutation and increased risk for neoplasia. Therefore, prenatal counseling and follow-up should be multidisciplinary.

1382W

Difficulty in Prenatal Diagnosis of Short Ribs with Polydactyly. *C. Hills[1,2], M.E. Pierpont[2,3].* 1) Children's Heart Clinic, Minneapolis, MN; 2) Children's Hospitals and Clinics of Minnesota, Minneapolis, MN; 3) University of Minnesota, Minneapolis, MN.

At 20 weeks gestation, a fetal ultrasound demonstrated a very narrow thoracic cage, shortened ribs, polydactyly, and reduced long bone growth. These results were consistent with Short Rib Polydactyly Syndrome (SRPS) type III, frequently a lethal condition. Parents declined amniocentesis. Minimal intervention was planned at delivery based on the poor prognosis. The parents are consanguineous (first cousins once removed). A paternal aunt had a stillborn infant with short limbs. After term delivery, the female infant was vigorous. The weight was 3400 g (50th centile), length 48 cm (20th centile) and OFC 36 cm (75th centile). Key physical findings included lack of respiratory distress, mild frontal bossing, depressed nasal bridge and hypertelorism. There was a central raphe from the nose to the lips. The upper lip gum was attached closely to the anterior alveolar ridge. The chest was narrow and appeared wider at the lower end of the thorax. Lungs were clear. A systolic murmur was heard. There were short limbs bilaterally with more proximal shortening than distal shortening and polydactyly of all 4 limbs. There were hypoplastic, dysplastic fingernails and toenails. An echocardiogram demonstrated a complete atrioventricular septal defect. A bone survey showed a skeletal dysplasia with narrowed thorax, shortened ribs, rounded metaphyses and lateral spikes associated with the distal aspect of the femurs and humeri. There was polydactyly and shortening of the long bones including the tubular bones of the hands and feet. The patient's clinical course and physical findings including her respiratory status, nail hypoplasia, appearance of the anterior alveolar ridge and the type of cardiac malformation were more consistent with Ellis-van Creveld syndrome (EVCS), but the patient's bone survey was interpreted as consistent with SRPS, type III. Mutations in the DYNC2H1 gene on chromosome 11q cause both SRPS type III and asphyxiating thoracic dystrophy (Jeune syndrome). EVCS is associated with mutations in the EVC1 and EVC2 genes on chromosome 4p. Cytogenetic testing showed a normal 46,XX karyotype. DNA analysis showed a homozygous deletion, c.1108delT in exon 10 of the EVC2 gene consistent with a diagnosis of EVCS. The clinical and radiological phenotypes of SRPS type III and EVCS have many similarities. This case illustrates the difficulty in making a definitive diagnosis prenatally and reviews the findings that led to the diagnosis of EVCS in this patient.

1383W

Fryns-like multiple congenital anomalies syndrome with 1,13 Mb chromosome 16p13.11 deletion detected by array-comparative genomic hybridization. *J. Martinovic[1,2], C. Fallet-Bianco[3], B. Simon-Bouy[4], J. Michel[5], E. Alix[5], D. Sanlaville[5].* 1) Unit of Fetal Pathology, Hosp Antoine Beclere, Clamart, France; 2) Unit of Fetal Pathology, Department of Pathology, Laboratoire Cerba, St Ouen L'Aumone, France; 3) AP-HP, Department of Neuropathology, Hôpital Saint-Anne, Paris, France; 4) Cytogenetics Laboratory, Centre Hospitalier de Versailles, Le Chesnay, France; 5) Cytogenetics Department, Hospices Civils de Lyon and UCBL1, Lyon, France.

We present a 27-week fetus with Fryns Syndrome (FS)-like phenotype who had submicroscopic chromosome 16p13.11 deletion of 1,13Mb detected by array-comparative genomic hybridization (CGH) after normal karyotyping with G-banded chromosome analysis. Fetal phenotype on fetopathological examination disclosed growth restriction, dysmorphic features including flat, coarse facies, depressed nasal bridge, long philtrum, retrognathia, hypoplastic ears, unilateral renal agenesis, uterine hemi-agenesis, costo-vertebral malsegmentation/fusion defects, hypoplastic terminal phalanges of fingers, pachygyria, hypoplastic corpus callosum and cerebellum. A 180,000-oligonucleotide microarray (Agilent Technologies, Santa Clara, CA) was used to analyze the fetal DNA. Array CGH revealed a 1.16 Mb 16p13.11 deletion in the fetus between genomic positions 15,039,244-16,174,807 (hg18). This deletion was confirmed by FISH using the RP11-368O18 BAC clone. This deletion occurred de novo, and should be presumed as a cause of this multiple congenital anomalies syndrome. This deletion includes several genes, in particular NDE1. Recurrent deletions in 16p13.11chromosomal region have been previously reported and have been associated with epilepsy, multiple congenital anomalies, and cognitive impairment. Recently, based on Nde1-null mice experiment, it was proposed that NDE1 have an essential role in human cerebral cortical neurogenesis. Moreover NDE1 deficiency causes both a severe failure of neurogenesis and a deficiency in cortical lamination. So, NDE1 haploinsuffiancy could explain cerebral malformation observed in the fetus. Nevertheless other malformations described in the fetus have not been previously reported in patients with this 16p13.11 deletion. We conclude that phenotypes similar to FS, however without diaphragmatic hernia, can be caused by a new clinically identifiable chromosome deletion syndrome at 16p13.11 including growth retardation, genito-urinary malformations, skeletal anomalies with costo-vertebral malseglmentation defects and terminal phalangeal hypoplasia, as well as cerebral anomalies with pachygyria, hypoplastic corpus callosum and cerebellum. This case expands the clinical phenotype described in patient with 16p13.11 microdeletion. We also conclude that array CGH should be performed prior to the definite diagnosis of FS in order to provide a causal explanation and an accurate genetic counselling to the family.

1384W

Retrospective study of 1,094,807 newborns showed the national average rate of preterm birth in China is 5.5%, which has been increased 14% from 2005 to 2009. *N. Zhong[1, 2], X-L. Zhao[2], China Preterm Clinical Research Consortium.* 1) Human Genetics, New York State Institute for Basic Research in DD, Staten Island, NY; 2) Peking University Center of Medical Genetics, Beijing, China.

Preterm birth (PTB), accounting for 5-12% newborns world-wide, is recognized as the leading cause for the neonatal death. However, the PTB rate in China was unknown. To determine the Chinese PTB rate, a retrospective study was performed by the China Preterm Clinical Research Consortium (CPCRC) headed by the Peking University Center of Medical Genetics. A total number of 1,094,807 newborns, delivered from January 2005 to December 2009 among 132 hospitals from 23 provinces, were studied based on hospital records. Our results showed that The PTB rate in each year is: 5.18% for year 2005, 5.36% for year 2006, 5.29% for year 2007, 5.74% for year 2008, and 5.92% for year 2009, giving rise to an increase of 14% from 2005 to 2009. The rates are noticed highly varied by geographic regions. The highest rate of 11.4% occurred at Henan province, which is located at center of China in the year of 2006. To assess the risk factors associated with PTB, environmental factors including education background, economic incomes, air pollutions, and geographical location were analyzed. Underdeveloped area was selected to compare to the advanced industrial area. Through our birth defect surveillance network, 40,000 newborns were crossed with 30,000 prenatal screenings, by which 1,919 pregnancies including 189 deliveries with twins were extracted. Our data showed that among 1,730 singletons, 8.5% was delivered before 37 weeks as the PTB in the industrial area, which is higher than the national average rate; 4.80% with weight less than 2,500 grams, as the low weight births (LBW), 1.67% had one or more structural dysmorphology, which were 13.6% (20/147) among PTBs but only 0.58% (9/1,558) in full-term births; and 1.67% stillbirths. To correlate to the prenatal screening, PTB at <32 weeks had 2.94%, 32-36 weeks had 6.19%, and >37 weeks had 3.47% of AFP>2MoM; <32 weeks had 11.76%, 32-36 weeks had 4.42%, and >37 weeks had 4.30.47% of HCG>3MoM; and <32 weeks had 44.1%, 32-36 weeks had 2.65%, and >37 weeks had 0.57% of dysmorphology. Among 189 twin-pregnancies, 46.5% had PTB and 5.29% had stillbirth. In addition, 32.39% had AFP>2MoM (compared to 26.73% in full-term birth), 21.02% had HCG>3MoM (compared to 13.37% in full-term birth), and 2.27% (compared to 0.5% in full-term birth) had physical abnormalities. Our study clearly provided significant evidence that increased level of mid-term prenatal screening of maternal AFP and HCG could be applied as a genetic signature for PTB.

1385W

Fetal hydrotorax - case report. *D. Albu[1, 2], C. Albu[1,2], E. Severin[1], M. Dumitrescu[3].* 1) University of Medicine and Pharmacy Carol Davila, Bucharest, Romania; 2) Alco San Clinic, Bucharest, Romania; 3) Marius Nasta Hospital, Bucharest, Romania.

Objective: To determine wether or not a fetus with hydrothorax has Down syndrome. Background: Fetal hydrothorax refers to a collection of fluid within the fetal thoracic cavity as a result of leakage or generalized fluid retention from a variety of causes. Case report: A 27-year-old Caucasian female, pregnant for the first time, was referred at 27 weeks' gestation for a routine prenatal ultrasound. The couple had normal general health and was not consanguineous. There was no family history of genetic disorders. Routine ultrasonography, triple test (AFP,uE3, hCG), selective ultrasonography for detection of fetal abnormalities, thoracocentesis and amniocentesis were performed. Results: Ultrasound examination at 27 weeks of gestation revealed a single fetus with a large hydrothorax (~200ccm) on the left side compressing the lung and pushing down the driaphragm. A sample of 70ccm pleural fluid was obtained at 28 weeks of gestation through fetal thoracocentesis by ultrasound guided puncture. Post procedural ultrasound examination revealed positive lung expansion. A sample of 40cmc amniotic fluid was also obtained for further analysis. The cesarean section was scheduled at 33 weeks of gestation after treatment with dexamethasone. Triple test was not sensitive to the presence of a possible trisomy. Genetic investigations were recommended because fetal hydrothorax is frequently associated with Down Syndrome. Karyotype and QF-PCR from both amniotic fluid and thoracic fluid indicated the presence of Trisomy 21. Conclusion: Prenatal diagnosis was useful in management, prognosis and detection of Down Syndrome through analysis of thoracic and amniotic fluid, in a fetus with hydrothorax.

1386W

Prenatal Ultrasound and Autopsy Findings in Costello Syndrome. *K. Chong[1], J. Jessen[1], K. Fong[2], S. Keating[3], P. Shannon[3].* 1) The Prenatal Diagnosis and Medical Genetics Program, Department of Obstetrics and Gynecology, Mount Sinai Hospital, University of Toronto; Toronto, ON, Canada; 2) Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, University of Toronto; Toronto, ON, Canada; 3) Department of Medical Imaging, Mount Sinai Hospital, University of Toronto; Toronto, ON, Canada.

Costello syndrome is an autosomal dominant genetic condition characterized by postnatal growth deficiency, dysmorphic facial features, mental retardation and cardiac defects including hypertrophic cardiomyopathy and arrhythmia. Prenatally, the phenotype for Costello syndrome has not been well recognized in pregnancy as the condition itself is rare; most of the published literature has been via case reports and studies compiled from parental surveys of liveborn children. We report a 37 year old G4P0SA3 woman who presented at 18 weeks gestation with a positive screen for Down syndrome. Amniocentesis revealed a normal male karyotype. Detailed fetal ultrasound at 25 weeks showed polyhydramnios, increased nuchal fold and discrepant limb/ head measurements. Fetal echocardiogram confirmed biventricular hypertrophy and possible hypoplastic aortic arch. Follow up fetal ultrasound at 30+weeks gestation showed large head and abdomen, bilateral pleural effusions, hepatomegaly, enlarged kidneys and generalized edema. Given the severity and poor prognosis of the cardiomyopathy, the pregnancy was induced and the baby died. Autopsy revealed hydrops fetalis, craniofacial dysmorphic features, hypertrophic cardiomyopathy and skeletal muscle changes suggestive of a congenital myopathy. Genetic testing revealed a common mutation in the HRAS gene confirming the diagnosis of Costello syndrome. The importance of fetal autopsy in pursuing a rare diagnosis like Costello syndrome in the setting of prenatal cardiomyopathy is discussed.

1387W

Interaction within the 10q24.2 region with in utero tobacco smoke exposure on asthma risk in Latino children. *S.S. Oh[1], D. Hu[1], C. Gignoux[1], L. Roth[1], L. Borrell[2], S. Sen[1], H. Farber[3], R. Kumar[4], F. Lurmann[5], P. Avila[6], D. Serebrisky[7], S. Thyne[8], W. Rodriguez-Cintron[9], J. Rodriguez-Santana[10], E. Gonzalez-Burchard[1].* 1) UC San Francisco, San Francisco, CA; 2) CUNY, New York, NY; 3) Texas Children's Hospital, Houston, TX; 4) Children's Memorial Hospital, Chicago, IL; 5) Sonoma Technologies Inc., Sonoma, CA; 6) Northwestern University, Chicago, IL; 7) Jacobi Medical Center, New York, NY; 8) San Francisco General Hospital, San Francisco, CA; 9) VA Medical Center, San Juan, Puerto Rico; 10) Centro de Neumologia Pediatrica, San Juan, Puerto Rico.

BACKGROUND Asthma is a complex disease with a multifactorial etiology. Asthma morbidity and mortality in U.S. Latinos is widely disparate (Puerto Rican children are among the most affected while Mexican American children are among the least affected), suggesting environmental and genetic influences. Smoking during pregnancy (i.e., in utero tobacco exposure) places the unborn child at risk for developing impaired lung function and asthma. We hypothesized that the joint exposure of in utero tobacco smoke and polymorphisms of asthma-related genes would synergistically increase asthma risk. **METHODS** We used a multi-center case-control study of Latinos aged 8-21 sampled nation-wide to investigate the association of asthma with top hits of SNPs identified from a meta-analysis of 9 GWA studies of asthma risk, 5 of which were conducted among Latinos. Allelic associations were assessed with a logistic regression model assuming additive inheritance. We also estimated the SNPs' interaction effects with in utero smoking on asthma risk by including interaction terms between in utero exposure and SNP genotype in logistic regression models. DNA from our sample of 1,049 asthmatic and 1,058 asthma-free Latino children was genotyped on an Infinium iSelect chip. **RESULTS** After adjusting for postnatal smoke exposure, Latino sub-ethnicity, global ancestry, age, and gender, we observed an enrichment of allelic association p-values from a 109kb region in 10q24.2, but only among children with in utero exposure to tobacco smoke. Among the 24 SNPs genotyped from this region, 24, 19, and 12 SNPs were significant at alpha levels of 0.05, 0.01, and 0.005, respectively. The SNP minor allele frequencies were not significantly different between cases and controls unexposed to in utero tobacco smoke, but among subjects exposed to in utero tobacco smoke, the minor alleles were present two to three times more frequently among cases than among controls, suggesting SNP-smoking interactions. In our adjusted logistic regression model, interaction terms for 24, 21, and 12 SNPs were significant at alpha levels of 0.05, 0.01, and 0.005, respectively. **CONCLUSION** Association of the 10q24.2 region with asthma is strongly modified by in utero tobacco smoke exposure, independent of postnatal tobacco smoke exposure. Future studies to replicate and extend these results will help identify mechanisms underlying the interactions.

1388W

Immune and fibrosis pathway genes are important in anti-SSA/Ro exposed neonates with congenital heart block and/or cardiomyopathy. *R.M. Clancy[1], P.S. Ramos[2], M.C. Marion[2], J.P. Buyon[1], C.D. Langefeld[2].* 1) New York University Langone School of Medicine, New York, NY; 2) Department of Biostatistical Sciences and Center for Public Health Genomics, Wake Forest School of Medicine, Winston-Salem, NC.

Fetuses exposed to maternal anti-Ro/SSA antibodies can develop congenital heart block and/or fibrotic cardiomyopathy (cardiac manifestations of neonatal lupus), which carry substantial mortality. Several lines of evidence support a fetal genetic risk, including increased recurrence rates, concordance in a third of monozygotic twins, and results from the first published genome-wide association study (GWAS). Since there may be a global enrichment of specific pathways overlooked if only the most significant associations are considered, enrichment of associations in specific pathway-related genes was herein further evaluated. Based on the proposed pathogenesis which invokes an injury cascade initiated by antibody binding to apoptotic cardiocytes and subsequent inflammation and tissue fibrosis, we posit that immune and fibrotic responses may promote cardiac injury in these children. To test this hypothesis, we used Ingenuity Pathway Analysis to compile a list of all genes genes with immune (n=1,993 genes) and fibrotic (n=327 genes) functions. We then tested for an enrichment of admixture and genomic control adjusted significant P-values (at alpha=0.001) in SNPs that met quality control criteria. We observed a strong enrichment of significant SNPs (P<1x10-7) in both immune and fibrosis-related pathways; the enrichment existed whether including (n=12,782 immune, 2,417 fibrotic SNPs) or excluding (n=12,472 immune, 2,387 fibrotic SNPs) the extended HLA region. As a control, genes with bone functions (n=568 genes) did not exhibit enrichment (P=0.08; 4,355 SNPs outside of the HLA). To narrow down the specific immune-related pathways, we tested for enrichment in specific candidate immune pathways: apoptosis (2,283 genes), T cell function (980 genes), cell infiltration (311 genes), innate immune cell function (1,381 genes), interferon (102 genes), TLRs (10 genes) and calcium channels (13 genes). Excluding the extended HLA region, the apoptosis- (P<1x107; 15,935 SNPs), T cell- (P=1.2x10-4; 5,768 SNPs), innate immune cell- (P<1x10-7; 8,730 SNPs), interferon- (P=6.4x10-5 5; 417 SNPs) and TLRs- (P=8.7x10-6; 46 SNPs) related pathways continued to show an enrichment of significance. These results suggest that immune- and fibrosis-related genes are likely involved in predisposing to cardiac manifestations of neonatal lupus.

1389W

Phenotype-Specific Adverse Effects of *XPD* Mutations on Human Prenatal Development Implicate Transcription Factor (TF)IIH in Regulation of Cellular Metabolism in Placenta. *R. Moslehi[1], A. Kumar[1], J.L. Mills[2], X. Ambroggio[3], C. Signore[2], A. Dzutsev[4].* 1) Epidemiology & Biostatistics, School of Public Health, and Cancer Research Center, University at Albany, Rensselaer, NY; 2) National Institute of Child Health and Human Development (NICHD), NIH; 3) National Institute of Allergy and Infectious Disease (NIAID), NIH; 4) National Cancer Institute (NCI), NIH.

Background Mutations in *XPD* (*ERCC2*), *XPB* (*ERCC3*) and *TTD-A* (*GTF2H5*), genes involved in nucleotide excision repair (NER) and transcription, can cause trichothiodystrophy (TTD). *XPD* mutations can also cause other DNA repair disorders such as xeroderma pigmentosum (XP). We hypothesize that abnormalities in TTD NER/transcription genes affect normal placental development and that fetal genotype and exact genetic abnormality are both relevant to the postulated mechanism. **Methods** We conducted a comparison of all reported XPD-associated TTD (n=43) and XP (n=37) cases with respect to frequencies of pregnancy complications including preeclampsia. We also mapped preeclampsia-associated mutations onto the available XPD crystal structures in order to delineate their biologic impact. Furthermore, we analyzed gene expression microarrays in placenta in order to decipher common biologic pathways of involvement of all TTD NER/transcription genes in normal human placental development. **Results** Compared to XP, TTD-affected pregnancies were associated with significantly higher incidence of preeclampsia (P=0.003), preterm delivery (<37 weeks gestation) (P<0.0001), low birth weight (<2500 grams) (P<0.0001), small for gestational age (SGA)<10thpercentile (P=0.006), SGA<3rd (P=0.003) and NICU admission (P<0.0001). Structural mapping localized the preeclampsia-associated mutations to a C-terminal motif and the helicase surfaces of XPD, regions implicated in binding of XPD with cdk-activating (CAK) and p44 subunits of Transcription Factor (TF)IIH. Time course, gene-gene correlation and pathway analyses suggested important functions for all TTD NER/transcription genes throughout gestation (from 1st to 3rd trimester) and implicated their involvement in regulation of cellular metabolism (via regulation of several nuclear receptors by CAK domain of TFIIH) in placenta. **Conclusion** Our results revealed that a specific subset of *XPD* mutations which lead to TTD, but are unrelated to XP, result in higher risk of preeclampsia and other gestational complications, possibly due to their adverse effects on binding of XPD with CAK and p44 subunits of TFIIH leading to impairment of TFIIH-mediated functions in placenta. Overall, our epidemiologic, molecular and microarray analyses implicated dysregulation of cellular metabolism in addition to transcription pathways, caused by abnormalities in TTD NER/transcription genes, in preeclampsia and other placental vascular complications noted in our study.

1390W

The fetal RHD genotyping and gender determination from cell-free fetal DNA circulating in maternal blood. *A. Stan[1], C. Dragomir[1], L. Savu[1], E. Severin[2].* 1) Genetic Lab , Bucharest, Romania; 2) Carol Davila Univ Med Pharm, Bucharest, Romania.

Background: The NIPD (non-invasive prenatal diagnosis) approach is of greatest interest to the researchers in the prenatal diagnosis field. We report the non-invasive fetal RHD genotyping and fetal gender determination performed in Romania. Objective: Although the prenatal diagnosis procedures currently employed in Romania are based exclusively on invasive sampling procedures of fetal cells our aim was to introduce a NIPD protocol in our country. Patients and Methods: We tested 68 pregnant RhD negative women with RhD positive partners who referred to our laboratory for prenatal diagnosis tests. For the cell-free total DNA extraction from maternal plasma we used the QIAamp® DSP Virus kit. The standard working protocol was modified. We developed two different PCR multiplex based reactions. The first included specific primers for two sequences in the exon 5 and respectively exon 7 of the RHD gene. To the second PCR reaction we added specific primers for fetal gender determination. Both reactions included the) -globin sequence detection as internal control. The PCR products were automated analyzed on the QIAxcel instrument using the QIAxcel DNA High Resolution Kit, QIAGEN. Results: We detected 48 fetuses with RHD positive genotype and 20 fetuses with RHD negative genotype. All 68 cases were analyzed in triplicate. For the last 12 samples we determined the fetal gender along with the RHD genotype; the results were confirmed by the QF-PCR method for aneuploidies detection from CVS or amniotic fluid. Conclusion: Our results confirm that this non-invasive approach is feasible and accurate and will improve the management of mother-fetus RhD incompatibility and avoid the unnecessary administration of anti-D immunoglobulin to mothers that does not undergo amniocentesis.

1391W

Non-Invasive Prenatal Detection of a novel 12p12.1-p11.22 Microdeletion in a Fetus By Next Generation Sequencing of Maternal Plasma DNA. *D. Peters, T. Chu, S. Yatsenko, N. Hendrix, W. Allen Hogge, U. Surti, K. Bunce, M. Dunkel, P. Shaw, A. Rajkovic.* Magee-Womens Res Inst, Univ Pittsburgh, Pittsburgh, PA.

Prenatal diagnosis of aneuploidies and genomic imbalances requires a pure sample of fetal genomic material, which must be obtained via invasive means such as chorionic villus sampling (CVS) or amniocentesis. Unfortunately, these invasive procedures carry a significant risk of fetal loss and parental anxiety. Alternative, risk-free, methods for the non-invasive analysis of fetal DNA in maternal plasma have shown promise for the detection of fetal aneuploidy. However, the non-invasive prenatal detection of chromosomal microdeletions and microduplications remains elusive. The goal of this study therefore is to demonstrate proof of concept for non-invasive detection of a chromosomal abnormality in a fetus with a novel 12p microdeletion. Having previously given birth to a daughter with a paternally inherited 4.2Mb deletion on chromosome 12p12.1-p11.22, a couple expecting their second child presented to the Center for Medical Genetics at the Magee-Womens Hospital of UPMC for genetic counseling. Amniocentesis performed at 21 weeks of gestation showed a normal male chromosome complement, but microarray analysis performed on DNA extracted from cultured amniocytes identified single-copy 12p12.1-p11.22 loss, the same deletion as identified in sibling and father. FISH analysis confirmed the interstitial deletion in the fetus. A maternal blood sample was drawn at 35 gestational weeks and DNA extracted from the plasma was then used as a substrate for single end read (35bp) Illumina DNA sequencing, which generated a total of 243,340,714 reads of which 75% (182,691,185) could be mapped uniquely and perfectly to the latest version of human reference genome, GRCh37. In addition, we obtained 7 maternal plasma libraries, where both the mother and fetus are known to be diploid for both chromosomes 12 and 14 as reference libraries for sequencing. When comparing the sequencing reads from the known 12p deletion library with those from the reference libraries, we were able to detect the single copy fetal loss of the 12p region in DNA from maternal plasma, achieving 100% sensitivity, with the test for the chr12.del region resulting in highly significant adjusted p values (<=0.05) in all 7 comparisons, and 100% specificity resulting in non-significant adjusted p values (>= 0.05) in all 7 comparisons. In summary, we have demonstrated proof of concept for non-invasive detection of a chromosomal abnormality in a fetus with a novel 12p microdeletion.

1392W

Runs of Homozygosity (ROH) analysis from GWAS data for Preterm Birth. *A. Uzun[2,3,4], B. Feenstra[5], ML. Marazita[6], M. Melby[5], JC. Murray[1], J. Padbury[2,4].* 1) University of Iowa, Department of Pediatrics,Iowa City, Iowa; 2) Women and Infants Hospital, Pediatrics, , Providence, RI; 3) Brown University, Center for Computational Molecular Biology, Providence, RI; 4) Brown Alpert Medical School, Providence, RI; 5) Statens Serum Institute, Department of Epidemiology, Copenhagen, Denmark; 6) University of Pittsburgh, Division of Oral Biology, School of Dental Medicine, Pittsburgh, PA.

Runs of consecutive homozygous markers may identify recessive traits/rare variants for complex diseases. This form of homozygosity mapping can be identified from polymorphic variance used for Genome Wide Association (GWAS) studies. We present an approach to identify patterns of homozygosity from GWAS data. We compare the outputs from cases and the controls from a large GWAS of preterm birth. The data was derived from the Gene Environment Association Studies initiative (GENEVA) funded by the trans-NIH Genes, Environment, and Health Initiative (GEI). The data from GENEVA consists of nearly 4000 Danish women and children. Genotype data from 1929 control and preterm women were studied. The gestational age at delivery varied from 20 to 40 weeks. We divided the patients into four groups. Group A: 40 weeks, group B: 37 and less weeks, group C: 34 and less weeks, group D: 30 and less weeks. We identified runs of homozygosity (ROHs) in whole genome by the ROH program implemented in PLINK version 1.07. In order to exclude short and very common ROHs that may occur in all individuals, the minimum length for an ROH was set at 1000kb, minimum number of consecutive SNP number was 100, density (kb/SNP) was 50 and largest gap was 1000kb. SNP data from each chromosome of each woman were used. We further defined the boundaries of shared regions of RoH using a custom program using Perl scripts and mapped these to the UCSC Genome Browser. We compared the block frequency and gene location between each gestational group. Mean number of blocks per patient did not differ, but the mean length of RoH is significantly greater in each of the younger gestational age groups, p<0.001. We found several RoH blocks covering preterm birth related genes (ASHG2010, Abstract # 1711). These regions were used to compare the abundance of RoH blocks in each gestational age group. For example on chromosome (chr) 1, RoH covering the MTF2, TMEM206, NENF, on chr 2 IL1R2, on chr 4 IL15, on chr 7 PON1, on chr 8 TRAM1, on chr 14 CKB, on chr 17 LLGL1 genes were significantly higher in group B than group A. We found 121 SNPs from preterm related genes which overlap between single gene variant association tests and the ROH block regions. Higher density coverage from GWAS data will increase the resolution and further define blocks. In combination with single variant association testing and haplotype analysis, RoH mapping may identify fruitful regions for targeted resequencing in specific groups.

1393W

Novel loci associated with birth weight provide biological links between intrauterine growth, postnatal growth and metabolic traits. *H. Yaghootkar[1], M. Horikoshi[2], D.O. Mook-Kanamori[3], U. Sovio[4,5], N.J. Timpson[6], M. Kaakinen[7], D.J. Berry[8], J.P. Bradfield[9], D.L. Cousmi[10], J.J. Hottenga[11], M.R. Jarvelin[4,20], M. Kerkhof[12], M. Kirin[13], E. Kreiner-Møller[14,15], V. Lagou[2], H.R. Taal[3], E. Thiering[16], N.M. Warrington[17], J.H. Zhao[18], S. van Wingerden[3], T.M. Frayling[1], M.I. McCarthy[2,19], R.M. Freathy[1], I. Prokopenko[2], EGG consortium investigators.* 1) IBCS, Peninsula College of Medicine and Dentistry, Exeter, Devon, United Kingdom; 2) WTCHG, University of Oxford, Oxford, United Kingdom; 3) Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands; 4) Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, United Kingdom; 5) Department of Medical Statistics, London School of Hygiene and Tropical Medicine, London, United Kingdom; 6) MRC Centre for Causal Analyses in Translational Epidemiology, University of Bristol, Bristol, United Kingdom; 7) Institute of Health Sciences, University of Oulu, Oulu, Finland; 8) Centre for Paediatric Epidemiology and Biostatistics, MRC Centre of Epidemiology for Child Health, University College of London, London, United Kingdom; 9) Center for Applied Genomics, The Children's Hospital of Philadelphia, Pennsylvania, USA; 10) Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland; 11) Department of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands; 12) Department of Epidemiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; 13) Centre for Population Health Sciences, University of Edinburgh, Edinburgh, Scotland, United Kingdom; 14) Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark; 15) The Danish Pediatric Asthma Center, Copenhagen University Hospital, Copenhagen, Denmark; 16) Helmholtz Zentrum Muenchen, German Research Centre for Environmental Health, Institute of Epidemiology, Neuherberg, Germany; 17) Centre for Genetic Epidemiology and Biostatistics, The University of Western Australia, Perth, Western Australia, Australia; 18) Division of Human Genetics, The Children's Hospital of Philadelphia, Pennsylvania, USA; 19) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, United Kingdom; 20) Institute of Health Sciences, Biocenter, University of Oulu, Finland.

The genetic loci influencing birth weight (BW) and its relationship to postnatal growth and adult disease are poorly characterized. Two loci have been reported, CCNL1 and ADCY5, which contain variants associated with BW. To identify additional loci associated with BW we performed a genome-wide association study using 26,864 European descent individuals from 18 studies and followed up 17 loci in up to 37,120 individuals. In each study, we analyzed the association between BW Z-score and SNP using linear regression, with sex and gestational age (when available) as covariates. We excluded multiple and premature births. We performed fixed-effects inverse-variance meta-analyses to combine results from all studies. We identified four novel and confirmed two loci containing variants associated with BW at p<9x10-8. These loci fell into three broad categories. First, two of the four strongest associations represented the same signals as known type 2 diabetes (T2D) signals - in or near ADCY5 (p=3.5x10-15, previously reported) and CDKAL1 (p=9x10-17). In both loci, the BW-lowering allele is also associated with greater T2D risk. This is consistent with the fetal insulin hypothesis, which proposes that common genetic variation influencing insulin secretion is a key link between lower BW and later life T2D risk. Second, three associations represented the same signals as known adult height signals, in or near HMGA2 (p=3x10-19), LCORL (p=1x10-8) and HHIP (p=9x10-8) genes. For all three loci, the height-increasing allele represented the same signal as the BW-increasing allele. Third, the previously reported association near the CCNL1 gene (p=2.9x10-28), represents the strongest association with BW (with percent effect sizes equivalent to the effect of the FTO locus on BMI). This locus has no known overlap with other traits and likely represents an effect specific to intrauterine growth. Of the 32 known adult-BMI loci, there was no evidence of association or enrichment of effects on BW. In a subset of studies (n= 8780), newborns with 9-12 BW-lowering alleles weighed 150g (95% CI: 116-185g) less than those with 5 or fewer BW-lowering alleles at 6 loci. This effect is equivalent to that of 5-6 cigarettes/day smoked in the 3rd trimester of pregnancy, or roughly half the effect reported for extreme maternal starvation. Our analysis of the genetic component contributing to BW highlights genetic loci contributing to growth processes and to metabolic trait regulation.

1394W

Noninvasive prenatal diagnosis of multiple fetal aneuploidies using massively parallel sequencing. *A.J. Sehnert[1], D.W. Bianchi[2], R.P. Rava[1].* 1) Research and Development, Verinata Health, San Carlos, CA; 2) Pediatrics, Obstetrics & Gynecology, Tufts University School of Medicine, Boston, MA.

Background: Recent advances in noninvasive prenatal diagnosis show that massively parallel sequencing of cell free fetal DNA in maternal blood detects fetal trisomy 21 (T21). We wished to determine whether techniques focused on T21 diagnosis would also detect other aneuploidies. Methods: Blood samples were collected from 1,014 pregnant women prior to prenatal procedure at 13 US clinics. DNA extracted from 119 samples underwent massively parallel sequencing. A training set of 71 samples was selected from the first 435 samples collected (Apr-Dec 2009). All subjects with an affected fetus in this series were included (26 abnormal karyotypes) and a random selection of non-affected samples. Utilizing normalized chromosome values (NCVs) from the sequencing data an algorithm was developed. The classification process was then evaluated on an independent test set of 48 samples (27 abnormal karyotypes) randomly selected from 575 samples (Jan-Jun 2010). Laboratory personnel were blinded to karyotype. Mapped sites for chromosomes of interest in the sequencing data from the training set were normalized individually by calculating the ratio of number of sites on a specified chromosome to number of sites on an optimized normalizing chromosome (or chromosome set). Threshold values for trisomy or sex chromosome classification were then established and a classification scheme was defined including a `no call' zone for each. Results: Sequencing of the independent test set lead to 100% correct classification of T21 (13/13) as well as T18 (8/8). One case of T13 was `no call' for Chr 13. In five samples with other complex karyotypes, 4 were correctly classified including 45,X (2/3) and a fetus with karyotype; 46,XY,+unidentified marker. The third 45,X sample was `no call' for sex. A rare Chr 9 abnormality was also unexpectedly detected. The sample, mosaic karyotype; 47,XX+9[9]/46,XX[6] was correctly classified for sex, and a relatively low NCV was observed for Chr 21. Since Chr 9 is in the denominator of our algorithm for calculating Chr 21 ratio, this lowered the overall NCV. Conclusions: Massively parallel sequencing is capable of detecting multiple fetal chromosomal abnormalities from maternal plasma. This may provide an advantage over serum analytes, which at present provide only indirect risk for T21 and T18.

1395W

Ancestral origins of African American women in the Healthy Pregnancy, Healthy Baby cohort. *M.L. Miranda[1, 2], M.E. Garrett[3], K.S. Quinn[3], G.K. Swamy[4], A.E. Ashley-Koch[3].* 1) Nicholas School of the Environment, Duke University, Durham, NC; 2) Department of Pediatrics, Duke University Medical Center, Durham, NC; 3) Center for Human Genetics, Duke University Medical Center, Durham, NC; 4) Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC.

African American women have greater risk than Caucasian women for adverse birth outcomes, even when controlling for social and demographic differences. However, many African American women have excellent birth outcomes, and a possible contributor to differential risk is the percentage of European admixture. The Healthy Pregnancy, Healthy Baby Study is a prospective cohort of pregnant women aimed at identifying genetic, social and environmental contributors to disparities in pregnancy outcomes in the US South. Clinical and genetic data were available for 848 non-Hispanic Black (NHB) women. Clinical outcomes analyzed included infant birth weight, pre-term birth (gestation < 37 weeks), birth weight less than 5th percentile for gestational age (SGA5), birth weight less than 10th percentile for gestational age (SGA10), and preeclampsia. 1509 SNPs were genotyped using the Illumina African American admixture panel. Six mitochondrial DNA (mtDNA) SNPs were genotyped via Taqman assays in order to construct ancestral mtDNA haplogroup (L0, L1, L2, L3, N, or R) for each subject. Among our NHB women, the genome-wide average percentage of European admixture was 17.9%. None of the clinical outcomes were associated with this estimate of European admixture. The distribution of subjects in each mtDNA haplogroup was: L0 (3.7%), L1 (18.5%), L2 (28.0%), L3 (39.9%), N (0.7%), R (9.3%). European admixture did not significantly differ among the 4 African mtDNA haplogroups (L0=17.3%, L1=17.4%, L2=17.6%, L3=16.7%). However, European admixture was higher among the women with mtDNA haplogroup R (24.4%, p<0.0001) compared with the African haplogroups. Clinical outcomes were also tested for association with African haplogroups. Women in the L3 haplogroup were more likely to deliver low birth weight (<1500g) babies than women in the L2 haplogroup (p=0.004). Women in the L3 haplogroup were also more likely to deliver SGA10 than women in the L2 haplogroup (p=0.016). These data suggest that NHB women in the L3 haplogroup, which originated in east Africa, are at greater risk for adverse birth outcomes than NHB women with the L2 haplogroup, which originated in western and sub-Saharan Africa. Thus, some measures of ancestral origin in African American women are correlated with differential risk for adverse birth outcomes. Additional analyses will be needed to determine whether the mtDNA haplogroups are a surrogate for mitochondrial function.

1396W

Differential impact of mtDNA mutations on the mtDNA copy number during human oogenesis and embryofetal development. *J. Steffann[1], S. Monnot[1], D.C. Samuels[2], N. Gigarel[1], P. Vachin[1], L. Hesters[3], P. Burlet[1], Y. Ville[4], M. Rio[1], A. Benachi[3], R. Frydman[3], AS. Lebre[1], A. Munnich[1], A. Rotig[1], N. Frydman[3], JP. Bonnefont[1].* 1) Paris Descartes University, Inserm unit U781, and Genetics Department, Necker Hospital, Paris, France; 2) Center for Human Genetics Research, Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, USA; 3) Department of Obstetrics and Reproductive Medicine, and Inserm unit U782, A Béclère Hospital, Clamart, France; 4) Obstetrics Department and Paris Descartes University, Necker Hospital, Paris, France.

Mitochondrial DNA (mtDNA) mutations cause serious disorders with maternal inheritance and a high transmission risk, resulting in common requests for preimplantation (PGD) or prenatal diagnoses (PND). These procedures are hampered by the lack of data on the pathophysiological impact of mtDNA mutations during human development. We previously showed in the frame of our PGD/PND activities that mtDNA mutations differentially modulate the bottleneck size in humans, and segregate according to a random genetic drift throughout the whole embryofetal development. There are however no available data regarding the putative impact of mtDNA mutations on the overall amount of mtDNA during human oogenesis and embryofetogenesis as well. In order to get an insight into this point, we collected oocytes (controls, n:19; m.3243A>G, n:12; m.8344A>G, n:5), preimplantation embryos (controls, n:9; m.3243A>G, n:12; m.8344A>G, n:12), and chorionic villi samples (CVS) at 11 GW (controls, n:7; m.3243A>G, n:14) and at delivery (controls, n:3; m.3243A>G, n:4). We devised a test enabling the simultaneous assessment of the mtDNA copy number (CN) and mutant load at the single cell level. The analysis of oocytes and embryos showed that the mtDNA CN i) is identical in control and mutant oocytes, suggesting that mtDNA mutations do not influence mtDNA replication during oogenesis, ii) is significantly increased in m.3243A>G vs control peimplantation embryos ($p<0.01$), suggestive of a mutation-dependent induction of mtDNA replication during early embryogenesis, iii) strongly correlates with the mutant load (range 0-80%) in m.3243A>G embryos ($R2=0.42$, $p<0.0013$), suggesting some process of compensation for the respiratory chain dysfunction, iiii) is identical in m.8344A>G vs control preimplantation embryos, indicating that the mtDNA replication induction observed in the m.3243A>G embryos is mutation-specific. The analysis of CVS showed that the mtDNA CN i) is significantly increased in m.3243A>G vs control CVS at 11-GW ($p<0.01$), ii) does not correlate with the mutant load (range 0-80%) at this gestation stage, iii) strongly decreases from 11 to 37-39 GW in both control (-50 %) and m.3243A>G CVS (-65%), and iiii) becomes then identical in both control and m.3243A>G CVS (heteroplasmy range: 5-45%). These data highlight the complex relationships between mtDNA mutations and the overall amount of mtDNA, depending on mutation types, mutant load values, cell types and human development stages.

1397W

Novel *NEB* mutation in an Ashkenazi Jewish infant with severe congenital Nemaline Myopathy. *A. Ludtke[1], R. Kornreich[1], T. Winder[2], L. Mehta[1].* 1) Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) Prevention Genetics Laboratory, Marshfield, WI.

Nemaline myopathy (NM) is a heterogeneous neuromuscular disorder characterized by variable onset and severity of muscle weakness. So far, mutations in 6 different genes encoding thin filament muscle proteins have been associated with NM. Mutations in the *NEB* gene cause autosomal recessive disease, including congenital forms of NM. The only known recurrent mutation in this gene is a 2502 bp deletion spanning exon 55, present in an estimated 1:108 individuals of Ashkenazi Jewish descent in the orthodox community. We report the case of a female infant who presented with severe muscle weakness, contractures, dysmorphic features and long bone fractures, consistent with fetal akinesia. The infant passed away at 3 days of age from respiratory failure. The pregnancy was complicated by polyhydramnios and bilateral club feet on fetal ultrasound at 20 weeks gestation. The parents were Ashkenazi Jewish, non-consanguineous, and with no relevant family history. The couple had been screened for Jewish genetic disorders through the Dor Yeshorim organization. The infant had normal blood chromosome analysis and array CGH. Due to the phenotype of a severe neuromuscular disorder, the parents underwent carrier screening for two common recessive, neuromuscular disorders, NM and spinal muscular atrophy. The mother was found to carry the common Ashkenazi Jewish NEB mutation R2478_D2512del, which was also present in the deceased baby's DNA. The father was negative for the mutation. Sequencing of all 183 exons including exon-intron junctions of the *NEB* gene in the father identified a novel heterozygous mutation c.9619-2A>G at the junction of intron 66 and exon 67. The same mutation was then identified in the child's DNA. Since the mutation is predicted to disrupt the intron 66 splice acceptor site, it is expected to be causative for NM in homozygous or compound heterozygous individuals. This case illustrates the phenotype of severe congenital NM which should be included in the differential diagnosis of fetal akinesia. Further, it highlights the limitations of targeted mutation panels for screening of recessive diseases - an important aspect of genetic counseling, where one member of a couple is identified to be a carrier and the other is negative for common mutations. Knowledge of the causative mutations in this setting helps to provide accurate genetic counseling and reproductive options, such as prenatal or preimplantation genetic diagnosis in future pregnancies.

1398W

Prenatal diagnosis of) -thalassemia using polymorphic marker revealed DNA recombination 5' to) -globin gene. *z. kainimoghaddam, M. Karimipour, A. Amiriyan, M. Taghavi, A. Sanjari, M. Chobini, S. Zeinali.* Molecular Medicine, Pasteur, Tehran, Tehran, Iran.

Introduction:) -thalassemia is an inherited autosomal blood disease and the most frequent single gene disorder in Iran. The prenatal diagnosis (PND) is an effective option for preventing the birth of an affected child Methods used for PND are direct mutations analysis and indirect method using DNA polymorphisms at B-globin gene cluster. There is always the possibility of error due to meiotic recombination between the polymorphic markers and the mutation in) -globin gene. In this study we report a family was referred to our clinic for PND with a major thalassemia child. Material and Methods: From each individuals (parents and affected child) 5ml of blood was collected in EDTA and DNA extracted by using sulting out method.CVS(chorionic wily sample) was obtained 10-12 weeks of gestation age.DNA was prepared from CVS by using standard methods. ARMS/PCR and PCR-RFLP were exploited for prenatal diagnosis in parallel. Results: In this study we found the mutation (-25bp deletion) for this family and five polymorphisc sites ((Hind III/. G, ,HincII/3'?) , AvaII/) , HinfI/)) were investigated .The fetus was normal for this mutation. AvaII/) polymorphic site was not informative but HinfI/) (-/-) showed the fetus was normal.The polymorphic sites (Hind III/ . G, ,HincII/3'?)) were similar each othe r(+/-) that indicated the fetus was heterozygous carrier. Discussion: These results show the fetus is normal for the mutation(-25bp deletion) associated the polymorphic site HinfI/) ,and other polymorphic sites are conversed .The finding of a recombination) -globin haplotype has important implication in the prenatal diagnosis of) -thalassemia disorder .Therefore for the PND of) -thalassemia by linkage RFLPs, it is important to carry out complete haplotype analysis of each chromosome in the family and to use polymorphic markers from both the 5' and 3' group whenever possible.

1399W

Preimplantation Genetic Diagnosis Based Detection of Uniparental Disomy on Embryos Undergoing IVF. *G. Ozgon[1], A. Fejzullahu[1,2], K. Senvardar[1,3], A. Biricik[4], F. Fiorentino[4].* 1) Nesiller Genetics Diagnosis Centre and R&D, Istanbul, Turkey; 2) Molecular Biology Genetics and Biotechnology Graduate Program, Institute of Science and Technology, Istanbul Technical University, Istanbul, Turkey; 3) Gebze Institute of Technology, Faculty of Science, Department of Molecular Biology and Genetics, Kocaeli, Turkey; 4) GENOMA, Molecular Genetics Laboratory, Rome, Italy.

In recent years, the clinical application of preimplantation genetic diagnosis (PGD) has provided an alternative approach for the prevention of affected pregnancies in couples at high reproductive risk. The main indications are advanced maternal age, repeated implantation failure, repeated miscarriage and severe male factor infertility. Therefore, the selection of euploid embryos for transfer by the help of preimplantation genetic screening (PGS) of chromosomally normal embryos from subfertile patients, has a strong impact in IVF efficiency as aneuploidies are the main cause of spontaneous abortions and implantation failures. Moreover, the technique diagnosis not only aneuploidies or unbalanced inheritance but also it allows the diagnosis of uniparental disomy (UPD). Although UPD, where both chromosomes are inherited from one parent and no chromosomes are inherited from the other, is exoterically a rare event, it was mentioned to be detected in higher proportion rate in chromosome 14. In view of these findings, the aim of this study is to investigate the association of UPD analyzed in aneuploidy and inv(9) conditions. In this study preimplantation genetic screening (PGS) was performed in 46 cycles for aneuploidy diagnosis subsequently in chromosomes 13, 18, 21 and XY. 365 blastomeres were analyzed by polymerase chain reaction (PCR) and nested polymerase chain reaction (Nested PCR) with specific markers used in aneuploidy screening. Couples with advanced maternal age, repeated implantation failure, repeated miscarriage and inv(9) indications were analyzed for aneuploidy screening (13, 18, 21 and XY). After PCR and Nested PCR reaction all products were analysed by fragman analysis on ABI 3100 Genetic Analyzer. The obtained results showed that the rate of UPD in couples with aneuploidy indication was approximately 3.6% (335 blastomere). More interestingly the rate of UPD in 35 blastomeres screened in couples with inv(9) indication was detected to be 34%. Inv(9) is a common (1-3%) heteromorphism in the general polulation categorized as a minor chromosomal rearrangement without abnormal phenotypes, however, it is correlated to be associated with subfertility and recurrent abortions. Although the UPD is a rare event, it is observed that there is a strong association between inv(9) and UPD in couples undergoing IVF. As a conclusion, further studies are needed to understand the cause of these genetic abnormalities in couples at high reproductive risk.

1400W

Pre-implantation genetic diagnosis offers a fair chance of having unaffected offspring for mitochondrial DNA disorders. *H.J.M. Smeets[1,2], D.M.E.I. Hellebrekers[1], R. Wolfe[3], A.T.M. Hendrickx[1], J.P.M. Geraedts[1,2], P.F. Chinnery[4], C.E. De Die[1,2], I.F.M. De Coo[5].* 1) Dept Clinical Genetics, Maastricht UMC, Maastricht, Netherlands; 2) Research School GROW, Maastricht UMC, Maastricht, Netherlands; 3) School of Public Health and Preventive Medicine, Monash University, Melbourne, Australia; 4) Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, UK; 5) Dept of Pediatric Neurology, Erasmus MC-Sophia Children's Hospital, Rotterdam, Netherlands.

Mitochondrial disorders are often fatal multisystem disorders, for a significant part caused by heteroplasmic mtDNA point mutations. Prenatal diagnosis is in general not possible for these maternally inherited mutations due to extensive variation in mutation load among embryos and the inability to accurately predict the clinical expression. We hypothesized that preimplantation genetic diagnosis (PGD) should be a better alternative, if a minimal mutant level existed below which the chance for an embryo of being affected was acceptably low, irrespective of the mtDNA point mutation. We performed a systematic review of muscle mutant levels, evaluating 159 different heteroplasmic mtDNA point mutations derived from 327 unrelated patients or pedigrees, but excluding three overrepresented mtDNA mutations. Mutation levels were included for familial mtDNA point mutations only, covering all affected (n=195) and unaffected maternal relatives (n=19) from 137 pedigrees. Mean muscle mutant levels were comparable between probands and affected maternal relatives and between affected individuals with tRNA- versus protein-coding mutations. Using an estimated a-priori prevalence of being affected in pedigrees of 0.477, we calculated the risk of being affected at varying mutant levels. A 95% or higher chance of being unaffected was associated with a muscle mutant level of 18% or less. Most mtDNA mutation carriers will have oocytes below this threshold. Our data provide for the first time carriers of all heteroplasmic mtDNA point mutations a fair chance of having healthy offspring, by applying PGD.

1401W

Monosomy 18p presenting prenatally as mild ventriculomegaly-case report and review of the literature. *K. Bajaj[1,2,3], M. Rosner[1,3], T. Goldwaser[1,3], J. Gebb[1,3], P. Dar[1,3], S. Klugman[1,3].* 1) Department of Obstetrics & Gynecology and Women's Health, Montefiore Medical Center, Bronx, NY; 2) Department of Obstetrics & Gynecology, North Bronx Health Network, Bronx, NY; 3) Albert Einstein College of Medicine, Bronx, NY.

BACKGROUND: Mild ventriculomegaly occurs in approximately 0.1% of pregnancies. Common causes include genetic aberrations, central nervous system (CNS) malformations, hemorrhage, or fibrosis due to infection. Aneuploidy is reported in 5-10% of cases. Most cases of isolated mild ventriculomegaly have normal developmental outcomes. CASE: 22 year-old nullipara with no significant family history presented for amniocentesis after anatomy sonogram at 20 weeks gestational age revealed a female fetus with mild bilateral ventriculomegaly (right ventricle = 11.2mm, left ventricle 10.5mm). Until then, the pregnancy had been uncomplicated, including a negative second trimester aneuploidy screen. The patient denied any fevers or infectious exposures. Fetal karyotype revealed a de-novo 46, XX, del(18)(p11.2); prenatal microarray confirmed this finding. Amniotic fluid was negative for toxoplasmosis and cytomegalovirus. Subsequent sonogram revealed low set gallbladder and right kidney and abnormally curved toes with overlapping 3rd and 4th digits in feet. After extensive counseling the patient decided to continue with the pregnancy DISCUSSION: Prenatal diagnosis of monosomy 18p was previously reported in association with increased nuchal translucency and holoprosencephaly. A critical region for holoprosencephaly, HPE4, is located on the distal portion of 18p. To our knowledge, this is the first report of monosomy 18p presenting prenatally with a subtle findings of mild ventriculomegaly. We review the cytogenetic/clinical features and management of isolated ventriculomegaly and monosomy 18p syndrome.

1402W

PRENATAL SCREENING OF SMN1 DELETION OR DUPLICATION SHOWED PREGNANCIES IN THE HIGH-RISK WOMEN HAD A HIGHER ABNORMAL COPY NUMBERS OF SMN1 ALLELES THAN THAT IN THE GENERAL PREGNANCIES. *Y. Chen, MJ. Liu, HB. Li, J. Mao, H. Li.* Center of Reproduction and Genetics, Suzhou Municipal Hospital, Suzhou, Jiangsu, China.

Because of the severity of spinal muscular atrophy (SMA) and the lack of effective treatment, a population-based screening of SMN1 deletion is promising for disease prevention. A TagMan probe-based real-time PCR assay, followed by multiplex ligation-dependent polymerase amplification (MLPA) as confirmation, was used to determine SMN1 gene deletion. 541 subjects including 458 pregnant women and 83 amniotic fluid samples that had been collected previously from a group of high-risk population for prenatal screening of trisomy 21, trisomy 18 or neural tube defect were studied. Our results showed that among the pregnant women, SMA carrier accounts for 2.18% (10/458=1/46). In addition, 1.75% (8/458) were identified having three copies of SMN1 allele and 0.22% (1/458) with 4 copies of SMN1allele, which brought the abnormal copy number up to 4.1%. The corresponding data with 1 copy and 3 copies in the amniotic fluid samples were 7.23% (6/83) and 1.20% (1/83), respectively, showing the abnormal copy number is 8.4% in the high-risk population that is significantly higher than the general population.

1403W

Early Delivery of Infants for Treatment of Retinoblastoma Diagnosed by Prenatal *RB1* Mutation Identification. *H. Dimaras[1, 2], E. Heon[3], J. Sutherland[3], M. Day[3], V. Khetan[3,4], J. Gardniner[5], H.S.L. Chan[1], B.L. Gallie[3].* 1) Hematology/Oncology, The Hospital for Sick Children, Toronto, ON, Canada; 2) Ophthalmology & Vision Sciences, The University of Toronto, Toronto, ON, Canada; 3) Ophthalmology & Vision Sciences, The Hospital for Sick Children, Toronto, ON, Canada; 4) Sankara Nethralaya Hospital, Chennai, India; 5) Ophthalmology & Vision Sciences, University of British Columbia, Vancouver, BC, Canada.

Purpose: To determine ocular outcomes of infants confirmed prenatally to carry their family's *RB1* mutation who underwent pre-term delivery to initiate treatment. **Methods:** A retrospective study of Sickkids medical records documented children born between 1 June 1996 and 31 May 2011, seen at Sickkids because of relation to a retinoblastoma proband. Information was collected on: relation to proband; sex; timing of and result of prenatal ultrasound (if performed); gestational age at birth; pregnancy or delivery complications; date of *RB1* molecular testing, type of sample tested and result; timing and location of first and all subsequent tumor appearance(s) in each eye; treatment history; International Intraocular Retinoblastoma Classification of each eye; laterality; Tumour Node Metastasis staging; date of last follow-up; and visual outcome. **Results:** Of 15 infants shown to carry their parent's *RB1* mutation, 9 were tested prenatally and 6 after birth. Of the infants tested prenatally, 7 were delivered at 36-37 weeks gestation and 2 were spontaneously premature. The infants that were not tested prenatally were born at full term. After birth, daily/weekly examination for tumors occurred. Vision-threatening tumors were present at birth in 22% (2/9) of infants delivered early or born prematurely, and 50% (3/6) of full-term infants; posterior tumors appeared age 1 to 6 months in 9 infants. All patients eventually developed tumors in both eyes. Good vision was maintained in all children delivered early; treatments included focal therapy (all); later chemotherapy (4). Full-term infants received focal therapy (6), chemotherapy (5), stereotactic radiation (2), and enucleation of one eye (2). **Conclusion:** Expedient intervention and optimal outcomes were facilitated by prenatal molecular detection coupled with early delivery.

1404W

Detection of aneuploidies from microdissected amniotic cells by quantitative fluorescent-polymerase chain reaction (QF-PCR). *R. Drouin[1], J. Lamoureux[1], A. Emad[1], E. BenDavid[1], K. Chun[2], C. Bouffard[1].* 1) Div Genetics, Dept Ped, Faculty Medicine & Health Sciences, Univ Sherbrooke, Sherbrooke, Quebec, Canada; 2) Genetics Program, North York General Hospital, Toronto, Ontario, Canada.

QF-PCR (Quantitative Fluorescent-Polymerase Chain Reaction) allows for the targeted detection of aneuploidies using fluorescently labelled PCR primers. In prenatal diagnosis, QF-PCR is an excellent alternative to fluorescence in situ hybridization (FISH) for rapid aneuploidy diagnosis (RAD) of chromosomes 13, 18, 21, X and Y, particularly in terms of cost and rapid turnaround time. In this project, we show that QF-PCR can be performed using the DNA of five laser-microdissected amniotic cells. To do so, cultured fetal cells from amniotic liquid sampling were split in two: one large aliquot for standard DNA extraction and another small one for laser microdissection. The fetal cells from the smaller aliquot were spread on membrane-mounted metal frame slides and five cells were isolated by laser microdissection. Then, whole genome amplification (WGA) was performed using extracted DNA from these microdissected cells. In parallel, DNA was extracted from the larger aliquot of cultured amniotic cells; these samples were used as controls for validation. PCR amplification of short tandem repeats (STR) specific for chromosomes 13, 18 and 21 as well as the sex chromosomes was performed on DNA from both the microdissected cells and the control amniotic cells using QF-PCR. Amplicons were separated by capillary electrophoresis and quantitatively analyzed to determine allele dosage and sexing. Results with control samples showed that QF-PCR is a very sensitive and specific technique for allele dosage and sexing. Results of QF-PCR on WGA-DNA from microdissected cells showed weaker signals of amplification as compared to that of control samples. However, allele dosage and sexing of samples correlated between control and WGA-DNA samples. To date, we have carried out studies on 20 samples with various chromosome constitutions, including 46,XX; 46,XY; 47,XY,+21; 47,XX,+21; 45,X; and 47,XXY. These results confirm that QF-PCR can be performed successfully on a small number of fetal cells (five cells). In addition to giving rapid, inexpensive and accurate results on amniotic fluid cells, QF-PCR is an efficient technique that could be used in non-invasive prenatal diagnosis for rapid detection of aneuploidies where a very small number of fetal cells would be available.

1405W

Clinical study of chorionic villous sampling for 10 years. *K. Lee[1], S. Kim[1], S. Shim[2], J. Baek[3], D. Cha[1,2].* 1) Dept OB/GYN, Kangnam-Gu, CHA General Hosp, CHA university, Seoul, Korea; 2) Genetics Laboratory, Fertility Center of CHA Gangnam Medical Center, CHA university, Seoul, Korea; 3) Department of Obstetrics and Gynecology, Bundang CHA Hospital, CHA university, Seoul, Korea.

Purpose : The purpose of this study was to evaluate the indications of chorionic villous sampling(CVS), the positive predictive value for fetal chromosomal abnormalities, the fetal loss rate of CVS at CHA medical center. Materials and methods : We reviewed medical records of 511 cases in whom the CVS for prenatal cytogenetic diagnosis were performed between 67 days and 120 days of gestation for the period of April 2000 to April 2010 in CHA medical center. Fetal karyotyping was obtained by direct and indirect culture methods. Results : The most common indications of CVS were abnormal ultrasonic findings including increased nuchal translucency (294/511, 57.5%). The positive predictive value of abnormal karyotyping according to the indication of CVS was highest in the cases showing parent abnormal karyotyping (14/21, 66.7%). Fetal loss rate was 1.2% (6/511). Conclusion : In our study, the complication rate of CVS was not much higher than second trimester amniocentesis. If CVS is performed by an expert operator, CVS is feasible and reliable procedure for prenatal genetic diagnosis.

1406W

Distribution of Fetal Skeletal Dysplasias in a Tertiary Care Centre: Genetic, Pathologic, and Radiological Findings in 91 cases. *U. Mohan[1], S. Keating[2], D. Chitayat[3,4], A. Toi[3,5], K. Chong[3,4], G. Tomlinson[6], P. Glanc[7].* 1) Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada; 2) Department of Pathology, Mount Sinai Hospital, Toronto, Ontario, Canada; 3) Center for Excellence in Obstetric Ultrasound, Mount Sinai Hospital, Toronto, Ontario, Canada; 4) Division of Clinical Genetics, Hospital for Sick Children, Toronto, Ontario, Canada; 5) Department of Medical Imaging, Mount Sinai Hospital, Toronto, Ontario, Canada; 6) Centre for Innovation in Complex Care, Toronto General Hosptial, Toronto, Ontario, Canada; 7) Department of Medical Imaging, Sunnybrook Health Sciences, Toronto, Ontario, Canada.

PURPOSE: The purpose of this study was to determine the distribution of various fetal skeletal disorders in a tertiary care centre, based on the 2006 Nosology and Classification of Genetic Skeletal Disorders[1]. The study provides additional information on rare skeletal disorders and further characterizes pathologic features of these disorders. **METHOD AND MATERIALS:** A retrospective review of 1806 perinatal autopsies from 2002-2009 at Mount Sinai Hospital, Toronto, ON was performed. The study population consisted of stillborns and terminated fetuses in their second and third trimesters as well as live born infants who died shortly after birth. The reports were reviewed for cases with a final diagnosis of a skeletal disorder, based on the 2006 Nosology[1]. Pathological, genetic, clinical and radiological information were gathered from the reports **RESULTS:** Of the 1806 perinatal autopsies performed, 91 (5.0%) received a diagnosis of a skeletal disorder. The 91 cases encompassed 15/37 groups according to the 2006 Nosology[1]. Out of the 91 cases, 75 (82.5%) had a complete autopsy (photos, x-rays, gross physical and histopathological analysis with or without biopsy) while limited autopsy (external exam, photos and x-rays) was performed in 16 (17.6%) cases. There was a slightly increased ratio of males to females (1.33:1). Prenatal screening was done in 14/91 (15.4%) cases and 7 had abnormal results. A diagnosis could be reached in 77/91 (84.6%) cases. The most common skeletal dysplasia was Thanatophoric dysplasia type 1 (17/91, 18.7%) followed by Osteogenesis imperfecta type 2 (15/91, 16.5%). **CONCLUSION:** Skeletal dysplasias comprised 5% of all perinatal autopsies at our tertiary referral centre. The most common skeletal dysplasias were Thanatophoric dysplasia type 1 and Osteogenesis imperfecta type 2. A combination of radiographic features, gross morphology, histopathology and DNA analysis provided a diagnosis in 77/91 (84.6%) cases while in 14/91(15.4%) cases a final diagnosis could not be determined. **CLINICAL RELEVANCE:** Familiarity with genetic and imaging findings and the distribution and lethality of various perinatal skeletal dysplasias is crucial in order to provide appropriate counselling for current and future pregnancies. 1. Nosology and Classification of Genetic Skeletal Disorders: 2006 Revision. A. Superti-Furga, S. Unger and the Nosology Group of the International Skeletal Dysplasia Society. Am J Med Gen. 143A:1-18;2007.

1407W

SNP array analysis in Prenatal Diagnosis: what do pregnant couples want to know? *G. Oudesluijs[1], S.R. Riedijk[1], M.I. Srebniak[1], A.M. Joosten[1], L.C.P. Govaerts[1], K.E.M. Diderich[1], M. Boter[1], D. Van Opstal[1], A. Tibben[1,2], R-J.H. Galjaard[1].* 1) Department of Clinical Genetics, Erasmus Medical Center, P.O. Box 2040, Rotterdam 3000 CA, The Netherlands; 2) Center for Human and Clinical Genetics, Leiden University Medical Center, P.O. Box 9600, NL-2300 RC Leiden, The Netherlands.

PND after detection of fetal abnormalities must be fast and accurate in case there is a limited legal time frame for TOP. Prenatal SNP array testing is faster (reporting time within a week) and detects much smaller aberrations (~0.15 Mb) compared to karyotyping (~ >5 Mb). However, CNVs leading to an (increased risk for) known disorders not related to the fetal anomalies could be encountered. These so-called incidental findings could lead to unsolicited knowledge about hereditary predispositions and carriership of all kinds of diseases at any age, apart from findings of uncertain clinical significance. Unexpected confrontation with unsolicited knowledge in a prenatal setting might be harmful for the decision process in the course of pregnancy. This has led to much debate concerning the use of microarrays in PND. Therefore pregnant couples need to be informed about the possible outcomes. We introduced a questionnaire that categorizes the kind of clinical information obtained from SNP array analysis. Pregnant couples can choose which clinical information they would like to receive about their unborn child. This was introduced during pre-test genetic counselling in 2009 in a selection of pregnant couples with fetal anomalies, which were referred to a clinical geneticist. Of all pregnant couples 25% indicated they only wished to be informed about results that (most probably) cause the fetal abnormalities. Another 25% also wanted to be informed about disorders that most probably affect health at childhood age. However, 50% chose to be informed of health problems at any age. Especially the latter choice does not comply with the generally accepted view that PST of children is not performed in general. This is because it is at odds with the future child's right to decide for her or himself whether or not to be informed of a (predisposition) of a genetic disorder. It also raises the question whether or not the prospective parents are truly able to make informed choices. Alternatively, a more general consent model could be more applicable in these situations. It is also questionable whether parents are fully entitled to choose not to be informed of a serious treatable genetic condition of their future child. We will present more data on these issues. These data are important to guide us in future proper genetic pre-test counselling in prospect of the higher chance of incidental findings when next generation sequencing is introduced as a diagnostic tool in PND.

1408W

Non-invasive Prenatal Diagnosis (NIPD) of Multiple Trisomic Chromosomes in a Triploid Fetus Using a Novel Single Nucleotide Polymorphism (SNP) Based Bioinformatics Approach. *M. Rabinowitz[1], G. Gemelos[1], M. Banjevic[1], J. Baner[1], B. Levy[1,2], M. Hill[1].* 1) Gene Security Network, Redwood City, CA; 2) Columbia University Medical Center, NY, NY.

Objective: Determine if trisomy is non-invasively detectable with high confidence on a triploid fetus, using novel informatics to analyze SNP loci of free floating fetal DNA in maternal plasma.

Design: Retrospective case study.

Materials and Methods: 20ML of blood was drawn from a pregnant patient following an abnormal ultrasound. After centrifugation, maternal DNA was extracted from the buffy coat (DNEasy, Qiagen); cell-free DNA was extracting from plasma (QIAamp, Qiagen). Targeted sequencing was applied to SNP loci on chromosomes 2 and 21 in both DNA samples. Maximum-Likelihood Bayesian estimation selected the most likely hypothesis from the set of all possible ploidy states. The method determines fetal DNA fraction, ploidy state and explicit confidences in the ploidy determination. No assumptions are made about the ploidy of a reference chromosome. The diagnostic uses a test statistic that is independent of sequence read counts, which is the recent state of the art.

Results: We accurately diagnosed trisomy of chromosomes 2 and 21. Child fraction was estimated at 11.9% [CI 11.7-12.1]. The fetus was found to have one maternal and two paternal copies of chromosomes 2 and 21 with confidence of effectively 1 (error probability<10^{-30}). This was achieved with 92,600 and 258,100 reads on chromosomes 2 and 21 respectively.

Conclusions: This is the first demonstration of non-invasive prenatal diagnosis of trisomic chromosomes from maternal blood where the fetus was triploid, as confirmed by karyotype. Extant methods of non-invasive diagnosis would not detect aneuploidy in this sample. Current methods rely on a surplus of sequence reads on a trisomic chromosome relative to disomic reference chromosomes; but a triploid fetus has no disomic reference. Furthermore, extant methods would not achieve similarly high-confidence ploidy determination with this fraction of fetal DNA and number of sequence reads. It is straightforward to extend the approach to all 24 chromosomes.

1409W

A Novel Method to Detect Fetal Trisomy in Pregnant Women Using a Selective Analysis of Cell-free DNA in Maternal Blood. *K. Song[1], A. Sparks[1], C. Struble[1], E. Wang[1], W. Barrett[1], R. Stokowski[1], C. McBride[1], J. Zahn[1], D. Hollemon[1], K. Lee[1], N. Shen[1], J. Sandler[1], J. Doshi[1], M. Sun[1], J. Garrison[1], P. Pattee[1], J. Stuelpnagel[1], A. Tomita-Mitchell[2], M. Mitchell[2], A. Oliphant[1].* 1) Tandem Diagnostics, San Jose, CA; 2) Medical College of Wisconsin, Milwaukee, WI.

We have developed a novel, highly accurate, cost-effective, high throughput method for non-invasive detection of fetal aneuploidy and sub-chromosomal copy number variation. Current prenatal testing for fetal aneuploidies such as Trisomy 21 (Down syndrome) is suboptimal in that screening blood tests and imaging are imprecise, and invasive testing, while diagnostic, carries the risk of fetal demise. To overcome these challenges, several groups have demonstrated that non-invasive analysis of cell free DNA (cfDNA) from maternal blood may be highly accurate for fetal aneuploidy detection. One approach utilizes next generation sequencing in which cfDNA from all chromosomes are analyzed. Because this method involves analysis of DNA from the entire genome, next generation sequencing costs and throughput constrain its efficiency and scalability. By contrast, our alternative approach utilizes specific assays developed against chromosomes or genomic regions of interest, thereby enabling sequencing of DNA from up to 96 different patient samples per Illumina HiSeq lane or up to 1500 patient samples per sequencing run. Our initial evaluation of selective cfDNA analysis involved 301 pregnant patient samples, including 39 confirmed Trisomy 21 and 7 confirmed Trisomy 18 pregnancies. Using 5-fold cross validation, we correctly classified 39/39 (100%) Trisomy 21 and 7/7 (100%) Trisomy 18 cases. One of 255 (0.4%) low to average risk samples was classified as Trisomy 21. We are currently conducting a larger study analyzing samples from pregnant women with Trisomy 13, Trisomy 18, and Trisomy 21 fetuses, and will present our findings. In addition to detection of chromosomal aneuploidies, our selective analysis of cfDNA in maternal blood has future use for analysis of sub-chromosomal region copy number changes in the fetus.

1410W

Impact of prenatal diagnosis on livebirth prevalence of infants with Down syndrome. *C. Stoll, Y. Alembik, B. Dott, M.P. Roth.* Genetique Medicale, Faculte de Medecine, Strasbourg, France.

Objectives: Down syndrome (DS) constitutes 8% of cases of registered congenital anomalies in Europe. The objectives of this study were to examine trends in the live birth prevalence of DS during 30 years in a well defined population in the light of trends in maternal age and prenatal diagnosis. Methods: The material for this study came from multiple sources on births and terminations of pregnancy (TOP) after prenatal diagnosis of DS in 402,532 consecutive pregnancies of known outcome. The study period was divided into 3 subgroups 1979-1988, 1989-1996 and 1997-2008.In the area under study prenatal diagnosis of DS is offered to all women more than 38 years. Maternal serum screening (triple test) is offered to all pregnant women since 1997 and fetal ultrasonographic scanning is routine practice. Results: Between 1979-1988, 1989-1996 and 1997-2008 TOP for DS was 16.2%, 46.8% and 72.3%, respectively. During the 3 time periods the livebirth prevalence per 10,000 of DS was 9.79, 10.26, and 6.08, respectively. The total prevalence of Down syndrome was 11.69, 19.31 and 23.10, respectively. The livebirth prevalence of DS has since 1997 increasingly diverged from the rising total prevalence. The main reason for these observations is the increase in maternal age, from 24.8 to 30.1 year. Conclusion: The rise of maternal age has brought with it an increase in the number of pregnancies affected by DS. The widespread practice of routine prenatal diagnosis and TOP has counteract the effect of maternal age in its effect on livebirth prevalence of DS. However the high total prevalence of fetal DS shows that more efforts on the primary prevention of chromosomal abnormalities are needed.

1411W

Future choices in prenatal diagnosis - How will women decide? *M.R. Susman[1], J. Halliday[1], J. Bayer[3], D. Amor[2].* 1) Public Health Genetics, Murdoch Childrens Research Institute, Parkville, Victoria, Australia; 2) Genetic Health Services Victoria, Murdoch Childrens Research Institute, Parkville, Victoria, Australia; 3) Centre for Community Child Health, Murdoch Childrens Research Institute, Parkville, Victoria, Australia.

Purpose: With the introduction of non-invasive prenatal diagnosis (NIPD) for Down syndrome and the simultaneous offer of arrays for molecular karyotyping, the complexity of women's choices will increase. There is considerable research into women's attitudes about testing for Down syndrome, but little information about other chromosome conditions. This study explores how women decide whether to have a hypothetical diagnostic prenatal blood test for four chromosome conditions, ranging from moderately severe to extremely mild, when given a short description of the outcome. **Methods:**Pregnant women, less than 20 weeks, recruited through ultrasound clinics, were sent a questionnaire based on the Theory of Planned Behaviour, encompassing measures of attitude (eg. believing that having the information is a good thing), subjective norm (eg. a partner influencing their decision) and perceived behavioural control (eg. wanting to test but it being too expensive). Each questionnaire included a description of one of the four conditions and each woman responded to only that condition. **Results:**Analysis of 230 questionnaires (62, 60, 51 and 57 respectively for each of the four conditions) showed that 73% of participants had a positive intention to test. There were no significant differences in demographics between the four groups, with 73% having a university education, 38% being in their first pregnancy, 90% having had prenatal screening for Down syndrome, and 11% utilising IVF or ICSI. Women's attitudes, subjective norms, and perceived behavioural control contributed 70% (R=0.84) of the variation in their intention to have a test. Attitude and subjective norm were both significant contributors to intention, with attitude being the best predictor () = 0.6). Significant differences between the four conditions were evident for attitudes, but not for subjective norms or perceived behavioural control. Women intending to test had significantly different attitudes from those not intending to test. **Conclusion:**This is the first quantitative study to explore women's attitudes to prenatal testing for less severe and milder chromosome conditions. A small, but important group of women differentiated between, but most wanted testing for all four conditions. This finding should be taken into account when developing policy and planning services related to NIPD.

1412W

Fractional DNA quantification by massively parallel shotgun sequencing — implications for fetal fraction measurement in maternal plasma. *J. Tynan[1], G. Hogg[1], M. Tang[1], L. Cagasan[1], J. Clemens[1], D. van den Boom[2], P. Oeth[1].* 1) Sequenom Center for Molecular Medicine, San Diego, CA; 2) Sequenom, Inc., San Diego, CA.

BACKGROUND: Presence of circulating cell free (ccf) fetal DNA in maternal plasma can be confirmed by detection of paternally inherited alleles in maternal plasma DNA, or alternatively, by detection of fetal specific methylation patterns in maternal plasma DNA. The fractional concentration of ccf fetal DNA in maternal plasma has been measured by paternal Y-chromosomal allele detection using digital PCR, and fetal specific methylation patterns of ccf fetal DNA in maternal plasma can be used to quantify the fetal fraction. Here, we demonstrate the ability to detect and quantify low fractional concentration single nucleotide polymorphism (SNP) alleles in mixed DNA samples using multiplexed PCR enrichment of SNP loci with simultaneous incorporation of sequences required for subsequent massively parallel shotgun sequencing (MPSS) in the absence of library generation. METHOD: We tested a high-level multiplexed SNP panel to amplify genomic loci of high minor allele frequency using a model system of genomic DNAs. Each of the forward and reverse PCR primers was within 36 base pairs of the SNP site and contained additional sequences to allow capture of the PCR product directly onto the flow cell surface for sequencing on the Illumina GAIIx or HiSeq2000. After cluster generation by bridge PCR, sequencing primers corresponding to the forward PCR primer sites were hybridized to the clusters for sequencing. The resulting clusters were sequenced for 36 cycles. The observed reads were compared to the expected 36 base reads based upon the amplicon PCR design, and the number of observed reads exactly matching the expected reads was determined. The number of matched reads for each of the expected SNP alleles was used to calculate the allele frequency of each SNP allele. RESULTS: In these initial experiments, each of the amplicon products was detected by sequencing. Based on observed allele counts, allele ratios matched genotypes of the DNAs used. Results of DNA mixtures at 2%, 6% and 20% fractions showed the ability to distinguish low fractional concentration alleles even in the 2% DNA mixture. CONCLUSION: This method demonstrates a proof-of-concept to apply MPSS of enriched targeted alleles to noninvasive prenatal diagnostics. Increased numbers of targets in such an assay are predicted to provide universal population coverage, and inclusion of molecular index sequences into the amplicon will allow multiplexing of samples into a single flow cell lane.

1413W

Discovery of the serum biomarker proteins in severe preeclampsia by Proteomic Analysis. *D.H. Cha[1], J.s. Park[2], S.J. Lee[2], Y.N. Kim[3], K.P. Kim[2].* 1) Dept OB/GYN, Kangnam CHA Hosp, Seoul, Korea; 2) Department of Molecular Biotechnology and Institute of Biomedical Science and Technology, Konkuk University, Seoul 143-701, Korea; 3) Department of Obstetrics and Gynecology, Inje University, Pusan Paik Hospital, Korea.

Preeclapsia (PE) is a severe disorder that occurs during pregnancy, leading to maternal and fetal morbidity and mortality. PE affects about 3~8% of all pregnancies. In this study, we conducted LC-MS/MS to analyze serum samples depleted of the six most abundant proteins from normal and PE-affected pregnancies to profile serum proteins. A total of 237 proteins were confidently identified with <1% FDR from the two groups of duplicate analysis. The expression levels of those identified proteins were compared semi-quantitatively by spectral counting. To further validate the candidate proteins with a quantitative mass spectrometric method, selective reaction monitoring (SRM) and enzyme linked immune assay (ELISA) of serum samples collected from pregnant women with severe PE (n=8) or normal pregnant women (n=5) was conducted. Alpha2-HS-glycoprotein (AHSG), Retinol binding protein4 (RBP4) and alpha-1-microglobulin/bikunin (AMBP) and Insulin like growth factor binding protein, acid labile subunit (IGFBP-ALS) were confirmed to be differentially expressed in PE using SRM ($p<0.05$). Among these proteins, AHSG was verified by ELISA and showed a statistically significant increase in PE samples when compared to controls.

1414W

Analysis of the relative contribution of genetic, developmental and environmental causes to the origin of birth defects. *R. Elespuru.* FDA/CDRH Div Biol, FDA Federal Labs at White Oak, Silver Spring, MD 20993.

Heritable damage to the genome is a concern along with cancer risk when considering the safety profile of radiation or chemotherapy, medical devices, drugs, and other products subject to regulatory risk/benefit analysis. Birth defects are a major adverse event monitored world-wide and could, potentially, be useful for assessing the extent of heritable effects. However, because information about the causes of birth defects is limited, these events generally have not been useful as genetic endpoints of environmental effects in humans. New genetic analyses and recent epidemiological studies are expected to provide a better understanding of these causes. In order to aid in prevention and risk assessment, the eleven most common birth defects (for the US listed on the Center for Disease Control web site; similar to the March of Dimes global listing) have been analyzed for likely causation. The categories include: developmental, mendelian genetic, multi-genic, and gene-environment - based alterations. Genetic causes are differentiated according to likely inherited or de novo alteration. All but the inherited genetic origins could be affected by external environmental exposures or by the combination of genetics and environment. Birth defects occur generally at an incidence of ~3-4% world-wide. The common anomalies analyzed represent ~one third of the total (i.e. ~1% of births). Those birth defects that are caused at relatively high frequency by inherited recessive alleles (cystic fibrosis, metabolic disorders), viruses (some eye defects), or developmental (gestational) effects (fetal alcohol syndrome, neural tube defects) are not likely to be informative as endpoints for heritable genetic alterations. Some birth defects appear to be caused by female based (Down Syndrome and other triploidies) or male based (Achondroplasia) deficiencies affected by germ cell age; these should be considered as target endpoints only when mechanistically appropriate. There is considerable evidence that some birth defects (e.g. cleft palate) have multiple contributing causes - developmental, mendelian genetic, multi-genic, and gene-environment. The causes of other major classes of birth defects (e.g. cardiovascular defects) remain largely unknown. However, emerging information may be added to a framework of categories representing different types of causes, some of which are informative and relevant to a given risk assessment perspective, and some of which are not.

1415W

Prenatal Onset of Neonatal Hemochromatosis and Infantile Cortical Hyperostosis: A Possible Mechanism for Disease Association. *N. Martin[1], G. Taylor[2], P. Whitington[3], H. Melin-Aldana[4], RS. Lachman[5], WR. Wilcox[5], A. Toi[6], R. Windrim[7], D. Chitayat[1,8], K. Chong[1]*. 1) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada; 2) Division of Pathology and Laboratory Medicine, Hospital for Sick Children, Toronto, ON, Canada; 3) Children's Memorial Research Center, Feinberg School of Medicine, Northwestern University, Chicago IL; 4) Dept of Pathology and Laboratory Medicine, Feinberg School of Medicine, Northwestern University, Chicago IL; 5) Medical Genetics Institute, Cedars-Sinai Medical Center, David Geffen School of Medicine at UCLA, Los Angeles, California; 6) Dept of Medical Imaging, Mount Sinai Hospital and University Health Network, University of Toronto, Toronto, ON, Canada; 7) Dept of Obstetrics and Gynecology, Mount Sinia Hospital, Toronto, ON, Canada; 8) Division of Clincal and Metabolic Genetics, Dept of Pediatrics, Hospital for Sick Children, Toronto, ON, Canada.

Neonatal hemochromatosis (NH) is characterized by hepatic failure in the newborn period with marked siderosis of extrahepatic tissues, including the heart, pancreas and mucosal glands of the respiratory system. Recent evidence suggests a congenital alloimmune disease between the maternal and fetal immune systems as a novel mechanism for NH. Infantile cortical hyperostosis (ICH) or Caffey disease consists of benign cortical thickening of affected bones and swelling of the contiguous soft tissues usually presenting in young infants. Prenatal onset is presumed to be autosomal recessive and associated with a high mortality rate. We present a fetus with ultrasound findings in the third trimester of marked polyhydramnios, deformed long bones with a small chest, possible brain abnormalities, and facial dysmorphisms. At birth, the baby had hypoglycemia, coagulopathy, and multisystem organ failure, which resulted in his death on day 18. Autopsy showed NH with liver damage. Immunostaining of the liver confirmed that the NH was alloimmune in origin. Skeletal findings were consistent with ICH. The pathogenesis of prenatal ICH is unclear, however, it has been hypothesized that myeloid hyperplasia in the fetal liver may be a cause for the lethality in these cases. Given the clinical and pathological findings of NH in this case, we suggest that prenatal ICH may be alloimmune in origin and that the inheritance pattern of this lethal condition may in some cases, not be the result of autosomal recessive inheritance.

1416W

Non-visualization of foetal gallbladder increases the risk of cystic fibrosis, when associated with echogenic bowel. *C. FEREC[1,2,3,4], I. DUGUEPEROUX[1,2,3,4], V. SCOTET[1,3,4], M.P. AUDREZET[1,2,3,4], A.H. SALIOU[5], M. COLLET[5], M. BLAYAU[6], S. SCHMITT[7], A. KITZIS[8], F. FRESQUET[8], F. MULLER[9]*. 1) Inserm U613, Brest, France; 2) C.H.R.U. Brest, Hôpital Morvan, Laboratoire de Génétique Moléculaire, Brest, F-29200, France; 3) Etablissement Français du Sang (EFS) - Bretagne, Brest, F-29200, France; 4) Université de Bretagne Occidentale, Faculté de Médecine et des Sciences de la Santé, UMR-S613, Brest, F-29200, France; 5) C.H.R.U. Brest, Hôpital Morvan, Service de Gynécologie-Obstétrique, Brest, F-29200, France; 6) C.H.R.U. Rennes, Laboratoire de Génétique Moléculaire, Rennes, F-35000, France; 7) C.H.R.U. Nantes, Laboratoire de Génétique Moléculaire, Nantes, F-44000, France; 8) C.H.R.U. Poitiers, Laboratoire de Génétique Moléculaire, Poitiers, F-86000, France; 9) A.P.H.P. Hôpital R. Debré, Laboratoire de Biochimie-Hormonologie, Paris, F-75019, France.

Objectives: Ultrasonographic examinations allow the detection of signs that may indicate abnormalities in the foetus. Among them, non-visualization of foetal gallbladder (NVFGB) or foetal echogenic bowel (FEB), may be possible clues for cystic fibrosis (CF). Cystic fibrosis is an autosomal recessive disease caused by mutations in the CFTR gene which lead, in its classic form, to severe chronic pulmonary infections and pancreatic insufficiency. The aim of our study was to evaluate the incidence of CF in cases referred for genetic diagnosis when the foetal gallbladder was not seen at ultrasonographic examination and correlate CF diagnosis with presence of echogenic bowel. Methods: We reviewed all consecutive cases of NVFGB diagnosed in Western France and referred for analysis of the gene responsible for CF over the 2002-2009 period. Results: Over the 8-year period, 37 foetuses with NVFGB had CFTR gene analysis. CF was diagnosed in five cases, leading to a CF incidence of 13.5%. All the CF foetuses had NVFGB associated with foetal echogenic bowel (FEB), and the risk of CF was still increased in foetuses with both these signs (5/11, 45.5%). The risk of CF was also 11.6-fold higher in foetuses with both these signs than in foetuses with FEB alone (45.5% vs. 3.9%; RR=11.6, 95% CI: [4.7; 28.8], p=0.0001]. Using Bayesian calculations, we estimated that the residual risk of CF was less than 1 in 68 (1.5%) when one mutation was identified by our molecular protocol. Nine of the non-CF foetuses had another disease evidenced (i.e. 28.1%) Conclusion: This study highlights the need to properly investigate the foetal gallbladder, especially when associated with FEB. Although it is not possible to accurately gauge the importance of NVFGB, it should be considered as an additional pointer to the need to conduct a CFTR gene analysis, especially when FEB is detected simultaneously.

1417W

Screening for the gr/gr Y chromosome Microdeletion in infertile Tunisian men. *A. HTIRA, M. GRIBAA, O. Mamay, I. Ben Charfeddine, A. AMARA, D. Hmida, M. AJINA, H. BEN ALI, S. TRABELSI, A. SAAD.* Laboratory of human cytogenetics, molecular genetics and reproductive biology in Farhat Hached University hospital, Street Ibn El Jazzar, 4000, Sousse, Tunisia.

As a common variation in the azoospermia factor c (AZFc) region of Y chromosome, the gr/gr deletion is regarded as a significant risk factor for spermatogenic impairment. But, no definitive conclusion has been established for its exact role. This deletion is caused by intra-chromosomal homologous recombination and removes half of the AZFc region genes. In our study we investigate the role of gr/gr subdeletion in spermatogenic failure. For this, a total of 82 idiopathic infertile Tunisian men referred to our laboratory for genetic investigations. They are affected by azoospermia or severe oligozoospermia which normal or elevated FSH level. All these patients have normal karyotype (46,XY) and show no subchromosomal microdeletions on Yq11 (AZFa, AZFb, AZFc). Analysis of gr/gr region on AZFc locus with specific STSs (sY1291, sY1161, sY1191 and sY1201) was performed on genomic DNA by multiplex PCR. Thirty two healthy fertile men with normal sperm were selected for control data. We identified four patients with gr/gr deletion (4.87%) and three in the group of control men (8.23%). No significant difference was found (P= 0,7). Our results showed that gr/gr deletion is a simple polymorphism and cannot be responsible of the spermatogenic failure in our patients. Otherwise, other studies found that this deletion can be responsible for a male infertility. By the way, the relationship between gr/gr deletion and male infertility can be related to the ethnicity and geographic region of the patients.

1418W

Role of genetic factors and oxidative stress in of idiopathic male infertility. *V. Sundararajan, T. Jayapalraja, R. Kumar, R. Dada.* All India Institute of Medical Sciences, New Delhi, New Delhi, India.

Introduction: Infertility is the major concern among the married couples and in atleast 50% of them; the male partner is the sole contributor to the problem. Majority of these men are diagnosed with no aetiology and hence they are idiopathic cases. Aim: The current study was planned to evaluate the role of genetic and molecular factors in 200 men with idiopathic infertility. Methods: The work involved cytogenetic and Y chromosome micro-deletion analysis. Patients with cytogenetic and Yq micro-deletion anomalies were excluded for further studies and screened for sperm mtDNA ATPase and NADH gene mutations. Seminal oxidative stress was estimated by chemiluminescence assay. Results: Out of 200 infertile men screened, 13% harbored cytogenetic abnormalities. This frequency is higher when compared to the previous report from Indian population. Azoospermia factor (AZF) microdeletions were detected in 9.5% infertile men, where AZFc deletion was predominant (6%) followed by AZFb (2%) and AZFa (1.5%) deletions. This is in accordance to the range reported in the world wide population. Level of ROS in both washed and neat semen was found to be significantly ($P<0.0001$) higher in infertile men compared to controls. However, no correlation between ROS level and the sperm parameters was observed. The ROS levels in raw semen showed a strong positive correlation ($r=0.842$, $p<0.0001$) with ROS levels in washed semen. Significantly higher frequency of nucleotide changes in mitochondrial ATPase6, ND4 and ND5 gene was observed in infertile OA men, who had significantly ($P<0.001$) higher ROS levels, compared controls. Majority of the mitochondrial nucleotide changes observed were found to be pathogenic by polyphen computational analysis. Conclusion: In conclusion, screening for cytogenetic and Yq anomalies are highly recommended in patients opting for ARTs as there is increased incidence of these anomalies in Indian population. Increased mitochondrial DNA mutations in infertile men may have better diagnostic and prognostic value in ART, as there is no paternal transmission of paternal mtDNA. It is also important to distinguish the genetic cause of male infertility, whether it is nuclear or mitochondrial, to counsel such couples prior to undergoing assisted reproduction.

1419W

High level of expression and rapid purification of recombinant human FSH in CHO cells. *S. Teimourain[1], M.H Modarresi[1], M.H Hedayati[2].* 1) Tehran University of medical sciences, Tehran, Tehran, Iran; 2) Pasteur Institute of Iran Research and Production complex 25 km west of Tehran Tehran-Karaj Autobahn Tehran,Iran Postal code:31599.

FSH is a glycoprotein which is clinically used for treatment of anovulation and in assisted reproduction technologies such as invitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). In this study,the genes encoding apha and beta-subunits of human FSH were sequense optimized by site directed mutagenesis and cloned in Pvitro-2- neo vector. After amplification of plasmid in Ecoli top 10 starin, plasmid was purified and then transfected into Chinese hamster ovary (CHO-K1) cells. In selective mediunm containing 800μg/ml G418, a CHO clone was isolated capable of secreting intact glycosylated FSH in the amount of 20pg/cell/48 hours. Roller bottel monolayer cells were incubated in DMEM medium containing 10μg/ml insulin. After three days, the supernatant harvested and purified by dye affinity chromatography(orange 1).The overal recovery was 55% as tested by Elisa and the purity was 90% as tested by SDS-PAGE .

1420W

Study of GTn-repeat expansion in Heme oxygenase-1 gene promoter as genetic cause of male infertility. *A. Aleyasin[1], E. Siasi[2], J. Mowla[3].* 1) Medicin genetic, National institute of genetic and biotechnology, Tehran, Iran; 2) Molecular and madicin genetic, Tarbiat modares university, Tehran, Iran; 3) Molecular genetic, Tarbiat modares university, Tehran, Iran.

Abstract Objective- The length of GT-repeats polymorphic region in the promoter of human Heme oxygenase-1 gene (HO-1) alters the level of its transcriptional activity in response to oxidative stresses. Decreased level of HO-1 protein in the seminal plasma has been reported to be associated with oligospermia and azoospermia in male infertility. This is the first study to investigate the association between GT-repeats expansion in promoter of HO-1 and male infertility. Methods- The allelic frequencies for different GT-repeats in the promoter of HO-1 gene were determined in 100 case and 100 normal control groups using PCR-PAGE, ABI fragment analysis genotyping and sequencing analysis. Results- All alleles were classified into S and L alleles. S alleles with < 27 specified as allele number 0 to 3 and L alleles > 27 repeats were specified as 4 to 6 alleles. The L allele frequency was significantly higher (54.5%) among case group than that in the normal controls (37.5%). Statistical analysis provide significant relationship between L alleles and male infertility ($P<0.001$). Conclusions- This study shows for the first time that GT-repeats expansion in promoter of the HO-1 gene is associated with oligospermia and azoospermia in male infertility among Iranian infertile cases.

1421W

Maternal smoking, gene variants of xenobiotic-metabolizing enzymes, and risk for anorectal atresia and stenosis. *M.M. Jenkins[1], J. Reefhuis[1], M.L. Gallagher[1], S.A. Rasmussen[1], D.A. Koontz[1], C. Sturchio[2], E.J. Lammer[3], M.A. Honein[1], National Birth Defects Prevention Study*. 1) Centers for Disease Control and Prevention, Atlanta, GA; 2) Battelle, Columbus, OH; 3) Children's Hospital Oakland Research Institute, Oakland, CA.

Maternal smoking during pregnancy has been suggested as a risk factor for anorectal atresia and stenosis in infants, but modest effect estimates might reflect an interaction between genetic risk factors and smoking. We analyzed 5 polymorphisms in 3 genes that code for enzymes involved in metabolism of chemicals in cigarette smoke (*CYP1A1*, *CYP1A2*, and *NAT2*) in mothers and their infants to assess their modifying effect on anorectal atresia and stenosis risk and the potential interaction with maternal smoking. Exposure assessment was based on maternal interviews, and DNA was extracted from cytobrush-derived buccal cells collected from infants and their parents as part of a multi-site, population-based, case-control study of major birth defects. Case families had infants with anorectal atresia or stenosis, and control families had live born infants with no major structural birth defects. Infants had estimated due dates between Oct 1, 1997 and Dec 31, 2003. Nineteen and one-half percent of mothers who completed the interview reported smoking in the month before pregnancy or during the first trimester. Whole genome amplification (WGA) was completed on samples with sufficient DNA (⁄ 5µg) using Sigma's GenomePlex® kit. Genotyping was completed on either genomic DNA or WGA products using Pyrosequencing® technology. Genotyping call rates were 99.4 to 99.6%. Data from 173 case families and 1706 control families with successful genotype results for ⁄ 4 variants from the infant, mother, or both, were included in these analyses. Independent effects of *CYP1A1*2A*, *CYP1A2*1C*, and *CYP1A2*1F* rapid oxidizer variants on anorectal atresia and stenosis risk were not observed in non-Hispanic White, non-Hispanic Black, or Hispanic mothers and their infants. Independent effects of *NAT2*5* and *NAT2*6* slow acetylator variants on anorectal atresia and stenosis risk were observed in Hispanic infants (odds ratio (OR)=2.7, 95% confidence interval (CI) 1.0-7.4 and OR=6.2, 95% CI 1.4-26.4, respectively); no significant effects were observed for non-Hispanic White (OR=0.7, 95% CI 0.4-1.3 and OR=1.4, 95% CI 0.7-2.9) and non-Hispanic Black (OR=1.4, 95% CI 0.2-12.3 and OR=3.6, 95% CI 0.6-20.5) infants; no significant effects were observed in their mothers. Further analyses stratified by periconceptional smoking status and adjusted for confounders will assess the interaction of maternal smoking and these xenobiotic-metabolizing enzyme gene variants on anorectal atresia and stenosis risk.

1422W

G5508A polymorphism of the *Sept12* gene may be associated with idiopathic male infertility. *M. Shahhoseini[1], M. Azad[1], M. Sabbaghian[2], K. Anisi Hemaseh[1], A. Amiri Yekta[1], M.H. Sanati[3], M.A. Sadighi Gilani[2], H. Gourabi[1]*. 1) Department of Genetics, Royan Institute for Reproductive Medicine, ACECR, Tehran, Iran; 2) Department of Andrology, Royan Institute for Reproductive Medicine, ACECR, Tehran, Iran; 3) National Institute for Genetic Engineering and Biotechnology, Tehran, Iran.

Introduction: Male infertility is a multifactorial disorder, which affects approximately 10% of couples at childbearing age with substantial clinical and social impact. Genetic factors are associated with the susceptibility to spermatogenic impairment in humans. Recently, *Sept12* is reported as a critical gene for spermatogenesis. This gene encodes a testis specific member of Septin proteins which belong to a family of polymerizing GTP-binding proteins. Septin 12 in association with other septins is an essential annulus component in mature sperm. **Material & Methods:** Seventy infertile men and thirty six normal controls referred to the Royan Institute were analyzed for 5 reported SNPs in the active site coding region of Sept12. Genomic DNA was extracted from periphery blood samples using standard techniques, and a 341bp DNA sequence was amplified by PCR and analyzed by sequencing. **Results:** Genotype analyses indicated that G5508A polymorphic *Sept12* alleles were distributed in three peaks of frequency in both control and diseases groups .Categorization of the alleles into (GG), (GA), (AA) types revealed a significant difference between azoo, asthenospermia and controls (*P* values less than 0.05 by chi-squre test). **Conclusion:** According to our finding we suggest that G5508A polymorphism in *Sept12* gene can affect on spermatogenesis in men, the opinion needs more investigation in different populations.

1423W

Association study of C109869T SNP in SLC6A14 gene with azoospermia and olidospermia in idiopathic infertile men. *E. Siasi[1], A. Aleyasin[2], J. Mowla[3]*. 1) Molecular and medicin genetic, Tarbiat modares university, Trhran, Iran; 2) medicin genetic, national institute of genetic and biotechnology, Trhran, Iran; 3) Molecular genetic, Tarbiat modares university, Trhran, Iran.

Back ground- C109869T polymorphism in SLC6A14 gene has been suggested to be associated with male infertility using Genome Wide SNP Association Study (GWAS). This SNP is an intergenic SNP located on the X chromosome 200 Kb from solute carrier family 6, member 14 (that is member of sodium and chloride dependent neurotransmitter transporters family). This SNP is functional variant responsible for the oligospermia phenotype by a regulatory capacity. Objective- This is the first study to evaluated association between C109869T SNP and idiopathic male infertilility in Iranian population. Methods- This relationship has been studied in 96 idiopathic infertile men with azoospermia and oligospermia and 100 normal control men. Primer pairs were designed to amplify 183bp fragment containing this polymorphism. Analysis of SNP was performed by PCR-RFLP and High Resolution Melt (real time PCR-HRM Corbett analysis). Amplified fragment was sequenced to determine the PCR product and allelic identify. Results- Genotype frequencies of the CC, CT and TT were as 85.5%, 5.2% and 9.3%, in the case group and 90%, 4% and 6% in the control group respectively. The prevalence of tested SNP was approximately similar in both infertile patients and fertile control groups. Statistical analysis showed significant relationship between C109869T SNP and idiopathic male infertility. Conclusion- This result indicated the (C109869T) SNP play role in infertile Iranian with oligospermia and azoospermia similar to that observed is Europian population.

1424W

Gene Expression Profiling of Uterine Prolapses. *H. Ak Celik[1], H.H. Aydin[1], B. Zeybek[2], N. Askar[2]*. 1) Dept. of Medical Biochemistry, School of Medicine, Ege University Bornova, Izmir, Turkey; 2) Dept. of Obstetrics & Gynecology, School of Medicine, Ege University Bornova, Izmir, Turkey.

Prolapses of the pelvic organs are a common disease affecting the live qualities of women. Age, menaupose, pregnancy, vaginal delivery, obesity, chronic constipancy, pelvic bone structure and hysterectomy are important risk factors. Additionally, population studies are shown that genetic factors play important role in prolapses of pelvis organs. Despite the high prevalence of the disease, very little is known its pathophysiology. In this study, discovery of new candidate genes which have role on developing of the disease in uterosacral ligaments of patients with uterus prolapses is aimed. For this purpose, whole genome gene expression profiling were carried out by using Affymetrix U133 2.0 plus GeneChip® in 10 controls and 15 uterine prolapses samples of uterosacral ligament. Statistical analyses were conducted by using Partek Genomic Suite (Partek Inc., MO, USA) and Ingenuity Pathway Analysis 8.7 (Ingenuity Systems Inc. CA, USA). Differences were considered statistically significant at P-value <0.05 and 2 fold change in gene expression between patient population and control subjects. There was a relationship between the uterine prolapses and increased expression of genes which are associated with biological adhesion, immune system and stimuli response processes. Among the genes; expressions of CREB5, FNDC1, HMCN1, VCAN, COMP, CTHRC1, DUSP10, THY1, CD44, ADAMTS16, SERPINE1, MLLT10, PAMR1, SYNC, GOLIM4 and PI16 were most significantly increased in uterine prolapses compared to the controls. However, ERAP2, HLA-DQA1, LOC572558 gene expressions were found decreased in the patients. Obtaining results provides to develop new strategies for new diagnostic and prognostic markers and understanding pathophysiology of uterine prolapses.

1425W

Exploration the tissue specific promoter of aromatase gene (*CYP19*) to SNP analysis in infertile men. *P. Afsharian, M. Khosravi, K. Fallah-Zadeh, M. Shahhoseini.* Department of Genetics, Royan Ins. for Reproductive Medicine, Tehran, Iran.

Aromatase is the key enzyme in biosynthesis of estrogens from androgens. It is encoded by the *CYP19* gene which is located in the chromosome 15q21.2 in human genome. Aromatase is expressed under different tissue specific promoters (TSPs) as an un-translated exon in mammals. In most mammals, *CYP19* expression is under the control of gonad- and brain-specific promoters, PII and PI.f respectively. In the human, however, there are at least eight additional promoters (totally 10 TSPs with the 93 kb in length) that seemed to have been recruited throughout the evolution possibly via alterations in DNA. One of the key mechanisms that permit the recruitment of such a large number of promoters seems to be the extremely promiscuous nature of the common splice acceptor site, since activation of each promoter gives rise splicing of an un-translated first exon onto this common junction immediately upstream of the translation start site in the coding region. These partially tissue-specific promoters are used in the gonads, bone, brain, vascular tissue, adipose tissue, skin, fetal liver and placenta for physiologic estrogen biosynthesis. PII is introduced as the specific promoter in human ovary tissue, although, it hasn't been directly launched as the main promoter in human testis, while there are some information about the aromatase promoter in rat testis (PII, PI.f & PI.tr). Since aromatase plays an important role in human reproductive endocrine system, genetic alterations of *CYP19* would be the critical parameter in fertility/sterility. Based on the recent studies that aromatase expression is declined through spermatogenesis failure; we study the promoter SNPs in infertile men referred to our institute. As the first step, we tried to screen the regulatory region of *CYP19* in order to find the testis-specific promoter in human testicular cell line (NT2). To achieve this purpose, the presence of RNApol-II in different candidate promoters (located in un-translated exon) was checked by the use of Chromatin Immunoprecipitation (ChIP) technique. Our results showed that among all known *CYP19* promoters, PII is the main promoter to study the aromatase SNPs in our patients.

1426W

Spontaneous pregnancies in patients with Turner syndrome **G. Bergvall MD, E. Stattin MD PhD Clinical Genetics, Umeå, Sweden.** *G. Bergvall, E. Stattin.* Laboratorymedicine, Clinical genetics, Umeå, UMEÅ, Sweden.

Introduction: Patients with Turner syndrome are in the majority of cases infertile and do not menstruate. Their ovaries degenerate and by birth or early childhood there is complete follicle depletion in the majority of cases, although in 5-20% of cases sufficient follicles survive to allow spontaneous menarche, followed by premature menopause. Spontaneous pregnancies in patients with Turner syndrome is uncommon, and completed pregnancies is rare. It has been suggested that it could be explained by a gonadal mosaicism, with presence of a normal celline. Here we want to report two cases with Turner syndrome who have completed four normal pregnancies without assisted reproduction. Methods We have in our laboratory and outpatient clinic investigated two women with Turner syndrome who have completed spontaneous pregnancies. Case 1 have completed one spontaneous pregnancy, while case 2 have completed three dito. All four children are healthy. We have performed a conventional cytogenetic analysis by G-banding on peripheral blood lymphocytes. We have done FISH-analysis on 200 cultured lymphocytes, as well as FISH-analysis on 500 imprinted oral buccal cells, obtained with brushpin. Results

	Karyotype	FISH/lymphocytes	FISH/ buccal cells	Age at childbirth
Case 1	45,X[24]	X/XX 198/2	X/XX/XXX 434/20/36	33
Case 2	45,X[24]/ 47,XXX[1]	X/XX/XXX 183/8/9	waiting for results	27, 29, 31

Conclusion: This casereport want to focus on the rare, yet possible situation where a women with Turner syndrome have regular menstruations and become spontaneously pregnant. Our investigations show normal sex chromosomes in 2%-4% of lymphocytes and 4%- ? of oral buccal cells. There is signs of low-grade mosaicims for normal cellines in these cases. Whether this is the situation in the gonads we can only speculate on.

1427W

PGD via array comparative genomic hybridization (aCGH) can be used for any translocation to simultaneously detect unbalanced embryos and aneuploidy. *P. Colls, T. Escudero, J. Fischer, G. Harton, S. Munné.* Reprogenetics, Livingston, NJ.

Objectives: 1) Determine the resolution of aCGH in detecting translocations; 2) Determine how many cases previously analyzed by FISH could be analyzed by aCGH; and 3) Validate aCGH for use in translocations in combination with aneuploidy testing. **Design:** Prospective validation of the technique and retrospective comparison with FISH. **Material and Methods:** Consenting translocation carriers underwent PGD via embryo biopsy with aCGH using 24sure-Plus microarrays. Embryos determined by aCGH to be abnormal were fixed and all cells analyzed by FISH with up to 15 probes to determine the error rate and resolution. Once the lower detection limit of aCGH was determined, we consulted our database of all previous PGD cases to determine how many would be analyzable by aCGH. **Results:** Nineteen (19) procedures of PGD for reciprocal translocations and 15 for Robertsonian translocations were performed with aCGH. ArrayCGH has the ability to detect fragments 4 Mb or greater, similar to the limit of conventional G-banded karyotyping. A total of 1317 procedures of PGD for reciprocal (n=931) or Robertsonian translocations (n=351), and inversions (n=35) previously analyzed by FISH were reviewed to determine if they could be diagnosed using aCGH based on the above resolution. 196 reciprocal translocations had one fragment smaller than 4 Mb, but none had two fragments smaller than 4 Mb. By detecting 3 fragments larger than 4 Mb, these cases are analyzable by aCGH. Similarly all Robertsonian translocations can be analyzed since whole q arms of acrocentric chromosomes are translocated. All inversions had a sufficient number of fragments large enough to be analyzed by aCGH. Twenty three embryos determined by aCGH to be unbalanced were reanalyzed by FISH and all (100 %) were found to be consistent with the aCGH diagnosis. To date, from 54 PGD procedures studied with aCGH, 22.7 % of embryos were normal or balanced, 19.9 % were unbalanced, 31.9 % were unbalanced and aneuploid, and 23.1 % were only aneuploid. **Conclusions:** Present results indicate that aCGH can simultaneously analyze any translocation detectable by regular karyotyping and any aneuploidy, even in cases in which one of the four chromosome fragments is below aCGH resolution. Using this method, the present error rate is 0 % for translocations, and 2 % for aneuploidy (see Gutierrez-Mateo et al. 2011). aCGH can detect 23 % more abnormalities than FISH performed only with probes for the translocation.

1428W

Familial X-Autosome translocation associated with Infertility. *L. Martelli[1,2], M.L.C. Moreira[2], R.M. Scarparo[2], C.G. Picanço[2], C.S. Pereira[1], C.H.P. Grangeiro[2], W.A.R. Baratela[2], J.M. Pina Neto[1,2], S.A. Santos[1], J. Huber[2], E.S. Ramos[1,2].* 1) Dept. Genetics, University of Sao Paulo, School of Medicine- Ribeirao Preto, SP, Brazil; 2) Medical Genetics Division, University Hospital, Ribeirao Preto, SP, Brazil.

Numerical and structural chromosomal aberrations are strongly correlated to infertility. Translocations involving the X chromosome and autosomes are rare rearrangements with an estimated occurrence of 1 to 3 per 10,000 live births. These translocations, although extremely rare, may affect reproductive capacity depending upon the location of breakpoints of the X chromosome. We report a 27 years old man, referred to the Medical Genetics Division (HCFMRP-USP) for male infertility. Familial history revealed one brother and two uncles on the maternal side with infertility. The patient is the second child of healthy, unrelated parents, with no history of miscarriages. He was born at term by Cesarean section after an uneventful pregnancy. He showed adequate psychomotor development and physical examination was normal with no dysmorphic features. After marriage and 3 years trying to have children without success, the couple was evaluated by the Human Reproduction Department, with final diagnosis of male factor infertility. Espermogram demonstrated azoospermy. Testicular biopsy showed that general architecture of testicles was preserved, with edematous stroma, and regular number of Leydig cells with mild thickening of seminiferous tubules. Conventional cytogenetic analysis by GTG high resolution banding revealed an apparently balanced translocation between X and 22 chromosomes with karyotype 46,Y,t(X;22)(p22.3;q11.2). Maternal karyotype showed the same translocation between Xp;22q. She presented a normal phenotype and three pregnancies: her first son (also diagnosed as azoospermic) and her daughter (22yo) also showed the same translocation. Infertility in X-autosome translocation carriers is more likely to arise because of meiotic disturbances leading to spermatogenic failure. Meiotic arrest is considered to result from a disturbance of the X chromosome condensation and inactivation process during male meiosis. This finding also has implications in female meiosis with increased risk of infertility. Therefore X inactivation studies were performed to predict premature ovarian failure in apparently unaffected sister who carries the same translocation.

1429W

Molecular genetics and cytogenetics investigation in a man with infertility and azoospermia. *H. Shabanloo[1], N. Khazamipour[2], E. Bagherizadeh[1], F. Behjati[1,3], M. Noruzinia[4].* 1) Genetic Department, Sarem Women Hospital, Tehran, Iran; 2) Sarem Cell Research Center, Sarem Women Hospital, Tehran, Iran; 3) Genetics Research Center, University of Social Welfare and Rehabilitation Science, Tehran,Iran; 4) Department of Medical Genetics, Tarbiatmodares university, Tehran, Iran.

Yq- microdeletions are the common cause of infertility. Three different loci (AZFa, AZFb, AZFc) involve the genes responsible for spermatogenesis mapped to these regions referred to as `azoospermia factors'. So molecular genetic analysis of Y-microdeletions is important in diagnostic procedure for nonobstructive infertile men. A 26 year old male with infertility was referred for genetic counseling. Paraclinical examination showed hypogonadotropic-hypogonadism. TESE confirms showed maturation arrest, leidig cell atrophy, tubular hyalinization and absence of spermatozoa. Chromosomal study was carried out using standard GTG banding technique. Molecular analysis performed by two multiplex PCR with the primer sets for ZFX/ZFY and SRY genes as internal control and primers for STSs in each AZF regions. (sY84 and sY86 in AZFa, sY127, sY134 in AZFb and sY254 and sY255 in AZFc regions). PCR products were electrophoresed on 1.8% agarose gel. Cytogenetic analysis showed that the patient was mosaic with a deletion of chromosome Yq described as45,X[8]/46,X,del(Y)(q11.23)[92]. Microdeletion analysis revealed no amplification in AZFb and AZFc STSs. But AZFa STSs as well as ZFX/ZFY and SRY regions are amplified. Deletion of any of `azoospermia factors' associated with spermatogenic arrest at a particular stage of germ cell development showed a characteristic testicular phenotype. The molecular and cytogenetic analysis for this patient confirm a microdeletion expanding AZFb and AZFc regions which can be the cause of his infertility.

1430W

Incidence of fra (16) (q22) and its Probable Consequences in a Worker of a Place with Extreme Chemical Pollution. *H. Pour-Jafari[1,2], K. Hasrak[1], B. Pourjafari[2], S. Ghahramani[3], E. Kamrani Saleh[3].* 1) Mol Med & Genetics, School of Medicine, Hamadan Un Med Sci, Hamadan, Iran; 2) Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran; 3) Genetic Lab., Sh. Beheshti Hospital, Hamadan, Iran.

The rare autosomal fragile site, fra (16) (q22), is the most common of all rare autosomal fragile sites and has a heterozygote frequency of about 5%. In some cases its familial types are reported. Also it is possible to experimentally induce fragile site on chromosome number 16 at q22 point treating with some chemicals in cell culture. Further studies on this fragile site indicate that fra(16)(q22) is a region with AT-rich and late replicating DNA. We report a family with two spontaneous abortions. Proband was a simple worker and clinically normal but fra(16)(q22) carrier. Also study of his sperm analysis has showed that majority of sperms were morphologically abnormal. We drew the pedigree, and studied his parent karyotypes. The karyotype of his father was normal but his mother was carrier for the mentioned fragile site. His wife had normal karyotype (46, XX) too. The important and notable point in their family history was that the proband has worked in a high risk work place with industrial chemical pollution in recent five years. The hazardous effects of such envivonmental work places are proved. Also the mutagenic and/or clustogenic effects of chemicals are shown in numerous research works. Sometime in genetic counseling at the first glance, it seems that the incidence of fragile site is direct result of environmental hazards. Although different evidence suggest that such polluted work place, without using special mask, as our case, could increase the risk of fetal deaths in progenies, but in our case the existence of fra (16) (q22) was from the mother side (familial) and probably the fetal deaths were its consequences . However genetic counselors have to consider the life style and work place situations of the families in their counseling procedures, paying attention to genetic factors, such as karyotyping, has its remarkable significance.

1431W

Identification of a hotspot for non-allelic homologous recombination (NAHR) centred on an Alusq sequence at the DPY19L2 locus. *C. Coutton[1, 2], F. Abada[1, 2], R. Harbuz[1, 2], D. Escalier[3], R. Zouari[4], R. Merdassi[5], M. Ben Khelifa[1, 2], F. Vialard[6], C. Triki[7], S. Hennebicq[8], P.S. Jouk[8], V. Satre[8], C. Arnoult[1], J. Lunardi[2], P.F. Ray[1, 2].* 1) Equipe Génétique, Infertilité et Thérapeutique, Laboratoire AGIM, CNRS FRE3405, Université Joseph Fourier, Grenoble, France; 2) UM de Biochimie et Génétique Moléculaire, CHU Grenoble, Grenoble, France; 3) UMR S933, Assistance Publique Hôpitaux de Paris, and Hôpital Armand-Trousseau, Paris, France; 4) Clinique de la Reproduction les Jasmins, 23, Av. Louis BRAILLE, 1002 Tunis, Tunisia; 5) Unité de Procréation Médicalement Assistée, Hôpital Aziza Othmana ,Tunis , Tunisia; 6) Department of Reproductive Biology, Centre Hospitalier Poissy Saint Germain, Poissy, France; 7) CMRDP, 5, rue Ibn Hazem 1002 Tunis Beledère, Tunisia; 8) Département de Génétique et Procréation, CHU Grenoble, 38700 Grenoble, France.

Globozoospermia is a rare syndrome of male infertility characterized by the presence of 100% round, acrosomeless spermatozoa in the patient's ejaculate. We demonstrated previously that a large majority of globobozoospermia patients had a 200 Kb deletion removing the totality of *DPY19L2,* a gene likely involved in spermatid polarization, and that the deletion probably occurred by Non Allelic Homologous Recombination (NAHR) between two homologous 28 Kb low Copy Number (LCR) located on each side of the gene. We have now analyzed 18 patients presenting with a homozygous *DPY19L2* deletion and 450 control subjects. One control subject was heterozygous for the deletion. Detailed analysis was carried out by DNA amplification and sequencing of the 37 deleted alleles identified. We demonstrate that the recombination always took place within a 1 Kb minimal region roughly located in the middle of the 28 Kb LCR. This region shows the highest sequence identity between the two LCRs (99% vs 97% for the rest of the sequence) and contains a canonical AluSq sequence located in the centre of the 1 Kb region. We observed three distinct breakpoints within this region indicating that the studied alleles (n=37) derived from at least three separate recombination events and not a single ancestral founding allele. These results thus show that the AluSq sequence might provide a favoured target site for homologous recombination within the LCRs. We also screened the 450 control subjects with inverted LCR PCR primers designed to amplify the recombined duplicated alleles. Three subjects carried a duplicated allele. Sequence analysis illustrated that the identified duplications corresponded to the reciprocal allele of two of the characterised deletions, thus confirming that these genetics events were indeed caused by homologous interchromatid or interchromosomal strand exchange. Overall this study shows that all recombination events identified at the *DPY19L2* locus occurred within a few hundred nucleotides of a canonical AluSq sequence indicating its likely involvement in the process, furthermore identification of perfect-match reciprocal deleted and duplicated alleles provide a textbook example of interchromatid or interchromosomal NAHR.

1432W

Screening for Copy Number Variations (CNVs) in Couples with Recurrent Pregnancy Loss (RPL). *J. Wen[1], M. Stephenson[3], W. Robinson[2], S. Martell[1], K. Calli[1], E. Rajcan-Separovic[1].* 1) Dept of Pathology, University of British Columbia, Vancouver, BC, Canada; 2) Dept of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 3) Dept of Obstetrics & Gynecology, the University of Chicago Medical Center, Chicago, IL, USA.

Background: Our recent whole genome array based studies of sporadic and recurrent miscarriages identified unique CNVs in ~50% of miscarriages with normal chromosome results. The majority of the CNVs were small (<250kb) in size and inherited from either partner. In addition, we identified CNVs in couples with RPL, which may predispose them to miscarriage. In order to determine if these candidate “miscarriage” CNVs are associated with RPL we screened 98 additional RPL couples and 90 fertile controls using Quantitative Multiplex PCR of Short fluorescent Fragment (QMPSF). Material and methods: 12 genes located within 12 “miscarriage” CNVs were selected for copy number assessment in RPL couples and in fertile controls. These genes included CTNNA3, TIMP2, RALA, PNPLA4, STX8, STX6, WDR37, NPAS3, CETN2, NSDHL, OFD1 and GPR180 . Results: Recurrence of copy number change was detected for 2/12 tested “miscarriage” genes so far. RALA (v-ral simian leukemia viral oncogene homolog A -ras related) gene copy number change was found in 13 subjects (9%) from 9 couples with RPL and in none of the controls. Interestingly, 4/9 couples had RALA gene copy number change detected in both partners. Duplications of RALA were more prevalent than deletions and occurred in 11/13 subjects. STX8 (Syntaxin 8) gene deletions occurred in 4 subjects (male and female) with RPL (3%) and in one control (1%). Conclusions: We identified two genes with higher frequency of copy number change in RPL subjects than controls. RALA is a multifunctional GTPase involved in a variety of cellular processes including cell migration, proliferation, and membrane trafficking. STX8 is a vesicle trafficking protein involved in cell cycle regulation. Transmission of copy number changes for these two genes from carriers to their miscarriages is under investigation to determine their role in recurrent pregnancy loss. In addition, frequency studies of all 12 “miscarriage” genes in 100 additional RPL couples and 100 additional idiopathic miscarriages using QMPSF is ongoing.

1433W

Genetic thrombophilic mutations among Iranian couples with recurrent spontaneous abortion. *A. Poursadegh Zonouzi[1], E. Sakhinia[2,3], A. Firozi[3], N. Karimi Ansari[3], N. Chaparzadeh[1], Y. Heshmat[3], M. Nouri[4], M. Sadaghian[4], L. Farzadi[4], A. Ghasemzadeh[4].* 1) Faculty of science Azarbaijan University of Tarbiat Moallem, Department of Cellular and Molecular Biology, Tabriz, Iran; 2) Faculty of Medicine Tabriz university of Medical Science, Department of Biochemistry Molecular Medicine Division, Tabriz, Iran; 3) Tabriz Medical Sciences University, Tabriz Genetic Analysis Centre (TGAC), Tabriz, Iran; 4) Tabriz University of Medical Science, Department of Obstetrics and Gynecology and women's reproductive health research center, Tabriz, Iran.

Introduction: Recurrent spontaneous abortion (RSA) is a frequent obstetric complication that affects 1-5% of couples. The pathophysiology of RSA is complex and remains largely unclear. Various investigations examined the prevalence of thrombophilic gene mutations in women with miscarriages. However, the role of these in RSA is still controversial. In order to determine the association of specific inherited thrombophilias and recurrent spontaneous abortion; 10 most common thrombophilic genes were investigated. Material & methods: A total of 139 women were included in this study: 89 women with a history of recurrent miscarriages and 50 fertile women with at least two live births from many different regions of North West of Iran. Genomic DNA was extracted from whole blood samples by a standard salting-out protocol. Two molecular genetics methods, Amplification Refractory Mutation System (ARMS-PCR) and restriction fragment length polymorphisms (PCR-RFLP) were then used for the identification of MTHFR (677C/T and 1298A/C), Factor V (1691G/A and 4070A/G), Prothrombin (20210G/A), PAI-1 (-675 I/D, 5G/4G), Factor XIII (Val34Leu), Beta-fibrinogen (-455G/A), ACE (intron 16 I/D), Factor VII (Gln353Arg), Glycoprotein Ia (807C/T), tPA (intron h D/I) gene mutations. Results: Between the two groups, no differences in the frequency of specific gene mutations were detected. However, the prevalence of homozygous mutations for MTHFR (677C/T and 1298A/C), Prothrombin (20210G/A), PAI-1 (-675 I/D, 5G/4G), Factor XIII (Val34Leu), Beta-fibrinogen (-455G/A), ACE (intron 16 I/D) and total gene mutations among patients with RSA was significantly higher than among controls. Conclusions: Compound thromboplilic gene mutations rather than a specific gene mutation can be a risk factor for first trimester RSA. However, further studies on a larger series are needed for a better understanding of the role of these gene mutations in this clinical setting.

1434W

Replication Study of 72 Single Nucleotide Polymorphisms Associated with Spontaneous Very Preterm Birth Using a State-Based Biobank. *D. Williamson[1], M. Pearl[2], J. Yang[2], GN. DeLorenze[3], CJ. Bean[4], WM. Callaghan[1], A. Grant[4], E. Lackritz[1], R. Romero[5], GA. Satten[1], H. Simhan[6], AR. Torres[7], J. Westover[7], R. Yolken[8], M. Kharrazi[9].* 1) Division of Reproductive Health, Centers for Disease Control and Prevention, Atlanta, GA; 2) Sequoia Foundation, La Jolla, CA; 3) Kaiser Permanente Division of Research, Oakland, CA; 4) Division of Blood Disorders, Centers for Disease Control and Prevention, Atlanta, GA; 5) Perinatology Research Branch, Eunice Kennedy Shriver National Institute of Child Health & Human Development, National Institutes of Health; 6) University of Pittsburgh School of Medicine, Pittsburgh, PA; 7) Center for Persons with Disabilities,UtahStateUniversity, Logan, UT; 8) School of Medicine, Johns HopkinsUniversity, Baltimore, MD; 9) Genetic Disease Screening Program, California Department of Public Health, Richmond, CA.

Background Preterm birth (birth < 37 weeks gestation) is one of the leading causes of infant mortality and morbidity. There are striking disparities in the rates and consequences of preterm birth across racial and ethnic groups. Research indicates that preterm birth is a multifactorial disease caused by genetic, social, and environmental factors, which most likely interact to increase risk. Identification of risk alleles may allow for early detection of women at high risk of preterm birth. Methods We identified 72 single nucleotide polymorphisms (SNPs) from manuscripts in the published literature that reported an association with preterm birth. We attempted to confirm these associations using data from 601 spontaneous very preterm births (<32 weeks for 207 whites and 217 Hispanics and <34 weeks for 177 blacks) and 796 controls identified from singleton live births born in southern California between 2000- 2007. Maternal blood specimens leftover from mid-pregnancy screening were genotyped using a Sequenom platform. Results We confirmed the association between 13 SNPs and spontaneous very preterm birth ($p < 0.05$). These included 5 among blacks (IFNG rs2430561, IL6R rs4845374, MMP1 rs1799750, TGFB1 rs1800469, TNFRSF1A rs740841), 2 among whites (IL10RA rs17121510, IL6R rs4845374), and 6 among Hispanics (IL10 rs1800896, IL15 rs10833, IL1B rs1143627, IL2RB rs228947, rs228954, rs3218315). Only one SNP was significant in two race-ethnicity groups (IL6R rs4845374) and no single SNP was significant across all three race-ethnicity groups. Conclusions: While the association between 13 previously-reported SNPs and very preterm birth were confirmed, we found differences in the genetic risk among each race-ethnic group.

1435W

Novel *FTO* locus Associated with polycystic ovary syndrome. *O.A. Garcia[1], P. Mutharasan[1], R.S. Legro[2], A. Dunaif[1], M. Urbanek[1].* 1) Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States of America; 2) Department of Obstetrics and Gynecology, Penn State Hershey College of Medicine, Hershey, Pennsylvania, United States of America.

Background: Polycystic ovary syndrome (PCOS) is a complex polygenic endocrine disorder that is the leading cause of infertility among women. PCOS not only affects the reproductive phenotype but is also associated with increased risk of obesity, insulin resistance and type 2 diabetes. Therefore the fat mass and obesity-associated *(FTO)* gene has been proposed as a candidate gene for PCOS. Recent studies have failed to demonstrate a strong association at traditional loci in the *FTO* gene. We tested the hypothesis that *FTO* contributes to the etiology of PCOS by tagging the entire *FTO* region and testing for association with PCOS. **Methods:** We genotyped 88 single nucleotide polymorphisms (SNPs) within *FTO* using the Infinium iSelect Beadchip technology (Illumina, San Diego, CA). The majority were haplotype-tagging SNPs (r^2>0.8 and minor allele frequency (MAF)γ 0.05); the remainder were known nonsynonymous coding SNPs. We tested for association in a European-derived cohort of 931 cases fulfilling NIH criteria and 961 minimally phenotyped controls. We adjusted for population stratification with 253 ancestry informative markers. Genetic analyses were carried out in PLINK using three models. Model 1 adjusted for population stratification using the first two principal components. Model 2 adjusted for population stratification and BMI. Model 3 adjusted for population stratification, BMI and age. **Results:** We found evidence for association at two regions of the gene. The first region maps to intron 1, encompassing the region previously found to be strongly associated with obesity and potentially associated with PCOS. The strongest association was observed at rs9939609 with Model 1 (OR=1.29, p=0.00021) but loses significance with Models 2 and 3. The second region maps to intron 8, an area not in linkage disequilibrium with region 1, with the strongest association at rs8056199. This marker was significantly associated with PCOS under all models tested but shows the strongest association with Model 3 (OR=1.29, p=0.00014). rs8056199 was not associated with BMI in the PCOS cases or in the complete cohort. **Discussion:** Previous studies have found evidence for association between variants within *FTO* and PCOS. However, these analyses have been limited to the region of *FTO* that is strongly associated with BMI, and this association was further weakened in PCOS cohorts after adjustment for BMI. Here we have identified a second region of *FTO* that is associated with PCOS but not BMI.

1436W

Maternal genetic variation in inflammatory response genes interact with a measure of air pollution exposure to influence infant birthweight in non-Hispanic black women. *A.E. Ashley-Koch[1], M.E. Garrett[1], K.S. Quinn[1], A.C. Buskwofie[1], S. Edwards[2], G.K. Swamy[3], M.L. Miranda[2, 4].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Nicholas School of the Environment, Duke University, Durham, NC; 3) Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC; 4) Department of Pediatrics, Duke University Medical Center, Durham, NC.

OBJECTIVE: To evaluate the contribution of maternal genetic variation and a measure of air pollution exposure to infant birthweight (BWT) among non-Hispanic black (NHB) women. **METHODS:** Healthy Pregnancy, Healthy Baby is a prospective cohort of pregnant women aimed at identifying genetic, social, and environmental contributors to racial disparities in pregnancy outcomes. English-literate women >18 yrs with a normal singleton pregnancy residing within Durham County, NC were enrolled prior to 28 weeks gestation. 673 NHB women were examined for BWT in this analysis. Maternal samples collected during inpatient admission for delivery were genotyped. Maternal residential address at enrollment was georeferenced and the distance to the nearest major roadway was calculated as a proxy for exposure to traffic-related air pollution. Haplotype tagging single nucleotide polymorphisms (htSNPs) were genotyped for 105 SNPs in 20 candidate genes using Taqman assays from Applied Biosystems Incorporated. Linear regression was used to examine the relationship between htSNPs and infant BWT, adjusting for parity, infant sex, maternal age, education, insurance, and smoking status. We also examined potential interactions between htSNPs and roadway proximity. **RESULTS:** Mean infant BWT was 3025 g (sd=653 g). Nominal evidence for main effects on infant BWT was detected with *CR1* (rs17047661, p=0.006), *IL10* (rs1518111, p=0.008), 2 SNPs in *IL8* (rs2227538, p=0.01; rs2227306, p=0.02), *IL12B* (rs2853694, p=0.03), *IL6* (rs2069840, p=0.03) and *IL12A* (rs568408, p=0.04). Evidence for SNPs interacting with roadway proximity to influence BWT was detected with two SNPs in *TLR4* (rs12344353, p=0.01; rs5030725, p=0.03), two SNPs in *IL4* (rs2227282, p=0.008; rs2243283, p=0.03) and one SNP in *INFG* (rs2069714, p=0.04). **CONCLUSIONS:** Similar to previous reports, genetic variation in inflammatory response genes provided evidence for main effects on infant BWT among NHB women in our study. Moreover, we provide the first evidence that some of these genes interact with air pollution exposure to influence infant BWT. Thus, maternal inflammatory response may be exacerbated by oxidative stress and particulate contamination due to exposure to air pollution during pregnancy.

1437W

***Hormad1*-deficiency causes azoospermia in males and pregnancy loss in females.** *H. Kogo, M. Tsutsumi, T. Ohye, H. Inagaki, H. Kurahashi.* Division of Molecular Genetics, ICMS, Fujita Health University, Toyoake, Aichi, Japan.

Infertility and pregnancy loss are commonly observed clinical problems in humans. Dynamic chromosome segregation processes, including meiotic recombination, synapsis and chiasma formation, are essential in meiotic prophase I. Genetic defects in the genes involved in these processes consistently cause reproduction failure in mice, suggesting that infertility in humans may be caused by defects in meiotic genes. To date, however, the molecular mechanism of mammalian meiosis is still insufficiently understood. In the present study, we investigated a hitherto uncharacterized meiotic gene, mouse *Hormad1* by gene targeting. *Hormad1*-deficient mice are infertile both in males and females. The mutant testes are hypoplastic due to the apoptosis of spermatocytes at the pachytene stage. The *Hormad1*-deficient spermatocytes show an extensive failure of homologous pairing and synapsis, which causes apoptosis due to the pachytene checkpoint. In contrast, unexpectedly, *Hormad1*-deficient ovaries contain a normal number of oocytes, despite extensive synapsis failure as in spermatocytes, and consequently produce aneuploid oocytes, indicating a failure of pachytene checkpoint function by the *Hormad1*-deficiency. The *Hormad1*-deficient oocytes can be fertilized, and the conceptus can occasionally survive after implantation, but fetal loss eventually results from aneuploidy. By analysis of *Hormad1*/*Spo11* double mutant female mice, the *Hormad1*-deficiency was found to completely abrogate the massive oocyte loss in the *Spo11*-deficient ovary. This result clearly demonstrates that HORMAD1 is required for a DNA damage-independent synapsis checkpoint. The double mutant spermatocytes also evade the arrest at the zygotene/pachytene transition caused by the *Spo11*-deficiency, although these cells are eventually eliminated at the pachytene stage due to the failure of meiotic sex chromosome inactivation, that is a male-specific phenomenon caused by extensive asynapsis. This additional male-specific surveillance mechanism for asynapsis will explain the sexually dimorphic consequence of meiocytes harboring extensive asynapsis in mammalian meiosis. Collectively, HORMAD1 is a key molecule required for multiple functions including homologous pairing, synapsis, and synapsis checkpoint in mammalian meiosis. Our results suggest that genetic defects affecting the HORMAD1-dependent function could be a candidate cause of azoospermia in males and pregnancy loss in females in human reproduction.

1438W

Association of overweight and obesity with diminished reproductive potential through change of sperm parameters; a pilot study. *M. najafi.* Zoology, University of Mysore, Mysore, karnataka, India.

Najafi M1, Yousefi A2, Sharath Kumar C3, Ravi Kumar S3 and Malini S.S1 1. Human Genetics Laboratory, Department of Studies in Zoology, University of Mysore, Manasagangothri, Mysore, Karnataka, India. 2. M.Sc Nutrition 3. Medivawe IVF and Fertility Research Hospital, Mysore, Karnataka, India. Corresponding authors; 1. Mohsen Najafi PhD. student, Human Genetics lab, Departemnt of studies in Zoology University of Mysore Email: mohsen.genomics@gmail.com 2. Dr. Suttur.S. Malini Assistant Professor DOS in Zoology, University of Mysore. Telephone: 08212419784 (O) E-mail: ssmalinisri@yahoo.co.in mohsen.najafi78@yahoo.com To investigate the link between increased BMI in overweight and obese men and diminished reproductive potential compare to control group in Karnataka, South India. In this pilot study a total 65 overweight and obese men (BMI⁄ 25), aged 25 to 45 years, were enrolled from different obesity clinics, infertility hospitals and gyms of Mysore city. Out of this number, 15 subjects were excluded from the study since either they were not convinced to donate semen sample or suffered from diabetes and other diseases. All cases and controls were evaluated for different semen parameters according to World Health Organization (WHO) standards. Statistical analysis was performed using the SPSS software, version 16.0. P-values less than 0.05 were considered statistically significant. Different conditions of infertility are observed among overweight and obese individuals enrolled in this study. Although sperm function test does not show statistical significant values in overweight and obese men when compared with control group but, variations in the semen parameters are significant in obese men compared to controls .In case of oligoasthenospermic subjects pH shows a significant increase compared to controls. Also the levels of Fructose show a significant decline in both azoospermic and oligoasthenosermic groups when compared to control group. Data obtained from this pilot study suggest a potential link between increased BMI in men and the semen quality which may ultimately diminish the chance of paternity. Key words: Obesity; BMI; Male Infertility.

1439W

Lessons from Chernobyl: Low dose irradiation at conception leads to a highly increased (epi) genetic risk. *K. Sperling[1], A. Koerblein[2], H. Neitzel[1].* 1) Inst Med & Human Gen, Charite - CVK, Berlin, Germany; 2) Untere Soeldnersgasse 8, D-90403 Nuernberg.

The international guidelines for radiological safety are primarily based on (a) studies of high doses of acute, external irradiation received by the Japanese atomic bomb survivors and (b) large-scale animal experiments, especially with mice. The genetic risk of low dose irradiation is generally estimated from high doses under the assumption of a linear model with no threshold. However, this model does not adequately consider the Chernobyl effect of internal exposure to radionuclides and, as outlined here, neglects the extreme sensitivity to ionizing radiation around conception. From an epidemiological point of view, the Chernobyl accident in April 1986 was unique, leading to a low dose radiological exposure of large parts of Europe, first due to the intake of short lived radionuclides. Exactly 9 months after the accident, there was a significant increase in the prevalence of Down syndrome (DS) in West-Berlin and Belarus. As more than 90% of DS cases are due to maternal meiotic nondisjunction, which takes place around conception, there is a highly distinct temporal relationship between exposure and the effect. This finding is paralleled by a significant increase in the sex ratio of newborn, beginning in January 1987 (Bavaria) and confined in 1987 to the exposed countries. This is explained by a loss of female embryos due to the extensive epigenetic modifications of the paternal genome, especially the paternal X, immediately after fertilization. Among the newborn with DS in West-Berlin, there was also a significant increase in the sex ratio. Since bias and relevant confounders could be excluded, a causal relationship between exposure and the increase both in the sex ratio and trisomy 21 remains the only explanation. In man, but not in mice, the maternal meiotic divisions around conception are extremely error prone and, perhaps, also the extreme epigenetic modifications of the paternal genome, explaining that even under optimal conditions more than 70% of all conceptuses are lost, mostly before clinical recognition of pregnancy. It is logical to assume that these naturally error prone processes can be easily disturbed by environmental factors, e.g. ionizing radiation. In this respect, extrapolation from mouse to human is of limited value and consequently there is an urgent need for analytical epidemiological studies with respect to other environmental hazards. Moreover, the International guidelines for radiological safety should be revised accordingly.

1440W

Outcomes of over 400 cycles of `transport' PGD for single gene disorders. *G. Harton[1], S. Jaroudi[2], M. Konstantinidis[3], J. Sanchez[1], S. Tormasi[1], R. Prates[1], N. Goodall[1], D. Wells[3].* 1) Reprogenetics, Livingston, NJ; 2) Reprogenetics UK, Oxford, UK; 3) University of Oxford, Nuffield Department of Obstetrics and Gynaecology, Women's Centre, John Radcliffe Hospital, Oxford, UK.

Introduction: Preimplantation genetic diagnosis (PGD) is offered to individuals at high risk of transmitting an inherited disorder to their offspring. PGD entails genetic testing of blastomeres biopsied from cleavage stage embryos from in vitro fertilization (IVF), and aims to ensure that embryos selected for transfer are unaffected by the familial disorder. The diagnosis should be sensitive, accurate and fast to allow for the greatest chance of pregnancy with the lowest risk of misdiagnosis. **Aim:** Evaluate the diagnostic and clinical outcomes of PGD cycles carried out for patients at high risk of transmitting a single gene disorder (e.g. cystic fibrosis). **Material & Methods:** A retrospective review of 405 cases of PGD performed between September 2008 and December 2010 was carried out. The maternal age ranged between 20 and 45 years (mean 33.28). IVF and embryo biopsy took place in more than 60 different fertility clinics, with blastomeres sent to a single specialist PGD laboratory for analysis. The majority of protocols involved a multiplex polymerase chain reaction (PCR) step to amplify the mutation site as well as linked short tandem repeat (STR) polymorphisms, followed by fragment size analysis. Further amplification of the mutation site was usually preformed by a nested PCR prior to minisequencing or other forms of DNA analysis. **Results:** PGD was performed for a total of 3376 embryos, with over 50 different disorders diagnosed. A diagnosis was obtained for 85% of cells tested. Amplification failure was observed for 10% of cells tested and allele dropout (ADO) was seen in approximately 11% of cells. The accuracy of the diagnosis was predicted to be greater than 98% in most cases. No misdiagnoses were recorded to date. The pregnancy rate (positive hCG) per embryo transfer was 59.7% and the implantation rate (number of gestational sacs/number of embryos replaced) was 48%. The pregnancy rate per transfer showed an influence of maternal age: 60% for patients under 35 years of age and 52.6% for patients over 35. **Conclusion:** Transport PGD allows patients to receive treatment in their local area, avoiding the need to travel to IVF clinics that have in-house PGD capability. This will reduce the financial burden and stress felt by patients and allows local clinics to retain cycles. The results of this large study showed good rates of diagnosis, accuracy and pregnancy, indicating that transport PGD is an effective treatment option for patients.

1441W

`No thanks'- why pregnant women choose not to have cystic fibrosis carrier screening. *L. Ioannou[1,2], J. Massie[3,4], S. Lewis[5], B. McClaren[6], V. Collins[1], M. Delatycki[1,2,4,7].* 1) Bruce Lefroy Centre , Murdoch Childrens Research Institute, Melbourne, Victoria, Australia; 2) Department of Medicine, Monash University, Melbourne, Victoria, Australia; 3) Department of Respiratory Medicine, Royal Children's Hospital, Melbourne, Victoria, Australia; 4) Department of Paediatrics, University of Melbourne, Melbourne, Victoria, Australia; 5) Public Health Genetics, Murdoch Childrens Research Institute, Melbourne, Victoria, Australia; 6) Genetics Education and Health Research, Murdoch Childrens Research Institute, Melbourne, Victoria, Australia; 7) Clinical Genetics, Austin Health, Melbourne, Victoria, Australia.

Introduction: A population-based cystic fibrosis (CF) carrier screening program was introduced in Victoria, Australia in 2006. It is offered for a fee by doctors to couples planning a pregnancy or in early pregnancy. The aim of this study was to assess the attitudes and opinions of women declining screening and compare these to those of individuals who accepted screening. Methods: Between December 2009 and May 2011, women who declined a direct offer of CF carrier screening by their obstetrician were invited to participate. The questionnaire explored knowledge, reasons for declining screening, decision making, attitude towards screening and satisfaction with information provided. Results: A total of 50 completed questionnaires were received. The majority of participants were aged 30-34 (54%), had a university qualification (78%) and earned a household income of more than $100,000AUD per annum (78%). Twenty-five participants (51%) answered 6 to 10 of the 15 knowledge questions correctly; however 29 (59%) incorrectly believed that couples who have a child with CF usually have a family history. Knowledge level was significantly lower in participants who declined screening, compared to those who accepted screening, for 9 of the 15 knowledge questions. The main reasons for declining screening were having no family history of CF and would not consider a termination of pregnancy for CF. Having a family history of CF (χ^2=54.86, p<0.01) or other genetic conditions (χ^2=53.68, p<0.01) had a significantly greater influence on the decision whether to have screening, for those who declined screening, compared to those who accepted it. Almost all of the women whom participated (96%) believe that CF carrier screening should be available to those who wish to be screened. Conclusion: There is no significant demographic difference between those who accepted screening and those who declined it. The main reason for declining screening is having no family history of CF, with those who declined screening stating that it had a greater influence on their decision compared to those who accepted screening.

1442W

Search for autosomal recessive prenatal lethal or semi-lethal mutations by genome-wide association study (GWAS) of reproductive traits. *J.X. Chong[1], G. Kosova[1], C. Ober[1,2].* 1) Human Genetics, The University of Chicago, Chicago, IL; 2) Obstetrics and Gynecology, The University of Chicago, Chicago, IL.

Spontaneous miscarriage is relatively common, with only ~25% of conceptuses surviving to term. Although most of these pregnancy losses are due to chromosomal abnormalities, some may be due to recessive lethal (or semi-lethal) mutations. Although many such mutations are known in mice, few autosomal recessive prenatal lethal mutations have been reported in humans. The Hutterites, a North American founder population, proscribe contraception and uniformly desire large families, making them particularly suitable for studies on the genetics of reproductive traits. In this study, we sought to identify recessive mutations that cause prenatal lethality in homozygotes by conducting a couple-based GWAS of family size and birth rate in ~260 Hutterite couples who had been genotyped for ~300,000 SNPs. Parental genotypes were used to calculate each couple's “risk” of having children homozygous for the minor allele at each SNP, based on Mendelian probabilities. Linear regression was performed to determine if risk at each SNP was a significant predictor of family size or birth rate. Two filters were then applied in series to all SNPs with p-values $\leq 10^{-4}$ with either phenotype. First, transmission disequilibrium tests were used to further select SNPs at which there was undertransmission of the minor allele (p<0.1) to children of heterozygous parents. Then, to look for deficiences of living homozygous offspring, genotype segregation ratios were examined among the offspring of couples in which both parents were heterozygous for the minor allele. We report a 154 kb region on chromosome 1 in which the minor alleles at multiple SNPs are associated with reduced family sizes (smallest p=8.3x10^{-5}) and under-transmitted in families of carrier parents (transmission:non-transmission ratio=282:333; p=0.04). Moreover, segregation ratios in 258 offspring of carrier couples were 85:118:55, significantly different than the expected 64.5:129:64.5 (p=0.01). This locus includes two candidate genes: *CNN3*, which regulates actin cytoskeleton remodeling that is required for trophoblast cell fusion during placenta formation; and *ALG14*, which is involved in the regulation of protein folding and stabilization and is required for growth in yeast. This is the first GWAS for prenatal lethal mutations, the results of which will add to our knowledge of genetic causes of pregnancy loss.

1443W

Determining the mutation underlying a novel 46, XY Disorder of Sexual Development (DSD) using next-generation sequencing strategies. *R.L. Hood[1,2,3], S. Douglas[3], C. Goldsmith[3], D.E. Bulman[2], K.M. Boycott[3].* 1) Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada; 2) Regenerative Medicine, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada; 3) Children's Hospital of Eastern Ontario Research Institute, University of Ottawa, Ottawa, Ontario, Canada.

The development of next-generation sequencing technologies has revolutionized modern genetics and enabled researchers to elucidate novel biological applications at unprecedented speeds. Through this technology one can experimentally determine the causative gene(s) or variants underlying a particular disease. We describe a family with a novel 46, XY disorder of sexual development (DSD) inherited in an autosomal dominant manner. Intrafamilial variability was evident with clinical presentations ranging from normal male (obligate carrier) to varying degrees of hypospadias to female external genitalia in the 7 affected male family members. The genetic cause of this disorder is currently unknown as mutations in genes known to be involved in the sexual development pathway, including SRY, NR5A1, DHH, NROB1, SOX9, WT1, and MAP3K1, were excluded via clinical testing and segregation analysis with linked markers. Association of the disorder in this family with a 1p31-p35 duplication encompassing WNT4 could not be excluded by linkage; however, normal copy number data using the Affymetrix 6.0 array made this very unlikely. Therefore, we plan on utilizing next-generation sequencing to experimentally identify the DSD gene and its causative mutation. It will then be possible to determine the biological significance this gene has in human sexual development. This study will elucidate additional molecular components and mechanisms underlying secondary sex-characteristics, thereby contributing to our overall understanding of sex differentiation and determination.

1444W

No evidence for aberrant placental DNA methylation in karyotypically normal recurrent miscarriage. *CW. Hanna[1], DE. McFadden[2], WP. Robinson[1].* 1) Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Department of Pathology, University of British Columbia, Vancouver, BC, Canada.

Recurrent miscarriage, 3 or more consecutive spontaneous abortions of pregnancy before 20 weeks gestation, occurs in 1-2% of couples trying to conceive. Chromosomal errors are observed in over 50% of miscarriages; however causes of karyotypically normal losses are less understood. Imprinted genes are expressed in a parent-of-origin specific manner and are particularly important in placental function. The mono-allelic expression of these genes is often associated with differential DNA methylation, alterations of which have been suggested to be a cause of pregnancy loss. We hypothesize that placental villi of karyotypically normal miscarriages from women with recurrent miscarriage will exhibit aberrant DNA methylation at imprinted loci and globally throughout the genome. DNA was extracted from placental villi of karyotypically normal miscarriages from women experiencing recurrent miscarriage (N=33) and chromosomally normal first trimester controls (N=16). Bisulfite pyrosequencing allowed the targeted interrogation of DNA methylation at the imprinted loci *PLAGL1*, *H19* ICR, *SGCE*, *TP73*, and *SNRPN*, as well as at LINE-1 elements, used as a surrogate measure of global methylation. Two of 33 (6.1%) recurrent miscarriage placentae showed a loss of methylation (defined as values greater than 1.5x the interquartile range) at any of the 5 imprinted loci, as did 1 of 16 (6.3%) control placentae. There was also no significant difference in average methylation at any of these imprinted genes after correcting for multiple comparisons. Genome-wide DNA methylation was also assessed in 20 samples (10 cases, 10 controls) using the Illumina Infinium HumanMethylation27 BeadChip, which interrogates 27,578 CpG sites. Significance Analysis of Microarrays was utilized to identify candidate genes, using a <1% False Discovery Rate and >10% absolute difference between groups. Fourteen candidate CpG sites were identified; however methylation at these sites was found to vary with gestational age of the pregnancy and therefore likely represents subtle differences in gestational age between the case and control groups. Finally, we found no difference in global methylation as measured by LINE-1 elements (p=0.61) or as an average of the 27,578 CpG sites (p=0.21). This study suggests that epigenetic errors in the placenta associated with imprinting or global DNA methylation do not represent a common contribution to karyotypically normal pregnancy loss in women with recurrent miscarriage.

1445W

A familial case of macrozoospermia caused by an abnormal splicing of *AURKC*. *M. Ben Khelifa[1,2], R. Zouari[3], R. Harbuz[1,2], L. Halouani[3], C. Arnoult[1], J. Lunardi[2], P.F. Ray[1,2].* 1) Laboratoire AGIM, FRE 3405 CNRS - UJF, Equipe Génétique Infertilité et Thérapeutique (GIT), campus santé de Grenoble, 38 700 La Tronche, France; 2) UM de Biochimie et Génétique , CHU Grenoble, Grenoble, France; 3) CPSR les Jasmins, 23, Av. Louis BRAILLE, 1002 Tunis, Tunisia.

The presence of close to 100% large headed multi-tailed spermatozoa in the ejaculate has been described as a rare phenotype of male infertility with a very poor prognosis. We demonstrated previously that most cases were caused by a homozygous mutation (c.144delC) in the Aurora Kinase C gene (*AURKC*) leading to the absence or the production of a non-functional protein. *AURKC* deficiency in these patients blocked meiosis and resulted in the production of tetraploid spermatozoa unsuitable for fertilization. We describe here the study of two brothers presenting with large-headed spermatozoa. Standard sperm analysis and molecular analysis of *AURKC* was carried out in two brothers presenting with a typical large headed spermatozoa phenotype. Both affected brothers were heterozygous for the c.144delC mutation. After complete sequencing of the gene, a new heterozygous variant, c.436-2A>G, was identified in both patients. This mutation is located in the acceptor consensus splice site of exon 5. AURKC transcripts were extracted from one of the patient's leukocytes and Reverse Transcription Polymerase Chain Reaction (RT-PCR) was realized showing 1) the presence of a truncated transcript indicating that c.436-2A>G leaded to the skipping of exon 5 ; 2) the absence of a normal size's band indicating that transcripts with c.144delC were subject to mRNA decay. We identified a new *AURKC* mutation in two affected brothers who were also carrying the recurrent c.144delC mutation. There was no phenotypic difference observed between the sperm of these patients compared with patients homozygous for the previously described mutation. RT-PCR analysis confirmed that the newly identified mutation altered the splicing of the mRNA, inducing the skipping of exon 5, thus confirming the pathogenicity of the mutation. These results indicate that *AURKC* molecular analysis of patients with large headed spermatozoa should not be stopped in the absence of a homozygous recurrent mutation on exon 3 but complete sequence analysis should be performed. This diagnosis is important since the identification of *AURKC* mutations in patients is strongly correlated with sperm chromosomal abnormality, precluding any attempt of intra-conjugal treatment by intra cytoplasmic sperm injection (ICSI).

1446W

Chromosomal segregation in spermatozoa of five Robertsonian Translocation carriers t(13;14). *S. Brahem, M. Mehdi, M. Mahjoub, M. Gribaa, S. Ibala, A. Saad.* Laboratoire de Cytogénétique, Biologie Moléculaire et Biologie de la Reproduction Humaines, Tunisia.

Purpose: To analyse the segregation of a Robertsonian translocation t(13;14) in five male carriers, and to verify a possible inter-chromosomal effect (ICE) of the Robertsonian translocation on chromosomes 18, X, and Y. Methods: The spermatozoa of these patients (n = 5) and of fifteen donors with normal semen parameters and 46,XY karyotype were analysed using triple colour FISH with locus specific probes for chromosomes 13, 14, and 21 and by triple colour FISH for chromosomes X, Y, and 18. Results: The frequency of balanced spermatozoa resulting from alternate segregation varied between 62.16 and 81.70% with a mean of 71.5%. The rates of unbalanced spermatozoa resulting from adjacent segregation varied between 13.4 and 25.1% with a mean of 18.26%. Triple colour FISH X-Y-18 showed a significant increase in disomy frequencies of these chromosomes in comparison with controls, indicating an ICE. Conclusion: In spite of the high number of normal/ balanced frequencies, there is still much unbalanced spermatozoa resulting from adjacent mode of segregation. This raises the question of the unbalanced chromosomal risk for the offspring of 45,XY, t(13;14) males and the importance of the genetic counselling prior to ICSI or IVF treatment for couples where the male is a Robertsonian translocation carrier.

1447W

Molecular diagnosis of male factor infertility using APEX microarrays. *E. Oitmaa[1,2], A. Tammiste[1], S. Suvi[2], M. Punab[3], M. Saare[4], M. Remm[1], A. Metspalu[1,5], A. Salumets[4,6].* 1) Institute of Molecular and Cell Biology, University of Tartu, Estonia; 2) Asper Biotech Ltd., Estonia; 3) Andrology Centre, Tartu University Hospital, Estonia; 4) Competence Centre on Reproductive Medicine and Biology, Estonia; 5) The Estonian Genome Center of University of Tartu, Estonia; 6) Department of Obstetrics and Gynecology, University of Tartu, Estonia.

Objective: Male factor infertility (MFI) issues are causative in approximately 15-20% of infertile couples. The unfavourable genetic background is thought to cause for 15-30% of male factor infertility cases. Therefore, the correct determination of genetic basis of infertility is very important for further treatment of patients. Here, we demonstrate a single-step Arrayed Primer Extension (APEX) based microarray assay for the MFI diagnosis. Design & Method: The MFI-APEX assay enables analysis of numerous genetic factors simultaneously, like AZF-microdeletions; Klinefelter syndrome (47,XXY); 270 mutations in cystic fibrosis transmembrane conductance regulator (CFTR) gene; 42 mutations/variations in genes involved in cryptorchidism, hypogonadism, spermatogenic failure and 6 polymorphisms in androgen receptor gene. MFI-APEX assay validation was performed in blinded study using 62 DNA samples. Ten DNA samples were obtained from men without infertility issues and 52 DNA samples obtained from MFI patients. Seven out of 52 DNA samples have microdeletions in the AZFc region, 2 DNA samples have microdeletions in the AZFb+c region, 14 DNA samples have 47,XXY and 29 DNA samples obtained from idiopathic infertile men. Analysis of the APEX genotyping data were performed with PicDBAutoScan 5.0 program of the Genorama Genotyping Software™ (Asper Biotech Ltd). Results: We developed MFI-APEX microarray assay for the detection of genetic factor of the male infertility. Analysis of the APEX results revealed that 1 idiopathic infertile patient was homozygous for p.Thr121ins (c.364_365ins3) in USP26 gene; AZFc microdeletion was correctly identified in 7 DNA samples; AZFb+c microdeletion was correctly identified in 2 DNA samples; 47,XXY was correctly identified in 13 DNA samples. Overall, the assay call rate was 99%, accuracy 95%, specificity 93% and sensitivity 97%. Conclusion: Our experiments demonstrated that MFI-APEX assay is suitable for the rapid single-step, robust, reliable and cost-effective detection of possible genetic cause for male infertility problem. Furthermore, MFI-APEX testing is recommended before the couple undergoes assisted reproduction in order to prevent the possible inheritance of the genetic lesion to the next generation.

1448W

***TP53* PIN3 and PEX4 polymorphisms are associated with recurrent in vitro fertilization failure and endometriosis.** *D.D. Paskulin[1,2], C.A.B. Souza[3], J.S. Cunha-Filho[3], P. Ashton-Prolla[1,2,4].* 1) Department of Genetics, UFRGS, Porto Alegre, RS, Brazil; 2) Genomic Medicine Laboratory, Experimental Research Center, Hospital de Clínicas de Porto Alegre, Brazil; 3) Department of Obstetrics and Gynecology, Hospital de Clínicas de Porto Alegre, Brazil; 4) Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Brazil.

Objective: p53 plays a crucial role in human fertility as it regulates leukemia inhibitory factor expression, a secreted cytokine that is critical for blastocyst implantation. Several polymorphisms in the *TP53* Pathway are thought to have significant effects on human fertility. We hypothesized that polymorphisms in the *TP53* Pathway that alter p53 function may be involved with recurrent *in vitro* fertilization (IVF) failure and endometriosis, and therefore, tested the association of functional polymorphisms in the *TP53* gene (PIN2, PIN3 and PEX4) as well as SNPs in *MDM2*, *MDM4*, *HAUSP* and *LIF* in women with these phenotypes. **Patients and Methods**: A total of 115 IVF patients with recurrent implantation failure (IVF Group), 98 infertile patients with endometriosis (END Group) and 130 fertile women (CON Group) were included in the study. DNA was obtained from leukocytes and ARMS-PCR or allelic discrimination using TaqMan Assays were performed for genotyping. Patients and controls were categorized according to genotype of each SNP and evaluated for baseline characteristics and pregnancy outcomes. *TP53* haplotype frequencies were calculated by direct count. **Results**: Baseline characteristics demonstrated that both IVF and END patients were younger and had reduced number of pregnancies, live births, cesarean and abortions when compared with the control group (all P<0.01). The prevalence of the *TP53* PIN3 A2 allele (P=0.042 for IVF and P=0.004 for END) and *TP53* PEX4 Pro allele (P=0.007 for IVF and P=0.009 for END) were increased in our IVF and END patients when compared with the control group. Haplotypes C-A2-Pro, C-A1-Pro were associate with high risk for IVF (P=0.038, OR= 1.6; P=0.004, OR=3.5 respectively) and END (P=0.018, OR=1.8; P=0.02, OR=4.11 respectively). No association was found between *MDM2*, *MDM4*, *HAUSP* and *LIF* SNPs and endometriosis or IVF. **Conclusion**: Our data confirms that the *TP53* PEX4 Pro allele is enriched in infertile women less than 35 years of age, and demonstrates that the Pro allele is also enriched in infertile women with endometriosis. In addition, the *TP53* PIN3 A2 allele was found, for the first time, to be associated with risk for these two clinical entities. Our results reinforce that *TP53* is crucial for human fertility and that polymorphisms that alter p53 expression patterns may be good biomarkers for infertility and endometriosis.

1449W

Genome-wide association study identifies novel candidate genes for male fertility in humans. *G. Kosova[1], N. Scott[1], C. Niederberger[2], G.S. Prins[2], C. Ober[1,3].* 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Department of Urology, University of Illinois at Chicago, Chicago, IL; 3) Department of Obstetrics and Gynecology, University of Chicago, Chicago, IL.

Despite the fact that hundreds of genes have been identified that affect fertility in animal models, relatively little is known about genes that influence natural fertility in humans. To broadly survey genetic variation affecting male fertility, we utilized a two-stage approach. First, we conducted a genome-wide association study (GWAS) for two measures of fertility, family size and birth rate, in 269 married men who are members of a founder population of European descent that proscribes contraception and desires large families. Associations between ~250,000 autosomal SNPs and the fertility traits were tested using a regression-based test, designed for large, complex families. Eighty two SNPs at 41 independent loci showed association at $P \leq 1x10^{-4}$ for either trait. At the second stage, the most significantly associated SNP at each of these 41 loci were taken forward to a validation study in 123 ethnically diverse men from Chicago who had previously undergone semen analysis. Nine SNPs that were associated with reduced fertility measures in the Hutterites were also associated with at least one of the 10 parameters of sperm quantity and/or motility. Overall, we observed 24 associations with *P*-values < 0.05, based on 5,000 permutations. The fraction of permutations yielding a higher number of significant associations in this data set was 0.0041, reflecting a significant enrichment for small *P*-values. Among the nine associated loci are outstanding candidates for male fertility genes, including *USP8*, an essential deubiquitinating enzyme that has a function in acrosome assembly, and genes with potential roles in regulation of innate immunity, such as cytokine induced *UBD* and *EPSTI1*, and the latent TGF-) receptor, *LRRC32*. We suggest that more severe mutations in some of these genes may account for some cases of unexplained infertility (or subfertility) in general population. Supported by NIH grant HD21244.

1450W

Successful application of preimplantation genetic diagnosis for aromatic L-amino acid decarboxylase deficiency by a combination of molecular approaches. *S.P. Chang[1,2], S.H. Wu[2], G.C. Ma[1,3], H.H. Wu[4,5], C.H. Wu[4,5], T.M. Chang[6], F.P. Tsai[7], M. Chen[1,2,4,8,9].* 1) Center for Medical Genetics, Changhua Christian Hosp, Changhua City, Taiwan; 2) Department of Life Sciences, National Chung-Hsing University, Taichung, Taiwan; 3) Institute of Biochemistry and Biotechnology, Chung Shan Medical University, Taichung, Taiwan; 4) Department of Obstetrics and Gynecology, Chung Shan Medical University, Taichung, Taiwan; 5) Department of Obstetrics and Gynecology, Changhua Christian Hospital, Changhua, Taiwan; 6) Department of Pediatrics, Changhua Christian Hospital, Changhua, Taiwan; 7) Poyuan Women's Clinic, IVF Center, Changhua, Taiwan; 8) Department of Life Sciences, Tunghai University, Taichung, Taiwan; 9) Department of Obstetrics and Gynecology and Department of Medical Genetics, College of Medicine and Hospital, National Taiwan University, Taipei, Taiwan.

Aromatic L-amino acid decarboxylase (AADC) deficiency is an autosomal recessive disorder, resulting in inborn error of neurotransmitter metabolism. Defects in the DDC gene, located in7p12.1p12.3, are known to be responsible for this condition. So far, less than 20 DDC mutants have been described in no more than 50 cases with clinical association worldwide. A Twainese couple experienced three neonatal deaths and two induced abortions because of AADC deficiency was referred to us and requested in vitro fertilization (IVF) treatment together with preimplantation genetic diagnosis (PGD) for an unaffected pregnancy. In a preliminary molecular evaluation, the couple was shown to carry the same heterozygous mutation in DDC, IVS6+4A>T, which has been reported as a hotspot in more than 80% of Taiwanese patients. Therefore, we designed and utilized a novel combination of amplification refractory mutation system and quantitative polymerase chain reaction (ARMS-qPCR), to screen and exclude the affected embryos from transfer. Preliminary testing showed that the ARMS-qPCR amplification rate of 60 single lymphocytes and 15 single blastomeres was 100% (60/60) and 93% (14/15), respectively. Direct sequencing of the PCR products proved that the accuracy was 100% (74/74). These results revealed that the protocol we used is feasible in both of blastomeres and lymphocytes. In PGD, five single cells obtained by blastomere biopsy in day-3 embryos were tested by means of ARMS-qPCR to detect the presence of the disease-causing mutation, and two of them were determined to be unaffected: one was homozygous wide-type and the other one was heterozygous carrier. Then, the two unaffected embryos were transferred in day 5. After 38 weeks of uneventful gestation, a healthy male baby was born successfully. Postnatal genotyping confirmed the baby to be a carrier with healthy phenotype. We concluded that ARMS-qPCR is a simplified and feasible technique for PGD. To our knowledge, this is the first successful application of PGD in AADC.

1451W

Thrombophilic Mutations and Susceptibility to Preeclampsia in a Population from Western Iran. *Z. Rahimi[1], Z. Rahimi[1], A. Vaisi-Raygani[1], A. Parsian[2].* 1) Biochemistry, Kermanshah University of Medical Sciences, Kermanshah, Kermanshah, Iran; 2) Division of Neuroscience & Behavior, NIAAA, National Institutes of Health, Rockville, Maryland, USA.

Aim. Preeclampsia is one of the most common and most serious complications of pregnancy. Inherited thrombophilias might be involved in the increased susceptibility to adverse pregnancy outcomes including preeclampsia. The aim of present study was to determine the possible association between factor V Leiden (FVL) mutation (G1691A) and prothrombin G20210A polymorphism with susceptibility to preeclampsia. Methods. We studied 198 women with preeclampsia including 128 women with mild and 70 women with severe forms and 101 age and parity matched healthy pregnant women with uncomplicated pregnancy from Western Iran. The sample was genotyped was done by polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) using MnI I and Hind III for FVL and prothrombin G20210A, respectively. Results. Among cases there were 23 women (19 with severe and 4 with mild preeclampsia) of early-onset pre-eclampsia (before 34 weeks gestation) and 175 cases of later-onset pre-eclampsia (after 34 weeks gestation). The frequency of heterozygous FVL mutation was 7.6% among all preecalmptic women (8.6% in mild and 5.7% in severe preeclamptic women) and 7.9% in healthy pregnant women (P>0.05). However, the prevalence of FV G1691A were 10.5 and 3.9% among severe preeclamptic women of early-onset and late-onset pre-eclampsia, respectively (P>0.05). The prevalence of prothrombin G20210A were 1.6, 2.9, and 3% among women with mild preeclamsia, severe pre-eclampsia and controls, respectively (P>0.05). The level of triglycerides (TG) was significantly higher among women with severe preeclampsia compared to mild preeclampsia that was not associated with the two thrombophilic mutations. Conclusions. Our results indicate that neither FVL nor prothrombin G20210A might be a risk factor for preeclampsia in our population. Key words. Thrombophilic mutations, FV Leiden, Prothrombin G20210A, Preeclampsia.

1452W

MTHFR genotype and haplotype analysis in couples with multiple miscarriages. *S. Stangler Herodez[1], B. Zagradisnik[1], A. Erjavec Skerget[1], V. Vlaisavljevic[3], N. Kokalj Vokac[1, 2].* 1) Laboratory of Medical Genetics, University Clinical Centre Maribor, Maribor, Slovenia; 2) Medical Faculty, University of Maribor, Maribor, Slovenia; 3) Department of Reproductive Medicine and Gynecologic Endocrinology, University Clinical Centre Maribor, Maribor, Slovenia.

INTRODUCTION. Multiple miscarriages may have adverse effects on the emotional and social aspects of infertile couples' life that wish to have a child. Infertility affects approximately 15% of married couples and is a worldwide reproductive health problem. Methylenetetrahydrofolate reductase (MTHFR) gene C677T and A1298C polymorphisms influence homocysteine metabolism which in turn may contribute to the unexplained embryo losses in early pregnancy and may be overrepresented in couples who have fertility problems or have experienced recurrent miscarriages. In this study we have performed the MTHFR genotype and haplotype analysis in couples with multiple miscarriages and healthy controls. METHODS. DNA was extracted from peripheral blood samples and allele specific PCR was performed for the detection of each mutation. Statistical analysis was performed with JavaStat software system. RESULTS. All possible MTHFR C677T/A1298C genotype combinations were represented in both tested groups. Combined MTHFR C677T/A1298C genotype distributions showed statistically significant differences between: couples with multiple miscarriages and controls ($p < 0.001$), and male probands with fertility problems and male controls without an infertility history ($p < 0.001$). The distributions of MTHFR C677T polymorphisms of couples with multiple miscarriages exhibited a significantly low frequency of the MTHFR 677CC genotype, compared to the control group, respectively, whereas no significant change in the couples MTHFR A1298C genotype frequency was observed. Further, only male probands with fertility problems exhibited significantly low frequency of the MTHFR 677CC genotype. Haplotype analysis of the two MTHFR polymorphisms showed a moderate difference in the distribution of the TC haplotype between couples group and controls. CONCLUSIONS. Significant difference in the prevalence of the combined MTHFR C677T/A1298C genotype distributions was observed in couples with multiple miscarriages when compared to controls without an infertility history. We found out that only male probands contributed to the association indicating that MTHFR mutation may be a gender specific factor which affects fertility of grown adults. The presence of combined MTHFR C677T/A1298C genotypes highly increased the risk of men infertility. Our presented data highlight the importance of MTHFR mutation screening in couples with multiple miscarriages, especially in male adults.

1453W

Higher clinical pregnancy rates are seen when performing 23-chromosome single nucleotide polymorphism (SNP) microarray preimplantation genetic screening (PGS) on blastocysts, versus Day-3 embryos. *A.T. Benner[1], P.R. Brezina[2], L. Du[1], M.C. Gunn[1], W.G. Kearns[3].* 1) Genetics, Center for Preimplantation Genetics, LabCorp, Rockville, MD; 2) Gynecology and Obstetrics Johns Hopkins Medical Institutions Division of Reproductive Endocrinology and Infertility, Baltimore, MD, United States; 3) Gynecology and Obstetrics, Genetics, Johns Hopkins Medical Institutions, Center for Preimplantation Genetics, LabCorp Rockville, MD, United States.

Background: We determined the clinical pregnancy rate using SNP PGS in women with recurrent pregnancy loss (RPL) undergoing in vitro fertilization (IVF). **Materials and Methods:** A retrospective review was conducted of all embryos that underwent PGS by 23 chromosome SNP microarrays from 1/1/2010 to 1/25/2011. Patients underwent standard in vitro fertilization (IVF) and preimplantation genetic screening (PGS) due to primarily a history of > 2 spontaneous miscarriages. Embryo biopsy was performed at either the cleavage or blastocyst stage. Sample DNA was amplified and analyzed using HumanCytoSNP-12 DNA beadchips and GenomeStudio and KaryoStudio software. Parental DNA was analyzed and compared to embryonic cells to determine the presence of benign copy number variations. Embryos derived from parents with known translocations or inversions were excluded from the study. Binomial confidence intervals for proportions were calculated. **Results:** 2,976 embryos from 317 clinical IVF cycles were tested. 2,704 (90.8 percent) of these embryos, derived from 249 (78.5 percent) IVF cycles, were at Day-3 of development (D3 group) when biopsy was performed. Conversely, 272 (9.2 percent) of embryos, derived from 68 (21.5 percent) IVF cycles, were at the blastocyst stage (D5 group) when biopsy was performed. On Day-3, per IVF cycle, an average of 8 total embryos were obtained of which an average of 3.1 were euploid and available for uterine transfer. At the blastocyst stage, per IVF cycle, an average of 4 total embryos were obtained of which an average of 1.9 were euploid. Of all embryos, 59 percent were aneuploid on Day-3 biopsy versus 41 percent aneuploidy in blastocyst biopsies. The clinical pregnancy rates were significantly higher (86 percent, $p<.05$) in the D5 group compared to the D3 group (65 percent). The miscarriage rate in the D3 and D5 groups were 6 percent and 9 percent respectively (not statistically different). However, both the D3 and D5 groups had miscarriage rates that were significantly lower than the background pregnancy loss rates seen in non PGS pregnancies from women undergoing IVF with a history of documented RPL (internal data). **Conclusion:** PGS using 23-chromosome SNP microarrays is practical in improving clinical pregnancy rates in couples with RPL. In addition, PGS performed on blastocysts, versus Day-3 embryos, results in significantly higher clinical pregnancy rates in similar patient populations.

1454W

23-chromosome single nucleotide polymorphism microarray (SNP) in embryos generated from parents with known chromosomal translocations also identifies a high rate of aneuploidy. *L. Du[1], P.R. Brezina[2], A.T. Benner[1], M.C. Gunn[1], W.G. Kearns[3].* 1) Genetics, Center for Preimplantation Genetics, LabCorp, Rockville, MD, U.S.A; 2) Gynecology and Obstetrics Johns Hopkins Medical Institutions Division of Reproductive Endocrinology and Infertility, Baltimore, MD, U.S.A; 3) Gynecology and Obstetrics, Genetics, Johns Hopkins Medical Institutions, Center for Preimplantation Genetics, LabCorp Rockville, MD, U.S.A.

Background: We determined the rate of aneuploidy in embryos from couples with balanced translocations or pericentric inversions undergoing in vitro fertilization (IVF) with preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS). **Materials and Methods:** A retrospective review was conducted of all embryos derived from parents with balanced reciprocal or Robertsonian translocations or pericentric inversions that were evaluated for PGD and PGS from 1/1/2010 to 1/25/2011. Patients underwent IVF and PGD/PGS. Embryo biopsy was performed at either the cleavage or blastocyst stage. Sample DNA was amplified and analyzed using HumanCytoSNP-12 DNA beadchips and GenomeStudio and KaryoStudio software. Parental DNA was analyzed and compared to embryonic cells to determine the presence of benign copy number variations. Binomial confidence intervals for proportions were calculated. **Results:** A total of 64 IVF cycles were evaluated of which the translocation or inversion was present in either the mother (43 percent) or the father (57 percent). 80 percent were reciprocal tranlocations, 18 percent were Robertsonian translocations, and 2 percent were pericentric inversions. These cycles generated 466 total embryos. Surprisingly, only 80 embryos (17 percent) were diagnosed with molecular karyotypes showing unbalanced translocations that were a result of segregation of known parental translocations. Of all the embryos evaluated, 174 (37 percent) were chromosomally normal for the translocation chromosomes and aneuploidy, while 149 (32 percent) were genetically balanced for the translocation chromosomes but possessed other aneuploidies. Sixty three (14 percent) of the samples evaluated were from highly fragmented embryos and resulted in failed amplification and no diagnosis. The average clinical pregnancy rate per IVF cycle was 58 percent and did not statistically differ regardless of if the parental translocation was of maternal or paternal origin, or if the translocation was of Robertsonian or reciprocal origin. **Conclusions:** Diagnosing viable embryos through 23 chromosome PGD/PGS is a promising strategy to maximize the pregnancy potential of patients with known genetic translocations or inversions. Aneuploidy on chromosomes unrelated to parental translocations or inversions was observed in 32 percent of embryos. Therefore, such parental chromosomal aberrations are likely an independent risk factor for aneuploidy.

1455W

In developing human embryos, chromosomal duplications (⁄ 200 kilobases (kb)) are more common than deletions ⁄ 200 kb as identified by 23 chromosome single nucleotide polymorphism (SNP) microarrays. *M.C. Gunn[1], P.R. Brezina[2], A.T. Benner[1], L. Du[1], W.G. Kearns[3].* 1) Genetics, Center for Preimplantation Genetics, LabCorp, Rockville, MD; 2) Gynecology and Obstetrics Johns Hopkins Medical Institutions Division of Reproductive Endocrinology and Infertility Baltimore, MD, United States; 3) Gynecology and Obstetrics, Genetics, Johns Hopkins Medical Institutions, Center for Preimplantation Genetics, LabCorp Rockville, MD, United States.

Background: We determined if, in the developing human embryo, the frequency of clinically significant chromosomal deletions (DEL) and duplications (DUP) ⁄ 200 Kilobases are similar as determined by SNP microarrays. **Materials and Methods:** A retrospective review was conducted of all embryos that underwent PGS by 23 chromosome SNP microarrays from 1/1/2010 to 1/25/2011. Patients underwent standard in vitro fertilization (IVF) and preimplantation genetic screening (PGS) due to primarily a history of > 2 spontaneous miscarriages. Embryo biopsy was performed at either the cleavage or blastocyst stage. Sample DNA was amplified and analyzed using HumanCytoSNP-12 DNA beadchips and GenomeStudio and KaryoStudio software. Parental DNA was analyzed and compared to embryonic cells to determine the presence of benign copy number variations. DELs and DUPs ⁄ 200 Kilobases were considered clinically significant. Embryos derived from parents with known translocations or inversions were excluded from the study. Molecular karyotypic interpretations were performed with the reader, the laboratory director, blinded to the identity of each sample. Binomial confidence intervals for proportions were calculated. **Results:** 1,902 embryos from 236 clinical IVF cycles were tested. 37.2 percent (707/1902) of all embryos were euploid without DUP or DEL. There was a high rate of morphologic fragmentation on some embryos which led to 9.8 percent (186/1902) embryos with no molecular diagnosis obtained. Of the remaining 1009 abnormal embryos, 5.7 percent (58/1009) had DUP or DEL with no aneuploidy and 27.4 percent (277/1009) had DUP or DEL coupled with aneuploidy. 66.8 percent (674/1009) had aneuploidy without DUP or DEL. In total, there were 470 DUP, 175 involving the p arm and 295 involving the q arm. In contrast, there were only 95 DEL, 35 involving the p arm and 60 involving the q arm. DUP and q arm abnormalities were significantly more commonly observed compared to DEL or p arm abnormalities ($p<.05$). **Conclusions:** DUP seem to be much more common than DEL in the developing embryo. Additionally, these DUP and DEL are more commonly found in the q rather than the p arm. All embryos survived to cleavage stage for PGS biopsy. Therefore, duplications or structural aberrations in the q arm might be more compatible with early embryo development than deletions or errors in the p arm.

1456W

Aneuploidy in human embryos is associated with clinically significant deletions and duplications identified by 23 chromosome single nucleotide polymorphism (SNP) microarrays. *W.G. Kearns[1], P.R. Brezina[2], L. Du[3], M.C. Gunn[3], A.T. Benner[3].* 1) Gynecology and Obstetrics, Genetics, Johns Hopkins Medical Institutions, Center for Preimplantation Genetics, LabCorp Rockville, MD; 2) Gynecology and Obstetrics Johns Hopkins Medical Institutions Division of Reproductive Endocrinology and Infertility, Baltimore, MD, United States; 3) Genetics Center for Preimplantation Genetics, LabCorp Rockville, MD, United States.

Background: We determined if clinically significant chromosomal deletions (DEL) and duplications (DUP)／ 200 kilobases in size are associated with aneuploidy. **Materials and Methods:** A retrospective review was conducted of all embryos that underwent PGS by 23 chromosome SNP microarrays from 1/1/2010 to 1/25/2011. Patients underwent standard in vitro fertilization (IVF) and preimplantation genetic screening (PGS) due to primarily a history of > 2 spontaneous miscarriages. Embryo biopsy was performed at either the cleavage or blastocyst stage. Sample DNA was amplified and analyzed using HumanCytoSNP-12 DNA beadchips and GenomeStudio and KaryoStudio software. Parental DNA was analyzed and compared to embryonic cells to determine the presence of benign copy number variations. DELs and DUPs／ 200 Kilobases were considered clinically significant. Embryos derived from parents with known translocations or inversions were excluded from the study. Positive predictive value and binomial confidence intervals for proportions were calculated. **Results:** 1,902 embryos from 236 clinical IVF cycles were tested. 37.2 percent (707/1902) of all embryos were euploid without DUP or DEL. There was a high rate of morphologic fragmentation on some embryos which led to 9.8 percent (186/1902) embryos with no molecular diagnosis obtained. Of the remaining 1009 abnormal embryos, 5.7 percent (58/1009) had DUP or DEL with no aneuploidy and 27.4 percent (277/1009) had DUP or DEL coupled with aneuploidy. 66.8 percent (674/1009) had aneuploidy without DUP or DEL. The presence of DEL or DUP was predictive of the presence of aneuploidy with a positive predictive value of 83 percent [95 percent CI = 76 percent -86 percent]. In only 3 embryos was a DUP or DEL found in the same chromosome as aneuploidy. The distribution of DEL and DUP was not normally distributed among all chromosomes, with 20.7 percent of all DEL and DUP occurring on either chromosomes 9 or 19 and only 1.9 percent of all DEL and DUP occurring on either chromosomes 15 or 21. **Conclusions:** The presence of DEL and DUP in developing embryos seems to be predictive of the presence of aneuploidy. These structural aberrations may disrupt normal cell cycle checkpoint mechanisms. This finding may further the understanding of developmental biology.

1457W

Preimplantation genetic diagnosis for recurrent pregnancy loss using array CGH. *S. Munne, r. prates, s. tormasi, n. goodal, g. harton, p. colls.* Reprogenetics, Livington, NJ.

Objective: To use single cell analysis by array CGH and screen the in-vitro fertilization generated embryos of patients suffering from recurrent pregnancy loss (RPL). The pregnancy results of this preimplantation genetic diagnosis (PGD) approach will be compared to the expected loss rate in the same patients. The error rate of the technique will be determined by reanalysis of the remainder cells of cleavage stage embryos determined by PGD to be abnormal. Material and Methods: 161 in vitro fertilization and PGD procedures of couples with idiopathic RPL (3 or more miscarriages) were included in the study. PGD was done using single cell biopsy at embryo cleavage stage followed by whole DNA amplification, and analysis by array CGH using a Bluegnome array. Each PGD patient was matched with their expected loss rate for RPL patients (Brigham et al. 1999). An ongoing pregnancy was defined as past second trimester. Some embryos classified as abnormal were later fixed for FISH analysis using probes for 12 chromosomes (X, Y, 8, 13, 14, 15, 16, 18, 19, 20, 21, 22) plus any other found abnormal in the PGD analysis. In addition, a small fraction of embryos had two cells analyzed by aCGH and that information was also used for validation purposes to determine the error rate of the technique. Results: In total 1520 embryos were analyzed, of which 33% were normal, 63% were abnormal, 3% had no nucleus in the cell biopsied or the DNA did not amplify, and 1% of cells had a chaotic profile that could not be analyzed. Of the abnormal embryos, FISH reanalysis of 108 embryos showed a discordance (error) rate with PGD results of 2.6%, and 102 embryos had 2 cells analyzed of which in 2.9% embryos one cell was normal and the other abnormal and thus considered discordant (errors). Of the 161 IVF procedures with PGD, pregnancy data was available for 138 procedures, of which 58 (42%) became pregnant and 53 cycles (38%) are ongoing past second trimester or delivered. We would expect a 34.3% miscarriage rate in this specific group of patients, but the miscarriage rate found was only 8.6% (5/58) ($p<0.001$). Conclusions: Current PGD results with single cell analysis of embryos indicate a very low discordance rate after reanalysis of the same embryos. In addition, PGD achieved a significant decrease in the miscarriage rate for idiopathic RPL patients. These results confirm that RPL is mostly caused by chromosome abnormalities in the embryos produced by these patients.

1458W

In women with a history of recurrent pregnancy loss (RPL) under 40 years of age, the rate of aneuploidy seen in embryos is relatively constant with advancing maternal age. *K. Richter[3], P. R. Brezina[1], A. T. Benner[2], L. Du[2], M.C. Gunn[2], B. Boyd[2], W.G. Kearns[4].* 1) Gynecology and Obstetrics, Division of Reproductive Endocrinology and Infertility, Johns Hopkins Medical Institutions , Baltimore, MD. U.S.A; 2) Genetics, Center for Preimplantation Genetics, LabCorp. Rockville, MD, U.S.A; 3) Research, Shady Grove Fertility Reproductive Science Center, Rockville, MD, U.S.A; 4) Gynecology and Obstetrics, Genetics, Johns Hopkins Medical Institutions, Center for Preimplantation Genetics, LabCorp Rockville, MD. U.S.A.

Background: We determined if patients undergoing in vitro fertilization (IVF) and preimplantation genetic screening (PGS) for RPL experienced increased rates of aneuploidy with increasing maternal age. **Materials and Methods:** A retrospective review was conducted of all embryos that underwent PGS by 23 chromosome SNP microarrays from 1/1/2010 to 1/25/2011. Patients underwent standard in vitro fertilization (IVF) and preimplantation genetic screening (PGS) due to primarily a history of > 2 spontaneous miscarriages. Embryo biopsy was performed at either the cleavage or blastocyst stage. Sample DNA was amplified and analyzed using HumanCytoSNP-12 DNA beadchips and GenomeStudio and KaryoStudio software. Parental DNA was analyzed and compared to embryonic cells to determine the presence of benign copy number variations. Embryos derived from parents with known translocations or inversions were excluded from the study. Data was split into four patient groups based on maternal age (<35, 35-37, 38-40, and >40). Binomial confidence intervals for proportions were calculated. **Results:** 1,902 embryos from 236 clinical IVF cycles were tested. Of embryos from women <35 years (N=594), 287 (48%) were aneuploid. Of embryos from women 35-37 years (N=268), 130 (49%) were aneuploid. Of embryos from women 38-40 years (N=626), 299 (48%) were aneuploid. Of embryos from women >40 years (N=414), 235 (57%) were aneuploid. No differences were observed among the rates of aneuploidy in the <35, 35-37, and 38-40 groups and these groups together had an average rate of aneuploidy of 48.1% [95% CI: 45.6%-50.7%]. The >40 group had an average rate of aneuploidy of 57% [95% CI: 52.0%-61.5%], significantly ($p=0.002$) higher than the pooled results of the <35, 35-37, and 38-40 groups. There was a high rate of morphologic fragmentation on some embryos which led to an approximate 9% of embryos with no molecular diagnosis obtained and this did not statistically differ between groups. **Conclusions:** Pregnancy loss rates increase steadily with advancing maternal age in the general population. Our data suggests that the underlying mechanisms that cause recurrent pregnancy loss may overshadow the gradual increasing rate of aneuploidy that accompanies increasing maternal age up to 40 years. After the maternal age of 40, age related aneuploidy begins to represent an additional pregnancy challenge for couples suffering from RPL.

1459W

Single nucleotide polymorphisms in angiogenesis regulating genes and the risk of preeclampsia and small for gestational age infants: evidence from parent-infant trios. *P.H. Andraweera[1,2], G.A. Dekker[1,3], S.D. Thompson[1], L.M.E. McCowan[4], R.A. North[5], C.T. Roberts[1] on behalf of the SCOPE consortium.* 1) Discipline of Obstetrics and Gynaecology, Research Centre for Reproductive Health, University of Adelaide, Adelaide, South Australia, Australia; 2) Human Genetics Unit, Faculty of Medicine, University of Colombo, Sri-Lanka; 3) Women's and Children's Division, Lyell McEwin Hospital, Elizabeth Vale, South Australia; 4) Department of Obstetrics and Gynaecology, University of Auckland, New Zealand; 5) Division of Women's Health, King's College London, London, UK.

Introduction: Altered endothelial integrity is demonstrated in pregnancies complicated by preeclampsia and small for gestational age infants (SGA). Both complications increase the risk of later life coronary artery disease and stroke. Angiopoietin-1(AGP1) is a pro-angiogenic growth factor and thrombospondin-1(TSP1) is an anti-angiogenic molecule. Two functional single nucleotide polymorphisms (AGP1 A1414T and TSP1 A2210G) are known to alter plasma AGP1 and TSP1 levels and also associate with stroke and coronary artery disease, respectively. We aimed to investigate these SNPs in preeclampsia and in SGA, in parent-infant trios. Method: We recruited 3234 nulliparous pregnant women, their partners and babies to a prospective multicenter cohort study in Adelaide and Auckland. Preeclampsia was defined as blood pressure／ 140/90 mmHg after 20 weeks gestation with proteinuria or any multisystem complication. SGA was defined as birthweight <10th customised centile adjusted for maternal height, weight, parity, ethnicity, gestational age at delivery and infant sex. Uterine and umbilical artery Doppler was performed at 20 weeks gestation. Average uterine artery resistance index >90th centile and the presence of bilateral notching of the uterine arteries were considered abnormal. DNA extracted from peripheral blood, buccal swabs, saliva or cord blood was genotyped using Sequenom MassARRAY. Logistic regression was used to compare the odds of preeclampsia and SGA between the genotype groups adjusting for potential confounders. Results: Amongst 2123 Caucasian pregnancies, 1185 (55.8%) were uncomplicated, 123 (5.8%) developed preeclampsia and 216 (10.2%) had SGA infants. Maternal AGP1 A1414T was associated with preeclampsia (aOR 3.2, 95% CI 1.5-6.4), increased mean uterine artery resistance index (p=0.029) and the presence of bilateral notching of the uterine arteries (p=0.017). Paternal (aOR 1.4, 95% CI 1.0-2.0) and neonatal TSP1 A2210G was associated with SGA (aOR 1.5, 95% CI 1.0-2.2). Maternal TSP1 A2210G approached significance for an association with SGA (p= 0.06, OR 1.3, 95% CI 0.9-1.9) and was associated with reduced maternal birthweight adjusted for gestational age at delivery (p=0.032). Conclusion: The SNPs previously shown as contributing to the risk of coronary artery disease and stroke are associated with preeclampsia and SGA infants. Inherited susceptibility to impaired endothelial integrity may underlie vascular disorders across the life course.

1460W

DNA Methylation Profile of Trisomy 22 Placentas. *J.D. Blair[1], D. Diego-Alvarez[1], M.S. Peñaherrara[1], D.E. McFadden[2], W.P. Robinson[1].* 1) Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada; 2) Department of Pathology, University of British Columbia, Vancouver, British Columbia.

Trisomy 22 (T22) is among the most common aneuploidies in clinically recognized pregnancies. All complete T22 end in miscarriage. The presence of an extra chromosome may affect gene expression directly through a dosage effect or indirectly through alterations in the epigenome such as DNA methylation. The placenta is the interface between the mother and fetus, thus changes in placental gene expression could have serious adverse effects upon the pregnancy. This study's intention is to investigate differences in DNA methylation in the placenta caused by the presence of T22. Genome-wide DNA methylation was assessed using the Illumina Infinium HumanMethylation27 BeadChip array, which analyzes 27, 578 CpG sites genome-wide. The samples tested were: first trimester T22 chorionic villi samples (n=5) and gestational age-matched chromosomally normal villi (n=7) as controls. Data analysis by Significance Analysis of Microarrays identified 48 differentially methylated candidate CpG sites, with a false discovery rate (FDR) of <10% and a Delta-) (absolute difference in average methylation) of >15%. Four of the top candidates (greatest Delta-) and 0% FDR) were chosen for follow up (SLC25A22, OR7A5, SLC6A11, CYP1A2). Pyrosequencing was used to validate the chip results in these genes using a larger sample size, (T22, n=6; FTC, n=24) while also surveying adjacent CpGs. Of the four sites examined, three, SLC25A22 (+12.9% greater average methylation in T22, p<0.001), SLC6A11 (-13.1%, p<0.001), and CYP1A2 (-28.1%, p<0.001) were all determined to have significant methylation differences between T22 samples and FTC samples. OR7A5 showed no significant methylation differences (p=0.52). The biological significance of these genes in miscarriage vary: The two SLC genes produce membrane-based ion transporters that are most prominent in the brain, while CYP1A2 is an estrogen metabolizer of particular interest to this project due to the complex hormonal regulation required for maintaining pregnancies. This study is important to gain insight into the specific epigenetic changes of T22 pregnancies, and more important, to develop T22 as a model to gain further insight into the genetic causes underlying a variety of pregnancy complications, including pregnancy loss.

1461W

Genomewide genetic analysis of spermatogenic failure. *D. Conrad[1], K.I. Aston[2], C. Ober[3], P.N. Schlegel[4], D.T. Carrell[2].* 1) Department of Genetics, Washington University School of Medicine, St Louis, MO, 63110, USA; 2) Andrology and IVF Laboratories, University of Utah School of Medicine, 675 Arapeen Drive, Ste 205, Salt Lake City, Utah, 84117, USA; 3) Department of Human Genetics, The University of Chicago, 920 E. 58th St. CLSC 425 Chicago, IL 60637, USA; 4) Department of Urology, Weill Cornell Medical College, New York Presbyterian/Weill Cornell Hospital, Starr 900, 525 East 68th Street, New York, NY 10065, USA.

The identification of multiple Y-linked loci responsible for spermatogenic failure is a canonical result of human genetics. Beyond these so-called Azoospermia Factor loci (AZF1 and AZF2) few convincing studies have shown a reproducible genetic basis for the disease elsewhere in the genome, despite the fact that spermatogenic failure has been observed in over 100 mouse knockout lines involving autosomal sequence. Here, we have used whole-genome SNP arrays to screen for evidence of dominant and recessive genetic factors influencing spermatogenic success in carefully selected individuals diagnosed with idiopathic non-obstructive azoospermia (n = 47) and severe oligozoospermia (n = 50), as well as normospermic individuals (n = 65) . We find reproducible signs of an increase in the amount of aneuploid sequence due to large (>100kb), rare CNVs on the X chromosome (204 total kb / sample vs 89 total kb / sample, p = 0.03) and autosomes (deletions only; 117 total kb / sample vs 56 total kb / sample, p = 0.02) in men with spermatogenic failure compared to normospermic individuals. A second signature, an enrichment of large chromosome segments apparently homozygous-by-descent (HBD), was identified in cases, supporting the existence of a recessive component to fertility, with as many as 5%-15% of cases showing patterns of HBD consistent with recent consanguinity. These genetic signatures of CNV burden and homozygosity are qualitatively similar between azoospermic and oligozoospermic samples, suggesting that it is useful to consider the two phenotypes as two regions along a continuous quantitative trait distribution, which has a polygenic basis waiting to be documented and integrated into clinical practice.

1462W

Self-Reported Reproductive Pathology in Women with Tuberous Sclerosis Complex. *E. Gabitzsch[1], M. Raia[1], M.K. Koenig[2], V.H. Whittemore[3], H. Northrup[1], S. Nader-Eftekhari[4], M.J. Gambello[1].* 1) Dept of Pediatrics, Division of Medical Genetics, University of Texas Health Science Center, Houston, TX; 2) Dept of Pediatrics, Division of Child and Adolescent Neurology, University of Texas Health Science Center, Houston, TX; 3) The Tuberous Sclerosis Alliance, Silver Spring, MD; 4) Dept of Obstetrics and Gynecology, Division of Reproductive Endocrinology, University of Texas Health Science Center, Houston, TX.

Tuberous Sclerosis Complex (TSC) is an autosomal dominant tumor suppressor disorder characterized by hamartomas in various organ systems. Inactivating mutations in either the TSC1 or the TSC2 gene cause most cases of TSC. Recently, the use of ovarian specific conditional knock-out mouse models has demonstrated a crucial role of the TSC genes in ovarian function. Mice with complete deletion of Tsc1 or Tsc2 showed accelerated ovarian follicle activation and subsequent premature follicular depletion, consistent with the human condition premature ovarian failure (POF). POF is defined in women as the cessation of menses before the age of 40 and elevated levels of follicle stimulating hormone (FSH). The prevalence of POF is estimated to be 1%, affecting a substantial number of women in the general population. Nonetheless, the etiology of most cases of POF remains unknown. Based on the mouse model results, we hypothesized that the human TSC1 and TSC2 genes are likely to be crucial for ovarian development and function. Moreover, since women with TSC already have one inactivated TSC gene, we further hypothesized that they may show a higher prevalence of POF or other reproductive pathology. To test this hypothesis, we surveyed 1000 women with TSC belonging to the Tuberous Sclerosis Alliance, a national support organization. We analyzed 182 questionnaires for information on menstrual and reproductive function, as well as TSC. This self-reported data revealed 8 women (4.4%) with possible POF, as determined by menstrual history report and additional supportive data in some of the respondents. This prevalence is much higher than 1% in the general population. Data from all women suggested other reproductive pathology associated with TSC such as a high rate of miscarriage (41.2%) and menstrual irregularity of any kind (31.2%). These results establish a previously unappreciated effect of TSC on women's reproductive health. Moreover, these data suggest that perturbations in the cellular pathways regulated by the TSC genes may play an important role in reproductive function.

1463W

Polymorphisms in LEP, LEPR, and PPAR. are associated with age at menarche and menopause in Korean women. *KZ. Kim[1], A. Shin[1], YS. Lee[2], SY. Kim[2], YJ. Kim[3], ES. Lee[4].* 1) Cancer Epidemiology Branch, National Cancer Center, Goyang-si, Korea; 2) Functional Genomics Branch, National Cancer Center, Goyang-si, Korea; 3) Cancer Early Detection Branch, National Cancer Center, Goyang-si, Korea; 4) Department of Breast and Endocrine Surgery, College of Medicine, Korea University, Seoul, Korea.

Purpose In this study, we explored the genetic effects of adiposity-related genes on timing of menarche and menopause, and total menstruation duration in lifespan among Korean women. Methods Ten single nucleotide polymorphisms (SNPs) in LEP, LEPR, and PPAR. genes were investigated to evaluate the genetic effects on menstruation among 400 breast cancer patients and 452 cancer-free healthy participants, from National Cancer Center in Korea. Associations between SNPs and age at menarche, age at menopause, and total menstruation duration were evaluated. Results Four SNPs (rs2167270 of LEP, rs7602 of LEPR, rs4684846, and rs3856806 of PPAR.) were associated with late menarche (⁄ 17 years old). Four SNPs (rs2167270, rs1801282 and rs2120825 of PPAR. , and rs3856806) were associated with early menopause (<40 years old) among postmenopausal women. In logistic regression model with covariate adjustment, subjects with GG genotype of rs7602 (LEPR) showed higher risk for late menarche (OR=1.91, 95% CI=1.07-3.40) compared to their counterpart with GA or AA genotypes. Although rs2167270 (LEP) was not associated with late menarche or early menopause, it was associated with total menstruation duration (<30 years; OR=0.56, 95% CI=0.31-0.99 for GG genotype) compared to GA or AA genotypes). Conclusion Our results propose that adiposity-related genes, LEP, LEPR, and PPAR. , might have a role in onset and cessation of menstruation, and total menstruation duration.

1464W

Risk assessment of ART and its related factors in the development of Prader-Willi syndrome. *K. Matsubara[1,2], N. Murakami[2], S. Sakazume[2], Y. Oto[2], T. Nagai[2], T. Ogata[1,3].* 1) Department of Molecular Endocrinology, National Research Institute for Child Health and Development, Tokyo, Japan; 2) Department of Pediatrics, Dokkyo Medical University Koshigaya Hospital, Koshigaya, Japan; 3) Department of Pediatrics, Hamamatsu University School of Medicine, Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Japan.

To examine whether assisted reproductive technology (ART) and its related factors can be a risk factor for the development of Prader-Willi syndrome (PWS), we studied 74 Japanese PWS patients who were born during the years 1997-2008 when statistical data on ART are available in Japan. Of the 74 patients, seven were born after ART (six after ICSI and one after IVF). Molecular studies revealed underlying genetic causes in all patients, including the seven patients born after ART of whom two had deletion of the paternally derived 15q imprinted region and five had trisomy rescue type upd(15)mat (TR-upd(15)mat). The maternal ages at birth were increased in PWS patients conceived after ART (36-45 years), especially in those with TR-upd(15)mat (38-45 years), consistent with the notion that the advanced maternal age is a risk factor for the development of TR-upd(15)mat. The frequency of births after ART was significantly higher in PWS patients than in the general population ($P=2.2\times10^{-9}$), even after adjusting maternal ages (⁄ 35 years) ($P=5.4\times10^{-6}$). Among PWS patients, however, although the relative frequency of TR-upd(15)mat was significantly higher in patients conceived after ART than in those conceived naturally ($P=0.008$), it became non-significant after adjusting maternal ages (⁄ 35 years) between the two groups (P=0.22). The results imply that ART and/or ART-related parental and environmental factors can be a risk factor for PWS in general. In addition, the relative predominance of TR-upd(15)mat in patients born after ART is primarily ascribed to an ART-related maternal age factor rather than ART itself.

1465W

Apoptosis is not the mechanism for embryo self correction of aneuploidy at the blastocyst stage. *B.R. McCallie[1], R. Loper[2], H. Buttermore[2], W.B. Schoolcraft[1,2], M.G. Katz-Jaffe[1,2].* 1) National Foundation for Fertility Research, Lone Tree, CO; 2) Fertility Laboratories of Colorado, Lone Tree CO.

Meiotic and mitotic errors are both responsible for chromosomal abnormalities observed during the preimplantation stages of embryo development. Studies comparing the chromosome constitution of early human embryos across developmental days have indicated the potential for “self-correction”, especially in the cases of trisomic embryos during development to the blastocyst stage. On average 50% of cleavage stage aneuploid embryos on day 3 are re-evaluated as euploid blastocysts on day 5 of embryonic development. One proposed mechanism of “self correction” is the extrusion of the trisomic chromosome, which would be expected to result in uniparental disomy (UPD). However, studies have shown that 0% of “corrected” chromosomes displayed UPD. An alternative mechanism for “self correction” is apoptosis of aneuploid cells within the embryo, as it is already known that apoptosis is an active pathway in human blastocysts. The aim of this study was to evaluate the function of apoptosis in association with chromosome aneuploidy of the day 5 human blastocyst. Surplus cryopreserved human blastocysts were donated with consent and IRB approval by infertile couples for research. Individual blastocysts underwent a trophectoderm biopsy after thawing to remove 4-6 cells for comprehensive chromosome screening of all 23 pairs of chromosomes using quantitative real-time PCR. Total RNA was isolated from individual euploid (n=8) and aneuploid (n=8) blastocysts using the Arcturus PicoPure RNA isolation kit (Applied Biosystems) followed by reverse transcription prior to pre-amplification with Taqman assays (Applied Biosystems). Quantitative real-time PCR was performed in triplicate for pro-apoptotic genes (BAX, BAD, BAK and BID), as well as for anti-apoptotic genes (BCL-2, BCL-W, BCL-XL and MCL-1), relative to two constant internal housekeeping genes; PPIA and ACTB. Results showed that all pro-apoptotic and anti-apoptotic genes were expressed in each of these 16 human blastocysts. Using GeneSpring GX11 software (Agilent Technologies) for statistical analysis, no significant differences in gene expression were observed for any of the pro-apoptotic or anti-apoptotic genes between euploid and aneuploid blastocysts. In conclusion, apoptosis is an active pathway at the blastocyst stage in human embryos. However, this pathway does not appear to be responsible for degradation of aneuploid cells on day 5 of embryonic development or embryo “self correction”.

1466W

PARP-3 polymorphism of an ADP-ribosyltransferase 3 (ART3) is associated with reduced sperm count in Czech dysfertile men. *P. Norambuena, P. Krenkova, A. Stambergova, M. Macek Jr., M. Macek Sr.* Dept. Biology and Medical Genetics , Charles Univ Prague- UH Motol, Prague, Czech Republic.

Poly(ADP-ribose) polymerase 3 (PARP-3) is a newly characterized PARP interacting with DNA damage response proteins, suggesting its contribution to the DNA damage response and epigenetic modification leading to gene silencing (1). The ART3 protein is expressed in testes in particular in spermatocytes, indicating that it exerts specific function only required at a particular stage of spermatogenesis (2). PARP-2 gene polymorphism T>A (SNP1-SNP4) is significantly associated with azoospermia secondary to meiotic arrest in Japanese men (3). The aim of our study was ascertainment of association of the G>A SNP rs6836703 polymorphism with impairment of spermatogenesis in Czech males. We genotyped DNA samples of 257 fertile men with proven paternity and 98 dysfertile men with the azoospermia, 29 with severe oligospermia (sperm count <5x106/ml) and with oligospermia (5-15.106/ml). The G>A polymorphism (rs6836703) was examined by high resolution melting of small amplicons. PCR was performed using the LightCycler480 Real-time PCR system. Melting profiles “G/G”, “G/A” and “G/A” were determined. Association studies were analyzed by odds ratio and chi-square test, where p-values >95 were considered significant. The “G”allele frequency in fertile males was 442/514 (86%), while for allele “A” 72/514 (14%). Azoospermic males did not differ from controls (31/36 - 86% of “G” alleles, 14% for “A” alleles in 5/36). In males with severe oligospermia only a tendency towards higher frequency of the “A” allele was observed (p=0.08; 22.4% versus 14% in controls). Highly significant increased frequency of allele “A” was revealed in oligospermia (p=0.004; 25.5% versus 14% in controls). Our original results suggest that PARP-3 gene polymorphism is associated with milder impairment of spermatogenesis in contrast to PARP-2 polymorphism associated with azoospermia (3). Our finding might also reflect the different sensitivity to epigenetic influences, due to the presumed PARP-3 impact on respective epigenetic modifications (1). In conclusion, the increased frequency of the “A” and of the “AA” genotype of the rs6836703 G>A PARP-3 polymorphism is associated with milder impairment of spermiogenesis in Czech dysfertile men. (1) Rouleau M et al. J Histochem Cytochem 2009; 57(7):675-685; 2) Friedrich M et al. Asian J Androl 2006; 8(3):281-287; (3) Sakugawa N et al. J Assist Reprod Genet 2009; 26(9-10):545-552. Supported by MZ0FZM2005, CZ.2.16/3.1.00/24022OPPK.

1467W

Differential expression of NELF splice variants in human and mouse immortalized GnRH neurons. *S.D. Quaynor[1,2], L.Y. Goldberg[1,2], H.G. Kim[1,2,3], L.P. Chorich[1,2,3], L.C. Layman[1,2,3].* 1) Institute of Molecular Medicine; 2) Section of Reproductive Endocrinology, Infertility, & Genetics, Department of Obstetrics & Gynecology, and; 3) Neuroscience Program, Georgia Health Sciences University, Augusta, GA.

Idiopathic hypogonadotropic hypogonadism (IHH) results from deficient gonadotropin-releasing hormone (GnRH) secretion or action. Kallmann Syndrome (KS) is due to migration failure of GnRH and olfactory neurons during development, resulting in IHH with anosmia. Nasal embryonic LHRH factor (NELF) is a nuclear protein that was differentially isolated from migratory GnRH neurons and mutations have been identified in IHH/KS. We previously found that although Nelf mRNA expression did not differ in mouse immortalized GnRH neuronal cells, NELF protein expression was greater in migratory compared with postmigratory cells. The goal of this study was to determine if mRNA/protein expression discordance was due to differential expression of splice variants (sv). We hypothesize that migratory GnRH neuronal cell lines (mouse GN11 and NLT; human FNCB4-hTERT) will have a different expression pattern of sv than postmigratory GT1-7 cells. PCR products from RT-PCR were cloned and individual colonies were analyzed by denaturing gradient gel electrophoresis (DGGE) and DNA sequencing. Preliminary results indicate five splice variants in mouse cells, and two splice variants in human cells. Human FNCB4-hTERT and all three immortalized mouse GnRH cells predominantly express variant 2 ranging from 72-78% in the cell lines. In addition, DGGE findings showed the presence of splice variants, which differed in specific cell types (pre-migratory and post migratory immortalized mouse GnRH neurons). Finally , we identified 3 novel splice variants in the immortalized mouse GnRH neuronal cells lines which have not been reported. Differential Nelf mRNA splice variant expression could play a role in GnRH neuron migration and/or function as well as in the human phenotype of IHH/KS.

1468W

High genetic heterogeneity in the causation of moles in patients with no NLRP7 mutations. *R. Reddy[1], J. Qian[1,2], R. Bagga[3], M.C Addor[4], J. Majewski[1,5], R. Slim[1].* 1) Department of Human Genetics and Obstetrics Gynecology, McGill University Health Centre, Montreal, Quebec, Canada; 2) Women's Reproductive Health Laboratory, Women's Hospital, Zhejiang University School of Medicine, Hangzhou, China; 3) Dept of Obstetrics & Gynecology, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India; 4) Division autonome de Genetique Medicale CHUV - 1011 Lausanne / Switzerland; 5) McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, Canada.

Background : Molar pregnancies are characterized by the absence of embryo and hydropic degeneration and proliferation of chorionic villi (OMIM 231090). Molar pregnancies affect 1 in 1000 pregnancies in North America and their incidences are much higher in Asia. To date, NLRP7 is the only known gene for hydatidiform moles (Murdoch et al., 2006). Genetic heterogeneity in the causation of recurrent moles has been documented based on the absence of linkage to 19q13.4, the region containing NLRP7, in one family (Slim et al., 2005) and the absence of mutations in this gene in several families. Therefore, more than one gene are involved in the causation of molar pregnancies. **Objective:** The goal of this study is to identify a second gene responsible for the causation of recurrent moles in cases with no NLRP7 mutations. **Methodology and Results:** To achieve this goal, a microarray SNP (Illumina 610 Quad) analysis had been performed on 8 affected patients and their unaffected relatives from 6 cases with no mutations in NLRP7. The Illumina data didn't show a common halpotype or homozygosity for the same chromosomal region in more than two patients indicating the high genetic heterogeneity in the causation of moles in these patients. Mutations in NLRP7 can explain 80-90% of the genetic cause of familial molar pregnancies, a situation analogous to Breast cancer where mutations in BRCA1 and BRCA2 explain 90% of the genetic heritability of Breast cancer while several other genes such as ATM, PTEN, CDH1, CHEK2 and p53 explain only 10% of the genetic heritability. Our Illumina data indicate the presence of more than one gene responsible for molar pregnancies in the small number of analyzed cases. Next generation Exome sequencing is ongoing on these patients and this approach may overcome the high genetic heterogeneity in patients with no NLRP7 mutations and lead to the identification of one or several new genes for recurrent moles.

1469W

Molecular study and familial pattern of internal apoptotic pathway BAX and BCL2 genes and mitochondrial genome in idiopathic repeated pregnancy loss. *S.M. Seyedhassani[1,2], M. Houshmand[1], A. Aflatoonian[2], S.M. Kalantar[2], G. Modabber[1].* 1) National institute of genetic engineering and biotechnology, Tehran, Iran; 2) Research and clinical center for infertility, Yazd, Iran.

Introduction: About 1 in 300 couples and 0.5-2% of women are involved in repeated pregnancy loss (RPL). Various etiological factors involve in RPL and the main part of them remains unknown. Among them the genetic factors are important. The apoptotic changes and the aberrant expression of many genes including apoptotic related genes were seen in RPL. Material and methods: Familial pedigrees of 335 consecutive couples suffering from RPL were initially evaluated at a primary stage. Among them, 96 women were screened as idiopathic at reproductive age. Molecular genetic variations in internal apoptotic related genes BAX, BCL2 and mitochondrial genome were investigated in comparison to control group. Results: The evaluation of familial pedigree of 335 RPL couples showed 120 cases of RPL in female relatives and 76 cases in male relatives. Other families with RPL were seen in two or three consecutive generations in 15.6% of female relatives. At least two cases of RPL in other consanguineous marriages were observed in 4.2%. There were familial marriages in 51.6% of RPL women and 21.8% of control group (P=0.0003). The mean of the D-loop mutations was 8.79 and 4.90 in RPL and control group respectively (ANOVA=0.0001). Among them, 22 mutations were significant in RPL group and the insertion of C in nucleotide 114 was novel. The G90C variation of BAX gene and the mean of mtDNA mutations were significant in second familial pattern consisted of familial marriages with RPL. Discussion: The high frequency of RPL in maternal pedigree implicates the maternal background of the disorder. On the other hand, there were higher rate of familial repeats of abortion, RPL repeats and consanguineous marriage in RPL pedigrees. Such evidences show the genetic background. However, pedigree analysis has critical role in the approach of RPL women. Our result indicates a supportive role of RPL for A(-179)G mutation in Bax gene, but two polymorphisms, G90C and G95A found in exon 1, provide a susceptible background for promoting miscarriages. These variations can have important roles in RPL, independently or as a part of haplogroups. An obvious role of inheritance and genetic background are detected by analysis of familial patterns.

1470W

Are ABO & Rh Blood Groups New Genetics Risk Factors for Endometriosis? *SMB. Tabei[1], K. Daliri[1], P. Rostami[2], A. Nariman[1].* 1) Department of Medical Genetics, Medical School, Shiraz University of Medical Sciences, Zand Street, Shiraz, Iran; 2) Dr. Rostami Infertility Center, Shiraz, Iran.

BACKGROUND: Endometriosis is a complex gynecologic disorder with a particular genetic background with recurrence risk 5-7% for first-degree relatives. It has been estimated that endometriosis occurs in roughly 5-10% of women in reproductive age and up to 50% of women with infertility. Up to now, associations between different malignancies and main blood groups are documented in the literature but, explanation for these associations is still unknown. A current hypothesis is increasing and facilitation of tumor cellular mobility by these antigens. ABO and Rh genes are located on 9q34and 1p36 chromosome respectively, therefore, these main blood groups may be assumed as important genetic risk factors for tumor or tumor-like diseases. Endometriosis shows some malignancy behaviors such as local invasion and genetic changes. Motivated by evidence that, ABO and Rh distribution in patients of United States, Turkey and South Korea have been revealed a controversial results we designed a case-control study in Southern population of Iran. Women with endometriosis also were divided into two groups fertile an infertile. METHODS: This case- control study was carried out, retrospectively. We used donor database of Shiraz Blood Transfusion Organization as control and medical records of 470 patients who have been diagnosed as endometriosis by laparoscopy and confirmed by pathology analysis who have been referred to Zeinab Hospital and Dr Rostami Infertility Center, Shiraz, Iran as cases. Statistical methods included chi square by Medcalc software. RESULTS And DISCUSSION: Our population is neighbor to Turkey (an Asian country) therefore, as we expected our results were similar to Demir et al in Turkish population, though Rh positivity in Turkish patients showed a significant difference compared with control. No significant difference was revealed between patients and control groups (ABO blood groups $P > 0.05$, Rh blood groups $P > 0.05$, 95% CI). Moreover, no significant difference was detected in ABO and Rh blood groups in women with endometriosis according to reproductive status (fertility or infertility). In conclusion, based on our results in Southern population of Iran and Demir et al from Turkey, ABO blood groups are not new genetics risk factors for endometriosis though Matalliotakis et al and Kim et al found significant relationship in USA and South Korea population, respectively.

1471W

Mutation in the *SYCP3* gene identified in a woman with recurrent pregnancy loss affect the synaptonemal complex conformation at meiotic prophase I. *M. Tsutsumi, H. Kogo, H. Inagaki, T. Ohye, H. Kurahashi.* Fujita Health University, Toyoake, Japan.

Meiotic chromosomal nondisjunction causes aneuploid conceptuses, and most of them result in early pregnancy loss. Events occurring in prophase during meiosis I are important for proper chromosome segregation. SYCP3 is one of the essential components of the chromosomal axes in the synaptonemal complex that is central to the interaction of homologous chromosomes called synapsis during prophase I. We had previously identified a heterozygous mutation in the *SYCP3* gene from a woman with recurrent pregnancy loss. This mutation was predicted to produce C-terminally truncated mutant SYCP3 protein that inhibited fiber formation by normal SYCP3 in a dominant negative manner when coexpressed in COS-7 cells. However, the behavior of the mutant protein in meiotic cells had been unclear.

In this study, we examined the effects of the mutant protein to meiotic chromosomes using a gene transfer system into mouse oocytes at prophase I combined with *in vitro* organ culture of ovaries. In brief, fetal mouse ovaries at 13 to 15 dpp were injected with a plasmid DNA carrying an epitope-tagged normal or mutant cDNA of the *Sycp3* gene with meiosis-specific promoter. The plasmids were introduced into oocytes by electroporation, and ovaries were *in vitro* cultured on the membrane insert for two to four days and analyzed by immunofluorescence. Chromosomal spreads of oocytes were prepared to analyze chromosomal axes and synapsis. When normal SYCP3 was expressed, the localization of the epitope-tagged SYCP3 was similar to that of endogenous SYCP3, and synapsis between homologous chromosomes marked with SYCP1 was normal at pachytene stage. When mutant was expressed, the epitope-tagged mutant SYCP3 also colocalized with endogenous SYCP3 along the chromosome axis, in some instances, intermittently. The chromosomal axes incorporated with more mutant SYCP3 appeared thicker and tended to show less synapsed. These data suggest that the mutant SYCP3 may disturb or delay the formation of synapsis. It is possible that, in the presence of mutant SYCP3, chromosomal axes alters its fine conformation and formation of synapsis or its robustness might be affected. Defects in synapsis may impair crossover events, which will cause nondisjunction in meiosis I and produce oocytes with aneuploidy leading to recurrent pregnancy loss.

1472W

ASSOCIATION OF CYTOKINE GENE POLYMORPHISMS AND RECURRENT MISCARRIAGES. *A. Kaur, N. Sudhir, A. Kaur.* Human Genetics, Guru Nanak Dev University, Amritsar, Punjab, India.

Cytokine gene polymorphisms in the promoter regions of TNF-(and IL-10 are associated with recurrent miscarriages (RM). The aim of present study was to investigate the association of the IL-10 -592C/A and TNF-(-308 G/A, promoter polymorphisms among women with at least three consecutive miscarriages. Among 80 couples studied, chromosomal anomalies were seen in 4 subjects (3 males and 1 female). Infections with TORCH test followed by structural uterine anomalies, endocrine defects and blood group incompatibility in couples emerged as important factors in the miscarriage cases. After cytogenetic investigations and analysis of clinical reports, genotyping was done in 50 women with RM and 50 controls for IL-10-592C/A and TNF-(-308G/A promoter polymorphism to see the association of these loci with miscarriages. Patients having anatomical abnormalities, hormonal disorders, hyperprolactinemia, erythroblastosis fetalis, chromosomal aberrations and TORCH infections were excluded from the polymorphism study. Allele frequencies were calculated for each genotype and the difference in allele frequencies between recurrent miscarriage and control women by using a Pearson $\chi 2$ test (SPSS Inc.10, Chicago, IL, USA) and expected genotype frequencies were calculated from the allele frequencies under the assumption of Hardy-Weinberg equilibrium. The mean maternal age was 25.9 years and that of controls was 26.4 years, mean miscarriages were 3.39 and mean gestational age was 3.6 months. IL-10-592C/A promoter polymorphism in the present case-control study did not show any association with the recurrent miscarriages (Chi-square value for genotypes = 0.491 and for alleles = 0.021 and $P > 0.05$). Statistical analysis further revealed that TNF-(-308G/A promoter polymorphism in the present sample was not associated with recurrent miscarriages (Chi-square value for genotypes = 1.413 and for alleles = 1.070 and $P > 0.05$). The results indicated absence of any association of a particular genotype or allele of IL-10-592C/A and TNF-(-308G/A promoter polymorphism with the recurrent miscarriages. The study is being carried out on a larger sample in our region.

1473W

Analysis of sperm telomeric length in idiopathic infertile men. *J. Thilagavathi, S. Venkatesh, M. Kumar, R. Kumar, R. Dada.* All India Institute of Medical Sciences, New Delhi, India.

Introduction: Telomeres are non-coding repeat hexanucleotide sequences that protect the ends of chromosomes and preserve the genome integrity within the cells. Telomeres shorten during each cell division in the absence of telomerase. When telomere length becomes critically short, cell senescence occurs. Telomeres in germline cells are balanced with each cell division and very less is known about its length in sperm and its association in male reproduction. Aim: The current study was aimed to estimate telomere length in the sperm from both infertile and control (fertile) men. Methods: Both infertile men (n=17) and controls (n=12) were evaluated for standard semen analysis and sperm DNA were isolated by separating the sperm from the semen by gradient separation technique. The average telomere length from the sperm DNA was quantified using a validated quantitative (Q-PCR) based assay. This method measures the average ratio of telomere repeat copy number to a standard single copy gene (36B4) copy number (T/S ratio) in each sample. Results: Infertile men had significantly ($P<0.0001$) lower sperm count and sperm motility compared to controls. Similarly normal morphology was also found to be significantly ($P<0.001$) lower in infertile men compared to controls. Relative quantification analysis showed that T/S ratio was significantly ($p<0.05$) lower in sperm samples from infertile men compared to controls (0.637 (0.617, 0.684) Vs 0.653 (0.631, 0.657). Conclusion: Altered telomere length is associated with many diseases however its role in male infertility is not explored. Our study for the first time confirms shortened telomere length in the sperm of infertile men. Therefore sperm telomere has functional role in fertilization apart from other disorders (cancer, and ageing) and needs to be confirmed with large number of samples.

325T

Genetic architecture of carotid artery intima-media thickness in Mexican Americans. *P.E. Melton, J.E. Curran, M. Carless, M.P. Johnson, T.D. Dyer, J.W. MacCluer, E.K. Moses, H.H. Goring, J. Blangero, L.A. Almasy.* Texas Biomedical Research Institute, San Antonio, TX.

Intima-media thickness (IMT) of the common and internal carotid arteries is a well established heritable risk factor for atherosclerosis and stroke. Often IMT is measured as the average of these two arteries yet they are believed to result from separate underlying biological mechanisms. The aim of this study was to conduct a family-based genome-association study (GWAS) in Mexican Americans to identify genetic polymorphisms influencing IMT and to determine if different carotid artery segments are influenced by different genetic components. IMT for the common and internal carotid arteries was determined through B-mode ultrasound in participants from the longitudinal San Antonio Family Heart Study (SAFHS). A GWAS utilizing 931,219 single nucleotide polymorphisms (SNPs) from Illumina (San Diego, CA) microarrays was undertaken with carotid artery IMT phenotypes utilizing an additive measured genotype model after adjustment for non-independence of families using kinship variance components. Among 29 families, 763 participants had IMT measured. Heritability estimates were 0.26 (±0.08) for average common carotid IMT and 0.45 (±0.07) for average internal carotid IMT. The genetic correlation between the common and internal carotid artery IMT was 0.51, indicative of some shared genetic components but also may suggest the potential for different contributing underlying biological pathways. The most robust association was detected for two SNPs (rs16983261, rs6113474, p=1.60x10-7) in complete linkage disequilibrium on chromosome 20p11 for the internal carotid artery near wall. The nearest gene to these SNPs is paired box 1 (PAX1). Heterozygotes with the minor allele (T) for these SNPS had significantly lower average (Δ=0.23 mm) internal carotid artery IMT. We also identify suggestive associations near genes (NAP5, EXOC3L2) that have shown genetic relationship with neurodegenerative disorders, including Alzheimers Disease, and schizophrenia. Further analysis of these results is being undertaken with full-genome sequencing in SAFHS participants. This study represents the first large scale GWAS of IMT in a non-European population and identified several novel loci. We also do not detect any common GWAS signals between common and internal carotid arterial segments indicating that they may not be completely influenced by shared biological components.

326T

New SLC10 mutations found in a Japanese patient with arterial tortuosity syndrome. *T. Morisaki[1,2], Y. Honda[3], A. Yoshida[1], K. Fujii[3], Y. Kohno[3], H. Morisaki[1].* 1) Dept Bioscience & Genetics, Natl Cerebr Cardiovasc Ctr Res Inst, Suita, Osaka, Japan; 2) Dept Mol Pathophysiol, Osaka Univ Grad Sch Pharm Sci, Suita, Osaka, Japan; 3) Dept Pediatircs, Chiba Univ Grad Sch Med, Chiba, Japan.

Arterial tortuosity syndrome (ATS) is a rare autosomal recessive connective tissue disorder characterized by tortuosity and elongation of the large and medium-size arteries, aneurysms or vascular dissection. ATS is caused by mutations in SLC2A10 gene, encoding for the facilitative glucose transporter 10 (GLUT10). So far, more than 30 families were reported to have SLC2A10 mutations world-wide, whereas no Japanese patient was reported. Here, we present the clinical and molecular characterization of a novel Japanese ATS patient. A 2 year-old boy was referred for virus infection and noticed to have symptoms for connective tissue disorders as well as annuloaortic dilatation and aortic tortuosity. There was no family history for similar symptoms nor parental consanguinity. Inguinal and diaphragmatic hernias and funnel chest were noted during his neonatal period. No developmental abnormality was observed so far. His skin was found to be loose and hyperextensible. Echocardiography showed normal aortic valve with three leaflets without stenosis or regurgitation, along with annular dilatation (the valve ring diameter:17 mm; sinus Valsalva diameter:27 mm; ST junction diameter: 16 mm). Also, he was found to have prominent tortuosity and elongation in both aorta and medium-sized arteries including cervical, intracranial, abdominal and iliac arteries. Based on his hyperextensible skin and arterial tortuosity, genetic analysis for SLC2A10 was performed and revealed that he has 2 novel point mutations in the exon 2 of this gene (p.Arg231Gln(c.692G>A) and p.Tyr239X(c.417T>A)). Genetic study of his parents revealed that the p.Arg231Gln mutation was derived from his father and the p.Tyr239X mutation from his mother and he was a compound heterozygote of these mutations. No mutation was identified in FBN1, TGFBR2, TGFBR1, ACTA2, or COL3A1 gene. This is the first report of a Japanese patient for ATS with compound heterozygotes of new SLC2A10 mutations.

327T

Distinct phenotypic differences between *TGFBR1* and *TGFBR2* gene mutation carriers in Loeys-Dietz syndrome. *H. Morisaki[1], A. Yoshida[1], H. Ogino[2], T. Morisaki[1,3], LDS clinical research group in Japan.* 1) Dept Bioscience & Genetics, NCVC Res Inst, Suita, Osaka, Japan; 2) Dept Cardiovascular Surgery, Natl Cerebr & Cardiovasc Ctr,Suita, Osaka, Japan; 3) Dept Molecular Pathophysiology, Osaka Univ Grad Sch Pharm Sci, Suita, Osaka, Japan.

Loeys-Dietz syndrome (LDS) is a systemic connective tissue disorder characterized by vascular and skeletal manifestations caused by mutations in *TGFBR1* or *TGFBR2* genes. It is generally considered that there are no phenotypic differences between patients with mutations in *TGFBR1* and those in *TGFBR2*, and the majority of LDS patients harbor mutations in *TGFBR2* gene. The nationwide research program to study clinical spectra of LDS in Japan started in 2009. Patients were recruited for genetic testing when their dysmorphic features or cardiovascular manifestations suggested of LDS. Fifty genetically-diagnosed patients are currently registered and complete clinical records were available from 40 of them. Using this cohort, we analyzed the phenotype-genotype correlations in Japanese LDS patients. The study cohort consisted of 18 *TGFBR1* mutation carriers (14 probands) and 22 *TGFBR2* mutation carriers (18 probands). Significant differences between patients with *TGFBR1* mutations and those with *TGFBR2* mutations were observed in regard to age at initial diagnosis of probands (31.2y vs. 10.1y, p=0.0001), first recognizable manifestation (skeletal / cardiovascular)(1/17 vs. 14/8, p=0.0002), cleft palate/abnormal uvula (22% vs. 73%, p=0.0015), hypertelorism (61% vs. 91%, p=0.02), strabismus (6% vs. 36%, p=0.039), positive wrist- & thumb sign (11% vs. 45%, p= 0.018), pectus deformity (6% vs. 32%, p=0.04), talipes equinovarus (0% vs. 23% , p=0.03), joint laxity (17% vs.73%, p=0.0004), and fulfillment of former Ghent MFS diagnostic criteria(11% vs.50%, p=0.009). Aortic involvement (83% vs. 91%, p=0.47) and tortuosity of cervical arteries (56% vs. 59%, p=0.82) were observed equally. In general, patients with *TGFBR2* mutations were diagnosed at younger age, usually suspected of Marfan-related disorders, showed more distinct dysmorphic features. In contrast, those with *TGFBR1* mutations are diagnosed at older age by cardiovascular event like AAE or TAAD, presenting less skeletal features. In this study, we found higher percentage of *TGFBR1* mutation probands (44%) compared to previous report (2-25%). This discrepancy may be explained by the difference in patient recruitment procedure, since initial diagnoses those with *TGFBR1* mutations were young-onset TAAD or familial TAAD in most of the cases. Since the risk of aortic involvement was similar between both genotypic groups, LDS should be considered as differential diagnosis of all familial TAAD patients.

328T

Coronary artery disease is associated with altered gene expression in human left ventricular myocardium. *J.D. Muehlschlegel[1], D. Christodoulou[2], K.Y. Kiu[1], J. Gorham[2], G. Lee[1], S.K. Shernan[1], S.F. Aranki[3], C.E. Seidman[2], J.G. Seidman[2], S.C. Body[1].* 1) Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA. 75 Francis St, CWN L1 Boston, MA 02115; 2) Department of Genetics Harvard Medical School NRB, 77 Avenue Louis Pasteur Boston, MA 02115; 3) Department of Surgery Division of Cardiac surgery Brigham and Women's Hospital 75 Francis St Boston, MA 02115.

Introduction: Cardiopulmonary bypass (CPB) with cardioplegic arrest is associated with ischemia leading to metabolic substrate depletion, reperfusion injury, apoptosis and necrosis. We assessed differences in gene expression in left ventricular (LV) tissue samples from patients with and without coronary artery disease (CAD) undergoing aortic valve replacement (AVR) with CPB prior to, and after cardioplegic arrest using whole-genome transcriptional profiling. Methods: Apical punch biopsies from the LV vent placement site were taken from 14 patients (8 with CAD, 6 controls without CAD) undergoing AVR with CPB at two time points; immediately after aortic cross clamping (pre-ischemia), and immediately before aortic cross clamp removal (post-ischemia). The Illumina HiSeq Analysis platform was used to quantify genome-wide mRNA expression. Lists of genes expressed differently in pre- vs. post ischemic tissue from patients with and without CAD were identified. These gene lists were subjected to Ingenuity Pathway Analysis 9.0 (IPA; Ingenuity Systems) to identify regulatory networks operant in these hearts. Multivariate modeling was used to adjust for age, sex and aortic cross clamp duration. Results: Median duration of aortic cross clamping was 119 mins and 80 mins for patients with and without CAD respectively. The top network represented genes involved in cellular growth and proliferation, cell death, and cancer. The majority of these genes were significantly up-regulated in CAD patients compared to no-change or down-regulated in non-CAD controls. Discussion: The human LV exhibits significant, yet variable changes in gene expression in response to ischemia during CPB depending on the presence of or absence of CAD. Variations in these pathways represent important contributors to the pathophysiology of ventricular ischemia, and thus, may have help guide efforts to reduce myocardial damage during surgery as well as to serve as potential biomarkers of injury.

329T

Identification of a new chromosomal locus for a mutation causing Left ventricular non-compaction with ventricular tachycardia cardiopathology. *E. Muhammad[1], A. Levitas[2], V.C. Sheffield[3], R. Parvari[1,4].* 1) Developmental Genetics, Ben-Gurion University of the Negev, Beer-Sheva, Israel; 2) Soroka Medcal Center, Ben Gurion University of the Negev, Beer Sheva, Israel; 3) University of Iowa, Iowa City, IA, United States; 4) National Institute of Biotechnology Negev, Beer Sheva, Israel.

Structural and functional disorders of the heart are important causes of morbidity and mortality. Three Bedouin patients at ages 14-18 years of a single large consanguineous Bedouin family presented with Left ventricular non-compaction and sustained Ventricular Tachycardia. They were evaluated by Echocardiography which showed severe left side enlargement, severely depressed left ventricular (LV) function with focal lacunas in the LV free wall, but normal origin of the coronary artery. The 2 older patients were treated with recurrent electrical cardio version and intravenous administration of amiodarone and on discharge have undergone implantable cardioverter-defibrillator (ICD) implantation. The recessive pattern of inheritance in the consanguineous family suggested homozygosity of the mutation inherited from a common founder. We have performed homozygosity mapping using the affymetrix SNP 5 array on the patients and found a single large chromosomal block of homozygosity on chromosome 11q24 shared by all of them. Verification of this block with VNTR markers in all available family members proved linkage. The Lod score analysis, under the model of a recessive trait with full penetrance, using the PedTool server, was 2.46 for 2 point and 4.59 for the multipoint analysis. This chromosomal locus does not contain any of the known genes causing this disease; efforts are now being done to identify the mutated gene.

330T

Association of MTHFR C677T Gene Polymophism in Children with Congenital Heart Defects. *H.P. Mundluru[1], S. Sunayana Begum[1], K. Srujana[1], K. Nageswar Rao[2], V. Sreedevi[1], G. Sandhya Devi[3], K. Vasudevan[2], K. Manohar[1], A. Jyothy[1].* 1) Genetic Toxicology, Institute of Genetics, Hyderabad, Andhra Pradesh, India; 2) Department of Pediatric Cardiology, Care Hospital, Banjara hills, Hyderabad; 3) Indo- American Cancer Hospital, Banjara Hills, Hyderabad.

Congenital heart defects (CHD) are the most common type of birth defects affecting 1% of new born children and results from incomplete development of the heart during the first 6 weeks of pregnancy. Shortness of breath, cyanosis, heart murmur, respiratory infections and stunted growth are the symptoms of congenital heart defects. The human body needs folate to synthesize, repair, and methylate DNA as well as to act as a cofactor in biological reactions. Maternal multivitamin supplementation containing folic acid reduces the risk of neural tube defects, and evidence suggests that it may be associated with other reproductive outcomes, including congenital heart defects. Methylenetetrahydrofolate reductase (MTHFR) catalyses the reduction of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, which is the circulating form of folate. 5-Methyltetrahydrofolate donates its methyl group to homocysteine, forming methionine and tetrahydrofolate. In the MTHFR enzyme, a common C→T substitution at position 677 (referred to as 677T) exists, resulting in a substitution of alanine to valine, causing impaired folate binding and reduces the activity of MTHFR enzyme. In the present investigation 45 children with congenital heart defects and 45 subjects belonging to the same age, sex and socio economic status were studied. The prevalence of heterozygotes were observed to be higher in patients when compared to controls. The study indicated that the heterozygous condition of MTHFR gene may be associated with the disease condition.

331T

The contribution of biogeographical ancestry and polymorphisms in the *CETP* and *LIPC* genes to dyslipidemia in HIV positive men receiving highly active anti-retorviral therapy. *M. Nicholaou, J. Martinson, L. Kingsley, Multicenter AIDS Cohort Study (MACS).* Infectious Diseases & Microbiology, University of Pittsburgh, Pittsburgh, PA.

Highly active anti-retroviral therapy (HAART) has been successful in delaying the progression to AIDS in HIV infected individuals. Exposure to HAART can result in metabolic side effects such as dyslipidemia in a subset of treated patients. We used a custom designed Illumina GoldenGate Genotyping assay to investigate the genetic susceptibility to dyslipidemia attributed to HIV infection and HAART treatment. We performed a literature search to identify SNPs that had been previously associated with dyslipidemia and/or cardiovascular disease (CVD) in the general population. 280 clinically relevant SNPs and 1,132 TagSNPs covering 75 gene regions were included on the GoldenGate array. 1,945 men were selected from the Multicenter AIDS Cohort Study (MACS) for genotyping and phenotypic analysis of serum lipid levels. This population was stratified by HIV/HAART status and biogeographical ancestry using a panel of 124 Ancestry Informative Markers (AIMs). Among men of European ancestry, those who were infected with HIV and receiving HAART had significantly lower serum low-density lipoprotein cholesterol (LDL, $P = 1.90 \times 10^{-4}$) and high-density lipoprotein cholesterol levels (HDL, $P < 1.00 \times 10^{-7}$), with significantly higher serum triglyceride (TRIG, $P < 1.00 \times 10^{-7}$) levels compared to HIV/HAART (-/-) controls. Among men of mixed African and European ancestry, those who were HIV/HAART (+/+) had significantly lower LDL ($P = 1.80 \times 10^{-4}$) levels compared to HIV/HAART (-/-) controls. Four SNPs; rs1532624 ($P = 1.66 \times 10^{-5}$), rs1532625 ($P = 2.36 \times 10^{-5}$), rs711752 ($P = 4.48 \times 10^{-5}$), and rs708272 ($P = 4.59 \times 10^{-5}$), located in the *CETP* gene region on chromosome 16 had statistically significant associations with serum HDL levels in HIV/HAART (+/+) European men. One SNP, rs261334 ($P = 6.53 \times 10^{-6}$), located in the *LIPC* gene region on chromosome 15 was associated with serum LDL levels and another SNP, rs4783961 ($P = 9.83 \times 10^{-6}$) located in the *CETP* gene region, was associated with HDL levels in HIV/HAART (+/+) men of mixed African and European ancestry. These results show that HAART-associated dyslipidemia varies depending on biogeographical ancestry and implicates two genes associated with serum lipid levels in these patients.

332T

***Il-6* and *TNF* variants interact with air pollution in modulating inflammatory blood markers and MI risk.** *F. Nyberg[1,2], S. Panasevich[1], K. Leander[1], P. Ljungman[1,3], T. Bellander[1], U. de Faire[1,4], G. Pershagen[1,5].* 1) Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; 2) AstraZeneca R&D Mölndal, Mölndal, Sweden; 3) Department of Cardiology, South Hospital, Stockholm, Sweden; 4) Department of Cardiology, Karolinska Hospital, Stockholm, Sweden; 5) Department of Community Medicine, Karolinska Hospital, Stockholm, Sweden.

Background: Air pollution exposure induces cardiovascular effects, possibly via systemic inflammation and coagulation misbalance. Genetic variation may determine individual susceptibility. **Objectives:** To investigate effect modification by inflammation (*IL6*, *TNF*) and coagulation (*fibrinogen B*) , *PAI-1)* gene variants on the effect of long- or short-term air pollution exposure on both blood marker levels and myocardial infarction (MI) risk. **Methods:** We studied 1192 MI cases and 1506 population controls aged 45-70 years from Stockholm. Selected *IL6*, *TNF*, *FGB* and *PAI1* SNPs/variants were genotyped. Retrospective emission inventories and spatial dispersion modelling was used to assess long-term air pollution exposure to traffic-NO2 and heating-SO2 emissions at home addresses up to 30 years back in time. Urban background NO_2, SO_2, PM_{10} and O_3 measurements were used to estimate variation in short-term air pollution exposure from hours up to 5 days back in time. Gene-environment interactions for short- and long-term air pollution on blood marker levels were studied in the population control sample, for long-term exposure on MI risk by comparing cases and controls, and for short-term exposure on MI onset, comparing the case onset time with control time points (case-crossover design). **Results:** We observed gene-environment interaction for several *IL6* and *TNF* SNPs in relation to inflammation blood marker levels. For example, for long-term air pollution, 1-year traffic-NO_2 exposure was associated with higher IL-6 levels with each additional *IL6-174C* allele, and 1-year heating-SO_2 exposure was associated with higher levels of TNF-(in *TNF-308AA* homozygotes vs. *-308G* carriers, with comparable findings for 5- and 30-year exposure metrics. Similarly for short-term air pollution exposure, we saw interaction with some *IL6* and *TNF* SNPs in relation to blood marker levels, most obviously for averaging over last 12-24 hours or 2 days. For each of these associations, the pattern of long-term MI risk or risk of acute MI onset followed closely the corresponding pattern of effect on blood markers across genotype groups. We observed no interactions for *FGB* or *PAI1*. **Conclusions:** Genetic variants in *IL6* and *TNF* may modify effects of both long- and short-term air pollution exposure on inflammatory marker levels, with a corresponding pattern of association with both long-term MI risk and acute MI onset.

333T

Cytokine (IFN-. , IL-6, TNF-(, TGF-) 1 and IL-10) genotyping in Turkish Children with cardiomyopathy. *S. Oguzkan Balci[1], N. Col Araz[2], O. Baspinar[3], T. Sever[1], A. Balat[4], S. Pehlivan[1].* 1) University of Gaziantep,Faculty of Medicine, Department of Medical Biology and Genetics, Gaziantep, Turkey; 2) University of Gaziantep, Faculty of Medicine, Department of Pediatrics, Gaziantep, Turkey; 3) University of Gaziantep, Faculty of Medicine, Department of Pediatric Cardiology, Gaziantep, Turkey; 4) University of Gaziantep, Faculty of Medicine,Department of Pediatric Nephrology, Gaziantep, Turkey.

Objectives of Study:Cardiomyopathy (CMP) is a cardiac muscle disease of an unknown etiology and defined by World Health Organization (WHO) as “diseases of the myocardium associated with cardiac disfonction”. Considering the role of cytokines in pathogenesis of CMP, and that cytokine gene polymorphism may affect the cytokine production, we investigated the association of CMP with IFN-. , IL-6, TNF-(, TGF-) 1 and IL-10 genes. Method: Twenty children with CMP, and 21 age-and sex-matched healthy controls were tested for 8 polymorphisms in 5 different genes. DNA was extracted from whole blood by standard salting out method. Cytokine genotyping was performed by polymerase chain reaction sequence-specific primer methods. The polymorphisms analyzed in the present study were IFN-. (+874), IL-6 (-174), TNF-((-308), TGF-) 1 (+10, +25) and IL-10 (-1082 , -819 , -592). The results were statistically analyzed by calculating odds ratios (OR) and 95% confidence intervals (CI) using chi-square test. Results: CMP patients had significantly higher frequencies of IFN-. (+874) polymorphism in both TT genotype (p=0.002, OR:11.6 [95 % CI:2.11-63.75]) and T allele (p=0.004, OR:3.34, CI: [1.35-8.27]). Our data also indicated a significant difference in C allele frequencies of IL-6 (-174) polymorphism between CMP patients and the controls (p=0.035, OR:2.36 [95 % CI:0.91-6.10]). In addition, GG genotype and G allele frequencies of TNF-((-308) polymorphism were lower in CMP patients than the controls (p=0.033, OR:0.20 [95 % CI:0.45-1.91], p=0.047, OR:0.26 [95 % CI:0.06-1.06], respectively). The haplotypes of TGF-) 1 and IL-10 were compared in terms of their expressions, and we found that the TT/GG, TC/GG haplotypes of TGF-) 1 had significantly decreased in the CMP (p=0.008, OR:0.16 [95 % CI:0.04-0.64]), whereas there were no statistically significant differences in the haplotypes of IL-10. Conclusion: The present study showed that TT/GG, TC/GG haplotypes of TGF-) 1, IFN-. (+874), IL-6 (-174) and TNF-((-308) polymorphisms were associated with CMP in Turkish children.We suggest that high expressions of IFN-. (+874) and TNF-((-308), and low or intermediate expressions of TGF-) 1 may be related to the pathogenesis of CMP. However, our results allow for only preliminary conclusions due to small sample size, therefore, further studies with larger samples are needed to address the exact role of this cytokines in childhood CMP.

334T

Down-regulation of Casz1 by siRNA injection into mouse tail-vein. *B. Oh, S.M. Ji, Y-B. Shin, S. Park, H. Lee, J-E. Lim.* Biomedical Engineering, Sch Med, Kyung Hee Univ, Seoul, Korea.

Blood pressure is regulated by many genetic and environmental factors. To identify genes that mediate this regulation, genome-wide association studies (GWASs) have been performed using large samples from various ethnicities. The Wellcome Trust Case Control Consortium (WTCCC), Amish study, KORA, KARE, the Global BPgen consortium, and the CHARGE consortium have conducted GWASs on hypertension and blood pressure, identifying 14 independent loci that govern blood pressure that reached genome-wide significance: 6 enzymes, 2 solutes channels, 2 transcription factors, 1 growth factor, 1 cell signaling protein, 1 structural protein, and 1 hypothetical gene. CASZ1, castor zinc finger 1, gene was found to be associated with blood pressure in both European descents and Japanese. CASZ1 was also known as survival-related gene (SRG). This gene encodes a putative protein of 172 amino acids, mainly located in the perinuclear region, and SRG is highly expressed in many human cancer cell lines although it is low in most tissues except liver and placenta. Also it was found that SRG-transfected cells are resistant to apoptosis induced by cytokine/serum deprivation, suggesting its role in controlling apoptosis and tumor formation. To attempt the down-regulation of Casz1 gene in mouse, we synthesized siRNA against mouse Casz1 and tested its efficiency in mouse NIH 3T3 cell lines. The siRNA reduced the transcript level by 58.53% in cells, and then the siRNA was injected into mouse tail-vein to reduce the expression of Casz1 in vivo, and the several tissues such as liver, kidney, heart, skeletal muscle, smooth muscle, spinal cord and brains were examined the level of Casz1 transcript 24 hours after the siRNA injection. The Casz1 transcripts in most tissues were seldom reduced but in kidney reduction upto 46% was repeatedly found. The reduction efficiency was at peak after 24 hours and the reduction seems to be decreased following 24 hours. Therefore we injected Casz1 siRNA everyday for three days to examine the phenotype change such as blood pressure, however the change of blood pressure could not be found.

335T

Functional consequences of heterozygous *ACTA2* mutations. *C.L. Papke[1], J. Cao[1], S. Lim[2], M. Rees[1], J. Chandra[3], A. Trache[2], W. Zimmer[2], D.M. Milewicz[1].* 1) Department of Internal Medicine, The University of Texas Health Science Center, Houston, TX; 2) Department of Systems Biology and Translational Medicine and Medical Physiology, Texas A&M Health Science Center College of Medicine, College Station, TX; 3) Department of Pediatrics - Research, Division of Pediatrics, The University of Texas MD Anderson Cancer Center, Houston, TX.

Mutations in ACTA2 (smooth muscle cell (SMC)-specific (-actin) cause a diffuse vasculopathy characterized by both thoracic aortic aneurysms leading to aortic dissections and occlusive vascular diseases, including early onset coronary artery disease and strokes. Occlusive lesions are characterized by medial and intimal SMC hyperplasia. *Acta2*$^{-/-}$ mice were used a model system to investigate the cellular pathways responsible for arterial dilatation and occlusion. These mice have normal vascular development but decreased arterial contractility. We found that *Acta2*$^{-/-}$ mice develop ascending aortic dilatation by 4 weeks of age, and the dilatation progresses with age. Aortic pathology in the *Acta2*$^{-/-}$ mice was characterized by thickening of the medial layer, increased number of elastic lamellae and SMCs and increased Mmp2 and proteoglycan expression (P<0.01). *Acta2*$^{-/-}$ aortic SMCs proliferated and migrated more rapidly than wildtype (WT) *in vitro* (P<0.05). *Acta2*$^{-/-}$ mice had greater neointimal formation with carotid injury (intima/media ratio of 2.19 vs 0.32 in WT; P<0.001). The MRTF:SRF axis was not responsible for the increased SMC proliferation. Assessment of focal adhesions (FAs) using quantitative Total Internal Reflection Fluorescence microscopy identified increased vinculin and activated focal adhesion kinase (pFAK) in FAs, and increased FA size in *Acta2*$^{-/-}$ SMCs compared to WT (P<0.01). Activation of FAs leads to increased expression of platelet-derived growth factor receptor-) (Pdgfr)), and increased expression and activation of Pdgfr) was confirmed in *Acta2*$^{-/-}$ SMCs compared to WT. SMC proliferation *in vitro* and neointimal formation *in vivo* in *Acta2*$^{-/-}$ mice was inhibited with imatinib mesylate (P<0.05). Media switching and PDGF-BB neutralizing antibody experiments demonstrated that Pdgfr) activation in *Acta2*$^{-/-}$ SMCs was ligand-independent. *Acta2*$^{-/-}$ SMCs had increased reactive oxygen species (ROS) compared to WT by flow cytometry, and treatment with either antioxidants or mitochondrial respiratory chain inhibitors decreased Pdgfr) activation. Thus, in *Acta2*$^{-/-}$ SMCs, loss of (-actin induces FA rearrangement with FAK activation and increased Pdgfr) expression, in addition to ligand-independent Pdgfr) activation due to increased ROS production. In summary, these data suggest that hyperplasia of SMCs contributes to occlusive lesions resulting from *ACTA2* mutations, and that imatinib could potentially be used to prevent these occlusive lesions.

336T

NMR-based fine mapping of lipoprotein subfractions strengthens association with genetic loci and provides information on their biological nature. *A.K. Petersen[1], K. Stark[2], M.D. Musameh[3,4], C.P. Nelson[3,4], W. Römisch-Margl[5], W. Kremer[6,7], J. Raffler[5,8], S. Krug[9], T. Skurk[9], M.J. Rist[10], H. Daniel[10], H. Hauner[9], J. Adamski[11,12], M. Tomaszewski[3,4], A. Döring[13,14], A. Peters[14], H.E. Wichmann[13,15,16], B.M. Kaess[2,3], H.R. Kalbitzer[6,7], F. Huber[6], V. Pfahlert[6], N.J. Samani[3,4], F. Kronenberg[17], H. Dieplinger[17], T. Illig[18], C. Hengstenberg[2], K. Suhre[5,8,19], C. Gieger[1], G. Kastenmüller[5].* 1) Institute of Genetic Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany; 2) Klinik und Poliklinik für Innere Medizin II, University of Regensburg, Regensburg, Germany; 3) Department of Cardiovascular Sciences, University of Leicester, Leicester, United Kingdom; 4) Leicester NIHR Biomedical Research Unit in Cardiovascular Disease, Glenfield Hospital, Leicester, United Kingdom; 5) Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, Neuherberg, Germany; 6) LipoFIT Analytic GmbH, Regensburg, Germany; 7) Institut für Biophysik und physikalische Biochemie, University of Regensburg, Regensburg, Germany; 8) Faculty of Biology, Ludwig-Maximilians-Universität, Planegg-Martinsried, Germany; 9) Else Kröner-Fresenius-Centre for Nutritional Medicine, Technische Universität München, Freising, Germany; 10) Molecular Nutrition Unit, Technische Universität München, Freising, Germany; 11) Institute of Experimental Genetics, Genome Analysis Center, Helmholtz Zentrum München, Neuherberg, Germany; 12) Lehrstuhl für Experimentelle Genetik, Technische Universität München, Freising-Weihenstephan, Germany; 13) Institute of Epidemiology I, Helmholtz Zentrum München, Neuherberg, Germany; 14) Institute of Epidemiology II, Helmholtz Zentrum München, Neuherberg, Germany; 15) Institute of Medical Informatics, Biometry and Epidemiology, Chair of Epidemiology, Ludwig-Maximilians-Universität, München, Germany; 16) Klinikum Grosshadern, München, Germany; 17) Division of Genetic Epidemiology, Department of Medical Genetics, Molecular and Clinical Pharmacology, Innsbruck Medical University, Innsbruck, Austria; 18) Research Unit of Molecular Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany; 19) Department of Physiology and Biophysics, Weill Cornell Medical College in Qatar, Education City - Qatar Foundation, Doha, Qatar.

Lipoproteins are highly heritable and risk factors for cardiovascular outcomes such as coronary artery disease, myocardial infarction, and stroke. So far, genome-wide association studies (GWAS) have revealed 95 lipid-associated loci when analyzing more than 100,000 samples. These 95 loci explain 10-12% of the interindividual variance of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG) and total cholesterol (TC). To discover more genes associated with a phenotype and thus explain more of the heritability of this phenotype, usually the sample size was increased. Instead of further raising the sample size, more refined phenotypes, namely lipoprotein subfractions, can be analyzed. We measured the concentration of 15 lipoprotein subfractions, termed L1-L15, in 1,791 plasma samples of the KORA study using diffusion-weighted NMR-spectroscopy and analyzed their association with the 95 known lipid loci. In addition, we conducted a cluster analysis to investigate the interrelationship of the lipoprotein subfractions in fasting samples as well as in samples during a lipid tolerance test. We found that eight loci (*LIPC*, *CETP*, *PLTP*, *FADS1-2-3*, *SORT1*, *GCKR*, *APOB*, *APOA1*) are associated with at least one of the 15 NMR-measured lipoprotein subfractions whereas only four loci (*CETP*, *SORT1*, *GCKR*, *APOA1*) are associated with serum lipid levels in the study. Statistical power analyses of the eight loci revealed a strengthening in association when analyzing lipoprotein subfractions. Accordingly, we also observed an increase in the variance explained by our regression models. The cluster analysis in fasting samples revealed five different groups of lipoprotein subfractions of which one only consisted of lipoprotein subfraction L1, which is only marginally captured by serum lipids. In conclusion, NMR-based fine mapping of lipoprotein subfractions strengthens the association with genetic loci and provides novel information on their biological nature.

337T

Lipoprotein lipase gene (*LPL*) resequencing and plasma lipid profile. *D. Pirim[1], F.Y. Demirci[1], S.C. Hughes[1], M. Irfan[1], Y. Wang[1], J.E. Hokanson[2], R.F. Hamman[2], C.M. Kammerer[1], M.I. Kamboh[1].* 1) Human Genetics, GSPH, Univ Pittsburgh, Pittsburgh, PA; 2) Epidemiology, Colorado School of Public Health, Univ Colorado Denver, Aurora, CO.

In the U.S., coronary heart disease (CHD) is the most common cause of death in both males and females. Epidemiological studies have identified several risk factors for CHD, including low high-density lipoprotein cholesterol (HDL-C) and elevated total cholesterol, low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG), but the genetic variations that cause predisposition to dyslipidemia still remain to be further explored. Lipoprotein lipase (*LPL*) is one of the major genes involved in lipid metabolism and its sequence variation has already been reported to be associated with the risk of CHD and other complex traits such as dyslipidemia, type 2 diabetes, essential hypertension, and Alzheimer's disease. In this study, we investigated the common and rare variation in *LPL* (located at 8p22) by resequencing individuals with HDL-C levels in the upper 5th percentile (n=47) and the lower 5th percentile (n=48) selected from a population-based non-Hispanic White (NHW) sample of 623 individuals. A total of 179 variants (substitutions or indels) plus one microsatellite were identified in 95 individuals by resequencing the entire *LPL* gene and flanking regions (about 30 kb), including 91 variants with minor allele frequency (MAF) <0.05 and 88 common variants with MAF ⁄ 0.05. Of the 91 relatively uncommon or rare variants, 21 were present only in the low HDL-C group and 25 were present only in the high HDL-C group. Overall, the prevalence of uncommon or rare variants was higher in the high HDL-C than the low HDL-C group. Thirty two common variants showed significant (p-value <0.05) allele frequency differences between the high and low HDL-C groups. Of the 12 common variants analyzed to date in the entire NHW sample, 7 showed significant association with the lipid levels. Our results suggest that both common and rare variants of this gene are associated with inter-individual variation in plasma lipid profile. The genotyping of remaining tag SNPs and relevant rare variants in the entire sample is about to be completed and their analysis will unravel the extent to which *LPL* genetic variation affects the regulation of plasma HDL-C levels and other lipids.

338T

Vestibular symptoms in long QT syndrome. *G. Poke, J. McGaughran.* Genetic Health Queensland, Brisbane, Australia.

This small case control study details self- and family-reported vestibular and auditory symptoms in a group of patients with LQTS 1 and 2 (both disorders of potassium channels). Their responses are compared with those of a control group with LQTS 3 (caused by a sodium channel defect). Potassium channels are vital for normal hearing, which depends on maintenance of a high endolymphatic potassium concentration. They are also necessary for normal cardiac conduction. Some disorders can affect both systems: for instance, in the heterozygous state, mutations in *KCNQ1* can cause Romano-Ward syndrome (nonsyndromic LQTS). Homozygous mutations in the same gene may result in Jervell and Lange-Nielsen syndrome (the combination of sensorineural hearing loss and LQTS). It is increasingly recognised that potassium channels may also be involved in vestibular function. This study was prompted by a number of LQTS patients in the cardiac clinic complaining of dizziness. We show that vestibular symptoms may be a hallmark of potassium-channel related LQTS, independent of cardiac rhythm disturbances.

339T

Familial Mutation In Kozak Sequence Of GATA4 Associated With ASD Type 2; GATA4 L325V Associated With Atrial Fibrillation. *A.V. Postma, R. Mohan, K. van Engelen, A. Ilgun, V.M. Christoffels, P. Barnett.* Academic Medical Center, Amsterdam, Netherlands.

The Gata family of transcription factors are critical in embryonic development, cell growth, and differentiation. They are defined by an evolutionarily conserved DNA-binding domain consisting of two zinc finger motifs. Gata4 is one of the earliest genes expressed by specified cardiac precursors at the cardiac crescent stage of mouse development. Missense mutations in GATA4 are associated with ASD type 2 and recently with atrial fibrillation. We screened 33 familial patients with ASD type2 and 17 with familial atrial fibrillation for mutations in GATA4 (all negative for Nkx2.5 and Tbx5 mutations). This led to the identification of a mutation at -6bp from the ATG start site, which co-segregates in a family with ASD type 2. This position is part of the strongly conserved Kozak consensus sequence which serves to initiate translation. Expression analysis showed that this mutation leads to a 50% decrease in the amount of protein, in effect a hypomorph, indicating that the -6bp is an essential part of the Kozak sequence. In a patient with familial atrial fibrillation without structural anomalies, we identified a L325V aminoacid change. The L325V mutant does not show a difference in activation of the ANF-luciferase reporter assays. However, qPCR analysis on H10 cells transfected with the mutant GATA4 gene, shows a significant increase in Cx40 and Cx43 expression and a decrease in Tbx3, in comparison to wildtype GATA4. Nuclear trafficking was normal for the L325V mutant. We are currently investigating the mechanism by which the L325V GATA4 mutation causes atrial fibrillation.

340T

Long QT Syndrome: A Preliminary Study. *N. Pratibha[1], A. Ali[1], FQ. Sameera[1], K. Thangaraj[2], A. Venkateshwari[3], C. Narsimhan[4].* 1) Department of Genetics, Osmania University, Hyderabad, India; 2) Center for Cellular and Molecular Biology, Habsiguda, Hyderabad; 3) Institute of Genetics and Hospital for Genetic Disorders, Ameerpet, Hyderabad; 4) Care Hospitals, Hyderabad.

Long QT Syndrome, a rare arrythmogenic disorder is characterized by delayed repolarization of the heart following a heartbeat, leading to the prolongation of the QT interval. Inherited LQTS is the prototype of the “primary cardiac arrhythmias” or “cardiac ion channelopathy”. LQTS is caused by mutations in genes encoding cardiac ion channels. One such disorder; “Jervell-Lange Nielsen Syndrome” is the outcome of mutations in KCNQ1 or KCNE1 genes resulting in defective IKs channels. PCR based SSCP analysis of KCNQ1 revealed band pattern variation in a proband. On sequencing a 4bp insertion was identified in the patient and his first degree relatives. This led to changes in the secondary structure of the mRNA. Insilco analysis revealed changes in splice site and binding sites of the SR and hnRNP proteins in the spliceosome complex. This change in binding sites could lead to alteration in processing of the mRNA during the maturation process which will be further discussed.

341T

Prenatal diagnosis for cardiomyopathy: genotype may not predict phenotype. *N. Quercia[1], S. Baxter[2], B.H. Funke[2,3], A. Dipchand[4], S. Bowdin[1].* 1) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, ON, Canada; 2) Laboratory for Molecular Medicine, Partners Healthcare Center for Personalized Genetic Medicine, Cambridge, MA; 3) Department of Pathology, Massachusetts General Hospital, Boston, MA; 4) Division of Cardiology, Hospital for Sick Children, Toronto, ON, Canada.

Our understanding of the natural history of hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) predisposing gene mutations is still emerging. Some genotype-phenotype correlations can be made however, caution must be used when using this data to counsel prenatal cases. In our experience, prenatal genetic diagnosis is rarely requested or available for isolated DCM/HCM. We present an infant female, diagnosed with DCM in infancy, the product of a first cousin Sri Lankan mating and prenatally predicted to be unaffected with DCM. Amniocentesis revealed a parentally inherited heterozygous MYBPC3 c.3628-41_3628-17del mutation that was predicted to result in mild or moderate HCM with later onset. Prenatal diagnosis was requested by her parents after genetic testing in their son, also diagnosed with DCM in infancy, was found to be homozygous for the MYBPC3 mutation. This deletion is common (2-8% of individuals) in the heterozygous form in the South Asian population. It is known to affect splicing and leads to exon skipping. It has been associated with mild hypertrophy in heterozygotes and has been reported to show severe and early presentation in homozygous individuals. Parental studies revealed they were both heterozygous for the mutation; both had normal echocardiograms and ECGs at 30 (mother) and 38 (father) years. Family history is remarkable only for a shared parental uncle who died suddenly at 30 years of suspected drowning. Prenatally, the couple was counselled that the presence of MYBPC3 mutation in the heterozygous state would likely result in mild to moderate HCM with later onset. Fetal echocardiograms at 15 and 20 weeks gestation were normal. The pregnancy and delivery were unremarkable. A postnatal screening echocardiogram at 3 weeks revealed DCM with a left ventricular ejection fraction of 36%. Postnatal sequencing of 19 DCM related genes, metabolic studies and karyotype were normal, aside from the known MYBPC3 heterozygous mutation. This case highlights that caution is needed when predicting the phenotype using prenatal genotype. Given that both heterozygous and homozygous individuals in this family presented with DCM in infancy, it is unlikely that the MYBPC3 familial mutation is the sole cause of DCM in this family; other modifying genes may play a role. Future genetic testing may reveal other currently unknown DCM predisposing gene mutation(s) that either alone or in conjunction with the MYBPC3 variant causes DCM.

342T

Effects of common and rare genetic variants of *APOC4* on HDL-cholesterol levels. *Z.H. Radwan[1], F.Y. Demirci[1], J.E. Hokanson[2], R.F. Hamman[2], C.H. Bunker[3], C.M. Kammerer[1], M.I. Kamboh[1].* 1) Human Genetics, GSPH, Univ Pittsburgh, Pittsburgh, PA; 2) Epidemiology, Colorado School of Public Health, Univ Colorado Denver, Aurora, CO; 3) Epidemiology, GSPH, Univ Pittsburgh, Pittsburgh, PA.

Coronary heart disease (CHD) is a major public health problem in Western countries as it continues to be a leading cause of premature mortality and morbidity. Dyslipidemia with low high-density lipoprotein cholesterol (HDL-C) and high low-density lipoprotein cholesterol (LDL-C) is one of the CHD risk factors. *APOC4* belongs to the *APOE/C1/C4/C2* gene cluster on chromosome 19q13 that has been implicated in regulation of different lipid traits in several studies. As compared to other members of this cluster, *APOC4* received relatively little attention in those studies. The primary aim of this study was to identify both common and rare variants in *APOC4* and flanking regions by resequencing about 5 kb-long genomic fragment in U.S. non-Hispanic White (NHW) and African Black individuals with HDL-C levels in the upper and lower 5th percentiles and examine their effects on HDL-C and other lipid levels. The analysis of sequencing data from individuals with HDL-C levels in the upper 5th percentile (47 NHWs and 48 African Blacks) and those with HDL-C levels in the lower 5th percentile (48 NHWs and 47 African Blacks) identified a total of 64 variants (substitutions or indels) plus one microsatellite, of which 13 were shared by both populations. Of 26 variants observed in NHWs, 12% were located in exons, 12% were indels, and 50% had ⁄ 5% minor allele frequency (MAF). Of 51 variants observed in African Blacks, 12% were in exons, 8% were indels, and 41% had ⁄ 5% MAF. All 3 exonic variants in NHWs and 4 out of 6 exonic variants in African Blacks were predicted to cause non-synonymous amino acid changes. Among NHWs, 31% of the individuals in the low HDL-C group had rare or relatively less common variants (MAF<5%) versus only 10% of those in the high HDL-C group. On the other hand, a reverse trend was observed in the Black sample (46% in the low HDL-C group versus 54% in the high HDL-C group). Screening of common variants in the entire sample set (623 NHWs and 788 Blacks) revealed significant or borderline associations with various lipid traits.

343T

G protein coupled Receptor Kinase 5 protein polymorphism and expression in Indian patients with Cardiac failure. *S. Ramalingam[1], T. Sairam[1], S. Ranjani[1], S. Narayanan[2], G. Rajendiran[2], J.S. Bhuvaneswaran[2], R. Sankaran[1].* 1) PSG Center for Molecular Medicine and Therapeutics , PSGInstitute of Medical Sciences and Research,Avanashi Road, Coimbatore, Tamilnadu, India; 2) Department of Cardiology,PSG Institute of Medical Sciences and Research,Avanashi Road, Coimbatore,Tamilnadu,India.

Purpose: Cardiovascular diseases are increasingly recognized as a major cause of mortality and morbidity globally and more so in the developing countries. Heart failure is a common consequence of coronary heart disease and with the increasing prevalence of heart diseases in India, cardiac failure has become one of the most important public health problems. Recent evidences reveal a protective effect of the GRK5Leu41 polymorphism in patients with cardiac failure. Studies have also shown that the expression of GRK5 in peripheral leucocytes is higher in mice models with cardiac failure and can be a surrogate marker to estimate the cardiac GRK expression. There are no studies in Indian population till date which looks at GRK5 polymorphism or its expression in cardiac failure patients. We studied the association of severity of cardiac failure with GRK5Leu41polymorphism and expression of GRK5 in peripheral blood in patients with cardiac failure. **Methods:** In this ongoing study we have recruited 32 patients with cardiac failure with class III or IV according to the New York Heart Association criteria. All patients were evaluated by a cardiologist and blood was collected for RNA and DNA isolation. Amplification was done using tetra primers and the SNP was detected using RFLP method. RNA (DNAase treated) was quantified using NANODROP 2000 and 500ng of RNA was used to synthesize cDNA using USB First strand cDNA Synthesis Kit (USB, USA) for Real Time PCR. **Results:** We present our interim results of this ongoing study. Of the 32 patients with cardiac failure, one patient had a homozygous GRK5Leu41 polymorphism and three had a heterozygous polymorphism respectively. The homozygous variant and two heterozygous variants presented with class III disease and one patient with heterozygous variant had class IV disease. The expression studies were carried out in 14 patients and there was an up regulation of GRK5 in 12 patients and down regulation in two patients. Those with downregulation had Class III symptoms with a mean duration of illness of 6 months, while those with upregulation had Class III (4patients) and class IV (8 patients), and a mean duration of illness of 2.6 years. The results of this study are consistent with physiological mechanisms operational in cardiac failure, and represent a study first of its kind in India.

344T

Genome-wide analysis of variability in normal cardiac conduction across multiple electronic medical record systems. *M.D. Ritchie[1], R.L. Zuvich[1], J.C. Denny[2, 3], D.C. Crawford[1], J.S. Schildcrout[4], A.H. Ramirez[2], J.M. Pulley[5], M.A. Basford[5], C.G. Chute[6], I.J. Kullo[6], C.A. McCarty[7], R.L. Chisholm[8], A.N. Kho[8], E.B. Larson[9, 10], G.P. Jarvik[9, 11], CHARGE. QRS GWAS Consortium[12], R. Li[13], D.R. Masys[3], J.L. Haines[1], D.M. Roden[2, 5].* 1) Molec Physiology & Biophysics, Center for Human Genetics Research, Vanderbilt Univ, Nashville, TN; 2) Department of Medicine, Vanderbilt Univ, Nashville TN; 3) Department of Biomedical Informatics, Vanderbilt Univ, Nashville, TN; 4) Department of Biostatistics, Vanderbilt Univ, Nashville, TN; 5) Office of Personalized Medicine, Vanderbilt Univ, Nashville, TN; 6) Department of Medical Informatics, Mayo Clinic, Rochester, MN; 7) Center for Human Genetics, Marshfield Clinic Research Foundation, Marshfield, Wisconsin; 8) Department of Medicine, Northwestern University, Chicago, IL; 9) Group Health Research Institute, Seattle WA; 10) Department of Medicine, University of Washington, Seattle, WA; 11) Department of Genome Sciences, University of Washington, Seattle, WA; 12) International Consortium; 13) NHGRI, NIH, Bethesda, MD.

The duration of the QRS interval, an index of conduction in the cardiac ventricle, varies ~2-fold in normal adults without cardiac disease. We used electronic medical records (EMRs) coupled to DNA samples to identify the genomic determinants of normal QRS duration in the electronic MEdical Records & GEnomics (eMERGE) network. The electronic phenotyping algorithm for normal QRS duration identified 6126 individuals of European-descent in five EMR systems across the network. These individuals had a first recorded ECG read as normal with no confounding heart disease, medications, or electrolyte abnormality at that time. After quality control, genome-wide association (GWA) analysis on 5272 individuals identified 108 QRS-associated SNPs with P values <10-5. Each of these were examined in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) QRS GWAS consortium meta-analysis, and 23 replicated with P values <1.1x10-11, while the remaining 85 had P values >0.003. Eighteen of the 23 replicating SNPs were in the chromosome 3 SCN5A-10A locus. The top SNPs/loci included rs6800541 in SCN10A with pE<8.54x10-6 (eMERGE) and pC<1.88x10-27 (CHARGE), rs2207790 in NFIA with pE< 8.70x10-5 and pC<6.31x10-18, and rs1321313 near CDKN1A with pE<8.61x10-5 and pC<4.60x10-25. To investigate potential pleiotropic effects of the 18 SCN5A-10A SNPs we conducted phenome-wide association (PheWAS) analysis examining clinical associations between these 18 SNPs across 744 diagnostic codes in the full eMERGE European-descent dataset (n=13,859). We identified diagnostic codes for atrial fibrillation and for cardiac arrhythmias (by definition absent at the time of index ECG recording) as the most common associated diagnoses. This result indicates that analyzing normal ECGs not only identified genomic predictors of normal conduction, but also allowed us to analyze clinical events in the EMR after the index ECG was recorded. We conclude that DNA banks coupled to EMRs not only provide a platform for GWAS, but also identify genotype-associated diagnoses in an unbiased fashion. A PheWAS approach can identify clinical conditions associated with SNPs of interest and in this case, demonstrated that sodium channel variants that influence QRS duration are also associated with arrhythmia outcomes.

345T

Antagonism of GxxPG-Fragments Ameliorates Manifestations of Aortic Disease in Marfan Syndrome Mice. *P.N. Robinson[1,2], G. Guo[1], B. Muñoz-García[1], C.E. Ott[1], J. Grünhagen[1], S. Mousa[3], A. Pletschacher[1], Y. von Kodolitsch[4], P. Knaus[5].* 1) Institute for Medical Genetics, Charité-Universitatsmedizin Berlin, Berlin, Germany; 2) Max Planck Institute for Molecular Genetics, Berlin, Germany; 3) Department of Anesthesiology and Operative Intensive Care Medicine,Charité-Universitätsmedizin Berlin, Berlin, Germany; 4) Centre of Cardiology and Cardiovascular Surgery, Department of Cardiology/Angiology, University Hospital Hamburg-Eppendorf, Hamburg, Germany; 5) Institut für Chemie und Biochemie, Freie Universität Berlin, Berlin, Germany.

Marfan syndrome (MFS) is an inherited disorder of connective tissue caused by mutations in the gene for fibrillin-1 (FBN1), with prominent clinical manifestations in the cardiovascular, skeletal and ocular systems. The complex pathogenesis of MFS involves changes in TGF) signaling, increased matrix metalloproteinase (MMP) expression, tissue fragmentation, and abnormal matrix-cell interactions. Fibrillin-1 and elastin have repeated Gly-x-x-Pro-Gly (GxxPG) motifs that can induce a number of effects including macrophage chemotaxis and increased MMP activity by induction of signaling by the elastin-binding protein (EBP). We have previously shown that aortic extracts from the mgR/mgR mice as well as a GxxPG-containing fibrillin-1 fragment significantly increased macrophage chemotaxis compared with extracts from wild-type mice or buffer controls. Fibrillin-1 underexpressing mgR/mgR mice were treated with weekly i.p. injections of the monoclonal antibody BA4 directed against elastin and fibrillin-1 GxxPG-containing fragments beginning at the third week of life and continuing over 8 weeks at a dose of 1 mg/kg, 5 mg/kg or 10 mg/kg. Following treatment, mice were sacrificed and compared to control antibody IgG treated mgR/mgR and wild type littermates. Treatment with BA4 at dosages as low as 1 mg/kg could already rescue elastin degeneration in mgR/mgR mice. However, no effects could be observed in IgG treated group. BA4 treatment additionally significantly reduced MMP-2, MMP-9, and pSmad2 activity, as well as fragmentation and macrophage infiltration in the aorta of the mgR/mgR mice. Primary cultured aortic vascular smooth muscle cells (vSMCs) were investigated for constitutive pSmad2 levels; there was a significant reduction of the constitutive pSmad2 activation in aortic vSMCs from the BA4 treated mgR/mgR mice. Pulmonary emphysema has been previously shown to develop in mgR/mgR mice. Treatment with BA4 prevented airspace enlargement and significantly reduced pSmad2 signaling. An additional treatment arm with the NSAID indomethacin similarly attenuated MMP and pSMad2 activity, macrophage infiltration, and fragmentation in the mgR/mgR aorta. Our findings emphasize the important role of secondary cellular events caused by GxxPG containing fragments and matrix-induced inflammatory activity in the pathogenesis of aortic aneurysm in mgR/mgR mice and suggest that antagonism of the effects of GxxPG fragments may be a fruitful therapeutic strategy in MFS.

346T

Pharmacogenetic of warfarin in Iranian patients (Polymorphisms Spectrum of VKORC1 and CYP2C9). *S. Saber[1,4], O. Aryani[1], F. Ghasemi[1], A.F. Fazelifar[3], M. Haghjoo[3], R. Kia[4], M. Houshmand[2].* 1) Dept Medical Genetics, Special Medical Center, Tehran, Iran; 2) National istitude for genetic engineering and biotechnology,Tehran,Iran; 3) Rajaie Cardiovascular Medical and Research Center, Tehran, Iran; 4) Sina Special Medical Center,cardiology ward,Tehran,Iran.

Background: Personalized medicine is a medical model emphasizing the systematic use of information about an individual patient to select or optimize that patient's preventative and therapeutic care. Pharmacogenetic is the field of study that examines the impact of genetic variation on the response to medications. Warfarin has been widely used for the prevention and treatment of thromboembolism. Warfarin therapy depends on interaction between physiological, environmental, and genetic factors. The Warfarin has narrow therapeutic index and widely variable therapeutic dose. Cytochrome P450 2C9 (CYP2C9) enzyme and Vitamin K epoxide reductase (VKORC1) conjointly determine the warfarin maintenance dose. Single Nucleotide Polymorphisms (SNPs) of these genes can cause to different amount of warfarin for each patients. Allele *1 is wild type and allele *2 (430A>C) 3*(1075A>C) are two well none SNPs that correlated to reduce warfarin dose at least 22% and 34% respectively. In the VKORC1 1639 (or 3673) SNP, the common G allele is replaced by the A allele. Because people with an A allele need to reduce warfarin dose at least 30%. Material and Methods: We checked index patients who need warfarin due to clinical disorders. Polymerase Change Reaction (PCR) was done on DNA samples and those mention SNPs were evaluated by Reverse Dot Blot method. Results: In our study prevalence of allele *1, 2*and 3* were 76.47%, 11.76 %and 11.76%, respectively. Initial doses were estimated by warfarindosing site and INR has been rechecked (international normalized ratio) for controlling the initial dose. Estimation of warfarin dosing in 45% of patients receive to INR: 2.5 after take the initial dose. 15% of patients need to higher dose and 40% need to lower dose than initial dose but we haven't received any reports of side effects in these patients. Conclusion: Prevalence of allele *2 and *3 is equal and it was 11.76%, in our study and 26.74 % of our group were carried A allele. Comparing with Caucasians was reported 10% and 6% for allele *2 and *3 and 37% of Caucasians carrying the A allele. We must expand our group and other relative genes for achieving to the best result in Iranian population, these studies are on the rest. Importance of pharmacogenetic has been shown for finding the best initial dose of warfarin. Further collaboration between physicians and geneticists need to improve well-time diagnostics, optimal treatment.

347T

Do ST-elevation and non-ST-elevation myocardial infarction have different genetic backgrounds? *P. Salo[1], J. Sinisalo[2], T. Hiekkalinna[1,3], J. Kettunen[1,3], A. Havulinna[4], M. Lokki[5], S. Ripatti[1,3], V. Salomaa[4], M. Nieminen[2], M. Perola[1,3,6].* 1) Public Health Genomics Unit, National Institute For Health And Welfare, Helsinki, Finland; 2) Division of Cardiology, Department of Medicine, Helsinki University Central Hospital, Finland; 3) Institute for Molecular Medicine Finland FIMM, University of Helsinki, Finland; 4) Chronic Disease Epidemiology and Prevention Unit, Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland; 5) Transplantation laboratory, Haartman Institute, University of Helsinki,Finland; 6) The Estonian Genome Center, University of Tartu, Estonia.

The most robustly replicated common risk allele for acute coronary syndrome (ACS) at 9p21.3 affects gene expression rather than the structure of a protein. This is consistent with the idea that differences in gene expression might explain a significant part of the genetic risk for common diseases. It is also believed that the genetic background differs for different subcategories of complex phenotypes. Taken together, this led us to test two hypotheses: First, many common expression quantitative trait loci (eQTL) are associated with ACS. Second, the effects of these loci are not equal for two subtypes of ACS, namely ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI).

We performed a GWAS in a sample consisting of acute coronary syndrome (ACS) patients and healthy controls (n=3373). We selected SNP's annotated as eQTL's in a public database and used non-parametric statistical methods to compare them with unlinked non-eQTL SNP's matched for allele frequency and proximity to the closest gene.

eQTL SNP's are more strongly associated with ACS than non-eQTl SNP's. This effect is significantly stronger for NSTEMI than for STEMI. The disparity is driven by eQTL SNP's at the MHC locus on 6p21.3. Exclusion of this locus diminishes the difference between eQTL and non-eQTL SNP's for NSTEMI and ACS.

Our results add to the notion that common differences in gene expression influence the risk for developing ACS. We show that the risk conferred by some eQTL's is conditional on a clinically relevant classification of ACS and identify a locus contributing to this difference. Furthermore, the results specifically suggest genetic variation affecting immune system function provides more risk for NSTEMI than for STEMI.

348T

Increased urinary globotriaosylceramide and previously undiagnosed Fabry patients are found in a non-selected heart disease patient population. *R. Schiffmann[1], S. Forni[1], C. Swift[1], X. Wu[2], D. Lockhart[2], S. Pond[3], M. Chee[3], K. Goss[4], K. Sims[4], E. Benjamin[2], L. Sweetman[1].* 1) Inst Metabolic Disease, Baylor Res Inst, Dallas, TX; 2) Amicus Therapeutics, Cranbury, NJ; 3) Prognosys Biosciences, Inc., La Jolla, CA; 4) Neurogenetics DNA Diagnostic Laboratory, Massachusetts General Hospital, Boston, MA.

We have initiated a large screening research protocol for the Fabry disease biochemical and genetic trait in a non-selected at-risk population of patients with cardiac disease. We hypothesized that (-galactosidase A deficiency is a genetic risk factor for non-specific cardiovascular complications that commonly occur in the general population. The verification of our hypothesis will lead to confirmation of (-galactosidase A deficiency due to *GLA* mutations as a general modifiable cardiovascular risk factor, allowing for better management and prevention of cardiac disease. Screening is performed by measuring urinary globotriaosylceramide (Gb3) in random samples of whole urine using tandem MS, measuring (-galactosidase A activity in dried blood spots, and looking for *GLA* gene mutations by parallel sequencing of the whole gene supplemented by conventional Sanger sequencing. Cardiac patients and non-cardiac controls are being tested. Thus far we have consented and sampled 1066 patients with a wide variety of cardiac abnormalities, the vast majority with coronary artery disease, and 185 controls. Urinary Gb3 in cardiac patients was 136±150 ng/mL (N=981) and 94±39 ng/mL (N= 166) in the controls (p<0.0001) - Gb3 upper limit of normal (99th percentile) is 200 ng/mL. In the first 60 patients sequenced we identified two women aged 35 and 72 years with *GLA* mutation (R118C and D83N). Both had normal urinary Gb3 levels and (-galactosidase A activity. Supraventricular tachycardia and atrial fibrillation were their primary cardiac abnormalities but also valvular disease and renal insufficiency in the older patient. We conclude first that urinary Gb3 is elevated in the general population of cardiac patients, the significance of which is currently being evaluated. However, urinary Gb3 is not an adequate screening biomarker for Fabry disease in this population because it is elevated in 15% of cardiac patients and overlaps with levels seen in patients with Fabry disease. Secondly, the general cardiac disease population may be significantly enriched in previously undiagnosed individuals with Fabry disease.

349T

Metabochip meta-analysis of > 190,000 individuals reveals 77 novel loci associated with blood lipid levels. *S. Sengupta[1], E.M. Schmidt[2], Global Lipids Genetics Consortium.* 1) Department of Biostatistics, University of Michigan, Ann Arbor, MI; 2) Bioinformatics Graduate Program, University of Michigan, Ann Arbor, MI.

The identification of genetic variants associated with lipid traits, which are heritable risk factors for cardiovascular disease, will give valuable insight into the biological basis of disease and may highlight new drug targets for cholesterol management or disease prevention. To date, genome-wide screening for common variants in >100,000 individuals of European ancestry has identified more than 95 loci significantly associated with various blood lipid phenotypes. Using a sample size of 99,137 individuals which nearly doubles the size of the original discovery setting, we conducted a two stage meta-analysis aimed at discovering additional lipid associated variants.

Plasma concentrations of the 4 blood lipid traits were measured in individuals from 39 cohorts, mainly of European ancestry. Individuals were genotyped for approximately 200,000 single nucleotide polymorphisms (SNPs) selected from genome wide meta-analyses of metabolic or cardiovascular traits on the Metabochip, a custom Illumina iSelect genotyping array. We carefully selected 4,763 SNPs for follow-up of high-density lipoprotein cholesterol (HDL-C), 4,780 for low-density lipoprotein cholesterol (LDL-C), 714 for total cholesterol (TC), and 4,759 for triglycerides (TG), as well as 28,927 specifically for fine mapping. By jointly meta-analyzing Metabochip results with our previous genome-wide association analysis of 23 cohorts, we identified 77 novel loci significantly associated with at least one of four lipid traits ($P<5 \times 10^{-8}$) in an interim analysis of 190,434 individuals. Of these 77 loci, 10 demonstrated a genome-wide significant association with TC, 18 with TG, 14 with LDL-C, and 35 with HDL-C. Highlights among our results include association between lipid traits and SNPs in a lipoprotein receptor gene, VLDLR ($P=9.4 \times 10^{-12}$ and $P=2.5 \times 10^{-11}$ for TC and LDL-C respectively) as well as VEGFA ($P=1.2 \times 10^{-14}$ for TG), implicated in atherogeneis, Vimentin ($P=2.0 \times 10^{-08}$ for TC), a filament responsible for transport of LDL-C from the lyosome for esterification, COPB1 ($P=4.6 \times 10^{-08}$ for HDL-C), involved in movement of lipid components to the endoplasmic reticulum, and PRKAG1 ($P=8.1 \times 10^{-09}$ for TC), responsible for inactivating HMGCR. Downstream analyses will include characterizing the proportion of variance explained by these loci and analyzing low frequency variation in previously identified loci and across the genome.

350T

Genomic and epigenetic alterations in heart development tissue of congenital heart defects. *C. Serra-Juhé[1, 2], I. Cuscó[1, 2], B. Rodríguez-Santiago[1, 3], T. Vendrell[4], F. Borràs[4], N. Torán[4], L.A. Pérez-Jurado[1, 2].* 1) Unitat de Genetica, Universitat Pompeu Fabra, Barcelona, Spain; 2) Centro de Investigación Biomédica en Red de Enfermedades Raras, CIBERER, ISCIII, Spain; 3) Quantitative Genomic Medicine Laboratories, Ltd (qGenomics), Barcelona, Spain; 4) Hospital Universitari Vall d'Hebron, Barcelona, Spain.

The mechanisms underlying the majority of sporadic and isolated congenital heart defects (CHD) are poorly understood. Accumulation of multiple genetic, genomic and/or epigenetic variants, along with possible somatic events, is thought to converge into deregulation of developmental networks leading to a morphologically abnormal heart. About 40% of patients with Down syndrome (DS) present CHD, implying the presence of additional risk factors other than the T21 in the etiology of this malformation. With the aim of identifying genomic and epigenetic alterations involved in CHD, we studied heart tissue samples of 33 fetuses with isolated CHD (ICHD) and 6 with DS and CHD by chromosome microarray analysis. In addition, samples of each group were screened for epimutations, using two groups (DS without CHD and normal fetuses) as controls. Methylation status was studied in 27,000 CpG using the Illumina Infinium Human Methylation Platform.

Potentially pathogenic CNVs were identified in 6 samples with ICHD but none in the other groups (other than T21 in DS). Those included a single *de novo* event (deletion of 0.36 Mb containing FOX gene cluster) and five rearrangements inherited from a normal parent: the recurrent 15q13.3 deletion, deletions in 13q21, 16q23 and 14q23 and one duplication in 5q35. Interestingly, all genomic abnormalities were identified in isolated left heart hypoplasia. Regarding the epigenomic studies, a total of 18 regions were identified as being differentially methylated (p<0.01) in CHD samples compared to the control group. A single region surrounding the GATA4 gene was found relatively hypermethylated in all patient samples. The remaining differentially methylated regions were present in either single or few samples, such as a region in 14q31 hypermethylated in two DS with CHD. Other tissues are being studied to determine whether the methylation marks are or not tissue-specific. Several genes potentially deregulated are good candidates for CHD based on predicted function or previously described animal models.

Our data provide additional dosage sensitive genes responsible for CHD, mainly left heart hypoplasia, and strongly suggest a relevant role of epigenetic alterations in CHD, either as the main cause of the malformation or as a modifier such as in DS.

351T

Novel identification of genetic risk factors in Koreans reveals association of PITX2 gene with lone atrial fibrillation. *D. Shin[1,3], A. Park[1], H. Hwang[2], N. Son[1,4], B. Park[1,4], J. Kwon[1], J. Lim[1], E. Shin[5], J. Lee[5], B. Joung[2], M. Lee[2], S. Kim[2], Y. Jang[1,2].* 1) Cardiovascular Genome Center, Yonsei Univrsity College of Medicine, Seoul, South Korea; 2) Division of Cardiology, Yonsei University Health System; 3) Yonsei University Research Institute of Science for Aging; 4) Department of Biostatistics, Yonsei University College of Medicine; 5) DNA Link Inc, Seoul, South Korea.

Atrial fibrillation (AF) is the most frequently and seriously encountered cardiac rhythm disorder in humans and is characterized by chaotic electrical activity of the atrial. Recent evidence indicates the phenotypes of lone AF are more likely to occur in patient with a genetic susceptibility. The PITX2 gene has a pivotal function in cardiac development and may be one underlying mechanism for AF development. The PITX2 gene expression identifies the left atrium during embryonic development, and also plays a crucial role in the differentiation, proliferation, and expansion of pulmonary myocardial cells. We examined a case-control study for a total of 754 Korean subjects, comprising of 354 cases with AF and 400 controls. Genotyping for variants was carried out by direct DNA sequencing and TaqMan® assay. We examined 5 tagging single nucleotide polymorphisms (tSNPs) in the PITX2 gene. We observed the significant differences in the allele and genotype distributions of rs2739200 and rs62338989 between patients with AF and control subjects (P<0.01). Logistic regression analysis represented that tSNPs, rs2739200 (-4257C/G) and rs62338989 (-4840C/T), in the promoter region confer the risk of lone AF. The significant associations were observed under a recessive model for GG genotype with a decreasing risk of AF (OR, 0.530; 0.378-0.744; p=0.0002). Whereas, PITX2 rs62338989 showed significant association in Korean AF condition under a dominant model with an increasing risk (OR, 1.885; 1.200-2.961; p=0.006). In haplotype analysis, haplotype CTCC was significantly associated with AF susceptibility (OR, 1.71; 1.246-2.347; p=0.0009). We provide evidence that PITX2 novel variants may contribute to protect or predispose Korean individuals to the risk of AF.

352T

A comprehensive approach assessing the contribution of polygenic variation to risk of cardiovascular disease. *M.A. Simonson[1,2], A.G. Wills[1], M.B. McQueen[2], M.C. Keller[1].* 1) Psychology and Neuroscience, Institute for Behavioral Genetics, Boulder, CO; 2) Integrative Physiology, Institute for Behavioral Genetics, Boulder, CO.

Background: Traditional genome-wide association studies are generally limited in their ability explain a large portion of genetic risk for most common diseases. We sought to use both traditional GWAS methods, as well as more recently developed polygenic genome-wide analysis techniques to identify subsets of single-nucleotide polymorphisms (SNPs) that may be involved in risk of cardiovascular disease, as well as estimate the heritability explained by common SNPs.

Methods: Using data from the Framingham SNP Health Association Resource (SHARe)., three complimentary methods were applied to examine the genetic factors associated with the Framingham Risk Score, a widely-accepted indicator of underlying cardiovascular disease risk. The first method adopted a traditional GWAS approach - independently testing each SNP for association with the Framingham Risk Score. The second two approaches involved polygenic methods with the intention of providing estimates of aggregate genetic risk and heritability.

Results: While no SNPs were independently associated with the Framingham Risk Score based on the results of the traditional GWAS analysis, we were able to identify cardiovascular disease-related SNPs as reported by previous studies. A predictive polygenic analysis was only able to explain approximately 1% of the genetic variance when predicting the 10-year risk of general cardiovascular disease. However, 20% to 30% of the variation in the Framingham Risk Score was explained using a recently developed method that considers the joint effect of all SNPs simultaneously.

Conclusion: The results of this study imply that common SNPs explain a large amount of the variation in the Framingham Risk Score and suggest that future, better-powered genome-wide association studies will uncover more risk variants that will help to elucidate the genetic architecture of cardiovascular disease.

353T

Interaction between GRK4 and BMI affects blood pressure. *R.S. Sobota[1], C.D. Schoeffel[2], R.M. Carey[2], H.E. McGrath[2], L.N. Gordon[1], M.J. Park[1], P.A. Jose[3], R.A. Felder[2], S.M. Williams[1].* 1) Human Genetics, Vanderbilt University, Nashville, TN; 2) Endocrinology and Metabolism, University of Virginia, Charlottesville, VA; 3) Pediatrics and Medicine, George Washington University, Washington, DC.

Essential hypertension is defined as high blood pressure with an unexplained etiology. Hypertension has numerous sequelae including predispositions to stroke, myocardial infarction, and aortic dissection The heritability estimates for this phenotype range from 30 to 40% and are believed to be a result of multiple genetic influences in the context of numerous environmental insults. Because of the high heritability and known physiological pathways that can affect blood pressure regulation we performed an association study between hypertension and 41 single nucleotide polymorphisms (SNPs) in 19 candidate genes from a Caucasian population that included 550 individuals. After genotyping we excluded SNPs with minor allele frequencies less than 10%. This left 23 polymorphisms in 15 candidate genes for subsequent analysis. Data on standard environmental phenotypes of gender, age and BMI were also collected for gene X environment modeling as well as epistasis analysis. Analyses were done using hypertension as a dichotomous trait, defined by systolic blood pressure more than 120 and/or diastolic blood pressure more than 80 or a previous diagnosis of hypertension. Blood pressure was also analyzed as a continuous trait for those individual who were not on anti-hypertensive medication (n= XXX). All logistic and linear models were adjusted for the gender, age and BMI. Logistic regression of hypertension status resulted in 4 SNPs being significant at the p <0.1 level, segregating in 2 genes (SNX19 and SLC4A5). Six of the 23 SNPs were significant at the 0.10 level in linear regression of both systolic and diastolic values (CAV1, DRD2 and SNX19). Explicit interactions between the genotypes and BMI were modeled with both logistic regression, which identified 3 significant SNPs (p < 0.1), and linear regression for SBP and DBP, 7 SNPs <0.1 (6 <0.05). Of note all 3 GRK4 SNPs assayed (R65L, A142V and A486V) showed a highly significant interaction term with BMI (P <0.01 in all cases). We identified an excess of associations over that expected by chance alone across all of our genes analyzed and our results identified an explicit interaction between GRK4 SNPs and BMI with blood pressure as a continuous trait. This result indicates that the action of GRK4 is BMI dependent, supporting the hypothesis that although genetics plays an important role in blood pressure regulation, its effects are strongly context dependent.

354T

Scanning whole exomes for variants associated with arrhythmogenic right ventricular cardiomyopathy. *L.F. Tang[1], K.J. White[1], J. Pons[1], J. Wojciak[2], J. Carroll[1], J. Olgin[2,5], R.L. Nussbaum[1,2,3], M.M. Scheinman[2,5], P.Y. Kwok[1,3,4].* 1) Cardiovascular Research Institute, University of California, San Francisco; 2) Department of Medicine, University of California, San Francisco; 3) Institute of Human Genetics, University of California, San Francisco; 4) Department of Dermatology, University of California, San Francisco; 5) Cardiac Electrophysiology, University of California, San Francisco.

Arrythmogenic Right Ventricular Cardiomyopathy (ARVC), a condition that often results in sudden cardiac death, is characterized by the loss of right ventricular cardiomyocytes and their replacement by fibrofatty tissue. Recent advances in genetics and identification of genetics mutations have improved our understanding of sudden cardiac death but ARVC remains a significant cause of death in the United States. At the Comprehensive Arrhythmia Genetics Center at UCSF, a diagnosis of ARVC was made on clinical and pathological grounds based on established ARVC task force criteria. As an initial genetics screen, we sequenced 32 genes (with 382 amplicons spanning 443 exons and the promoters, totalling 185 kB of sequence) that are known to cause or might be associated with ARVC. At present, we have identified 191 variants (21 novel) in this set of 32 genes. In addition, we sequenced the whole exomes of ARVC patients without deleterious mutations in common ARVC susceptibility genes. In all, we captured and sequenced 201,121 exons from 20,794 genes as well as UTRs and miRNAs using the Illumina HiSeq 2000 platform. Confident variant calls were obtained for each individual with approximately 35,000 SNPs found in the targeted regions at 40X average coverage. Around 2,000 novel variants were found by this high throughput sequencing approach. Further analysis is now under way to identify variants that are likely to be causative for ARVC. This study allows a comprehensive catalog of genetic variants for future diagnostic purpose.

355T

Apolipoprotein B Synthesis Inhibition by Mipomersen Reduces LDL-C When Added to Maximally Tolerated Lipid-Lowering Medication in Patients with Severe Heterozygous Hypercholesterolemia. *J. Tardif[1], R. Ceska[2], L.J. Burgess[3], H. Soran[4], I. Gouni-Berthold[5], G. Wegener[6], S. Chasan-Taber[6], M. McGowan[7].* 1) MHI Research Center, Montreal Heart Institute, Montreal, Quebec, Canada; 2) Center for Preventive Cardiology, General Teaching Hospital and First Medical Faculty of Charles University, Prague, Czech Republic; 3) Stellenbosch University and Tygerberg Hospital, Parow, South Africa; 4) Central Manchester Foundation Trust, University of Manchester, Manchester UK; 5) Department of Internal Medicine II, University of Cologne, Cologne, Germany; 6) Genzyme Corporation, Cambridge, MA; 7) Cholesterol Treatment Center, Concord Hospital, Concord, NH.

Synopsis: In 2 prior studies, mipomersen (MIPO) 200 mg sc weekly reduced LDL-C in patients with homozygous and heterozygous familial hypercholesterolemia (HeFH) by 25% and 28% respectively when added to lipid lowering therapies (LLTs), including high-dose statins. Patients with severe hypercholesterolemia (SH) often fail to normalize LDL-C despite potent LLTs. **Objective:** This randomized, double-blind, placebo-controlled, multi-center, phase 3 trial examined the safety and efficacy of MIPO in patients with SH, >90% of whom were HeFH. **Methods:** Adults (≥ 18 years) with LDL-C ≥ 300 mg/dL or LDL-C ≥ 200 mg/dL with coronary heart disease or other forms of clinical atherosclerotic disease on a maximally tolerated statin and at least one other class of LLT were enrolled. Patients continued prescribed LLT regimen and were randomized (2:1) to MIPO 200 mg sc weekly or placebo (PBO) for a 26-week period. The primary end point was percent reduction in LDL-C from baseline to week 28 or 2 weeks after the last dose for those not completing dosing. **Results:** Fifty-eight patients were randomized (39 MIPO, 19 PBO) at 26 clinical centers in 6 countries. Forty-five (78%) patients completed the treatment period. Twelve (31%) MIPO and 1 (5%) PBO patient(s) withdrew (8 of the MIPO and the 1 PBO withdrawals were due to an adverse event [AE]). LDL-C was reduced by 36% from a mean baseline level of 276 mg/dL in the MIPO group vs. an increase of 13% from a BL of 249 mg/dL in the PBO group (p<0.001). This represents a 101 mg/dL mean reduction of LDL-C in the MIPO group. Similarly, MIPO produced highly significant (p<0.001) reductions in other atherogenic lipoproteins [e.g., apo B and Lp(a)]; there was no change in HDL-C. The most common on-treatment AEs were injection site reactions (90% MIPO, 32% PBO) and flu-like symptoms (46% MIPO, 21% PBO). Six (15%) MIPO patients and no PBO patients had ALT ≥ 3×ULN on consecutive measures at least 7 days apart. One MIPO patient had a transient ALT ≥ 10× ULN. ALT elevations were not associated with clinically significant increases in bilirubin. **Conclusions:** MIPO treatment significantly reduced LDL-C and apoB in patients with severe hypercholesterolemia. The data support the potential utility of MIPO as a pharmacologic option for additional LDL C lowering in patients with heterozygous hypercholesterolemia not adequately controlled under existing LLTs.

356T

Thoracic aortic disease in two patients with juvenile polyposis and hereditary hemorrhagic telangiectasia (JPS-HHT) due to *SMAD4* mutations. *P. Teekakirikul[1,3,4], D.M. Milewicz[2], D.T. Miller[5,7,10], R.V. Lacro[6,10], E.S. Regalado[2], A.M. Rosales[8,10], D.P. Ryan[9,10], T.L. Toler[1,10], A.E. Lin[1,10].* 1) Genetics Unit, MassGeneral Hospital for Children, Boston, MA; 2) Department of Internal Medicine, The University of Texas Health Science Center at Houston, Houston, TX; 3) Department of Genetics, Harvard Medical School, Boston, MA; 4) Cardiovascular Division, Brigham and Women's Hospital, Boston, MA; 5) Division of Genetics, Children's Hospital Boston, Boston, MA; 6) Department of Cardiology, Children's Hospital Boston, Boston, MA; 7) Department of Laboratory Medicine, Children's Hospital Boston, Boston, MA; 8) Pediatric Cardiology Unit, MassGeneral Hospital for Children, Boston, MA; 9) Department of Pediatric Surgery, MassGeneral Hospital for Children, Boston, MA; 10) Harvard Medical School, Boston, MA.

Background: Dilation or aneurysms of the ascending thoracic aorta can progress to acute aortic dissection (TAAD). Mutations in genes encoding proteins in the TGF-) signaling pathway cause both syndromes and inherited predispositions to TAAD, including *TGFBR1, TGFBR2* and *SMAD3*. *SMAD4* mutations are associated with both juvenile polyposis (JPS) and hereditary hemorrhagic telangiectasia (HHT), now viewed as a single JPS-HHT syndrome. A JPS-HHT family was recently reported to have TAAD and mitral valvulopathy. We report two additional patients with JPS-HHT and *SMAD4* mutations who also have thoracic aortic disease. **Case 1:** An 11 yo white male (BSA 1.29 m^2) without Marfan features had JPS and a *de novo SMAD4* mutation (c.1340_1367dup28). Echo showed mild-mod dilation of the aortic annulus (2.40 cm, Z-score +4.19) and aortic root (3.29 cm, Z +4.04, normal range 1.87-2.80), and mild dilation of the sinotubular junction (2.44 cm, Z +2.13) and ascending aorta (2.72 cm, Z +2.63). Cardiac CT confirmed aortic dilation without aortic tortuosity and tiny pulmonary AVMs. Medication was not prescribed; follow-up echo 7 mos later showed no progression in aortic dilation. Exercise test showed no arterial oxygen desaturation. He had one blanching lesion on his forearm and is being watched for possible HHT. **Case 2:** A 34 yo white female (BSA 2.0) with JPS-HHT and Marfan-like skeletal features was found to have a *SMAD4* mutation (c.1245_1248delCAGA); mutational analysis of *TGFBR1* and *TGFBR2* was negative (*FBN1* testing declined). She had a reduced upper/lower segment ratio, narrow palate, visible veins and multiple striae, pes planus, scoliosis, joint hypermobility, and arachnodactyly. Echo showed mild aortic root dilation. The annulus, root, sinotubular junction, and ascending aorta measured 2.0 cm, 3.8 cm, 3.2 cm, 3.5 cm, respectively. She subsequently developed a pulmonary AVM and hepatic focal nodular hyperplasia. Her father also had JPS-HHT with Marfan-like features and died of an acute aortic dissection at age 60. **Conclusion:** These two new cases confirm the association of thoracic aortic disease with JPS-HHT resulting from *SMAD4* mutations. Case 2's affected father died of an acute aortic dissection. Thus, we propose that the thoracic aorta should be screened and monitored in patients with *SMAD4* mutations to prevent untimely deaths due to dissection. This report also confirms *SMAD4* as another protein in the TGF-) signaling pathway that, when mutated, predisposes to TAAD.

357T

Functional Relationship of the COL4A1/COL4A2 Locus on Chromosome 13q34 to Coronary Artery Disease (CAD). *A. Turner, P. Lau, O. Jarinova, R. McPherson.* University of Ottawa Heart Institute, Ottawa, Canada.

Type IV collagen triple helices constitute the major structural component of basement membranes, consisting primarily of 2 COL4A1 chains arranged with 1 COL4A2 chain. *COL4A1* and *COL4A2* also have important functional roles in angiogenesis, and mutations are associated with diverse vascular abnormalities. The *COL4A1* and *COL4A2* genes on chromosome 13 are arranged in a head-to-head conformation, and uniquely share a common, bidirectional promoter. In the Ottawa Heart Study (OHS) and as part of CARDIoGRAM, a large meta analysis of genome-wide association studies for CAD (>22,000 CAD cases & >64,000 controls), we identified the COL4A1/COL4A2 locus as one of 13 novel regions associated with CAD (Nature Genetics 2011). The index SNP at this locus (rs4773144) has a minor allele frequency of 0.4 and is associated with an increased risk of CAD (allele specific odds ratio=1.21 in OHS). In a search for functional genetic variants, we resequenced the bidirectional *COL4A1/COL4A2* promoter in 500 CAD cases and 500 controls and identified four novel SNPs, in promoter/enhancer regions essential for *COL4A1* and/or *COL4A2* gene expression and in strong linkage disequilibrium with several OHS risk SNPs. In a luciferase assay, the risk allele for the rs35466678 SNP, in the 5'UTR of *COL4A2*, reduced promoter activity by 13% ($p<0.005$). Furthermore, the linked risk alleles for the rs7327528 and rs117410570 SNPs, both within an enhancer essential for *COL4A2* transcription, reduced *COL4A2* promoter activity by 17% ($p<0.0005$). Reduced *COL4A2* promoter activity *in vitro* due to these SNPs will be investigated further to determine effects on protein levels and basement membrane structure/function. These findings are important because misregulation of *COL4A1* and *COL4A2* could have important consequences relevant to CAD, including effects on basement membrane integrity and angiogenesis.

358T

GLUT10 connects TGF) signaling to cellular metabolism in cardiovascular development. *Z. Urban[1,2], A. Willaert[2,3], S.M. Khatri[1], B.L. Callewaert[2,3], P.J. Coucke[3], S.D. Crosby[4], B.L. Loeys[3], M. Tsang[5], A. De Paepe[3].* 1) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Department of Pediatrics, Washington University School of Medicine, St. Louis, MO; 3) Department of Medical Genetics, Ghent University Hospital, Ghent, Belgium; 4) Department of Genetics, Washington University School of Medicine, St. Louis, MO; 5) Department of Developmental Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Growth factor signaling results in dramatic phenotypic changes in cells, which require commensurate alterations in cellular metabolism. Such growth-metabolic coupling is well understood in the case of the IGF/PI3K/AKT/mTOR pathway, however, for other growth factors it has been a matter of conjecture. Recent studies showed that mutations in *SLC2A10*/GLUT10, a member of the facilitative glucose transporter family, were associated with altered transforming growth factor-) (TGF)) signaling in patients with arterial tortuosity syndrome (ATS). The objective of this work was to test if *SLC2A10*/GLUT10 can serve as a link between TGF) -related transcriptional regulation and metabolism during development. In zebrafish embryos, knockdown of *slc2a10* by antisense morpholino oligonucleotide injection caused a wavy notochord and cardiovascular abnormalities with a reduced heart rate and blood flow, and incomplete and irregular vascular patterning. These effects were phenocopied by treatment with a small-molecule inhibitor of TGF) receptor (tgfbr1/alk5). Array hybridization showed that the changes at the transcriptome level caused by the two treatments were highly correlated, revealing that reduced tgfbr1 signaling is a key feature of ATS in early zebrafish development. Interestingly, a large proportion of the genes, which were specifically dysregulated by knocking down the *slc2a10* gene and not by tgfbr1 inhibition play a major role in mitochondrial function. These experiments show that *slc2a10*/glut10 is essential for the development of the cardiovascular system and connective tissues by facilitating cellular respiration and TGF) signaling.

359T

Maternal origin of PEAR1 gene locus affects platelet response to collagen. *D. Vaidya[1], R.A. Mathias[1], L.R. Yanek[1], R. Qayyum[1], N. Fadaday[2], B.G. Kral[1], D.M. Becker[1], L.C. Becker[1].* 1) Medicine, Johns Hopkins University, Baltimore, MD; 2) Anesthesiology, Johns Hopkins University, Baltimore, MD.

Background: We have previously shown that the A allele of intronic SNP rs12041331 of the PEAR1 gene on Chr 1 is associated with reduced ex vivo platelet aggregation in response to collagen. A previous case series report suggested that a parentally imprinted genetic region in Chr 20 affects platelet phenotypes. We thus examined whether platelet aggregation is also affected by parental origin of rs12041331 alleles.

Methods: We used data from European ancestry samples from GeneSTAR, a family study of the genetics of platelet reactivity. Maximal platelet aggregation in platelet rich plasma was measured after stimulation with collagen 2μg/mL. We included offspring whose parents had informative genotypes such that the parent of origin of alleles could be inferred (n=613 from 406 families, 53% female, 36 ± 9 yrs of age). We used linear mixed models to examine the association of platelet aggregation with maternally or paternally transmitted rs12041331 alleles, after adjusting for age and sex.

Results: The mean and standard deviation of percent platelet aggregation by the parental origin of the A and/or G allele is tabulated below.

Mean (SD) collagen-induced % platelet aggregation by rs12041331 allele origin

Paternal G, maternal G, N= 576	Paternal A, maternal G, N= 20	Paternal G, maternal A, N= 12	Paternal A, maternal A, N= 6
65.1 (29.1)	72.2 (25.1)	31.1 (37.4)	31.2 (32.0)

Possessing an A vs. G allele of maternal origin had stronger association with lower levels of platelet aggregation (beta = -36.3, $p=4.2*10^{-7}$) as compared to an A vs. G allele of paternal origin (beta = 7.5, p=0.22; p-value of the difference between beta coefficients=$3.2*10^{-5}$).

Conclusion: We found evidence of genetic imprinting of the parent of origin of the PEAR1 locus for ex vivo collagen-induced platelet aggregation. Genetic loci for platelet function should be examined as candidates for parental imprinting.

360T

Genome-Wide Association Study of Pulse Pressure and Mean Arterial Pressure Identifies Novel Loci Associated with Cardiovascular Disease and Stroke. *C.M. van Duijn[1], L.V. Wain[2], G.C. Verwoert[1,3], P.F. O'Reilly[4], G. Shi[5], T. Johnson[6], A.D. Johnson[7], M. Bochuld[8], K.M. Rice[9], P. Henneman[10], A.V. Smith[11], G.B. Ehret[12], N. Amin[1], M.G. Larson[13], V. Mooser[15], D.I. Chasman[14], M. Jarvelin[4], A. Chakravati[12], G.R. Abecasis[16], V. Gudnason[11], C. Newton-Cheh[17], D. Levy[7,13], P.B. Munroe[6], B.M. Psaty[18, 19], M.J. Caulfield[6], D.C. Rao[20], M.D. Tobin[2], P. Elliott[21], International Consortium of Blood Pressure Genome-wide Association Studies.* 1) Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands; 2) Departments of Health Sciences and Genetics, University of Leicester, University Road, Leicester, UK; 3) Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands; 4) Department of Biostatistics and Epidemiology, School of Public Health, Imperial College London, Norfolk Place, London, UK; 5) Division of Biostatistics and Department of Genetics, Washington University in St. Louis, School of Medicine, Saint Louis, Missouri, USA; 6) Clinical Pharmacology and The Genome Centre, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK; 7) National Heart, Lung and Blood Institute, Bethesda, MD, USA; 8) Centre Hospitalier Universitaire Vaudois and University of Lausanne, Lausanne, Switzerland; 9) Department of Biostatistics, University of Washington, Seattle, Wa, USA; 10) Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands; 11) Icelandic Heart Association and University of Iceland, Reykjavic, Iceland; 12) Center for Complex Disease Genomics, McKusic-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland,USA; 13) Framingham Heart Study, Framingham, Massachusetts, USA; 14) Division of Preventive Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA; 15) Genetic Division R&D, GlaxoSmithKline, King of Prussia, Pennsylvania, USA; 16) Center for Statistical Genetics and Department of Biostatistics, University of Michigan School of Public Health, Ann Arbor, MIchigan, USA; 17) Center for Human Research Center, Massachusetts General Hospital, Boston, and Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA; 18) Group Health Research Institute, Group Health Cooperative, Seattle, Washington, USA; 19) Cardiovascular Health Research Unit, Departments of Medicine, Epidemiology, and Health Services, University of Washington, Seattle, Washington, USA; 20) Division of Biostatistics and Departments of Genetics, Psychiatry, and Mathematics, Washington University in St. Louis, School of Medicine, Saint Louis, Missouri, USA; 21) MRC-HPA Centre for Environment and Health, Imperial College London, London, UK.

Previous genome-wide association studies of blood pressure have identified 29 loci associated with systolic and diastolic blood pressure. We undertook a genome-wide association study of two further blood pressure phenotypes: pulse pressure (PP, the difference between systolic and diastolic blood pressure), a measure of stiffness of the main arteries, and mean arterial pressure (MAP), a weighted average of the systolic and diastolic blood pressure. Both PP and MAP are predictive of hypertension and cardiovascular disease. A total of 74,064 individuals of European ancestry were included in the discovery stage and 48,607 individuals were included in the follow-up stage. We studied 2.5 million directly genotyped or imputed single nucleotide polymorphisms. Seven novel loci showed genome-wide significant association with PP or MAP (P= 2.7x10-8 to P=2.3x10-13). Of these, four novel loci were found to be associated with PP but not to systolic or diastolic blood pressure (at 4q12 near CHIC2/PDGFRAI, 7q22.3 near PIK3CG, 8q24.12 in NOV, 11q24.3 near ADAMTS-8), highlighting the relevance of studying PP as an alternative measure of blood pressure phenotypes. In our study of persons of European descent, we found a locus associated with both PP and MAP (at 2q24.3 near FIGN), which was recently associated with systolic blood pressure in east Asians. We examined the clinical impact of the novel loci on morbidity in independent large disease consortia including Cardiogram, PROCARDIS, ECHOGEN, AortaGEN, CKDgen, KidneyGEN and Decode. These analyses included 6788 cases with hypertension, 30,657 with coronary heart disease, 2708 with stroke, 2526 with heart failure and 5807 with kidney disease. Adjusting for multiple testing, the PP risk score was associated with hypertension (P=7.9x10-6), incident stroke (P=4.9x10-4) and coronary heart disease (P=4.3x10-4) and the MAP risk score was associated with hypertension (P=5.1x10-16), coronary heart disease (P=4.0 x10-20), stroke (P=0.0019) and left ventricular wall thickness (P=2.1x10-4). Our findings demonstrate that analysis of alternative blood pressure phenotypes provide further insights into the genetic architecture of blood pressure and highlight pathways that may differentially influence systolic and diastolic blood pressure.

361T

Gene expression signatures of left ventricular mass and stroke volume change in response to endurance training. *D.D. Vance[1, 3], L. Nathanson[1, 3], M. Stoutenberg[2], G. Chen[3], R. Myerberg[3], K. Jacobs[2], J. Clark[2], A. Perry[2], D. Seo[1, 3], E. Rampersaud[1, 3], P.J. Goldschmidt-Clermont[1, 3].* 1) John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL, USA; 2) Department of Kinesiology and Sports Science, University of Miami, Miami, FL, USA; 3) Miller School of Medicine, University of Miami, Miami, FL, USA.

Changes in cardiac morphology including increased left ventricular (LV) mass and stroke volume (SV) have been well documented among athletes. Although the pathophysiology of these processes is well described, the biological mechanisms for these changes are unknown. To further explore this, we examined the genomic profiles of a cohort of 20 men (29.3 ±1.0yr) a subset of which experienced significant changes in LV mass and SV after participating in a 17 week half marathon training program. Overall, LV mass increased from 186.6±37.4 g to 209.2±30.0 g (p=0.009) and SV increased from 72.6±14.0ml to 91.6±20.9ml (p=0.001). Fasting blood samples and data from echocardiograms were collected at baseline and at the completion of the training program. We compared the global gene expression in the leukocytes of men who experienced ⁄ 14% increase in LV mass and ⁄ 35% increase in SV to those with less significant changes (upper quartile). Leukocyte samples were collected, stored and total RNA was isolated using the Leukolock system (Ambion). Gene expression profiling was performed using the Affymetrix GeneChip Human Gene ST array and analyses were performed with ANOVA implemented in Partek. Twelve genes were overexpressed (p<.01;fold change (FC)⁄ 1.5) among men with ⁄ 14% increase in LV mass. These genes are involved in inflammatory response and two genes mapped to the NOD-like receptor signaling pathway. In contrast, two genes (CCL3L1; RFC1) had lower expression among the men with greater increase in LV mass (p<.01; FC; ≤-1.5); CCL3L1 is a cytokine involved in immunoregulatory and inflammation. Seven genes were overexpressed (p<.01;FC⁄ 1.5) in the men with greater SV increase and map to pathways involved in inflammatory immune response, angiogenesis, and endothelial cell signaling. Validation of these data is in progress. In summary, we have identified subsets of genes that are differentially expressed in men who experienced significant changes in LV mass and SV following 17 weeks of endurance training. A common theme for the genes identified appears to be their involvement in inflammatory and tissue repair processes. Our study suggests that utilization of genomic approaches is a potentially powerful approach for understanding exercise-induced changes to cardiac morphology.

362T

microRNA-152 mediates DNMT1-regulated DNA methylation in the estrogen receptor (gene. *Y. Wang[1], W. Chou[1], K. Chen[1], H. Cheng[1], R. Lin[2], S. Juo[1].* 1) Department of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Department of Neurology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.

Introduction: Estrogen receptor ((ER( ) has been shown to protect against atherosclerosis. Methylation of the ER(gene can reduce ER(expression leading to a higher risk for cardiovascular disease. Recently, microRNAs have been found to regulate DNA methyltransferases (DNMTs) and thus control methylation status in several genes. Results: (1) The ER(expression was decreased and ER(methylation was increased in LPS-treated human aortic smooth muscle cells (HASMCs) and the aorta from rats under a high-fat diet. (2) Among 1,087 surveyed miRNAs, the expression level of miR-152 was decreased by 2.3-fold when HASMCs were treated with LPS. Subsequent real-time PCR experiment confirmed that LPS can decrease miR-152 expression at 48 h and 72 h (P = 0.0015 and 0.006). (3) The significant increases of DNMT1, the miR-152 targeting gene, mRNA expression and protein levels in HASMCs were found at 48 h and 72 h after the treatment of LPS. These results indicate that the reduced miR-152 can lose an inhibitory effect on DNA methyltransferase, which leads to hypermethylation of the ER(gene and a decrease of ER(level. (4) When DNMT1 was inhibited by RNA inference, the mRNA and protein levels of ER(were simultaneously increased by 4.33- and 2.32-fold, respectively. These results clearly show that ER(gene can be silenced by DNMT1. Conclusion: The present study identified a novel mechanism to regulate ER(expression in the context of epigenetic therapy in the context of atherosclerosis and cardiovascular disease. The discovery of miR-152 and ER(methylation in this study may let us gain more insight to the development of atherosclerosis. Furthermore, miR-152 can also be a therapeutic agent for atherosclerosis.

363T

Pediatric cardiomyopathy: genetic causes and utility of testing. *S.M. Ware, M. Tariq, S.J. Kindel, T. Le, J.A. Towbin.* Molec Cardio Biol/Human Gen, Cincinnati Children's Hosp, Cincinnati, OH.

Cardiomyopathy is a disease of heart muscle causing systolic and diastolic dysfunction, or both, and, although rare, is a common cause of cardiac failure in children. Major phenotypic subgroups include dilated (DCM), hypertrophic (HCM), restrictive (RCM), left ventricular noncompaction (LVNC) and arrhythmogenic (ARVC) forms. In the pediatric population, causes include sarcomeric mutations, genetic syndromes, and inborn errors of metabolism. Despite progress in identifying various genetic causes for pediatric cardiomyopathy, in the majority of children no cause can be determined. Our inability to identify and assign causal genes to individual patients represents a clear obstacle to our ability to understand the implications of specific genotypes and precludes the integration of genotype and phenotype data for individual patient risk stratification. The aim of this study was to determine the utility and diagnostic yield of PCR-based high throughput sequencing for the diagnosis of pediatric cardiomyopathies. A cohort of 84 pediatric patients under 10 years of age was enrolled in this study including 19 RCM, 18 HCM, 19 DCM, and 23 LVNC and 5 mixed cardiomyopathy patients. Screening was performed using PCR-based next-generation sequencing (Illumina GAII) covering more than 31 sarcomeric and metabolic genes. Disease-causing mutations were identified in 40 patients (48%; 10 with RCM, 9 with HCM, 7 with DCM, 13 with LVNC and 1 mixed), including ~15% with a family history of disease. Twenty-eight percent of patients had mutations in more than one gene (compound-heterozygotes), most frequently in sarcomeric genes MYBPC3, MYH7, TPM1, and TNNT2. An additional 21 patients (25%) had variants of uncertain significance or potential disease associated alleles. This study provides large-scale, genetic mutation prevalence data for a pediatric population with systolic or diastolic dysfunction and demonstrates the importance of high-throughput technologies to identify multiple mutations in a single patient. These data also suggest that pediatric cardiomyopathy genetic testing panels should encompass a wider array of genes than currently available via clinical testing in order to account for the contribution of metabolic and syndromic causes in this population.

364T

9p21 region is associated with brain white matter lesions. *L.R. Yanek[1], P.A. Nyquist[1], B.G. Kral[1], D. Vaidya[1], R.A. Mathias[1], B. Suktitipat[2], T.F. Moy[1], L.C. Becker[1], D.M. Becker[1].* 1) Medicine, Johns Hopkins University, Baltimore, MD; 2) Genometrics Section, Inherited Disease Research Branch, National Human Genome Research Institute, Baltimore, MD.

Background: The 9p21 locus has been associated with vascular disease phenotypes including coronary artery disease (CAD), abdominal aortic aneurysm, peripheral arterial disease, and intracranial aneurysm. We therefore evaluated the association of the 9p21 region with ischemic white matter disease represented as the ratio of white matter lesion to total brain volume (LBV). Methods: We examined adult relatives of probands with early onset CAD. Brain MRI (3T) was performed, and the total white matter lesion and total brain volume were measured; the ratio of white matter lesion to brain volume was calculated and used for analysis. Participants with a history of CAD, stroke, or transient ischemic attacks were excluded. We examined the association with all SNPs with >1% MAF genotyped in the 9p21 region on the Illumina 1Mv1_c chip in European (EA) and African Americans (AA) separately. Associations with LBV were performed under an additive model or dominant model based on genotype frequencies, adjusted for age, sex, family clustering, and population stratification. Results: Participants included 308 EA from 172 families and 184 AA from 115 families (41% male, 51.8 ± 10 years of age). In EA 64 SNPs were examined and 84 in AA. Five SNPs were associated with LBV at the p<0.05 level: in EA, rs3731238 (beta= 0.6529, p=0.0097), rs10965243 (beta=0.4027, p=0.0328), and rs2383208 (beta=0.446, p=0.0034); in AA, rs3217986 (beta=-0.7040, p=0.0402) and rs10811658 (beta=0.4214, p=0.0100). Significant SNPs were either in the 9p21 region previously found with intracranial aneurysms, but not in LD with previously reported SNPs; or, in the opposite end of the 9p21 region near/ in the ANRIL locus, where we previously reported an association with incident CAD in this same high risk family population. Conclusions: The 9p21 region appears to be associated with LBV, a measure of ischemic white matter disease, in a high risk population. Further studies of the 9p21 region may parse out locus-specific variations that are related to cerebrovascular disease.

365T

An integrative pathway analysis using gene expression, single-nucleotide polymorphism and environmental factor successfully predicts disease status of hypertension. *H.-C. Yang[1], C.-W. Lin[1], K.-M. Chiang[2], Y.-J. Liang[1], C.-W. Chen[1], S.-M. Hwang[3], K.-S. Lynn[4], J.-W. Chen[5], W.-H. Pan[2].* 1) Institue of Statistical Science, Academia Sinica, Taipei, Taiwan; 2) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 3) Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan; 4) Institute of Information Science, Academia Sinica, Taipei, Taiwan; 5) National Yang-Ming University School of Medicine and Taipei Veterans General Hospital, Taipei, Taiwan.

[**Background**] Renin-angiotensin-aldosterone system (RAAS) pathway, calcium-signaling (CS) pathway and calcium-channel (CC) functional group are known as the relevant biosystems targeted by antihypertensive drugs such as angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and calcium channel blockers. In each of these biosystems, several dozens of genes are involved. If we can not consider them simultaneously, we may lose sight of the whole picture. Genetic marker and gene expression data each has its unique biological meaning. [**Methods**] Our analysis pipeline began with a biosystem enrichment analysis to verify the significance of the three biosystems based on gene expressions, single-nucleotide polymorphisms (SNPs) and a major environmental factor [body mass index (BMI)] of 200 matched case-control pairs. Gene expressions of 334 transcript probes on 248 genes and genotypes of 7,368 SNPs on the 228 genes involved in the three biosystems were collected from the experiments with Phalanx Human OneArray and Illumina 550K SNP BeadChip, respectively. Then we carried out a 10-fold cross-validation procedure for a statistical classification analysis to establish a hypertension prediction model with the highest testing accuracy. Furthermore, we evaluated the prediction model by using independent samples of 53 patients and 50 controls from the same Han Chinese population. [**Results**] A biosystem enrichment analysis verifies the significance of the three biosystems, RAAS pathway ($p < 0.0001$), CS pathway ($p < 0.0001$) and CC functional group ($p < 0.0001$). Statistical classification analysis derives the best classification model with a training accuracy of 80.1% and a testing accuracy of 85%. In the model, BMI provides a net accuracy of 52.5% in predicting hypertension. Prediction accuracy is accumulated to 60% after including two optimally selected SNPs, rs1543208 and rs7921117, and then accumulated to 85% after further including gene expressions of four best selected genes, *CACNB1*, *NOS3*, *CACNA1C* and *CACNA1G*. Finally, the derived prediction model with seven key biomarkers receives a prediction accuracy of 85.1% in an independent validation dataset of 103 samples. [**Conclusions**] RAAS, CS and CC are validated as the important biosystems relevant to hypertension in the Taiwan Han Chinese population. Gene expressions and SNPs involved in the three biosystems and environmental variable BMI provide an informative biosignature for predicting hypertension.

366T

BRCA2 Variants and Cardiovascular Disease in a Multi-Ethnic Cohort. *K. Zbuk[1,2], M. Heydarpour[1], G. Pare[1,3], D. Davis[4], R. Miller[4], M. Lanktree[5], S. Yusuf[1,6], D. Saleheen[7], J. Danesh[7], R. Hegele[5], S.S. Anand[1,6].* 1) Population Health Research Institute, Hamilton Health Sciences, Hamilton, Ontario, Canada; 2) Dept. of Oncology, McMaster University, Hamilton, Ontario, Canada; 3) Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada; 4) Six Nations Health Services, Ohsweken, Ontario, Canada; 5) Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada; 6) Departments of Medicine and Epidemiology, McMaster University, Hamilton, Ontario, Canada; 7) Department of Public Health and Primary Care, Cambridge University, Cambridge, UK.

Background: Germline mutations of BRCA1/2 are associated with hereditary breast and ovarian cancer. Recent data suggests excess mortality in mutation carriers beyond that conferred by neoplasia, and recent in vivo and in vitro studies suggest a modulatory role for BRCA proteins in endothelial and cardiomyocyte function. We therefore tested the association of these markers with clinical cardiovascular disease (CVD). Methods: Using data from 1,170 individuals included in two multi-ethnic population-based studies (SHARE and SHARE-AP), the association between BRCA2 variants and CVD was evaluated. 21 SNPs in BRCA2 with minor allele frequencies (MAF)>0.01 were genotyped using the Illumina CVD array. 115 individuals (9.8%) reported a CVD event, defined as myocardial infarction (MI), angina, silent MI, stroke, and angioplasty or coronary artery bypass surgery. Analyses were adjusted for age, sex, ethnicity, and other traditional CVD risk factors as covariates. A single SNP (rs11571836) was subsequently genotyped using Sequenom technology in 1045 cases of incident MI and 1135 controls from the South Asian subset of an international case-control study of acute MI (INTERHEART), and imputed in 4686 cases of MI and 4500 controls from the Pakistan Risk of Myocardial Infarction Study (PROMIS) study. Results: Two BRCA2 SNPs, rs11571836 and rs1799943, both located in untranslated regions, were associated with a lower risk of prevalent CVD (OR 0.52 p=0.001 and OR 0.50 p=.002 respectively) in the SHARE studies. Analysis by specific ethnicities demonstrated an association with CVD for both SNPs in Aboriginal People, and for rs11571836 only in South Asians. No association was demonstrated in the European and Chinese subgroups. A non-significant trend towards an association between rs11571836 and lower risk of MI (OR=0.87 p=0.06) was observed in South Asians from the INTERHEART study, but was not evident in PROMIS (OR= 0.96 p=0.22). The combined p value of the replication cohorts was p= 0.10 (Stouffer's z trend) and meta-analysis resulted in a combined OR=0.94 (p=0.06 fixed effect model). These results suggest a possible role for BRCA2 in the etiology of CVD, however much larger studies are needed to confirm or refute this hypothesis. More comprehensive interrogation of BRCA gene variation, including functional analyses, is required to fully understand whether BRCA family members are involved in CVD pathogenesis.

367T

Risk Variants of Coronary Artery Disease in the Chromosome 9p21 Region are associated with *ANRIL* Expression in GENOA. *W. Zhao[1], J.A. Smith[1], M. Fornage[2], P.A. Peyser[1], S.T. Turner[3], S.L.R. Kardia[1].* 1) Department of Epidemiology, University of Michigan, Ann Arbor, MI; 2) Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center, Houston, TX; 3) Department of Nephrology and Hypertension, Department of Medicine, Mayo Clinic, Rochester, MN.

Recent genome-wide association (GWA) studies have shown that single nucleotide polymorphisms (SNPs) in the chromosome 9p21 region are associated with coronary artery disease (CAD). Most of the SNPs identified in this region by GWA studies are non-coding SNPs, suggesting that they may influence gene expression by cis or trans mechanisms to affect disease susceptibility. Since all cells from an individual have the same DNA sequence variations, levels of gene expression in immortalized cell lines can reflect the functional effects of heritable DNA sequence variations that influence or regulate gene expression. To evaluate the functional consequences of the risk variants in the chromosome 9p21 region on gene expression, we examined the association between 137 SNPs in this region and both gene-level and exon-level RNA expression of the adjacent genes *CDKN2A*, *CDKN2B*, *ANRIL* and *C9orf53* from transformed beta-lymphocytes in 812 Caucasian participants from The Genetic Epidemiology Network of Arteriopathy (GENOA) study. Linear mixed effects modeling was used to test the association between each SNP and RNA expression using age-adjusted RNA expression as the outcome, SNP as the predictor, and pedigree as the random effect. We found that the exon-level RNA expression of *ANRIL* was significantly associated with multiple SNPs (111 SNPs) after Bonferroni correction. More importantly, 72 out of the 74 CAD-associated SNPs identified in a recent GWAS conducted by the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) consortium were all associated with *ANRIL* expression. The remaining two SNPs were also significantly associated with *ANRIL* expression before Bonferroni correction. The magnitude of change in the RNA expression for significant SNPs ranges from 11% to 40%. No significant association was found between these SNPs and the other three genes. These results suggest that the effect of risk variants in chromosome 9p21 region on susceptibility to CAD is likely to be mediated through *ANRIL* expression.

368T

Identification of 132 new mutations of the *FBN1* gene in patients with suspected Marfan syndrome. *W. Zou, H. Wang, B.A. Westerfield, L.S. Pena, A. Do, G.A. Rodriguez, D.J. Penny, Y. Fan.* John Welsh Cardiovascular Diagnostic Laboratory, Section of Cardiology, Department of Pediatrics, Baylor College of Medicine, Houston, TX.

Marfan Syndrome (MFS) is a heritable disorder of connective tissue with multisystemic manifestations, which typically involves the skeletal, cardiovascular and ocular systems. It is usually caused by defects of fibrillin-1 (*FBN1*) gene and its diagnosis requires the presence of Ghent criteria. *FBN1* encodes a large secreted 350-kDa glycoprotein that is multidomain, highly repetitive, and known to be a major component of 10-12 nm microfibrils. *FBN1* gene mutations are associated not only with the MFS but also with a spectrum of conditions phenotypically related to MFS, including dominantly inherited ectopia lentis, severe neonatal Marfan syndrome and aortic aneurysm. There appear to be no mutation hot spots in the *FBN1* gene since the identified mutations were distributed throughout the entire gene. However, a clustering of mutations associated with the most severe form of Marfan and neonatal Marfan syndrome has been noted in a region encompassing exons 23 to 32. To date, more than 700 mutations have been identified in the *FBN1* gene in MFS patients and related diseases. We conducted FBN1 mutation analysis in a large cohort of 1025 cases with suspected MFS referred to our center for genetic analysis. Peripheral blood-derived genomic DNA was used to amplify the 65 coding exons of the *FBN1* gene by polymerase chain reaction (PCR) using intron-based and exon-specific primers. The PCR products were purified and analyzed by bidirectional sequencing using Big Dye terminator chemistry (v3.1) and ABI3730XL genetic analyzer. A total of 199 mutations were identified in 1025 unrelated cases with suspected MFS. Of these, 132 mutations were not reported previously, to our knowledge, which included 82 missense mutations, 13 predicted splicing site alterations that were expected to result in exon skipping or activation of a cryptic splice site, 6 nonsense mutations and 31 deletion/insertion mutations that were predicted to cause a frameshift and lead to the MFS phenotype via *FBN1* haploinsufficiency. These mutations were distributed throughout the entire *FBN1* gene and approximately 13% of the mutations occurred in two or more MFS patients. In summary, our study further expanded the mutational spectrum of the *FBN1* gene in patients with MFS. This data is expected to considerably improve genetic counseling for and medical care of MFS patients.

369T

The genetics of Brugada Syndrome : a new approach. *S. Le Scouarnec[1], P. Lindenbaum[2], V. Portero[2], A. Derevier[2], J-B. Gourraud[2], C. Scott[1], J. Barc[3], H. Le Marec[2], A. Wilde[3], V. Probst[2], C. Bezzina[3], E. Schulze-Bahr[4], N. Carter[1], J-J. Schott[2], R. Redon[2].* 1) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 2) L'institut du thorax, Nantes, FR; 3) Academic Medical Center, Amsterdam, NL; 4) University Hospital Muenster, DE.

Brugada Syndrome (BrS) is an inherited form of cardiac arrhythmia characterized by ST-segment elevation on the electrocardiogram, associated with a higher risk of sudden cardiac death (SCD) in young individuals. So far, genetic investigations have often been restricted to ion channel genes and the great majority of cases are still unexplained. We are combining high-throughput, genome-wide methods (exome sequencing, array-CGH, and SNP genotyping) to reveal genetic defects in familial cases of BrS and to improve molecular diagnosis of cardiac arrhythmias and prevention of SCD. Twenty families with at least 3 affected patients and a history of SCD have been included in the study, after exclusion of the most frequently involved gene, *SCN5A*. Exome sequencing and high-resolution array-CGH have been performed for one patient per family. By array-CGH, we have identified deletions and duplications absent from public databases and containing entire genes in some cases. Among these is a large de novo 670 kb deletion encompassing seven genes and adjacent to, but excluding, a gene already linked to arrhythmias, suggesting the possible deletion of a regulatory region of this gene. Exome enrichment reaches 58% on average and the mean sequencing depth per patient ranges from 84X to 136X, around 90% of the targeted sequence being covered by at least 10 reads. More than 30,000 simple nucleotide variants per patient have been identified by this approach. After excluding non-coding and synonymous variants as well as variants found by other studies and in databases such as dbSNP and the 1000 genomes project, candidate genes have been prioritized and are now followed-up. SNP genotyping of all affected family members is in progress to determine regions likely to be identical-by-descent. This will allow us to prioritize candidate variants according to their co-segregation with the BrS phenotype.

370T

Identifying at-risk genetic variants for sudden cardiac death from whole exome sequence data in the *ClinSeq™* study. *D. Ng[1], J.J. Johnston[1], F.M. Facio[1], S.G. Gonsalves[1], C. Krause[1], J.K. Teer[1,2], J.C. Mullikin[1,2,3], L.G. Biesecker[1,2], National Institutes of Health Intramural Sequencing Center, NIH, Bethesda, MD.* 1) Genetic Disease research Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 2) NIH Intramural Sequencing Center, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 3) Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

U.S. estimates of sudden cardiac death (SCD) range from 180,000 to >450,000/yr. Ten to fifteen percent of SCD is due to cardiomyopathies and 1-2% to channelopathies. Identifying at-risk gene variants for SCD in asymptomatic individuals can potentially save lives. *ClinSeq™* aims to identify medically actionable genetic variants and return results to participants for monitoring, prophylaxis, and treatment. We looked for deleterious changes in channelopathy and cardiomyopathy genes in an adult cohort not ascertained for arrhythmia, cardiomyopathy or SCD. METHODS: 401 whole exome sequences were annotated for variants in 39 cardiomyopathy and 24 channelopathy genes with an algorithm that filtered results based on genotype quality, allele frequency, mutation type, and information in locus-specific databases. Variants were assigned pathologic scores ranging from 0-5 (0= poor genotype quality, 1=not pathogenic (probability <0.001), 2=likely not pathogenic (0.001-0.049), 3=uncertain (0.05-0.949), 4=likely pathogenic (0.95-0.99), 5=definitely pathogenic (>99); Plon et al. 2008). RESULTS 234 variants were found in 24 channelopathy genes. Forty-one variants were excluded (3 scored 0, 38 score 1 due to high frequency), leaving 193 variants. Human Gene Mutation Database and locus-specific databases were reviewed. Eighteen variants were designated not pathogenic (score 1); 173 were defined as variants of uncertain significance (score 2 [n=8], score 3 [n=148], score 4 [n=17]); and two variants were designated pathogenic (score 5). *SCN3B* p.Leu10Pro is associated with atrial fibrillation and Brugada syndrome. Participant's baseline ECG showed left bundle branch block and prolonged QTc 493ms. *KCNH2* p.Arg312Cys is associated with long QT syndrome type 2. Baseline ECG showed sinus bradycardia 56 bpm, 1° AV-block, and normal QTc 402ms. Neither participant reported a family history of SCD. *ClinSeq™* participants with variants scored 4-5 will be invited to the NIH for cardiology evaluation to delineate their risk for SCD. Results of cardiomyopathy gene analysis will be reported. Conclusion Whole exome sequencing has the potential to identify clinically relevant variants. Much work is needed to define genotype-phenotype risk for SCD. Sequencing costs have dropped, but clinical annotation remains costly and time consuming. Combining existing knowledge into a centralized database is crucial in achieving the goals of personalized medicine.

371T

Genetic screening of long QT syndrome (LQTS) in Sweden. *A. Norberg[1], K. Cederquist[1], J. Jonasson[1], B.A. Jonsson[1], A. Rydberg[2,3], S.M. Jensen[3,4], E.L. Stattin[1,3].* 1) Clinical Genetics, Umeå University Hospital, Umeå, Sweden; 2) Department of Clinical Sciences, Pediatrics, Umeå University Hospital; 3) Centre for Cardiovascular Genetics, Umeå University Hospital; 4) Heart Centre, Umeå University Hospital.

Background: Long QT syndrome (LQTS) is an inherited disorder characterized by prolongation of the QT interval on ECG and risk of syncope and sudden death. The most common genes implicated in LQTS are the cardiac ion channels *KCNQ1, KCNH2, SCN5A, KCNE1* and *KCNE2*, accounting for about 70% of the identified mutations in patients with a clinical diagnosis. The symptoms are very variable in LQTS patients, and genotype influences the clinical course. **Objectives:** This study aims to report the spectrum and prevalence of LQTS mutations in Sweden. Knowledge of founder mutations or certain subdiagnostic criteria for different mutations will facilitate the molecular genetic analysis. **Methods:** A cohort of 200 consecutive, unrelated patients (137 females, average age 33 years, range 0 to 79 years) referred to Umeå for LQTS clinical genetic testing between March 2006 and October 2009 have been evaluated. Coding sequences and splice sites of the five genes have been screened by DHPLC and/or DNA sequencing. MLPA has also been performed to detect any large deletions or duplications. Furthermore, 36 selected patients have been screened for mutations in *RYR2*, a gene known to be involved in the clinically overlapping disease catecholaminergic polymorphic ventricular tachycardia (CPVT). **Results:** Overall, in 102 of 200 (51%) probands, we could detect a disease-causing mutation. These genotype-positive cases stemmed from 61 distinct mutations, of which 17 (28%) were novel to this Swedish cohort. The majority (79%) of the distinct mutations were found in a single case, whereas 13 mutations (21%) were observed more than once. Two Swedish founder mutations, *KCNQ1*-R518X and *KCNQ1*-Y111C, accounted for 25% of the genotype-positive probands. Among the genotype-positive patients, 100 had a single mutation, whereas only 2 patients had more than one definitely pathogenic mutation. However, several probands carried rare variants that are difficult to interpret since they are referred to in the literature as both mutations and functional polymorphisms. Although they might contribute to the phenotype, they were not considered as disease-causing mutations, since careful interpretation of genetic test results is critical in clinical use. **Conclusion:** Two Swedish founder mutations account for about one-fourth of the identified mutations. This cohort increases the publicly known LQTS-associated mutations, of which approximately one-third of the detected mutations continue to be novel.

372T

Mysteries of the past - Genetic evaluation of families with unexplained sudden cardiac death has revealed inheritable heart diseases. *A. Wisten[1, 2], I. Boström[3], S. Mörner[2], E. Stattin[3].* 1) Department of Internal Medicine, Sunderby Hospital, Luleå, Sweden; 2) Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden; 3) Department of Medical Biosciences, Medical and Clinical Genetics, Umeå University, Umeå, Sweden.

Background: Sudden cardiac death (SCD) in the young is a rare and tragic event. Standard forensic autopsy is often unsuccessful in determining the cause of SCD. Post mortem genetic testing or molecular autopsy has shown that these deaths can be caused by inherited arrhythmic disorders. Objectives: The purpose of this study was to determine the cause of sudden death, in subjects with normal autopsy findings. Subjects were identified in a national Swedish study of SCD in 15 to 35 year olds from 1992-1999. Methods and results: We performed molecular genetic analysis of first degree relatives to 25 subjects who suffered sudden unexplained death. Coding exons of the KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, KCNJ2, RyR2, MYBPC3 and MYH7-genes were screened. We identified the causative underlying disease in five subjects [LQTS (3), SQTS (1), HCM (1)], and molecular genetic analysis provided confirmation in three families with LQTS. Target mutation analysis of autopsy tissue confirmed the diagnosis in two of the deceased subjects. Polymorphisms were identified in relatives. In the SQTS- and HCM-families, there were two siblings, who both died from SCD. Conclusions: It is possible to identify a mutation in a next-of-kin and to verify the mutation in autopsy tissue many years after death. However, this investigation should start directly. To prevent more deaths in the families, prompt genetic and clinical evaluation should be performed. Blood or appropriate tissue for DNA extraction ought to be obtained at the post-mortem examination. We found a plausible or verified diagnosis in 20% of the families. Familial cascade screening revealed five asymptomatic carriers at risk. Effective evaluation of relatives, guided by genetic testing, can identify asymptomatic carriers and hopefully prevent further deaths in the family. Common polymorphisms may possibly act as modifier gene alleles increasing arrhythmia susceptibility.

373T

The Spectrum of *SCN5A* Gene Mutation in Singapore Brugada Syndrome Patients. *R.Y.Y. Yong[1], M. Uttamchandani[1], B.Y. Tan[2], J.L. Neo[1], S.H. Yap[1], L.S.H. Gan[1], W.Q. Wong[2], R. Liew[2], C.K. Ching[2], D.T.T. Chong[2], C.W.F. Chong[3], T.W. Lim[3], W. Chow[4], S. Moochhala[1], E.P.H. Yap[1], S.C. Seow[3], W.S. Teo[2].* 1) Defence Medical & Environmental Research Institute, DSO National Laboratories, 27 Medical Drive #12-00, Singapore 117510; 2) National Heart Centre, Singapore General Hospital, Mistri Wing, 17 Third Hospital Avenue, Singapore 168752; 3) The Heart Institute, National University Hospital, Cardiac Department, 5 Lower Kent Ridge Road, Singapore 119074; 4) Headquarters Medical Corps, Singapore Armed Forces, HQ SAF Medical Corps, 701 Transit Road, #04-01, Singapore 778910.

Brugada syndrome (BrS) is an inherited arrhythmia syndrome with an increased risk of sudden death, resulting from polymorphic ventricular tachycardia and/or ventricular fribrillation in the absence of gross structural abnormalities. BrS is characterized by right bundle bock and ST-segment elevation in the right precordial ECG leads. The incidence of clinical event, syncope and sudden cardiac death, depends on ethnicity, with a higher rate of sudden death in patients of Asian origin. Mutations in the *SCN5A* gene, which encodes the pore-forming subunit of the cardiac voltage-gated sodium channel, are found in 20% to 30% of BrS patients in Caucasian population. We analysed the sequence of *SCN5A* gene in 40 unrelated BrS patients (36 males, 90%) from Singapore. Bi-directional Sanger sequencing was carried out on approximately 20Kb of all the exonic cum flanking sequence of *SCN5A*, including 2.8Kb of its promoter region. All DNA variants of interest identified by sequencing were verified with genotyping on Sequenom platform. The minor allele frequency of all potential mutations in normal controls were determined by genotyping 105 healthy Singapore Chinese controls. Mutation was found in 7 of the 40 patients (17.5%), with 2 patients harbouring 2 mutations. A total of 8 mutations were identified, comprising of 5 missense, 1 non-sense, 1 in-frame deletion/insertion and 1 intron mutation that is expected to affect the splice site. Of the 8 mutations, 6 had not previously been described (75%). Several polymorphisms in the coding regions, both synonymous and non-synonymous, were also identified. Of the 40 patients, family samples were available for 5 probands. Two of the probands with mutation identified demonstrated Mendelian segregation of the mutation with BrS phenotype within the family, providing further support to the DNA mutation been causal. The study is on-going. To our knowledge, this is the most comprehensive study on mutation spectrum in BrS patients in Singapore to date.

374T

Effects of Variation in the Haptoglobin Gene on Subclinical Cardiovascular Disease in the Diabetes Heart Study. *J.N. Adams[1,2,3], A.J. Cox[2,3,4], B.I. Freedman[5], J.J. Carr[6], D.W. Bowden[2,3,4].* 1) Program in Molecular Genetics and Genomic, Wake Forest School of Medicine, Winston Salem, NC, USA; 2) Center for Human Genomics, Wake Forest School of Medicine, NC, USA; 3) Center for Diabetes Research, Wake Forest School of Medicine, NC, USA; 4) Department of Biochemistry, Wake Forest School of Medicine, Winston Salem, NC, USA; 5) Department of Internal Medicine - Nephrology, Wake Forest School of Medicine, NC, USA; 6) Department of Radiologic Sciences, Wake Forest School of Medicine, NC, USA.

Identification of heritable risk factors for cardiovascular disease (CVD) and other complications of type 2 diabetes mellitus (T2DM) are important to help understand individual risk for disease. Haptoglobin (HP) is an acute phase protein that binds to freely circulating hemoglobin. Genetic variants in HP have previously been reported as associated with an increase in CVD events and mortality in individuals with diabetes. Haptoglobin has two distinct alleles, Hp 1 and Hp 2, with Hp2 arising from a duplication of approximately a 1700 base pair region encompassing exons 3 and 4. The aim of this study was to examine the association of the HP genotypes with measures of subclinical CVD in a sample enriched for T2DM. Haptoglobin genotypes were determined in 1208 European Americans from 474 families in the Diabetes Heart Study (DHS). Measures of subclinical CVD included coronary artery, carotid artery, and abdominal aortic calcified plaque determined by CT scanning and carotid artery intima media thickness (IMT) determined by B-mode ultrasound. Haptoglobin genotyping was performed by PCR amplification followed by resolving products by agarose gel electrophoresis specific to each HP allele. Association between HP genotypes and clinical traits was assessed using variance components analysis in SOLAR. Additive genetic models were assessed with age, sex, and T2DM affection status included as covariates. Statistical significance was accepted at $p<0.05$. This analysis revealed an association between HP genotypes and IMT ($p=0.01$); with the HP 2-2 genotype being the risk genotype. We did not observe an association between HP genotypes and either coronary ($p= 0.82$), carotid ($p= 0.12$) or abdominal aortic calcified plaque $p= 0.81$). Adjustment for other known CVD risk factors including BMI, smoking, lipid and anti-hypertensive medications did not affect the outcomes. In conclusion, this study revealed evidence for association between HP genotypes and carotid IMT in European Americans with T2DM. Lack of association with atherosclerotic calcified plaque may reflect differences in the pathogenesis of these CVD phenotypes. HP variation may contribute to the heritable risk for cardiovascular complications in T2DM.

375T

Novel Mutations in MYBPC3 in Cardiomyopathy - An Indian Study. *A. Ali[1], T.R. Reena[1], R. Advithi[1], C. Narsimhan[2], N. Pratibha[1].* 1) Department of Genetics, Osmania University, Hyderabad, Andhra Pradesh, India; 2) Care Hospitals, Hyderabad, Andhra Pradesh, India.

Cardiomyopathy is a group of disorders characterized by malfunctioning of the myocardium and is clinically identified by heart failure, arrhythmias and sudden death .The Indian population is considered to be at high risk for cardiovascular diseases. Based on the pathophysiology, cardiomyopathies are classified as HCM, RCM, ARVD/C and DCM, with the etiologies related to sarcomeric proteins, desmosomal proteins and Ryanodine receptor components of the cardiomyocytes. Hypertrophic cardiomyopathy (HCM) is a complex muscular disorder of the heart, inherited as an autosomal dominant disease with variable penetrance in at least 50% of cases. The prevalence of HCM has been reported to be one in 500 in a population of young adults. The clinical course is variable and unpredictable, ranging from a benign asymptomatic course to severe heart failure and sudden cardiac death. The present study includes clinically confirmed 97 HCM patients referred to various hospitals in India. Mutational Screening of MyBPC3 was carried out using SSCP method followed by sequencing. The following novel mutations were identified in our cohort:- a) frameshift mutation Ins A 11577 ^11578 in Exon 19 in a patient. b) A novel SNP C >T at 1093 codon in exon 31 in a proband. In silico analysis revealed structural and functional alterations of the MyBPC3 protein. The present study is an attempt to highlight the unique genetic variations in Indian population indicating hotspot variations and different gene implications from those of western populations, which will be further discussed.

376T

Network-driven integrative genomics analysis of the CARDIoGRAM GWAS reveals key drivers and subnetworks of coronary artery disease. *T.L. Assimes[1], T. Huan[2,3], S. Maouche[4], J. Zhu[2], B. Zhang[2], J. Erdmann[4], C. Nelson[5], K.I.E. Snell[5], T. Quertermous[1], N. Samani[5], H. Schunkert[4], X. Yang[1], Coronary ARtery DIsease Genome wide Replication and Meta-analysis (CARDIoGRAM).* 1) Department of Medicine, Stanford University School of Medicine, Stanford, CA; 2) Sage Bionetworks, Seattle, WA; 3) The National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, MA; 4) Department of Medicine, University of Lübeck, Lübeck, Germany; 5) Department of Cardiovascular Sciences, University of Leicester, Leicester, UK.

Objective: Through meta-analysis of 14 GWAS, CARDIoGRAM identified 25 loci for coronary artery disease (CAD). However, the molecular mechanisms underlying these associations remain unclear and susceptibility loci with more subtle effects were undoubtedly missed. We hypothesize that genetic variation with both strong and subtle effects drives gene subnetworks that in turn affect risk of CAD. **Methods:** We surveyed CAD-associated molecular interactions by integrating CARDIoGRAM association results, expression SNPs (eSNPs), and gene networks constructed from six tissues of orthogonal mouse and human studies. A total of 18 pre-selected coexpression network modules along with 12 CAD-related gene sets or expression signatures (positive controls) from public databases and the literature were first mapped to eSNPs to derive the corresponding eSNP sets. For each eSNP set, the significance of enrichment for low p value associations to CAD in CARDIoGRAM was evaluated using both Fisher's exact and Kolmogorov-Smirnov (KS) tests. The gene sets that were significantly enriched for CAD-association eSNPs were in turn integrated with tissue-specific Bayesian networks and a protein-protein interaction (PPI) network to identify central network nodes (key drivers or KDs) that drive these CAD-related gene sets. The top KDs were then used as seeds to derive subnetworks that link the KDs to reveal the CAD network structure. **Results:** Eleven of 12 positive control gene sets and 12 of 18 coexpression network modules were significantly enriched for eSNPs with low p value association to CAD in CARDIoGRAM at a Bonferroni-corrected $p<0.05$ for both tests. We identified both tissue-specific KDs and KDs common to multiple tissues and found them to be enriched for CAD-related biological processes such as circulation, inflammatory response and coagulation. The top KDs across tissues are IL1RN, EGR2, NCF2, and LDLR, and the tissue-specific KDs include LPL and APOE from liver, ALOX5AP and ACE from kidney, F7 and ANAX2 from adipose, BACH1 and FER1L3 from blood, and CD36 and PPARG from the PPI network. The KDs were found to be highly connected in the networks and representative subnetworks of the top KDs (21 known and 27 novel) were derived. **Conclusions:** Our network-driven integrative analysis not only identified known and novel CAD risk genes but also defined a network structure that sheds light on the molecular interactions of CAD risk genes within, between and across tissues.

377T

Impact of genetic polymorphisms of lipoprotein lipase and the susceptibility to hypertension. *Y. Chen[1], Y. Wang[1], R. Wang[1], C. Chen[1], F. Wu[1], T. Wu[1,2].* 1) Department of Public Health, China Medical University, Taichung, Taiwan; 2) Graduate Institute of Biostatistics, China Medical University, PhD.

Hypertension is an important risk factor leading to cardiovascular diseases, such as stroke, myocardial infarction, atherosclerosis and heart failure, etc. Hyperlipidemia is not only the major risk of ischemic stroke but also associated with the severity of hypertension. Hyperlipidemia is affected by vitamin B6 and lipoprotein lipase activity. In addition, genetic factors also have been associated with hypertension development. This study investigated whether LPL genotype a factor associated with hypertension. Study subjects consisted of 136 hypertensive patients and 195 non-hypertensive subjects in Nan-tou County. Each participants completed the demographic and lifestyle questionnaire. Blood samples were collected and we used Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) to determine LPL gene S447X genotype. All statistical analyses were conducted using statistical package SAS for Chi-square test, ANOVA test, and logistic regression. Results showed that LPL gene of S/X genotype in hypertension patients had significant differences between blood pressure level ($p < 0.0001$). Triglyceride levels and LPL gene of the S/X polymorphisms were significantly different ($p = 0.0090$) between case and control. Logistic regression analysis showed that individuals with the SX or XX genotype had a much lower risk of hypertension than those with SS genotype. (Odds ratio 0.1, 95% Confidence interval, 0.05-0.19) LPL gene S/X polymorphisms are significantly associated with hypertension. Individuate with SS genotype at higher risk of stroke.

378T

Adiponectin SNPs do not associate with coronary artery disease in Filipino diabetics. *M.L.G. Daroy[1], M.A. Luz[1], K.A. Pasion[1], V.C. Lacuesta[1], M. Mararang[1], M.V. Mendoza[1], J.T. Asi[1], A.A. Maliglig[1], J.A. Alfon[1], S.J. Soriano[1], C.A. Mapua[1], R.R. Matias[1], F.E.B. Posas[2].* 1) Research and Biotechnology Div, St. Luke's Medical Center, Quezon City, NCR, Philippines; 2) Heart Institute, St. Luke's Medical Center, Quezon City, NCR, Philippines.

Two SNPs in the adiponectin gene, SNP 45T>G and SNP 276G>T, previously reported to be associated with the development of coronary artery disease in Type 2 diabetics, were studied in Filipinos who were examined for CAD at the Heart Institute of St. Luke's Medical Center, Philippines. Lack of association (p-value= 0.910) between SNP 45T>G and CAD (>10% stenosis) was obatained from 894 cases and 119 normal controls studied. A similar result (p-value= 0.607) was reached from SNP 276G>T in 653 cases and 102 controls. Further analyses of association across varying degrees of CAD severity (10%, 40%, >70%) likewise showed no association. While SNP 45T>G was not associated with cases of prior heart attack, a slight but not significant association of SNP 276G>T was obatained for 141 cases that had a prior heart attack (p-value= 0.075). The results of this study suggest that genetic determinants associated with CAD in Filipino diabetics are significantly different from other Asian population groups (Japanese, Korean) in which these two SNPs in the adiponectin gene are shown to be significantly associated. Hence, more powerful GWAS should be conducted in this this group of Asian Filipinos for coronary artery disease and Type 2 diabetes.

379T

A multi-ethnic association study of C-reactive protein levels using the ITMAT Broad-CARe cardiovascular gene SNP array. *J. Ellis[1], J. Walston[2], J. Dupuis[3], J. Baumert[4], E. Larkin[5], M. Barbalic[6], B. Keating[7], P. Durda[8], E. Fox[9], C. Palmer[10], Y. Meng[10], T. Young[10], R. Schnabel[11], C. Marzi[12], J. Bis[13], V. Ramachandran[11], J. Pankow[14], G. Lettre[15], E. Lange[1], C. Ballantyne[16], M. Gross[14], J. Wilson[9], N. Nock[17], G. Papanicolaou[18], W. Koening[19], R. Tracy[8], A. Reiner[13], E. Benjamin[11], NHLBI Candidate Gene Association Resource (CARe) Consortium and collaborating cohorts.* 1) University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Johns Hopkins University, Baltimore, MD; 3) Boston University School of Public Health, Boston, MA and NHLBI Framingham Heart Study, Framingham, MA; 4) Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany; 5) Vanderbilt University, Nashville, TN; 6) University of Texas Health Science Center at Houston, Houston, TX; 7) University of Pennsylvania School of Medicine, Philadelphia, PA; 8) University of Vermont, Burlington, VT; 9) University of Mississippi Medical Center, Jackson, MS; 10) The Broad Institute, Cambridge, MA; 11) Boston University School of Medicine, Framingham, MA; 12) Research Unit of Molecular Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany; 13) University of Washington, Seattle, WA; 14) University of Minnesota, Minneapolis, MN; 15) Département de Médecine, Université de Montréal, Montréal, Canada; 16) Baylor College of Medicine, Houston, TX; 17) Case Western Reserve University, Cleveland, OH; 18) National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health, Bethesda, Maryland; 19) University of Ulm Medical Center, Ulm, Germany.

INTRODUCTION: C-reactive protein (CRP) is a heritable biomarker of systemic inflammation and a predictor of cardiovascular disease (CVD). We sought to uncover novel genetic variants and further characterize previously identified loci associated with circulating levels of CRP in a multi-ethnic sample. METHODS: Using the ITMAT Broad-CARe (IBC) SNP array, a custom 50,000 SNP gene-centric array having dense coverage of over 2,000 candidate genes for CVD pathways, we performed a meta-analysis of up to 26,065 participants of European descent and 7,584 African Americans for association with log-CRP level. Samples were from the Candidate gene Association Resource (CARe) cohorts (Atherosclerosis Risk in Communities Study, Framingham Heart Study, Cardiovascular Health Study, Coronary Artery Risk Development in Young Adults Study, Multi-Ethnic Study of Atherosclerosis Study, Jackson Heart Study and Cleveland Family Study), the Women's Health Initiative, and the KORA study. RESULTS: We observed IBC-wide significant evidence for association ($p\text{-value} < 2.2 \times 10^{-6}$) for 12 loci. Seven of these loci (CRP, APOE, TCF1, LEPR, GCKR, IL6R, IL1RN) have previously been established as being associated with CRP in multiple studies. Two of these loci (NLRP3; HNF4A) were recently identified in a large scale genome-wide association (GWA) meta-analysis that included a subsample of the present study. An independent replication analysis in the subsample of 15,637 participants that were not included in this previously published GWA study was supportive for association (NLRP3, $p=8.8 \times 10^{-5}$; HNF4A, $p=1.4 \times 10^{-3}$). Three loci meeting IBC-wide significance in the total sample have not previously been reported to be associated with CRP (ARNTL, $p=2.3 \times 10^{-7}$; FTO, $p=1.6 \times 10^{-6}$; RPS6KB1, $p=2.0 \times 10^{-6}$). An analysis restricted to subjects of European descent resulted in less significant p-values for 9 of the 12 loci meeting IBC-wide significance in the multi-ethnic meta-analysis. Finally, we observed evidence for 4 independently associated loci meeting IBC-wide significance within CRP and 2 independently associated loci within IL6R based on a conditional meta-analysis. CONCLUSION: This large multi-ethnic association meta-analysis identified 3 novel loci associated with serum CRP levels in the ARNTL, FTO, and RPS6KB1 genes and confirmed two others in the HNF4A and NLRP3 genes. These results may identify additional pathways that influence chronic inflammation.

380T

ROCK2 gene confers a risk to arterial stiffness and is translationally modulated by miR-1183. *Y.C Liao[1,2,3], K.C Chen[3], W.W Chou[3], I.W Wang[3], E. Hsi[2,4], S.H.H Juo[3,4].* 1) Section of Neurology, Taichung Veterans General Hospital, Taichung, Taiwan; 2) Graduate Institute of Medicine,College of Medicine , Kaohsiung Medical University, Kaohsiung 807, Taiwan;; 3) Department of Medical Genetics, College of Medicine , Kaohsiung Medical University, Kaohsiung 807, Taiwan;; 4) Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung 807, Taiwan;.

Background: Rho-associated kinase (ROCK) has been showed to be involved in the pathogenesis of atherosclerosis. We hypothesized that single nucleotide polymorphisms (SNPs) at ROCK2 gene, the major isoform in muscle cells, are associated with susceptibility of arterial stiffness.

Methods and Results: Stiffness parameters including beta ()), elasticity modulus (Ep) and pulse wave velocity (PWV) were obtained by carotid ultrasonography. Seven tagging SNPs of ROCK2 were genotyped in 856 subjects from the general population. Two functional SNPs, miRSNP rs978906 and non-synonymous SNP rs9808232, showed significant associations with the stiffness parameters. We then conducted a serial of cellular experiments with constructs carrying different alleles at rs978906 and rs9808232. We found that miR-1183 targeting at the sequences around rs978906 was able to prohibit ROCK2 expression. Suppression of miR-1183 levels accompanied with over-expression of ROCK2 protein was noted in smooth muscle cells treated with ox-LDL. Although rs9808232 caused an amino acid substitution, there was no significant difference in the ROCK2 activity between two alleles of rs9808232. The association between rs978906 and stiffness parameters was replicated in another group of 527 high risk subjects.

Conclusions:ROCK2 polymorphism rs978906 was associated with stiffness parameters in Taiwanese population. ROCK2 expression could be modulated by miR-1183, indicating a potential role of miR-1183 during atherosclerosis process.

381T

Genome-wide linkage and regional association study of obesity-related phenotypes. *A. Liu[1], T. Kelly[1], D. Rao[2], J. Hixson[3], L. Shimmin[3], C. Jaquish[5], D. Gu[4], D. Liu[4], J. He[1].* 1) Epidemiology Dept, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA; 2) Division of Biostatistics, Washington University School of Medicine, St Louis, MO; 3) Human Genetics Center, School of Public Health, University of Texas, Houston, TX; 4) Chinese Academy of Medical Sciences, Beijing, China; 5) National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD.

Background The genetic mechanisms underlying obesity remain largely unknown. The objective of the current study was to conduct a genome-wide linkage scan and regional association study of obesity-related phenotypes.

Methods Height, weight, waist circumference and other important covariables were collected from 3,142 participants from 633 Han, Chinese families of the Genetic Epidemiology Network of Salt-Sensitivity (GenSalt) Study. Lymphocyte DNA samples were also collected and used for genotyping microsatellite markers and single nucleotide polymorphisms (SNPs). Multipoint quantitative trait linkage-analysis was performed using body mass index (BMI) and waist circumference (WC) phenotypes. Additive associations between each SNP in the linkage region and BMI and WC were assessed using a mixed linear regression model to account for family dependencies. Phenotypes were adjusted for the fixed effects of age, gender and field center in all analyses.

Results Maximum multipoint LOD scores of 3.13 and 1.89 were observed for WC and BMI, respectively, at 22q13.33. Within this region, novel marker rs16996195 [minor allele frequency (MAF) = 0.019)] was significantly associated with the WC phenotype. Based on its physical position, marker rs16996195 is located in the TBC1 domain family, member 22A gene (TBC1D22A), a novel gene never implicated in obesity. Compared to those who were homozygous for the major C allele of this variant, carriers of the minor T allele had significantly decreased WC [80.67 (80.09, 81.2) versus 77.55 (76.00, 79.10) cm; p-value = 3.86E-5; FDR p-value= 0.048]. Although not significant after adjustment for multiple comparisons, a similar trend was observed for the BMI phenotype. Additional adjustment of obesity-related phenotypes for rs16996195 revealed that some of the observed linkage could be explained by this marker.

Conclusions Our findings suggest that genomic region 22q13.31-22q13.33 harbors important QTLs for WC and BMI. Furthermore, a novel variant in the TBC1 domain family, member 22A gene was important predictors of obesity-related phenotype. Futther research into the TBC1 domain family, member 22A gene is warranted.

382T

Primary osteoarthritis of the hip and knee shows linkage to chromosome 2q21 in Finnish families. *M. Taipale[1], E. Jakkula[1], O. Kämäräinen[1], S. Barral[2], I. Kiviranta[3], H. Kröger[4], J. Ott[5], L. Ala-Kokko[1,6], M. Männikkö[1].* 1) Oulu Center for Cell-Matrix Research, Biocenter Oulu and Department of Medical Biochemistry and Molecular Biology, University of Oulu, Oulu, Finland; 2) Gertrude H. Sergievsky Center, College for Physicians and Surgeons, Columbia University, New York, USA; 3) Department of Orthopaedics and Traumatology, Helsinki University Hospital, Helsinki, Finland; 4) Department of Orthopaedics and Traumatology, Kuopio University Hospital, Kuopio, Finland; 5) Institute of Psychology, Chinese Academy of Sciences, Beijing, China; 6) Connective Tissue Gene Tests, Allentown, Pennsylvania, USA.

Studies with twins and families have shown that hereditary factors have a major role in the development of osteoarthritis (OA). Several genome-wide linkage scans have indicated suggestive linkage to most of the chromosomes, but no major disease causing variants have been identified so far, even though many variants in several genes have been implicated through previous linkage scans and following association analyses. We identified a novel susceptibility locus for primary hip and knee OA in Finnish families. Genome wide linkage analysis was performed using ten independent families (225 individuals) originating from Central Finland. Additional five families with 54 individuals were included in the following fine mapping. Genome wide analysis identified two potential loci on chromosomes 2 and 11 with suggestive logarithm of odds (LOD) ⁄ 1.5. Fine mapping confirmed the susceptibility locus on chromosome 2q21 with a multipoint LOD score of 3.96 peaking at the marker D2S1260 using a recessive model of inheritance. Two-point analysis confirmed linkage to the same locus with a LOD score of 3.63. Based on the observation that only one family (family 10) contributed significantly to the LOD score, one healthy and two affected family members from the family 10 were chosen for targeted resequencing. The entire 15.5 Mb segment of chromosome 2 consisting the linkage peak and neighboring regions was targeted by NimbleGen Sequence Capture 2.1M array (Roche) and the sequencing was performed by The Genome Analyzer IIx (Illumina) in the Institute for Molecular Medicine Finland (FIMM, Helsinki, Finland). Targeted resequencing resulted in 27 964 high quality variant calls (reference sequence GRCh37). Two affected family members shared 4006 variant calls, of which 241 (6%) were not previously annotated in dbSNP. Out of these 4006 variant calls 760 variant calls were found under the linkage peak of chromosome 2 (3.2 Mb), of which 46 (6%) were absent from dbSNP. In order to reveal OA associating sequence variant in family 10, these 46 variants will be genotyped in all family members for whom DNA is available by Sequenom SNP genotyping method.

383T

A high density screening of the MHC region identified two independent signals for susceptibility to ulcerative colitis. *P. Goyette[1], G. Boucher[1], P-A. Gourraud[2], A. Latiano[3], C. Lagacé[1], V. Annese[3,4], S.L. Hauser[2], J.R. Oksenberg[2], J.D. Rioux[1,5].* 1) Montreal Heart Institute, Montréal, Canada; 2) Department of Neurology, School of Medicine, University of California San Francisco; 3) Lab. Research Gastroenterology Unit, IRCCS "Casa Sollievo della Sofferenza" Hospital, San Giovanni Rotondo, Italy; 4) Azienda Ospedaliero Universitaria (AOU) Careggi, Unit of Gastroenterology SOD2, Florence, Italy; 5) Université de Montréal, Montréal, Canada.

The major histocompatibility complex (MHC) region has been associated to several autoimmune and inflammatory diseases. It encompasses 7.6Mb on chromosome 6p21.3 and contains over 400 genes, 40% of which encode proteins with immunological functions. Among these, the classical HLA class I (*HLA-A, -B, -C*) and class II (*HLA-DP, -DQ, -DR*) gene clusters, involved in antigen processing and presentation, are well characterized in terms of structure, variability and function. Ulcerative colitis (UC) is a chronic inflammatory disease of the colon and rectum showing inflammation primarily limited to the mucosal layer. Both genetic and non-genetic risk factors are believed to play a role in disease susceptibility. Several linkage and genome-wide association studies have demonstrated an important role for the MHC in UC susceptibility, but none have clearly identified the origin of the signal(s) due to the low density of markers tested, extensive allelic variation and linkage disequilibrium (LD) in the region. While the main association signal detected in UC is located in the class II gene cluster, it is unclear whether this represents association to specific HLA alleles or to neighboring loci. We have typed a total of 993 UC cases and 630 controls for SNPs tagging the variability of the MHC region, and performed HLA typing on a subset of these samples (516 UC cases and 490 controls). First, we selected 6411 SNPs capturing the variability of the MHC, and screened a cohort of 386 UC cases and 630 controls. Our preliminary association results and conditional analyses indicate the presence of 3 independent signals distributed across the MHC, including signals near the *IER3* gene (nominal P= 7.04E-6), the *HLA-A* gene (nominal P=5.37E-5) and the *HLA-DQA2* gene (nominal P=1.92E-5). The addition of 607 cases typed at lower density (800 SNPs) increased the significance for 2 of these signals (nominal P=1.93E-7 for the *HLA-A* locus and 1.02E-6 for the *HLA-DQA2* locus), while the *IER3* signal was not captured by the lower density SNP panel. We used results from direct typing of HLA alleles on a subset of the samples to determine whether the association signal originated primarily from HLA alleles or from neighboring markers. Our results indicate that HLA alleles do not represent the main association signal in either class I or class II regions. Our data confirms the involvement of MHC in the pathogenesis of UC and identifies at least two independent signals in the region.

384T

Association study of celiac disease genes in Finnish inflammatory bowel disease patients. *A. Parmar[1,2], M. Lappalainen[1,3], P. Paavo-Sakki[3,4], L. Halme[5], M. Färkkilä[4], U. Turunen[4], K. Kontula[1,3], A. Aromaa[6], V. Salomaa[6], L. Peltonen[7,8], J. Halfvarson[10], L. Törkvist[11], M. D'Amato[9], P. Saavalainen[1,2], E. Einarsdottir[1,2].* 1) Research Program for Molecular Medicine, University of Helsinki, Helsinki, Finland; 2) Department of Medical Genetics, Haartman Institute, University of Helsinki, Finland; 3) Department of Medicine, Helsinki University Hospital, Helsinki, Finland; 4) Department of Gastroenterology, Helsinki University Hospital, Helsinki, Finland; 5) Department of Surgery, Helsinki University Hospital, Helsinki, Finland; 6) National Institute for Health and Welfare, Helsinki, Finland; 7) Institute for Molecular Medicine Finland, Helsinki, Finland; 8) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 9) Karolinska Institutet, Department of Biosciences and Nutrition, Stockholm, Sweden; 10) Örebro University Hospital, Department of Medicine, Örebro, Sweden; 11) Karolinska Institutet, Department of Clinical Science Intervention and Technology, Stockholm, Sweden.

Crohn's disease (CD) and ulcerative colitis (UC) constitute the two major forms of inflammatory bowel disease (IBD), characterised by remitting and relapsing chronic inflammation of the gastrointestinal tract. Finland, Sweden and Denmark are among high incidence areas in Europe and epidemiological studies show that IBD incidence in these countries is still increasing. A number of genes predisposing individuals to IBD have been identified. These genes may be involved in the regulation of innate-or adaptive immune responses, autophagy and the regulation of cell death. As in the case of IBD, celiac disease (CelD) is an immunological disease which primarily affects the gastrointestinal tract. A recent genome-wide association study (GWAS), consisting in part of Finnish samples, identified a number of celiac disease susceptibility loci harbouring genes involved in the regulation of the immune system. A subset of these CelD risk loci may also be associated with increased risk for other autoimmune diseases, including CD and UC. In the current study, we investigated whether the novel CelD risk loci may also affect susceptibility to IBD in the Finnish population. In total, we tested the association of 45 SNPs with CD, UC, and IBD as well as subphenotypes of CD and UC. A meta-analysis on a combined Finnish-and Swedish UC dataset was also performed. None of the tested markers showed significant association (uncorrected p<0.01) to CD, whereas rs2298428, downstream of the gene UBE2L3, was associated with early onset of CD (0-18 years), p_{corr} 0.008, OR 2.51 (1.55-4.06). Furthermore, rs917997, downstream of the IL18RAP gene, was associated with structuring phenotype in CD (p_{corr} 0.035, OR 1.78 (1.27-2.49). Rs864537, within the CD247 gene, showed some association to UC, p_{uncorr} 0.007, OR 0.80 (0.68-0.94). Rs6974491, within the gene ELMO1, was associated with early onset of UC (0-18 years), p_{corr} 0.016, OR 2.19 (1.43-3.36). All of the loci in the current study are strong candidates for celiac disease susceptibility, and some of the markers associated with CD and/or UC or their subphenotypes in the current study have been shown previously to be associated with IBD (CD or UC). Studying these polymorphisms in the Finnish materials is thus likely to help us understand the aetiology of IBD, as well as give us a better understanding of the risk genes that are relevant to IBD patients in Finland.

385T

Association analysis of 71 susceptibility loci for European Crohn's disease in the Japanese population. *K. Yamazaki[1], A. Hirano[1,2], J. Umeno[1,2], M. Takazoe[3], S. Motoya[4], T. Matsui[5], T. Matsumoto[5], Y. Nakamura[6], N. Kamatani[7].* 1) Laboratory for Genotyping Development, Center for Genomic Medicine, RIKEN, Kanagawa, Japan; 2) Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; 3) Department of Medicine, Division of Gastroenterology, Social Insurance Chuo General Hospital, Tokyo, Japan; 4) Department of Gastroenterology, Sapporo Kosei Hospital, Sapporo, Japan; 5) First Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo, Japan; 6) Laboratory of Molecular Medicine, Institute of Medical Science, The University of Tokyo, Japan; 7) Director, Center for Genomic Medicine, RIKEN, Kanagawa, Japan.

Inflammatory bowel disease (IBD) represents the two common types of chronic intestinal disorders, Crohn's disease (CD) and ulcerative colitis. The success of genome-wide association studies (GWAS) revolutionized the field of complex disease genetics through the identification of many susceptibility loci. On 2010, a meta-analysis of six GWAS for CD reported 71 susceptibility loci in European descents. Since we found apparent ethnic differences in the CD susceptibility loci between European and Japanese populations, it is unclear whether these susceptibility loci are also associated with CD in Japanese population. To investigate the association of CD susceptibility loci in Japanese CD patients, we genotyped 71 reported SNPs using a total of 701 CD patients and 3,389 controls. After excluding two SNPs with HWE *P* value of less than 0.01, we found 19 SNPs that showed nominal association with Japanese CD patients (uncorrected $P<0.05$). Among them, five SNPs showed significant association after adjustment of multiple testing by Bonferroni correction; rs3810936 in *TNFSF15* on 9q32 ($P=1.46\times10^{-18}$), rs415890 near *CCR6* on 6q27 ($P=4.38\times10^{-5}$), rs6738825 in *PLCL1* on 2q33 ($P=8.35\times10^{-5}$), rs6556412 near *IL12B* on 5q33 ($P=1.03\times10^{-4}$) and rs4409764 near *NKX2-3* on 10q24 ($P=6.61\times10^{-4}$). One of these candidates, rs2476601 in *PTPN2* showed the opposite direction of effect as compared to European population and further studies were required to examine association. *IL23R* and *NOD2*, the two strongest susceptibility loci for CD in European population, were not associated with Japanese CD patients because risk alleles in these genes were very rare or monomorphic in the Japanese population (risk allele frequency of rs2076756 in *NOD2* was 0.0007 and that of rs11209026 in *IL23R* was 0, respectively). In addition, SNPs in *SP140* and *TYK2* were also monomorphic in Japanese. These results indicate that there exists different genetic architecture for CD susceptibility between European and Japanese populations. In conclusion, we examined 71 CD susceptibility loci identified by European GWAS meta-analysis in a Japanese population. We found 19 common susceptibility loci for both European and Japanese populations, however, *IL23R* and *NOD2* were not associated in Japanese. We are applying these results for pathway-based analysis to clarify the differences in the pathogenesis of CD between European and Japanese.

386T

Gene-environment interaction effects of 17q21 variants and rhinovirus wheezing illness on risk for childhood-onset asthma. *M. Caliskan[1], D.A. Loisel[1], G. Du[1], D.J. Jackson[2], J.E. Gern[2], R.F. Lemanske[2,3], D.L. Nicolae[1,4,5], C. Ober[1,6].* 1) Department of Human Genetics, The University of Chicago, Chicago, IL, USA; 2) Department of Pediatrics, University of Wisconsin-Madison, Madison, WI, USA; 3) Department of Medicine, University of Wisconsin-Madison, Madison, WI, USA; 4) Department of Medicine, The University of Chicago, Chicago, IL, USA; 5) Department of Statistics, The University of Chicago, Chicago, IL, USA; 6) Department of Obstetrics and Gynecology, The University of Chicago, Chicago, IL, USA.

The 17q21 asthma locus, including the *ORMDL3* and *GSDML* genes, represents the most highly replicated asthma susceptibility locus and is one of the most significant genetic risk factors for childhood asthma. However, the biology of this locus and its role in asthma are still largely unknown. To characterize the role of this locus in childhood asthma, we conducted a candidate gene association study of 6 previously associated 17q21 SNPs and asthma in 247 children participants in the Childhood Origins of ASThma (COAST) birth cohort and the RhinoGen Study, both in Madison, Wisconsin. We also tested for gene-environment interactions between 17q21 genotypes and common environmental risk factors for asthma: rhinovirus wheezing illness (RV-WI) or respiratory syncytial virus wheezing illness (RSV-WI) in early childhood in 185 COAST children (67 with asthma), in whom information on WI with RV and RSV infections in the first 3 years of life was collected prospectively. Genotypes at all 6 SNPs were associated with childhood onset asthma (p=0.00045-0.0036); however, the association with asthma was significant only in the 61 COAST children who had RV-WI (p=0.0011) and not in the 124 children who did not have RV-WI in childhood (p=0.78), resulting in a significant gene-environment interaction (GEI) effect on asthma risk (17q21 genotype x RV-WI, interaction p = 0.014). No such interaction was observed for RSV-WI ($p > 0.40$). Moreover, genotypes at this locus were not associated with the occurrence of RV-WI in the first 3 years of life, number of RV-WI, duration of RV-WI or cold symptom burden during viral illness (p=0.05-0.91). We interpret these findings to indicate that variants at the 17q21 locus do not influence the risk for RV infection per se, or persistence and severity of RV infection. Instead, we propose that children with 17q21 risk variants may respond differently to RV-induced damage, which increases their subsequent risk for asthma. Our study is the first to specifically attribute 17q21 locus-associated risk for asthma to early-life WI with RV infection, which provides clues as to role of the 17q21 variants in the etiology of asthma.

387T

Analysis of gene-environment interaction for plasma CRP level in a Korean cohort. *E.P. Hong[1], J.G. Seo[1], D.H. Kim[2], J.W. Park[1,*].* 1) Dept. of Medical genetics; 2) Dept. of Social Medicine, Hallym University, College of Medicine, Chuncheon, Gangwon-do, Korea.

High sensitivity C-reactive protein (hs-CRP) level is a well-known inflammatory biochemical marker to predict a variety of age-related disease such as cardiovascular disease. The plasma CRP level differs by age, gender, and ethnic groups. Multiple genetic and non-genetic factors influence the plasma hs-CRP level. We evaluated gene-environment interaction affecting the plasma CRP level using a six-year follow up data obtained from a community-based cohort study in Korea. We firstly performed linear regression analyses to identify risk factors for the elevated plasma hs-CRP level among 32 clinical and environmental variables. We genotyped 969 Koreans aged above 46 for 50 single nucleotide polymorphism (SNP) markers located near or in 6 candidate genes (i.e. APOC1, CRP, HNF1A, KIAA0182, NAV2, and ULK4). We performed single SNP analyses under three genetic models (i.e. additive, dominant, and recessive models) and compared the hs-CRP level by genotype of each SNP using a cox proportional hazard model. Finally, we evaluated gene-environment interaction using the method of survival dimensionality reduction. We identified risk factors such as a history of smoking (HR = 1.7, p = 0.0004) and a high waist-hip ratio (HR = 18.4, p = 0.0218) for the elevated hs-CRP level. We found evidence for gene-environment interaction; for example, a NAV2 gene variant (11p15.1) significantly interacted with cigarette smoking to result in the elevated hs-CRP level. The evidence for gene-environment interaction in the determination of plasma CRP level shown in this study may warrant further study.

388T

Liability threshold modeling of covariates increases power in case-control association studies. *A. Price[1], N. Zaitlen[1], S. Lindstrom[1], B. Pasaniuc[1], M. Cornelis[2], G. Genovese[3], A. Barton[4], D. Bowden[5], S. Eyre[4], B. Freedman[5], D. Friedman[3], L. Groop[6], B. Henderson[7], P. Hicks[5], L. Kolonel[8], C. Langefeld[5], L. Le Marchand[8], K. Waters[7], C. Haiman[7], D. Hunter[1], R. Plenge[9], J. Worthington[4], D. Schaumberg[1], D. Chasman[2], D. Altshuler[9], B. Voight[9], P. Kraft[1], N. Patterson[9].* 1) Harvard School of Public Health, Boston, MA; 2) Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 3) Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; 4) Manchester Academic Health Science Centre, The University of Manchester, Manchester, U.K; 5) Wake Forest University School of Medicine, Winston-Salem, NC; 6) Scania University Hospital, Lund University, Malmö, Sweden; 7) University of Southern California Keck School of Medicine, Los Angeles, CA; 8) Cancer Research Center, University of Hawaii, Honolulu, HI; 9) Broad Institute, Cambridge, MA.

Genetic case-control association studies often include data on covariates, such as body mass index (BMI) or age, that may modify the underlying genetic risk of case or control samples. For example, in type 2 diabetes, odds ratios estimated from low-BMI cases are larger than those estimated from high-BMI cases. An unanswered question is how to optimally use this information to maximize statistical power. In this study we show via simulation that our approach to fitting liability threshold models and computing association statistics, which accounts for disease prevalence and non-random ascertainment, can use this information to increase power. Our method outperforms standard case-control association tests, case-control tests with covariates, tests of gene x covariate interaction, and tests that restrict to a subset of samples. We investigate empirical case-control studies of type 2 diabetes, prostate cancer, breast cancer, rheumatoid arthritis, age-related macular degeneration, and end-stage kidney disease over a total of 78,256 samples. In these data sets, liability threshold modeling outperforms logistic regression for 104 of the 140 known associated variants investigated (p-value < 10-9). The improvement varied across diseases with a 17% median increase in test statistics, corresponding to a greater than 25% increase in power. Application of liability threshold modeling to future case-control association studies of these diseases, or other diseases with analogous effects of covariates on genetic risk, will yield a substantial increase in power for disease gene discovery.

389T

Detection of Intergenerational Genetic Effects: A Plea for Pedigrees. *J.S. Sinsheimer[1], E.J. Childs[2], E.M. Sobel[1], C.G.S. Palmer[1].* 1) Department of Human Genetics, Univ California, Los Angeles, Los Angeles, CA, 90095; 2) Department of Epidemiology, Johns Hopkins University, Baltimore, MD, 21218.

Background: In most cases, genome-wide association studies (GWAS) have failed to account for more than a modest amount of the disease risk attributed to genetic variants. Intergenerational genetic effects provide one potential source of the remaining risk. In general, GWAS using unrelated individuals cannot detect intergenerational genetic effects contributing to disease. Thus, alternative study designs and statistical methods are needed.

Methods: To detect intergenerational genetic effects, we improve the extended maternal fetal genotype (MFG) incompatibility test to estimate any combination of maternal effects, offspring effects, and their interactions at polymorphic loci or multiple SNPs, using any size pedigrees. We apply our improved EMFG test to simulated and actual pedigree data.

Results: We show that intergenerational effects can explain a substantial portion of the heritability of a trait but that GWAS of unrelated individuals are poorly powered to detect these effects. Through simulated data, we explore the advantages of using extended pedigrees rather than nuclear families. In particular, we find that using the EMFG test with extended pedigrees increases power and precision, whereas partitioning extended pedigrees into nuclear families can underestimate intergenerational effects. Application of the EMFG to data from families affected by schizophrenia demonstrates that intergenerational effects may play an important role in complex disease susceptibility. Alternative explanations such as ascertainment and mate selection biases cannot explain our results.

Conclusions: Our results demonstrate the power of the EMFG test to examine intergenerational genetic effects, highlight the importance of pedigree rather than case/control or case-mother/control-mother designs, and demonstrate that pedigrees provide an opportunity to examine alternative, non-causal mechanisms.

390T

Localization of rare variants influencing complex traits using lineage-specific linkage analysis in extended pedigrees. *H.H.H. Goring, J.W. Kent Jr., E. Drigalenko, T.D. Dyer, J. Blangero.* Dept Gen, Texas Biomedical Research Institute, San Antonio, TX.

Large-scale genome-wide association studies (GWAS) have successfully identified common SNPs associated with many complex traits, but the identified loci collectively explain only a small fraction of estimated heritability. This has led to the speculation that rare variants may explain much of the “missing heritability”. A key problem when seeking to identify rare variants is their rarity, necessitating either enormous sample sizes or various collapsing strategies. Both of these two limitations can be overcome by studying extended families. The reason is that a rare allele, if present in a founder individual of one of the available pedigrees, will often be present in multiple copies in a study, for the simple reason that the allele will have been passed on randomly in meiosis from the founder to offspring and further downstream generations. The expected number of copies of an allele from a founder, no matter its population rarity, is given by the sum of the kinship coefficients of all descendants with that founder. If the observed copy number is sufficiently high, then rigorous statistical evaluation of co-segregation of rare allele and complex trait is possible. Based on this idea, we have developed a mixed model variance components approach to localize rare variants of strong individual effect in extended pedigree samples. We first identify founders with a sufficiently many descendants (⁄ 10) such that linkage analysis of a single lineage may yield a genome-wide statistically significant signal. We then perform a genome-wide linkage analysis on each of the two haplotype lineages of a given founder, with the lineages changing along the genome at the positions of recombination events. Ultimately, each significant linkage peak comprises a candidate region where the DNA sequence of that lineage's founder is examined for putatively causal sequence variants. We have evaluated the performance of this approach via simulation of rare variants, demonstrating that the method is able to obtain significant linkage peaks in specific lineages even if linkage analysis of the entire dataset, with or without a locus-heterogeneity component, fails to do so. We applied this approach to quantitative traits (including expression profile data) in the San Antonio Family Heart Study. Our results indicate that classical quantitative trait linkage analysis misses many potential rare QTLs that can be detected using our efficient lineage-specific localization method.

391T

A mutation in *BMP3* Contributes to Canine Brachycephaly. *E.A. Ostrander[1], S. Hutchinson[2], A. Byers[1], B. Carrington[3], D. Faden[1], R. Sood[3], A. Boyko[4], J.W. Fondon 3rd[5], R.K. Wayne[6], C.D. Bustamante[4], B. Ciruna[2], J.J. Schoenebeck[1].* 1) Cancer Gen Br, Bldg 50, NHGRI/NIH, Bethesda, MD; 2) Program in Developmental Biology and Stem Cells, The Hospital for Sick Children and the University of Toronto, Toronto, ON, Canada; 3) National Human Genome Research Institute Zebrafish Core, Bethesda, MD; 4) Department of Genetics, Stanford School of Medicine, Stanford, CA; 5) Department of Biology, University of Texas at Arlington, Arlington, TX; 6) Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA.

Cephalic disorders occur with a frequency greater than 1 in 2,500 live births, yet the genetic underpinnings of most cephalic disorders remain unknown. For many purebred dogs, skull shapes reminiscent of cephalic disorders such as brachycephaly, dolichocephaly, and hydrocephalus are breed-defining features. Geometric morphometric analysis of 348 purebred dog skulls from 93 breeds was used to quantify skull shape. After correcting for size, we used principal components analysis to characterize remaining shape variance. Fifty-four percent of variance was explained by the first principal component (PC1), whose factor loadings included changes in rostrum length, cranial vault depth, and width of the zygomatic arches. Thus, PC1 is a quantitative measure of morphological changes pertinent to brachycephaly (rostrocaudal skull shortening). A genome-wide association scan of PC1 revealed numerous, highly significant QTLs. Detailed analysis of the association on chr32: 8-8.5 Mb (best SNP, $P<3x10^{-44}$) revealed a depression in heterozygosity (H_o) among brachycephalic dogs. Loss of H_o is a hallmark of selective sweeps and can be indicative of a locus experiencing strong, selective pressure over generations of planned breedings. Fine mapping on chr32 revealed a missense F->L mutation in the predicted signaling portion of canine *BMP3*. Among the TGF) superfamily, the amino acid mutated in *Bmp3* is invariably aromatic (Phe or Tyr), leading us to hypothesize that such a mutation could lead to loss of function. To test our hypothesis, we turned to zebrafish as an experimental surrogate for testing *Bmp3* function. Our data show that during development, zebrafish *bmp3* is expressed in neurocranial tissues. Furthermore, morpholino knockdown of *bmp3* led to loss of cartilaginous head structures. Overexpression of wild type and mutant canine Bmp3 RNA demonstrates that only the first is biologically active in fish. Together, these experiments reveal a conserved role for *Bmp3* during vertebrate cranioskeletal development and suggest that the canine F->L mutation renders this molecule inactive. Identification of *Bmp3* as a contributor to canine brachycephaly demonstrate the power of studying dog genetics to uncover factors and pathways likely to be relevant to human cephalic disorders, thus enabling genetic diagnostics and new treatment paradigms.

392T

Architecture of glaucoma endophenotypes in heterozygotes carrying the myocilin K423E mutation. *P. Belleau[1], S. Dubois[1], K. Lebel[1], R. Arseneault[1], E. Shink[1], G. Côte[2], M. Amyot[3], V. Raymond[1,2].* 1) Laboratory of Molecular Genetics of Sensory Systems, CHUL (Université Laval Hospital) Research Centre, Québec City, QC, Canada; 2) Dept of Ophthalmology, Université Laval, Québec City, QC, Canada; 3) Dept of Ophthalmology, Université de Montréal, Montréal, QC, Canada.

Chronic open-angle glaucoma (COAG, as defined in Shields Textbook of Glaucoma, 6th Ed., 2011) is characterized by optic nerve degeneration and visual field impairments. Potential disease mechanisms implicate elevated intraocular pressures (IOP, a known risk factor), optic nerve hypersensitivity to stressful events and/or inflammation. COAG is a complex genetic disease that can also segregate in a Mendelian fashion in some families. We studied the CA family, a huge French-Canadian pedigree, in which autosomal dominant COAG was caused by the myocilin (MYOC) K423E mutation. The disorder showed a very wide range of ages at onset (AAO) and, elevated IOPs were detected before optic nerve degeneration. The goal of this study was to establish if any of its endophenotypes could be controlled by modifier genes. To do so, we assessed seven (7) traits potentially implicated in its disease mechanisms. We also analyzed if WDR36, a putative modifier gene, had any effect on the endophenotypes. Quantitative traits values (QT) were extracted from the records of 150 $MYOC^{K423E}$ heterozygotes. Traits relative to intraocular pressures were: age of the 1st IOP $\geq$ 22 mm Hg (age at onset: AAO), maximal IOP and number of years with at least 1 IOP value $\geq$ 22 mm Hg. Traits relative to optic nerve hypersensitivity were: cup to disk ratio (C/D), first C/D $\geq$ 0.7, variation of the C/D per time period (ΔC/D) and a novel index defined as ΔC/D pondered by IOP measurements. We further analyzed the heritability (h^2) of these QT. To evaluate the effects of WDR36 variations on QT, we compared QTs displayed by double mutants who simultaneously carried 1 $MYOC^{K423E}$ mutation and 1 WDR36 variation versus the medians of QT displayed by $MYOC^{K423E}$ carriers who were WDR36 wild-type and shared a kinship coefficient of $\geq$ 0.0625 with the double mutants (at most 1st degree cousins). In our heterozygotes, AAO and IOP max were characterized as 2 heritable traits relative to IOP since of their h^2 values were both $\geq$ 0.4. On the other hand, the other 4 QTs relative to optic nerve hypersensitivity showed no significant h^2. The majority of WDR36/ $MYOC^{K423E}$ double mutants displayed a younger AAO and higher IOP max when compared to their respective controls. In conclusion, our study strongly suggests that MYOC-related modifier genes primarly act on mechanisms leading to elevated intraocular pressures. Our data also suggest that WDR36 may act as a modifier by altering similar mechanisms leading to elevated IOPs.

393T

Hepatic steatosis, hepatic inflammation and the *CPN1-ERLIN1-CHUK-CWF19L1-BLOC1S2* gene cluster in the NHLBI Family Heart Study (FamHS). *M.F. Feitosa[1], M.K. Wojczynski[1], K.E. North[2], J. Wu[1], M.A. Province[1], J.J. Carr[3], I.B. Borecki[1].* 1) Div Statistical Genomics, Washington Univ Sch Medicine, St Louis, MO; 2) Dept Epidemiology, Univ of North Carolina, Chapel Hill, NC; 3) Dept Radiology, Wake Forest University School of Medicine, Winston Salem, NC.

Nonalcoholic fatty liver disease (NAFLD) is a common disease and ranges from simple steatosis, to nonalcoholic steatohepatitis, to cirrhosis. CT measured liver attenuation (Hounsfield Units) is inversely related to the amount of fat in the liver (FL), and represents a non-invasive measure of steatosis. Serum ALT levels are indicators of the inflammatory severity of hepatic steatosis and have been used as a biochemical inflammatory marker of NAFLD. The phenotypic correlation between CT measured FL and ALT levels was 27% ($p<0.0001$) in FamHS. A correlated meta-analysis (CMA) approach can improve power to detect variants that have pleiotropic effects on correlated traits. We investigated whether genetic variants accounted for concomitant variation in FL and ALT in 2,679 subjects of European descent using a CMA approach. We excluded heavy drinkers and subjects with hepatitis C. FL and ALT levels were adjusted for age, sex, clinical center, alcohol consumption, genotype batch effects, family relatedness, and population stratification. FL was also adjusted to an external calibration standard. We performed univariate genome-wide association (GWA) scans on ~2.5 million imputed SNPs, and used the univariate GWA results in a CMA, correcting for correlations between FL and ALT GWA scans. We found evidence for association between variants of the *CPN1-ERLIN1-CHUK-CWF19L1-BLOC1S2* gene cluster on 10q24-q25 with concomitant variation in FL and ALT levels. Among the 42 SNPs in this gene cluster (CMA-p $<7.7\times10^{-6}$), seven SNPs in complete LD were strongly associated with the CMA phenotype of FL and ALT ($9.50\times10^{-11} \leq$ CMA-p $\leq 1.68\times10^{-9}$) as compared with suggestive univariate GWA of FL and ALT (e.g. CMA-p=9.50×10^{-11} vs. FL p=4.18×10^{-7} and ALT p=9.20×10^{-6}). To investigate whether the 42 variants were associated with "inflammatory severity of hepatic steatosis", we created a categorical trait using ALT ($\geq$ 40 U/L) and/or FL ($\geq$ 40 HU) to define steatosis-inflammatory affection status. The results endorsed evidence of association between the 42 variants with steatosis-inflammatory affection ($1.9\times10^{-2} < p < 1.1\times10^{-4}$). This gene cluster is involved in transcription factors, cell proliferation, lipid-raft-like domains, liver-expressed proteins, and inflammatory mediator-protein of hepatic and systemic insulin resistance. Our findings suggest that variants of *CPN1-ERLIN1-CHUK-CWF19L1-BLOC1S2* gene cluster influence both hepatic steatosis and hepatic steatosis with inflammation.

394T

Body mass index-related single nucleotide polymorphisms (SNPs) associations with pubertal timing in 55,203 women: the ReproGen Consortium. *L. Fernández-Rhodes[1], K.K. Ong[2], E.W. Demearath[3], J. Dreyfus[3], J.M. Murabito[4], C.E. Elks[2], D.I. Chasman[5], K.L. Lunetta[6], T. Esko[7], C. He[8], T. Corre[9], K.E. North[1, 10], D.J. Hunter[11], J.N. Hirschhorn[12], N. Franceschini[1] on behalf of the ReproGen Consortium.* 1) Department of Epidemiology, UNC Gillings School of Global Public Health, Chapel Hill, NC, USA; 2) MRC Epidemiology Unit, Cambridge, United Kingdom; 3) Division of Epidemiology and Community Health, University of Minnesota School of Public Health, Minneapolis, MN, USA; 4) Boston University School of Medicine and the Framingham Heart Study, Framingham, MA, USA; 5) Brigham and Women's Hospital and Harvard School of Medicine, Boston, MA, USA; 6) Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA; 7) Estonian Genome Center, University of Tartu, Estonia; 8) Department of Public Health, Indiana University School of Medicine, Indianapolis, IN, USA; 9) Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milan, Italy; 10) Department of Genetics and Carolina Center for Genome Sciences, UNC Chapel Hill, Chapel Hill, USA; 11) Departments of Nutrition and Epidemiology, Harvard School of Public Health, Boston, MA, USA; 12) Department of Genetics, Harvard School of Medicine, Boston, MA, USA.

Background: Obesity is of global health concern. Recent genome-wide association studies (GWAS) of age at menarche identified several variants that had been previously associated with body mass index (BMI). Well-described associations between childhood obesity, earlier pubertal timing in girls, and adult obesity, may be in part due to genetic factors. **Methods:** To systematically test if known genetic variants for BMI are also associated with pubertal timing in girls, we conducted a meta-analysis of the association of 68 single nucleotide polymorphisms (SNP) with age at menarche (ranging from 9-17 years) in 55,203 women of European descent from 23 studies in the ReproGen Consortium. Each study performed a separate analysis using additive models and adjusting for birth year, site (as appropriate), and population stratification. Between-study heterogeneity was investigated using meta-regression techniques. **Results:** Eight BMI loci were previously reported to associate with age at menarche in this dataset. We identified novel associations with age at menarche at four out of 18 recently published BMI loci (*TNNI3K, RBJ, STK33, TMEM160*). In each case, the BMI-increasing allele decreased age at menarche (between 0.41 to 0.58 weeks per allele). **Conclusions:** We identified several novel associations of age of menarche with recently reported BMI GWAS variants. Our findings suggest complex genetic relationships influencing both menarche and obesity, which are consistent with a role of these genes in growth and development.

395T

Modeling genome-wide association SNP impact on metabolic diseases through structural equations. *R. Karns[1], P. Succop[1], G. Zhang[2], G. Sun[1], S. Indugula[1], S. Missoni[3], Z. Durakovic[3], R. Chakraborty[4], P. Rudan[3], R. Deka[1].* 1) Environmental Hlth, Univ Cincinnati, Cincinnati, OH; 2) Human Genetics Division, Cincinnati Children's Hospital, Cincinnati, OH; 3) Institute for Anthropological Research, Zagreb, Croatia; 4) Center for Computational Genomics, University of North Texas Health Science Center, Fort Worth, TX.

This study provides structure and context to the complex relationships between genome-wide association (GWA) variants, biochemical and anthropometric traits, metabolic syndrome, and cardiovascular diseases through latent variable analysis and structural equation modeling. We have performed GWA of fifteen age- and gender-adjusted quantitative traits indicative of cardiovascular health in an isolated population of the eastern Adriatic (n=1325). From each GWA, the most significant SNPs were extracted for inclusion as manifest predictors in a structural equation model of traits involved in the development of metabolic syndrome (MetS) and cardiovascular diseases. In our final model, eleven metabolic and anthropometric traits were distilled into a single manifest variable (HbA1c) and three latent factors (described as obesity, blood pressure, and lipid factors), each with loadings of at least 0.4, that predicted MetS (all *P*-values<0.05). MetS was most strongly associated with the obesity factor (*P*<0.0001), and in turn predicted increased risks of gout and coronary heart disease (*P*-values<0.0001 and 0.002, respectively). Coronary heart disease was also predicted by HbA1c (*P*<0.0001). Odds for kidney disease were predicted by type-2 diabetes and coronary heart disease (*P*-values<0.0001). Type-2 diabetes was also associated with increased risk for stroke (*P*<0.0001), and gout predicted increased odds for type-2 diabetes (*P*=0.003). Additionally, thirty-one GWA-extracted variants were significantly associated with the latent factors and HbA1c. One HDL-related variant, rs16855289, was directly and significantly associated with MetS (*P*=0.002). Two glucose-related variants (rs7100623 and rs12243326) were associated with type-2 diabetes (*P*-values 0.002 and <0.0001, respectively), which was also predicted by HbA1c (*P*<0.0001). The derived structural equations model provides a context for the whole-system impact of GWA variants and clarifies the relationships between anthropometric and biochemical traits indicative of cardiovascular health. In addition, we provide further support of the functionality of MetS as a predictor of the development of cardiovascular diseases. Supported by NIH grants R01-DK069845 and T32-ES010957.

396T

Gene set enrichment analysis with distal *cis*-regulatory elements in genetics of vascular dementia. *Y. Kim, J. Ryu, M. Kong, C. Lee.* Soongsil University, Seoul, Korea.

Vascular dementia (VD) has been greatly concerned to the elderly and their families as the second most common dementia after Alzheimer's disease. Knowledge on its genetic basis has been quite limited from association studies conducted with candidate genes. Recently, a genome-wide association study (GWAS) was conducted for susceptibility to VD. Nevertheless, genetic associations have not been observed with any nucleotide sequence variants. This might be attributed not only to a quite conservative significance threshold of Bonferroni multiple test but also to a considerable complexity for genetic architecture of VD. In the current study, a gene set analysis by functional annotation including distal *cis*-regulatory sequences was employed using a variant set with potential false negative associations from the previous GWAS. As a result, the variants were significantly enriched near genes involved in critical biological processes to VD such as associative learning ($P = 2.29\times10^{-11}$) and memory ($P = 1.73\times10^{-7}$). Since we could not obtain such results by gene-based annotation analysis with the sequences from 5kb upstream to 1kb downstream, this study suggested that distal regulatory elements might play considerable roles in susceptibility to VD. Further examination on functions of the distal regulatory elements using chromatin immunoprecipitation (ChIP) data revealed potential enhancers regulating their target genes.

397T

Geographic differences in genetic susceptibility to IgA nephropathy: GWAS replication study and geospatial risk analysis. *K. Kiryluk[1], Y. Li[1], M. Rohanizadegan[1], S. Sanna-Cherchi[1], M. Choi[2], F. Scolari[3], L. Gesualdo[4], S. Savoldi[5], A. Amoroso[6], B. Julian[7], R. Wyatt[8], J. Novak[7], B. Stengel[9], L. Thibaudin[10], F. Berthoux[10], F. Eitner[11], J. Floege[11], U. Panzer[12], J. Nagy[13], R. Lifton[2], A.G. Gharavi[1].* 1) Dept Medicine, Columbia University, New York, NY; 2) Department of Genetics, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT, USA; 3) University of Brescia and Second Division of Nephrology, Montichiari Hospital, Montichiari (Brescia), Italy; 4) Department of Biomedical Sciences, University of Foggia, Foggia, Italy; 5) Nephrology and Dialysis Unit, Ciriè Hospital, Torino, Italy; 6) Department of Genetics, Biology and Biochemistry, University of Torino, Torino, Italy; 7) Departments of Microbiology and Medicine, University of Alabama at Birmingham, Birmingham, AL, USA; 8) Children's Foundation Research Center at the Le Bonheur Children's Hospital, and the Division of Pediatric Nephrology, University of Tennessee Health Sciences Center, Memphis, TN, USA; 9) Inserm, Centre for Research in Epidemiology and Population Health, Villejuif, France; 10) Nephrology, Dialysis and Renal Transplantation Department, University North Hospital, Saint Etienne, France; 11) Department of Nephrology, University of Aachen, Aachen, Germany; 12) III Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; 13) Nephrology Center and 2nd Department of Internal Medicine, Medical Faculty, University of Pécs, Hungary.

IgA nephropathy (IgAN) is a major cause of kidney failure worldwide. It is common among Asians, moderately prevalent among Europeans and rare in Africans. In a recent GWAS, we localized 5 IgAN susceptibility loci, including 3 independent loci in the MHC, a common deletion CFHR1,3Δ, and a 5th locus on chr.22q12. The goals of this study were: (1) to replicate the GWAS loci in independent cohorts, (2) to assess differences in the effects of these loci in diverse populations, and (3) to model the distribution of risk alleles in the worldwide populations. We studied the five IgAN risk loci in an independent cohort composed of 5 nationalities (French, Italian, German, Hungarian, and African-American, totaling 1,120 cases and 1,665 matched controls). After stringent QC measures, the association analysis was performed individually in each cohort followed by a joint analysis with 4 cohorts from the original GWAS (Beijing, Shanghai, Italian and North American; combined N=8,687). Heterogeneity was assessed using Cochrane's Q-test and het. index (I2). A genetic risk score was calculated and after validation, the risk prediction model was applied to 1,042 HGDP individuals (57 world populations). Spatial interpolation of the risk trend surface was used to construct topographical disease risk maps across 6 continents (Spatial and Maps packages, R v.2.9).

Four out of 5 loci demonstrated significant direction-consistent replication (ORs 0.70-0.86, p-values 9x10-8-1x10-2) with minimal heterogeneity (I2<25%, Q-test p>0.05). Heterogeneity was observed only for the TAP1/2-PSMB8/9 locus within the MHC region (I2=75%, Q-test p<0.01). This locus replicated in Italians and Germans (OR=0.60 and 0.67, P=5x10-3 and 2x10-2), but not in French, Hungarians, or African-Americans. However, in the combined analyses, all 5 loci remained highly significant with minor alleles conferring protective effects (ORs 0.66-0.81, p-values 4x10-27-7x10-10). The risk score model explained ~5% of the total variance in disease risk. In the geospatial analysis, the genetic risk of IgAN increases sharply with distance from Africa (P<2x10-16), and thus closely parallels the geographic distribution of IgAN.

The five IgAN risk loci explain a significant proportion of the risk of IgAN. These data suggest that variation in risk allele frequencies in part contributes to the known variation in disease prevalence among different ethnicities.

398T

Phenotype mapping: An approach for integrating multidimensional genetic and phenotypic data. *E.R. Martin, D. Ma, M.L. Cuccaro.* Dr. John T. Macdonald Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL.

Complex human diseases are marked by both genetic and phenotypic multidimensionality; that is, we expect that several genetic variants lead to the disease, which itself is often composed of several phenotypic dimensions. For example, autism spectrum disorder is a common neurodevelopmental disorder characterized by impairments in social, communicative, and behavioral dimensions. Dealing with such genetic and the phenotypic multidimensionality simultaneously in analysis is challenging. Traditionally, the mapping of complex diseases begins by reducing the phenotype to a simple composite trait (often a binary disease diagnosis) to allow for thorough analysis of multiple genetic variants, thereby mapping genetic variants to the composite trait. We suggest an alternate strategy that begins with reducing genetic variants to a composite genotypic value, which allows for more thorough analysis of phenotypic dimensions. This strategy provides a framework for mapping phenotypic dimensions to a composite genotype value. The Phenotype Mapping (PM) approach examines genetic variants in a gene/set of genes already known (or hypothesized) to be associated with the disease. It is designed to test the hypothesis that genotypic associations (from one or a combination of variants) with the disease are explained by their association with a specific phenotypic dimension (or combination of dimensions). The PM procedure involves two steps: (1) Given a set of genetic variants, stepwise logistic regression is conducted in cases and controls to determine best model for predicting affection status from genotypes. This model then is used to determine fitted (genotypic) values for each individual. (2) General linear regression is conducted in cases only to test for association between the genotypic values from step 1 (as the response variable) and the phenotypic dimensions (as main effects and interactions). A benefit of this approach is that there is no penalty for selecting the best model in step 1 since the analysis in step 2 is independent. Using computer simulations, we explore several genotype-phenotype models to demonstrate the best uses of the PM approach and compare it to traditional approaches where possible. The PM approach provides an approach to simultaneously incorporate genetic and phenotypic data into an integrated analysis. This approach has the power to reveal more homogeneous phenotypic subsets that will improve the success of replication and follow-up studies.

399T

Gene-expression Guided Selection of Candidate Loci and Molecular Phenotype Analyses Enhance Genetic Discovery in Systemic Lupus Erythematosus. *T. Niewold[1], Y. Koldobskaya[1], A. Kumar[1], S. Agik[1], J. Arrington[1], B. Franek[1], M. Kumabe[1], T. Utset[1], R. Mikolaitis[2], M. Jolly[2], A. Skol[3].* 1) Section of Rheumatology, Univ Chicago, Chicago, IL; 2) Section of Rheumatology, Rush University, Chicago, IL; 3) Section of Genetic Medicine, Univ Chicago, Chicago, IL.

Objective: Systemic lupus erythematosus (SLE) is a highly heterogeneous autoimmune disorder characterized by differences in autoantibody profiles, serum cytokines, and clinical manifestations. This study aims to use gene expression data to rationally select novel candidate genes associated with molecular phenotypes in SLE. Methods: We devised an algorithm to select additional candidate SNPs from an existing case-case genome-wide association study of SLE patients stratified by autoantibody profile and serum interferon alpha. The top 200 SNPs from the GWAS were searched in the SCAN database, which compares genome-wide expression data to genome-wide SNP genotype data in HapMap cell lines. SNPs were chosen if they were associated with differential expression of 15 or more genes at a significance of p<9x10-5. This resulted in 11 SNPs which were genotyped in 453 SLE patients and 526 matched controls. Logistic regression models were used to detect associations between the candidate SNPs and serum IFN-(and autoantibodies in SLE patients. Results: A Q-Q plot demonstrated clear deviation from the null distribution in favor of positive findings in our autoantibody-SNP association results (p=3.2x10-37). 6 of 11 SNPs showed associations with specific autoantibody classes within either the European- or African-American ancestral backgrounds (p<0.05), and three of these six would withstand a Bonferroni correction for multiple comparisons (p<4.5x10-3). Two SNPs were associated with serum IFN-(, and one of these was independent of any autoantibody association (p=1.5 x 10-3). Case-control analysis showed no large differences in allele frequencies. Conclusions: This study illustrates the utility of gene expression data to rationally select candidate genetic loci implicated in complex genetic diseases such as SLE. Stratification by molecular phenotypes should be useful in the discovery of additional genetic loci and in elucidating pathogenic pathways in SLE.

400T

Increasing association mapping power and resolution in mouse genetic studies through the use of meta-analysis for structured populations. *E. Kang[1], N. Furlotte[1], A. Van Nas[2], A. Lusis[2], E. Eskin[1,2].* 1) Department of Computer Science, University of California, Los Angeles, CA, USA; 2) Department of Human Genetics, University of California, Los Angeles, CA, USA.

Mouse models have played an integral role in the discovery and understanding of the mechanisms underlying many human diseases. The primary mode of discovery has traditionally been linkage analysis applied to crosses between two inbred strains. Although this method results in high power to identify regions that effect traits, resolution is often low, making it difficult to identify the causal variant. More recently, a panel of mice termed the hybrid mouse diversity panel (HMDP) has been developed to combat this issue. By utilizing population structure correction and genome-wide association study methodologies, mapping results within this panel tend to have both high resolution and high statistical power. However, power in the HMDP is limited by the number of available inbred strains. In this paper, we introduce a study design based on the concept of meta-analysis, in which HMDP data is combined with F2 cross data, in order to achieve high power and increase resolution. Due to the drastically different genetic structure of F2 crosses and the HMDP, the best way to combine results from the two studies for a given SNP is dependent on the strain distribution pattern of the SNP in each study. We present a method based on meta-analysis that takes into account the genetic structure when combining results and show that it significantly outperforms standard meta-analysis techniques. We apply our method to simulated data and show that by combining results we obtain increased power and resolution over traditional F2 mapping and mapping with HMDP.To further increase resolution, we also introduce a fine mapping method, which utilizes the linkage disequilibrium structure between SNPs in order to find the most likely causal SNP within a candidate region. We show through simulation that this method can increase resolution even further than combining F2 and HMDP results. We use our method to map HDL cholesterol and show that we are able to identify loci that are known to be associated with HDL cholesterol.

401T

Discovering Pleiotropy in Complex Phenotypes by Multivariate Latent Modeling. *A.T. Kraja[1], D.C. Rao[2], D.K. Arnett[3], I.B. Borecki[1], S.C. Hunt[4], M.A. Province[1].* 1) Div of Statistical Genomics, Center for Genome Sciences & Systems Biology, Washington Univ Sch of Med, St Louis, MO; 2) Div of Biostatistics, Washington Univ Sch of Med, St Louis, MO; 3) Dep of Epidemiology, Univ of Alabama Sch of Pub Health at Birmingham, AL; 4) Div of Cardiovascular Genetics, Univ Utah Sch of Med, UT.

Nowadays abundant SNPs and next generation sequencing (NGS) products represent great resources in juxtaposition with the existing state of phenotypes in statistical prediction models. A search on PubMed using keywords “PRINCIPAL COMPONENT ANALYSIS” / “STATISTICAL FACTOR ANALYSIS” / “CLUSTER ANALYSIS” / “DISCRIMINANT ANALYSIS” / “STATISTICAL NEURAL NETWORKS” and “SNP” / “SEQUENCING” produced respectively 14,228/ **92**/ **98**; 16,608/ **5**/ **18**; 32,643/ **162**/ **1,289**; 12,281/ **10**/ **17**; and 3,043/ **9**/ **7** citations. These citation numbers reflect the fact that multivariate statistical methods pervade medical literature, but their use lags in relation to NGS. We provide theoretical statistical reasoning why multivariate latent modeling of phenotypes represents an attractive methodology for discovering polymorphisms' pleiotropic effects. We also worked on a number of complex traits considered as risk factors for metabolic syndrome and focused on a special region of chromosome 3p25-26 in humans. We had already reported for this region a linkage peak of a LOD score above 3 for obesity-insulin factor in the HyperGEN study; we provide also linkage findings for each contributing trait. Association results based on mixed models, with latent modeling of phenotypes, for the above referred chromosomal region with data from the African Americans and whites of the HyperGEN study, the whites of Family Heart Study and of the Framingham Heart Study support two significant association peaks. One, with SNPs related to *CNTN6-CNTN4* and the other to, SNPs that are part of a region of genes *IL5RA*, *TRNT1*, *CRBN*, and *LRRN1*. In this study, we discern the relation among the linkage peak and association test results, by additionally simulating such a region and SNPs pleiotropic effects on simulated correlated traits. We conclude that multivariate latent modeling of phenotypes represent a useful statistical methodology that empowers pleiotropy detection.

402T

A wavelet-based nonparametric approach to association analysis of functional data. *H. Shim[1], M. Stephens[1, 2]*. 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Department of Statistics, University of Chicago, Chicago, IL.

High-throughput sequencing technologies are now routinely applied at a genome-wide scale to collect a variety of phenotypic data. For example, ChIP-Seq, RNA-Seq, and DNase-Seq are used to assay transcription factor binding, gene expression and chromatin accessibility, respectively. Here we consider the development of statistical methods for testing for differences in these types of "functional" data between two or more groups. This problem arises both in genetic association studies (e.g. in testing for genetic variants that affect chromatin accessibility) or more generally in testing for differences among multiple treatment groups. In this work we introduce a Bayesian nonparametric approach to this problem, based on a wavelet regression known to be well suited for modeling spatially heterogenous functional data. Specifically, the functional data is projected into the wavelet space by a wavelet transformation and then the transformed wavelet coefficients are tested for association in a Bayesian regression model. We impose a shrinkage prior on the wavelet coefficients, which results in denoising of the signal in the wavelet space where the signal tends to be contained in a small subset of the coefficients. In addition to testing for association, our approach aims to provide a better interpretation of the analysis such as which parts and features of the functional data are associated with a given variant. We illustrate the proposed method on DNase-Seq data from 70 HapMap Yoruba lymphoblastoid cell lines and compare its performance with an analysis previously performed on the same data using a heuristic procedure.

403T

Pain-free conditional and joint association analysis from meta-analysis summary statistics uncovers additional variants for complex traits. *J. Yang[1], M.E. Goddard[2,3], P.M. Visscher[1], DIAGRAM consortium, GIANT consortium*. 1) Queensland Institute of Medical Research, Brisbane, Queensland, Australia; 2) Department of Food and Agricultural Systems, University of Melbourne, Victoria, Australia; 3) Biosciences Research Division, Department of Primary Industries, Bundoora, Victoria, Australia.

Genome-wide association studies (GWAS) have been successful in identifying variants, genes and pathways involved in genetic variation for human complex traits and diseases. For many traits such as height and body mass index (BMI) and diseases such as type 2 diabetes and breast cancer, more and more associated genetic variants have been identified by meta-analyses of a large number samples. Here we present a "pain-free" conditional and joint association analysis that uses summary-level GWAS statistics from a meta-analysis and estimated linkage disequilibrium (LD) from a reference sample with individual-level genotype data. We analysed the summary data of the GIANT meta-analysis for height and BMI with the LD structure estimated from genotype data of two independent cohorts. We identified 32 loci with multiple association signals (33 primary and 44 additional SNPs) for height, which cumulatively explain ~3.5% of variance at these loci compared to ~2.1% if we consider only the leading SNPs at each locus. We did not find any loci showing multiple signals for BMI. The method is also applicable to case-control data and we demonstrate this by examples from the DIAGRAM meta-analysis of type 2 diabetes. Our new method is computationally fast and facilitates fine-scale genetic mapping even when only summary statistics are available in the discovery sample.

404T

Understanding the Genetics of Vesico-Ureteric Reflux: from Mouse Models to a Human Cohort. *C.L. Watt[1], J. El Andalousi[2], I.R. Gupta[1,2]*. 1) Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Pediatrics, Research Institute of the McGill University Health Center, Montreal, Quebec, Canada.

Introduction: Vesico-ureteric reflux (VUR) is the retrograde flow of urine from the bladder to the kidneys due to a defect of the uretero-vesical junction (UVJ). It occurs in 1% of the population and is associated with recurrent urinary tract infections, hypertension and end stage renal disease. Most affected children have kidneys that are normal in size and form. VUR is genetically heterogenous and is associated with multiple genes and loci in humans. To identify mouse models with VUR and normal kidneys, we screened inbred mouse strains for these phenotypes and found that the C3H and DBA mice have a 100% and 38% incidence of VUR respectively, while the C57BL/6 (B6) mouse does not reflux. All of these strains have kidneys of normal size. Genetic characterization of the C3H mouse has revealed that VUR is recessive and maps to a locus on chromosome 12: *Vurm1*. This region is orthologous to a VUR susceptibility locus previously identified in humans. Complementation analysis between C3H and DBA mice suggests *Vurm1* also confers VUR susceptibility in DBA mice. Statement of purpose: To refine the *Vurm1* locus using existing C3H and newly generated DBA backcross (N2) mice and to apply the findings from mouse models to a human cohort with VUR. Methods: F1 hybrids derived from crossing refluxing DBA mice and non-refluxing B6 mice were backcrossed to DBA mice to generate N2 mice. All progeny were tested for VUR and kidney surface areas were obtained. To establish a human cohort, children with VUR are being recruited from the Montreal Children's Hospital. Saliva samples are taken for DNA extraction and medical histories are obtained from affected children and their immediate family. Grade of VUR, kidney length by ultrasound and glomerular filtration rate (GFR) of affected children are recorded. Results: DBA X B6 F1 hybrids do not reflux, confirming VUR is recessive. DBA N2 mice exhibit a 51% incidence of VUR (n=116). C3H and DBA N2 mice have kidneys of normal size by planar surface measurements. To refine *Vurm1*, DBA and C3H N2 mice will be genotyped for SNPs spanning chromosome 12 and will be subject to a linkage analysis. To date, 137 children have been recruited: 22 of them have a sibling or another family member with confirmed VUR. 95% of the patients have phenotypically normal kidneys (confirmed by kidney length and GFR). As *Vurm1* is refined in the mouse, candidate genes will be prioritized and examined in both mouse models and the human cohort.

405T

Next-generation sequencing of *IL23R* reveals a novel low-frequency non-synonymous SNP that is associated with ankylosing spondylitis in a Han Chinese population. *S.I. Davidson[1], L. Jiang[2], E.A. Glazov[1], A. Cortes[1], M. Donskoi[1], P.A. Danoy[1], G.P. Thomas[1], H. Xu[2], M.A. Brown[1]*. 1) Human Genetics Group, The University of Queensland Diamantina Institute, Woolloongabba, Queensland, Australia; 2) Shanghai Changzheng Hospital, The Second Military Medical University Hospital, Shanghai, China.

Aims: Ankylosing spondylitis (AS) is a highly heritable common inflammatory arthritis affecting 0.5% of white Europeans that predominantly targets the spine and sacroiliac joints of the pelvis, causing pain and stiffness leading eventually to joint fusion. *IL23R*, a cytokine receptor gene known to play a role in regulating inflammation and autoimmune disease, is strongly associated with AS in white Europeans. We performed an association study in Han Chinese AS cases and healthy controls to assess whether *IL23R* is associated in this ethnically distinct population in which the disease has similar prevalence and presentation. No association was found, suggesting that *IL23R* either plays no role in Han Chinese or novel genetic variants contribute. We therefore screened *IL23R* to identify novel rare variants associated with AS.

Methods: A 170kb region containing *IL23R* and its flanking regions was sequenced in 50 cases and 50 controls, as well as the 30kb region of peak association in white Europeans in 650 cases and 1300 controls. Sequencing of pooled overlapping 5kb and 10kb amplicons was performed using an Illumina GAII sequencer. Validation genotyping using the ABI OpenArray platform was undertaken in 872 cases and 1397 controls.

Results: We identified 1007 variants, of which 713 are novel variants not found in dbSNP b130. Several novel potentially functional variants in *IL23R* were identified, including one nsSNP Gly149Arg (chr1:67421184 GA) which is predicted to be functionally deleterious by SIFT, one splice site variant, and one 5' UTR and seven 3' UTR variants. Validation genotyping showed that the Gly149Arg variant is associated with AS in Han Chinese (P=1.07x10^{-3}, OR=0.57), with five further low-frequency variants showing association at P<0.05.

Conclusions: This is the first study to implicate rare variants in *IL23R* in AS pathogenesis, and has identified a rare, novel nsSNP with deleterious effects that is associated with AS in Han Chinese. This suggests that decreased IL-23R function protects against AS. These findings confirm disease associations with different SNPs in the same gene in ethnically remote populations, indicating the importance of performing genetic studies in multiple populations when searching for associated loci.

406T

Initial results from the UK10K project to study the effects of rare variants by whole genome and exome sequencing in 10,000 phenotyped samples. *R. Durbin[1,2] on behalf of the UK10K Project Consortium.* 1) Wellcome Trust Sanger Inst, Cambridge, United Kingdom; 2) www.uk10k.org.

The UK10K project was launched in 2010 to investigate the role of low frequency and rare genetic variants in health and disease. We are in the process of sequencing whole genome at low coverage (average 6x) 4,000 samples from two longitudinal cohorts with rich clinical and molecular phenotype data, TwinsUK and ALSPAC, and by exome pull-down 6,000 samples selected for extreme phenotypes in neurodevelopmental disorders (autism and schizophrenia), severe obesity, and a range of other more rare disorders. The project is a collaboration of the Wellcome Trust Sanger Institute, and multiple UK clinical collaborators from across the country.

Samples are being analysed using pipelines developed from those used for the 1000 Genomes Project. At the time of abstract submission, we are analyzing data from approximately 800 whole genome samples from TwinsUK, and a similar number of exome samples. More have been sequenced and await analysis, and we are on track to complete whole genome sequencing around the end of 2011, and exome sequencing in mid 2012. In the neurodevelopmental arm cases have been weighted in favor of a number of “high value” criteria, including multiply affected families, and depth of additional phenotype data. By the time of the meeting 280 autism and 870 schizophrenia cases are estimated to be sequenced. We are implementing automated pipelines for the efficient and effective annotation of variants to facilitate detection association with the medically-relevant phenotypes of interest. We already have some promising indications of functional association in rare disease samples.

All primary data and variant call sets will be released to the EGA at the EBI for access for research purposes under a Data Access Agreement. An extensive consultation and discussion process led to an Ethical Governance Framework document http://www.uk10k.org/ethics.html which in particular lays out a management pathway for the return of `pertinent' individual findings to research participants. This policy document constitutes a work in progress but is a first step at finding a practical solution to some of the ethically challenging issues that are raised by whole genome and exome sequencing, and has proved useful in multiple contexts by people associated with the project.

407T

Estimating Genetic Effects and Quantifying Missing Heritability for Rare Variant Complex Trait Association Studies via Sequence Data. *S.M. Leal[1,2], D.J. Liu[1,2].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of statistics, Rice University, Houston, TX.

Complex trait rare variant association studies using next generation sequence data are being performed for a wide variety of phenotypes. It has been demonstrated that analyzing rare variants individually is extremely underpowered. Therefore many powerful methods have been developed specifically to analyze rare variant data, which are all based upon jointly analyzing multiple rare variants within region, which is usually a gene. After a significant association is identified, it is also of great importance to estimate genetic parameters of interest and quantify the proportion of heritability explained by the gene. Although rare variant association methods are powerful a drawback is that within the statistical testing framework it is not possible to tease apart causal from non-causal variants. Consequently, the causative-variant-effect is not estimable. Alternatively, we describe how it is possible to efficiently estimate the locus-average-effect which is defined by the mean quantitative trait difference between rare variant carriers and non-carriers. Due to the presence of non-causative variants, genetic variance explained by the locus-average-effect will underestimate but provide a lower bound for the true underlying locus genetic variance. In addition, incorporating assumptions on the effect size distributions of rare variants for complex traits, an estimated upper bound for the true locus genetic variance can also be obtained. An additional problem is when the locus-average-effect is estimated using the same dataset where a significant association is detected, the naïve estimator can be seriously inflated due to the winner's curse. The bias is quantified under a rigorous population genetic model and complex trait phenotypic models. A bootstrap-sample-split algorithm is applied to correct for the winner's curse and can be used for any rare variant test. It is shown through extensive simulations that the boot-sample-split procedure can greatly reduce the bias of the estimates, even for poorly powered studies (e.g. ~30%). Not only are these methods vital for estimating the amount of missing heritability due to rare variants, but they are also important for designing replication studies and risk prediction.

408T

Whole exome sequencing in affected members of a large, multigenerational spina bifida family. *C.A. Markunas, B. Rusnak, A.E. Ashley-Koch, S.G. Gregory.* Center for Human Genetics, Duke University, Durham, NC.

Neural tube defects (NTDs) encompass a range of birth defects that result from a failure of the neural tube to close during the 3rd and 4th weeks of development. The incidence of NTDs is estimated to range between 1 and 10 per 1000 births worldwide, with the two most common forms being anencephaly and myelomeningocele, which is more commonly referred to as spina bifida. Anencephaly occurs when the neural tube fails to close in the cranial region, while myelomeningocele occurs when the neural tube fails to form in the spinal region. NTDs are thought to have a complex etiology, resulting from both genetic as well as environmental factors. In order to identify genetic factors, we performed whole exome sequencing on four, distantly related family members diagnosed with myelomeningocele from a large multigenerational, Mendelian-like family. Agilent's SureSelect Human All Exon 50Mb kit was used for whole exome capture and Illumina's HiSeq 2000 was used to generate 100 bp paired end reads. On average, we achieved 89x coverage within the target interval across our samples. Sequencing reads were aligned to the human reference (1000 genome's b37) using BWA. Following alignment, duplicate removal (Picard), and assessment of data quality (Picard), GATK was used for further processing and multi-sample variant calling (target +/- 200 bp window). A series of variant quality filters were applied to the raw call set, leaving a total of 88,780 variants. Out of the quality filtered variants, 55.9% were intronic, 16.2% were coding-synonymous, 14.9% were missense, 6.9% were intergenic, and the remaining 6% included variant classes such as nonsense. By further restricting these variants to autosomal and setting a relatively low minimum genotype quality threshold for each individual ($N_{remaining}$=78,362), we found that 4.3% and 10.7% of the variants were shared across all 4 individuals in a heterozygous and homozygous state, respectively. The application of additional filters, such as allele frequency and variant class, produced several potentially interesting genes, including one which may be involved in cell polarity.

409T

Identification, replication, and fine-mapping of loci associated with adult height in individuals of African ancestry. *A. N'Diaye[1], G.K. Chen[2], C.D. Palmer[3,4], B. Ge[5], B. Tayo[6], R.S. Cooper[6], T. Pastinen[5], B.E. Henderson[2], J.N. Hirschhorn[3,4,7], G. Lettre[1,8], C.A. Haiman[2].* 1) Montreal Heart Institute, Montreal, Canada; 2) Department of Preventive Medicine, Keck School of Medicine and Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA, USA; 3) Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, USA; 4) Divisions of Genetics and Endocrinology and Program in Genomics, Children's Hospital Boston, Boston, MA,, USA; 5) Department of Human Genetics, McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, Canada; 6) Department of Preventive Medicine and Epidemiology, Loyola University Chicago Stritch School of Medicine, Maywood, IL, USA; 7) Department of Medicine, Harvard Medical School, Boston, MA, USA; 8) Département de Médecine, Université de Montréal, Montréal, Québec, Canada.

Adult height is an ideal phenotype to improve our understanding of the genetic architecture of complex diseases and traits: it is easily measured and mostly influenced by genetics (h2~0.8). More than 180 single nucleotide polymorphisms (SNPs) are associated with height. They were mostly identified in populations of European descent, convey modest effects and explain ~10% of the variance in height. To search for novel loci for height in populations of African ancestry, and to explore the replication of known height loci in other ethnic groups, we combined height genome-wide association results at 3,310,998 genotyped or imputed SNPs from nine studies (20,809 African Americans). Association analysis was performed using linear regression. Results were combined using the inverse variance meta-analysis method. From our meta-analysis we prioritized 153 SNPs with P<1x10-5 for in silico replication in up to 16,436 African Americans. After combining data, 40 SNPs from 11 different genomic regions reached genome-wide significance (P≤5x10-8), including two novel loci (rs12393627, P=3.4x10-12 and rs4315565, P=1.2x10-8). rs12393627 on chromosome Xp22 is located 3.2 kb upstream of the arylsulfatase E (ARSE) gene. Mutations in this gene cause X-linked brachytelephalangic chondrodysplasia punctata, a congenital disorder of bone and cartilage development also characterized by short stature. rs4315565 on 2p14 is located at 189 kb upstream of the bone morphogenetic protein 10 (BMP10) gene, a member of the TGF-) signaling pathway implicated in human growth. We tested for replication in the African height meta-analysis of the 180 European height SNPs using a procedure based on linkage disequilibrium (LD) differences between populations of European and African descent. We found strong overall evidence of replication (P≤3.1x10-4), indicating a shared genetic basis for height in populations separated since the out-of-Africa event. We also used LD differences to attempt to fine-map the European height association signals in African-ancestry individuals, and developed an experiment using allelic gene expression phenotypes in the HapMap YRI cell lines as functional readouts. The fine-mapping results showed an enrichment of SNPs that are associated with expression of nearby genes when compared to the index European height SNPs (P<0.01). Our results highlight the utility of genetic studies in non-European populations to understand the etiology of complex human diseases and traits.

410T

Whole-genome sequencing to identify the genetic basis for resistance to HIV infection. *K. Pelak, K.V. Shianna, D. Ge, D.B. Goldstein, National Institute of Allergy and Infectious Diseases Center for HIV/AIDS Vaccine Immunology (CHAVI).* Center for Human Genome Variation, Duke University School of Medicine, Durham, NC, 27708, USA.

Variants in the human (host) genome have been shown to play a role in determining the course of infection after a person is exposed to a number of different infectious diseases, including HIV-1. Of particular interest for this study are the genetic variants that can protect individuals from HIV-1 infection. Individuals who are homozygous for a 32bp deletion in the CCR5 gene, which encodes a coreceptor that HIV uses to enter the cell, are resistant to infection from most strains of HIV-1. About 1% of people of northern and western European descent are homozygous for this deletion, and the proportion of CCR5d32 homozygotes increases in European populations that are at high risk but have remained seronegative (HRSN). However, much of the “HIV-resistance” observed in the rest of the HRSN population cannot be explained by host or viral genetic variants. In this study, we seek to identify additional host genetic variants that may provide resistance to HIV infection. We have done whole-genome sequencing on 44 HRSN individuals with hemophilia who were highly exposed to contaminated blood products between 1979 and 1984, but who did not become infected by HIV-1. I have compared the whole-genome sequences from these individuals to the whole-genome sequences of 43 white population controls. Based on this comparison, we have selected 1,390 variants for follow-up genotyping on an Illumina iSelect chip. All of these variants show some amount of enrichment in the HRSN individuals as compared to the population controls. Among the variants chosen for follow-up are 692 variants that were homozygous in one or more of the HRSN cases and had a MAF<0.08 in the controls. Another 307 variants were chosen because they were enriched in the cases according to an allelic or recessive Fisher's Exact Test. An additional 84 stop gain variants were chosen because they were at a higher frequency in the cases than in the controls. These variants are being genotyped in 361 other high-risk seronegative individuals, in 3200 population controls, and in 1000 HIV+ individuals. We would expect a potentially protective variant to be heavily depleted or entirely absent in the HIV+ individuals. We would also expect a potentially protective variant to continue to show enrichment in the other HRSN samples, and to occur at low frequency in the population controls.

411T

Sequencing Workflow at a Medium-Scale Genomics Center. *J. Romm, B. Marosy, B. Craig, K. Hetrick, H. Ling, M. Barnhart, S. Griffith, E. Pugh, K. Doheny.* Center for Inherited Disease Research (CIDR), Johns Hopkins University, Baltimore, MD.

The Center for Inherited Disease Research (CIDR) provides high quality next-gen sequencing (NGS), genotyping and statistical genetics consultation to investigators working to discover genes that contribute to disease. CIDR has developed a high-throughput NGS lab, workflows and an automated data processing/analysis QC pipeline designed to provide high quality NGS data including variant calls, supporting data, and QC metrics. We aim to achieve 90% of on-target bases at a minimum sequencing depth of 8X. Our dedicated Project Management team initiates contact with PI's and serves to answer questions throughout the process. DNA samples are received in 2D barcoded tubes and enter our Sample Handling LIMS. Samples are evaluated for degradation on 2% e-gels and quantitated on the Nanodrop® ND-1000 Spectrophotometer. All samples intended for whole-exome sequencing (WES) are genotyped on a GWAS array to perform checks such as gender, unexpected duplicates, IBD sharing and concordance with previous genotyping prior to processing. The GWAS array also enables a data quality check with the sequencing data results (concordance and sensitivity). Lab processing is tracked in the Exemplar™ LIMS modified in-house. The lab work is a modification of the Broad Institute's 100ng with-bead protocol (Genome Biology 2011, 12:R1). The protocol was optimized by CIDR to work with the Agilent© SureSelect™ XT kits for WES. Shearing of the DNA is performed with the Covaris™ E210, library prep with the Beckman Coulter Biomek® 2000 or Perkin Elmer® Multiprobe® II HT PLUS, and QC'ed with the Agilent Bioanalyzer™. After the 24 hour capture, post-hyb processing is performed on the Agilent Bravo™, quantitated on the Agilent Bioanalyzer and normalized to run on the Illumina® HiSeq 2000™. We currently index 4 samples per lane using TruSeq v3 chemistry and 600GB runs. Data QC includes evaluating batch and individual sample level metrics such as array concordance, sensitivity, Ts/Tv, % in dbSNP, % on target, % duplicates and library size. CIDRSeqSuite was developed in-house to process our sequencing data in order to obtain QC metrics and release-ready files. Samples are demultiplexed and aligned with BWA; local realignment and base call quality recalibration using the Genome Analysis Toolkit (GATK) from the Broad; duplicates flagged with Picard; SNVs and indels called and QC filtered using SAMtools then annotated using ANNOVAR.

412T

Pathway-based genetic association analysis for exome sequencing data. *G. Wu[1], K. Wang[2], D. Zhi[1].* 1) Biostatistics, University of alabama at Birmingham, Birmingham, AL; 2) Zilkha Neurogenetic Institute, Department of Psychiatry and Department of Preventive Medicine, University of Southern California, Los Angeles, CA.

Exome sequencing emerges as a powerful approach for the detection of coding variants, enabling genetic association studies for both common and rare variants. While a plethora of rare variant association methods are developed, most methods are gene-based and have insufficient power for genome-wide scale analysis. Pathway association analysis can detect biological pathways significantly enriched for high-ranking variants and genes, even when few of them reach genome-wide significance individually, and thus is more appropriate for exome sequencing data. However, existing variant-level pathway association analysis methods are targeted to genome-wide association studies (GWAS) data, and cannot tackle rare variants. In this study, we develop a new practical approach for pathway-based association for exome sequencing data. We adopt a penalized hierarchical generalized linear model that takes into account biological information at variant-, gene-, and pathway-levels. Estimation is achieved by using an efficient algorithm for sparse group lasso. In simulations with 200,000 rare and common variants in 20,000 genes and 200 pathways, with some realistic assumptions on the genotype allele frequency distributions and on the underlying genetic model, our approach can generate an estimate in less than a second. More importantly, we show that our approach is powerful in terms of identifying truly causal pathways. When pathway-wide heritability is high (50%), our method can achieve a power of 60% for a sample size of merely n=200. Furthermore, we find that identifying causal genes and variants are much more challenging, suggesting that pathway-based association might be a more powerful and practical approach for exome sequencing data analysis at the current stage.

413T

Replication of GWAS Candidate Genes in Four Independent Populations Confirm the Role of Common Variants and Identifies the Contribution of Rare Variants in PAX7 and VAX1 in the Etiology of Non-syndromic CL(P). *A. Butali[1], S. Suzuki[1,2,6], M.A Mansilla[1], E. Dragan[1], Y. Suzuki[2], T. Niimi[2], M. Yamamoto[6], G. Ayanga[9], T. Erkhembaatar[9], H. Furukawa[2,6], K. Fujiwawa[2], H. Imura[2], A.L Petrin[1], E. Leslie[1], J. L'Heureux[1], A.C Lidral[10], M.E Cooper[4], N. Natsume[2], T.H Beaty[3], M.L Marazita[4,5], J.C Murray[1,7,8].* 1) Pediatrics, University of Iowa, Iowa city, IA; 2) , Division of Research and Treatment for Oral and Maxillofacial Congenital Anomalies, School of Dentistry, Aichi-Gakuin University, Japan; 3) Johns Hopkins University, School of Public Health, Baltimore, Maryland, USA; 4) , Center for Craniofacial and Dental Genetics, Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA 15260; 5) Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA 15219; 6) Faculty of Psychological and Physical Science, Aichi-Gakuin University, Japan; 7) University of Iowa, Departments of Pediatric Dentistry, Epidemiology and Biology, Iowa City, IA 52242; 8) College of Nursing, University of Iowa, Iowa City, IA 52242; 9) and Children's Health Research Center Hospital, Ulaanbaatar, Mongolia; 10) Department of Orthodontics, College of Dentistry, University of Iowa.

Background:Genome wide association studies (GWAS) of cleft lip with or without cleft palate (CL(P)) have identified several significant and near-significant genetic associations for non-syndromic CL(P)(Beaty et al.,2010).To replicate two of the near-significant GWAS signals, the present study investigated the role of both common and rare variants in the PAX7 and VAX1 genes. Methods:Direct sequencing was used to search for sequence variations in coding regions and conserved non-coding regions in and around the PAX7 and VAX1 genes in 1454 individuals of European and Asian (Filipino,Mongolian,Japanese)ancestry.TaqMan genotyping was carried out using GWAS markers in VAX1 and PAX7 on 5,421 individuals,and TDT was used to investigate family based association in each population.Case-control comparisons were carried out using Fisher's exact test. Results:Nineteen new variants were found in PAX7 (eight missense, three synonymous and eight non coding variants) Eleven new variants were found in VAX1(two missense mutations,two synonymous mutations and seven non- coding mutations).A statistically significant difference(p= 0.007)was observed between cleft cases and controls from the Philippines for a VAX1 new variant in the 3'UTR (chr10_118880130).TDT analysis showed strong associations with markers in VAX1 (rs7078160, p=1.46E-06 and rs475202 p=0.0008) in both Mongolian case and Japanese case-parent triads. Analysis using PLINK analyses suggested a possible maternal genotype effect for a VAX1 marker in Mongolian and Japanese combined CL(P)cases versus controls (rs7078160,p=9.7E-05,OR=2.33).A significant association with CL(P) was also observed in the Philippines case-parent triads for rs70781860(p= 0.03).Furthermore,CL(P) males were mostly responsible for the effects in both Japanese and Mongolian populations (rs7078160, p=5.2E-05, OR= 3.40).Note, no significant association was observed for these markers in VAX1 for the Iowa CL(P)case-parent triads,the T-allele at rs6659735 trinucleotide marker in PAX7 was significantly under -transmitted(p=0.02). Conclusions:Our study replicated previous GWAS findings for markers in VAX1 across three independent Asian populations, and identified rare variants in PAX7 that may contribute to the etiology of CL(P).The role of these rare variants warrants further investigation through deep sequencing around these and other candidate genes. Grant: NIH DE-08559, DE016148 , KAKENHI 20791560(Aichi-Gakuin),,U01 DE-20057 and U01-DE-018993.

414T

Comparison of "synthetic associations" and "natural associations" in human population genetic data. *D. Chang, A. Keinan.* Cornell University, Ithaca, NY.

Genome-wide association studies (GWAS), based on the Common Disease-Common Variant hypothesis, have been highly successful in associating thousands of loci to many complex traits and diseases. While they have greatly expanded our knowledge, for most phenotypes associated markers explain only a small fraction of heritability and the leap from association to causality has been limited. Rare variants have been suggested as one possible cause underlying these shortcomings due to their exclusion in most GWAS, potentially higher effect sizes, and differing patterns of linkage disequilibrium. Recent studies have attempted to quantify the nature of associations due to underlying rare variants ("synthetic associations"). While these simulation-based studies offered key insights into synthetic associations, a study accounting for the empirical genetic variation patterns in humans, and their impact on signals of associations, is warranted to provide a more complete picture of the phenomenon in light of human evolutionary history. Hence, we studied the characteristics of synthetic associations as compared to associations due to common causal variants ("natural associations") in real data. Considering rare and common risk alleles in HapMap3 sequencing data, we assigned individuals as cases or controls, and performed an association test using HapMap3 genotyping data. We found that the median distance from an association to the furthest causal variant is at least 1.5-fold greater for synthetic associations than natural associations. However, our results predict that this increased distance of synthetic associations is much less pronounced than previously suggested, thus significantly reducing the length of the region required for fine mapping to discover most underlying rare causal variants. In further analysis, we discovered that this increased distance can partially be explained by the age of the mutation, with more recent rare mutations introducing further synthetic associations. Additionally, we found that independent synthetic associations are twice as likely as independent natural associations. Despite these clear trends, the characteristics vary greatly between different populations and regions of the genome. This heterogeneity testifies to the effect of evolutionary history and locus-specific attributes on GWAS signals. Overall, our results recount a more complex relationship between rare and common variation than depicted by simulations alone.

415T

The MHC association in celiac disease maps to a few amino acid polymorphisms in HLA-DQ. *J. Gutierrez-Achury[1], G. Trynka[1], K.A Hunt[2], J. Romanos[1], C. Wijmenga[1], D. van Heel[2], P.I.W de Bakker[3, 4, 5].* 1) Department of Genetics, University Medical Hospital Groningen - University of Groningen, Groningen, Netherlands; 2) Blizard Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, London E1 2AT, United Kingdom; 3) Brigham and Women's Hospital, Boston, MA; 4) Broad Institute of Harvard and MIT, Cambridge, MA; 5) University Medical Center, Utrecht, The Netherlands.

Background: Variants in the major histocompatibility complex have been widely associated to many inflammatory and infectious diseases. However, the analysis of this region presents particular challenges because of its characteristic genetic structure. In order to identify independent effects in celiac disease, we have used a custom genotyping array (Illumina immunochip) to genotype >10,000 SNPs within the MHC in 11,850 cases and 12,109 controls. Methods: Using a reference panel of European ancestry, we imputed classical alleles of the *HLA-A*, *B*, *C*, *DQA1*, *DQB1*, *DRB1*, *DPA1* and *DPB1* genes at 2 and 4-digit resolution as well as polymorphic amino acid positions in those gene products. We used conditional analysis to identify independent associations. Results: Of all variants tested, the strongest association was at amino acid position 55 in the DQ) 1 protomer (χ^2= 6280, df=2, -log(p)=1364), which is located in the binding groove and likely interacts with the gluten antigen. Adjusting for position 55, we also found significant associations for positions 57 in DQ) 1 (-log(p)=56) as well as 25 and 215 in DQ(1 (-log(p)=20 and -log(p)=294, respectively). The haplotypes formed by these four positions recapitulate the known risk-conferring effects of the DQ2.5 (OR=12.21, -log(p)=1662), DQ2.2 (OR=3.4 , -log(p)=1710), and DQ8 (OR=4.7, -log(p)=1701) alleles. After controlling for these HLA-DQ effects, we found >10 markers associated across the MHC. The association within class I was located around *HLA-A*, whereas the associations in class II mainly clustered near *TAP1* and *TAP2*. Conclusions: Although the DQ2 and DQ8 molecules are well-established genetic risk factors for celiac disease, we have pinpointed specific amino acids of HLA-DQ that can explain the bulk of the observed MHC association. Our analysis also provides evidence for multiple additional independent effects outside HLA-DQ that require further study.

416T

Genome-wide association study to identify variants associated with amphetamine sensitivity in humans. *A.B. Hart[1], B.E. Engelhardt[2], M. Wardle[3], A. Skol[4], M. Stephens[1,5], H. de Wit[3], A.A. Palmer[1,3].* 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Department of Computer Science, University of Chicago, Chicago, IL; 3) Department of Psychiatry and Behavioral Neuroscience, University of Chicago, Chicago, IL; 4) Department of Medicine, University of Chicago, Chicago, IL; 5) Department of Statistics, University of Chicago, Chicago, IL.

Humans vary in their response to the drug *d*-amphetamine, and this variation has been shown to have a genetic basis through twin studies. Previous studies to identify genetic polymorphisms associated with this variability have focused on genes that have known, related functions or that have been previously implicated in similar phenotypes, such as response to methylphenidate. Instead, we ran a genome-wide association study with a cohort of 381 non-drug-abusing individuals. The subjects were given a capsule over three double-blind sessions of randomized order (placebo, 10 mg, and 20 mg of *d*-amphetamine) during which subjects were phenotyped at six time points, utilizing established drug response questionnaires (POMS, DEQ, ARCI) and physiological measures (blood pressure, heart rate). We used sparse factor analysis (SFA) to summarize the resulting complex phenotype data, projecting this large set of phenotypes down to a reasonable number of interpretable, smoothed phenotypes. SFA generated eleven biologically meaningful factors that were able to explain the bulk of the phenotypic variance; these factors represented different types of drug response measures and pre-drug personality and physiological characteristics. Subjects were genotyped on the Affymetrix 6.0 array at 906,598 SNPs, and imputation was used to expand coverage to approximately 8 million SNPs. Association mapping was performed in Bayesian and frequentist frameworks with software packages BIMBAM and SNPTest, respectively. From these analyses, we identified numerous potential associations with interesting biological implications. Potential associations include the association of a SNP (rs248797) in the *SRD5A1* gene, which is involved in the synthesis of the known GABA(A) receptor agonist allopregnanolone, with feelings of pre-drug friendliness, and the association of a SNP (rs73036321) in the *ZNF30* gene with mood response to 10 mg and 20 mg doses of the drug. By taking advantage of rigorous phenotyping to capture drug response and sparse factor analysis to project the survey responses to a small number of interpretable phenotypes, we have identified novel genetic associations with both pre-drug personality traits and amphetamine sensitivity in humans.

417T

Genetics of Allergy and Related Phenotypes in Participant Driven and Cross Sectional Cohorts. *D.A. Hinds[1], G. McMahon[2], A.K. Kiefer[1], C.B. Do[1], N. Eriksson[1], M. Curran[3], M. Loza[3], D. Talantov[3], N.J. Timpson[2], D.M. Evans[2], B. StPourcain[4], S.M. Ring[4], K.C. Nadeau[5], D. Miralles[3], G. Davey-Smith[2], J.Y. Tung[1].* 1) 23andMe, Inc., Mountain View, CA, USA; 2) MRC CAiTE Centre/School of Social and Community Medicine, University of Bristol, Bristol, United Kingdom; 3) Pharmaceutical R&D, Johnson & Johnson, San Diego, CA, USA; 4) School of Social and Community Medicine, Oakfield House, Oakfield Grove, Bristol, United Kingdom; 5) Division of Allergy and Clinical Immunology, Department of Pediatrics, Stanford University, Stanford, CA, USA.

We have collected survey information from the 23andMe participant cohort covering a range of immune hypersensitivity phenotypes including allergy, asthma, and eczema. We describe results of genome-wide association analyses of these traits, representing a combined total of nearly 16,000 cases. We find genome-wide-significant associations (P<5E-08) for variants upstream of *HLA-C* with grass allergies (rs9266270: P=3.7E-09, OR=0.81) and for a non-synonymous variant in *TLR1* with seasonal allergies (rs4833095: P=1.5E-09, OR=1.15), as well as suggestive associations for several additional loci with established biological roles in immune response, including *CLEC16A* (rs725613) and *IL1R1*/*IL1R2* (rs11674302). In specific lookup analyses, the *TLR1* and *HLA* associations specific to grass are corroborated in a GWAS of allergy skin prick response tests from the Avon Longitudinal Study of Parents and Children (ALSPAC), with the *TLR1* finding reaching genome-wide significance for allergy to mixed grasses (P=9E-09, OR=1.7). The *TLR1* variant has been reported to affect responsiveness to bacterial antigens, and the *CLEC16A* variant has been previously associated with type 1 diabetes and multiple sclerosis. We also replicate many known associations with asthma, including *IL18R1*, *HLA-DQB1*, *IL33*, *SMAD3*, *GSDMB*, *GSDMA*, and *IL2RB*, with effect sizes consistent with the original reports. Our results further support the existence of a shared genetic etiology for these conditions, as well as distinct patterns of association across phenotypes. Our findings also demonstrate that self report is an effective method for collecting phenotypic information for genetic analysis of allergy related phenotypes.

418T

High Through-Put Genotyping at CIDR. *M. Hurley, M. Zilka, C. Oncago, J. Romn, K. Doheny.* CIDR, Johns Hopkins Univeristy, Baltimore, MD.

The Center for Inherited Disease Research (CIDR) provides high quality next gen-sequencing (NGS), genotyping and statistical genetics consultation to investigators working to discover genes that contribute to disease. CIDR began production level genotyping in 1996 with Human STRP linkage panels (~400 markers per sample). Today CIDR is running arrays with greater than 2.5 million polymorphisms per sample. Even with the recent advent of NGS, the demand for genotyping has remained high. CIDR releases data for an average of 1 study per week. Over the 1st 5 months of 2011, we have completed 19 studies involving 38,000 samples. 85% of studies were genome-wide association studies (GWAS), 12% custom SNP, 2% linkage and 1% methylation. All samples received at CIDR are pretested using an AIMS focused 96-SNP GoldenGate® SNP "barcode" panel to check unexpected duplicates, gender, sample quality and relationships. To verify that samples are not degraded, a 2% e-gel is run. After pretesting is complete, the PI is given the opportunity to replace samples or fix sample information based on the results. Throughout the entire process, samples are tracked using a customized sample handling LIMS that integrates with both the Illumina® LIMS (used for GoldenGate® processing) and an in-house developed Infinium® LIMS. Using three Illumina® iScans™ with autoloaders and the first dual HiScan™ autoloader, CIDR is able to process over 100,000 samples a year. CIDR can process 2,000 samples per week based on the Omni 2.5-Quad and 4,000 GoldenGate® samples. All GWAS experiments are analyzed using an automated pipeline for the generation of quality control statistics. These QC metrics are stored and available for daily QA/QC of data produced in the lab. All assay failures are repeated one time. Genotype cluster positions for GWAS studies are generated by re-clustering the data using a project's samples before release and a technical filter is performed on the data to identify gross assay failures. CIDR performs a manual review of the clustering for each SNP for GoldenGate® and custom iSelect® up to 1,000's of SNPs. While processing high numbers of samples, CIDR has been able to maintain high quality standards through the use of automation, rigorous QC measures and strict LIMS control. Overall, the cumulative GWAS error and missing rate are 0.015% and 0.2% respectively and the custom genotyping error and missing rate are 0.009% and 0.18%.

419T

A Genome-Wide Association Analysis for chroidal neovascularization in highly myopic eyes in Japanese. *M. Miyake[1,3], K. Yamashiro[1], H. Nakanishi[1,3], H. Hayashi[1,3], I. Nakata[1,3], Y. Kurashige[1,3], A. Tsujikawa[1], M. Moriyama[2], K. Ohno-Matsui[2], M. Mochizuki[2], T. Kawaguchi[3], R. Yamada[3], F. Matsuda[3], N. Yoshimura[1].* 1) Department of Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan; 2) Department of Ophthalmology and Visual Science, Tokyo Medical and Dental University Graduate School of Medicine, Tokyo, Japan; 3) Center for Genomic Medicine/Inserm U.852, Kyoto University Graduate School of Medicine, Kyoto, Japan.

Myopia is the most common type of ocular disorder in the world. Pathological myopia, also called high myopia, is one of the leading causes of legal blindness in developed countries. The main cause of visual acuity loss in highly myopic eyes is a development of choroidal neovascularization (CNV). The CNV formation leads to serous retinal detachments and subretinal hemorrhage with fibrotic membrane formation, which results in irreversible visual impairment. To identify genetic determinants associated with the development of CNV in highly myopic eyes, we conducted a 2-stage-genome-wide association study (GWAS). In the first stage, we genotyped 481 patients with high myopia (axial lengths >28.0 mm in both eyes) using Illumina HumanHap 550k or 660K arrays. We divided them into 2 groups whether they have CNV (n=171) or not (n=310), and performed case-control study for each SNPs. We selected 11 SNPs that showed P-values smaller than 5.0×10-5 in the first stage and evaluated the association to CNV formation in the second stage analysis with 351 patients with high myopia (axial lengths > 26.0 mm in both eyes) using Taqman SNP assay. Patients with CNV (n=139) are older (62.6 ± 13.3 vs 54.8 ± 16.2; p=0.02) and more female-dominant (Male:female=31:108 vs 72:140; Odds ratio 0.56; 95% confidence interval 0.34-0.91) than patients without CNV (n=212). To adjust the age and gender difference, we used multivariate logistic regression analysis. Out of the 11 selected SNPs, no SNP showed a significant association with CNV occurence in the second stage. Most correlated SNPs are rs4284034 (Adjusted Odds ratio 1.41; p=0.12) and rs4556082 (Adjusted Odds ratio 1.34; p=0.15). In the present study, we could not find a locus that associates with the development of CNV in highly myopic eyes. Further studies with larger cohort may reveal the genetic background of CNV development.

420T

Allele-specific enhancer variants in open chromatin at the *GALNT2* human high-density lipoprotein cholesterol locus. *T.S. Roman[1], M.P. Fogarty[1], S. Vadlamudi[1], A.F. Marvelle[1], K.J. Gaulton[1], A.J. Gonzalez[1], Y. Li[2], K.L. Mohlke[1].* 1) Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Biostatistics, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Epigenomic maps of predicted regulatory regions offer a promising approach to identify functional variants at genome-wide association (GWA) signals. We used regulatory maps from HepG2 cells to predict functional variants associated with HDL-C level within intron 1 of *GALNT2*, encoding an N-acetylgalactosaminyltransferase. We tested for SNP association with *GALNT2* expression using eQTL and allelic expression imbalance (AEI) experiments in primary human hepatocytes, and we examined allele-specific regulatory activity of all 23 variants in strongest linkage disequilibrium (r^2>.8) with the HDL-C-associated index SNP. In eQTL experiments using hepatocytes from 50 individuals, we found that the HDL-C-increasing alleles showed suggestive evidence of association with increased *GALNT2* mRNA expression. In our AEI experiments, hepatocyte RNA from 36 individuals heterozygous for an HDL-C-associated SNP showed a 7% increase (P= $5.4x10^{-7}$) in *GALNT2* intron RNA expression correlated with the HDL-C-increasing alleles. Based on the observation that the HDL-C-associated variants are correlated with *GALNT2* expression, we hypothesized that one or more of these associated variants would affect *GALNT2* expression. Of the 23 variants considered, 16 overlap HepG2 open chromatin, histone modification, and/or transcription factor ChIP-seq peaks. Transcriptional reporter assays in HepG2 cells detected enhancer activity at 5 variants, which are located in epigenomic-predicted regulatory regions. Of these 5, allele-specific enhancer activity was observed with a 3-variant haplotype and one additional SNP, rs2281721. Site-directed mutagenesis experiments showed that at least two of the SNPs in the haplotype (rs4846913 and rs2144300) act additively to increase transcriptional activity. Our data thus suggest that rs4846913, rs2144300, and rs2281721 are the most likely HDL-C-associated candidate SNPs to influence *GALNT2* expression. These eQTL, AEI, and transcriptional enhancer data all show a consistent direction of association; HDL-C-increasing alleles are correlated with increased *GALNT2* expression. This direction of effect differs from a study of *Galnt2* overexpression in mouse liver that led to decreased plasma HDL-C levels. Epigenomic data helped discern regulatory regions and may have aided identification of the functional variants underlying this GWA signal; however, the link between *GALNT2* expression and HDL-C level through DNA variants remains unclear.

421T

Allelic expression as a guide for functional fine-mapping in SLE. *J.K. Sandling[1], G. Nordmark[2], M-L. Eloranta[2], I. Gunnarsson[3], E. Svenungsson[3], L. Padyukov[3], G. Sturfelt[4], A. Jönsen[4], A.A. Bengtsson[4], L. Truedsson[5], C. Eriksson[6], S. Rantapää-Dahlqvist[7], C. Sjöwall[8], J. Kere[9,10,11], L.A. Criswell[12], R.R. Graham[13], T.W. Behrens[13], T. Pastinen[14,15,16], L. Rönnblom[2], A-C. Syvänen[1].* 1) Molecular Medicine, Department of Medical Sciences, Uppsala University, Uppsala, Sweden; 2) Section of Rheumatology, Department of Medical Sciences, Uppsala University, Uppsala, Sweden; 3) Rheumatology Unit, Department of Medicine, Karolinska Institutet/Karolinska University Hospital, Stockholm, Sweden; 4) Section of Rheumatology, Department Clinical Sciences, Lund University, Lund, Sweden; 5) Department of Laboratory Medicine, Section of MIG, Lund University, Lund, Sweden; 6) Department of Clinical Immunology, Umeå University Hospital, Umeå, Sweden; 7) Department of Rheumatology, Umeå University Hospital, Umeå, Sweden; 8) Rheumatology AIR, Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden; 9) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 10) Folkhälsan Institute of Genetics, Helsinki, Finland; 11) Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden; 12) Rosalind Russell Medical Research Center for Arthritis, Department of Medicine, University of California, San Francisco, CA; 13) Genentech Inc., South San Francisco, CA; 14) Department of Human Genetics, McGill University, Montréal, Quebec, Canada; 15) McGill University and Genome Québec Innovation Centre, Montréal, Quebec, Canada; 16) Department of Medical Genetics, McGill University, Montréal, Quebec, Canada.

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease affecting mainly women. There are currently about 30 confirmed SLE susceptibility loci, most of which have been identified through genome-wide association studies (GWAS). Typically the associated variants reside in non-coding DNA, and few causal alleles have been identified to date. However, these are often assumed to affect gene regulation. By mapping differences in allelic expression (AE) it is possible to identify *cis*-acting regulatory variants affecting gene expression. We have utilized AE-mapping to identify regulatory variants of relevance in SLE by intersecting AE-mapping data from cell lines with data from an US SLE GWAS. The AE data was generated by quantifying the allelic ratios between transcripts in 53 CEU lymphoblastoid cell lines on Illumina Human1M BeadChips (Ge *et al.* Nat Genet. 2009) and the SLE GWAS was performed in 1,310 US Caucasian SLE patients and 7,859 controls genotyped on the Illumina 550k SNP platform (Hom *et al.* N Engl J Med 2008). Sixty regions were identified to have overlapping AE-mapping ($P_{mapping}$ <5x10^{-5}) and SLE GWAS signals ($P_{association}$ <5x10^{-3}). In these regions candidate functional variants were selected using information from the ENCODE project (www.genome.gov/10005107). A panel of 214 SNPs, including the potentially regulatory variants and the SLE GWAS SNPs, for these loci were then genotyped using a custom Illumina GoldenGate assay in two SLE case-control cohorts: one from Sweden and one from Finland, to investigate their relevance for SLE susceptibility. We analyzed a total of 1,300 SLE patients and 2,300 controls, and found that the association statistics for the GWAS and candidate regulatory variants were highly correlated (r^2=0.8), indicating that the candidate regulatory variants could be responsible for the observed SLE association signals. Additional functional follow-up will be required to validate the exact functional role these *cis*-regulatory variants play in SLE susceptibility.

422T

Identification of novel genes that contribute to both asthma and COPD, with replication in a large population-based cohort. *J. Smolonska[1,2], G.H. Koppelman[2], C. Wijmenga[1], J.M. Vonk[3], P. Zanen[4], M. Bruinenberg[5], L. Franke[1], H.M. Groen[2], H.M. Boezen[3], D.S. Postma[2].* 1) Genetics, University Medical Center, Groningen, Netherlands; 2) Pulmonology, University Medical Center Groningen, the Netherlands; 3) Epidemiology, University Medical Center, Groningen, Netherlands; 4) Pulmonology, University Medical Center, Utrecht, the Netherlands; 5) LifeLines, University Medical Center Groningen, the Netherlands.

In 1961, Orie postulated the Dutch hypothesis stating that asthma and chronic obstructive pulmonary disease (COPD) share genetic and environmental risk factors, ultimately leading to either asthma or COPD depending on the timing and type of environmental exposure. Genome wide association studies (GWAS) have been performed in asthma and in COPD, but no overlapping genes have been identified. We aimed to establish whether there are common genes in asthma and COPD in two populations from the same Northern region in the Netherlands. We performed GWA studies on 921 asthma patients characterized by hyperresponsiveness and lung function (reversibility) with 3246 controls from LifeLines, a general population cohort, and on 1030 COPD cases (FEV1/FVC<70% and at least 20 packyears smoking) with 1799 controls. After meta-analysis, all SNPs with p<0.001 were replicated in an independent set of LifeLines samples (asthma: 534 cases and 2569 controls, COPD: 711 cases and 1854 controls). Results were combined using Fischer's exact method. We found one SNP with p= 9.96*10e-09, and 19 SNPs with suggestive p <1*10e-05. All of them point to three loci located on chromosomes 2, 5 and 13, however only one contains a gene. Together with a gene upstream locus associated on chromosome 2 it is seen in a protein network with NFKB2 This study identifies novel loci associated with both asthma and COPD development, contributing to the hypothesis that these respiratory diseases share some genetic origins. We replicated these genes in a large cohort of asthma and COPD patients and controls from the general population.

423T

Statistical approaches for assessing missing heritability. *M. Stephens, X. Zhou, P. Carbonetto.* Statistics & Human Gen, Univ Chicago, Chicago, IL.

Although recent genome-wide association studies have identified numerous loci associated with complex traits, in most cases the identified loci account for only a small proportion of total phenotypic variation, usually substantially smaller than previous estimates of the ``heritability'' of these traits. This gap has been referred to as the "missing heritability", and it has attracted considerable research interest. One source of missing heritability is genetic variants with small effect sizes that lie below the genome-wide significance threshold used in typical GWAS. Evaluating the likely combined contribution of these small-effect variants to the missing heritability could help in planning future studies (e.g. deciding whether larger studies of the same genetic variants might yield further helpful insights, or whether it might be important to look at different genetic variants, by sequencing for example). Although these individual small-effect variants cannot be identified with confidence, their combined effects (specifically the total proportion of phenotypic variance explained, or PVE) can nonetheless be estimated by statistical modeling approaches. There exist at least two published statistical modeling approaches along these lines: one based on linear mixed models (LMM), and the other based on Bayesian variable-selection regression (BVSR). These two approaches make very different assumptions: in particular the BVSR is motivated by a sparsity assumption, whereas LMM is motivated by a model with a very large number of causal SNPs with small effects. They also have very different computational properties: LMM is computationally fast genome-wide, whereas existing implementations of BVSR are generally tractable only if the total number of genetic variants contributing to the trait is limited (eg <200). Here we compare and contrast these methodologies on simulated phenotype data with real genotypes. We show that when the number of causal SNPs is small BVSR provides more accurate estimates for the PVE. However, when the number of causal SNPs is large the LMM is more accurate. To take advantage of the merits of both methods, we introduce a hybrid approach. We demonstrate its effectiveness in estimating the PVE in both scenarios regardless of the number of causal SNPs and their effect sizes. This hybrid approach thus provides a useful tool for estimating the PVE and contributes to understanding the genetic architecture underlying complex traits.

424T

Genome-wide association study of rheumatoid arthritis in an isolated population. *K.E. Taylor[1], Y.S. Aulchenko[2], J. Nititham[1], B. Oostra[2], P.K. Gregersen[3], C.M. van Duijn[2], L.A. Criswell[1].* 1) Rosalind Russell Medical Research Center for Arthritis, University of California San Francisco, San Francisco, CA; 2) Dept. of Epidemiology and Biostatistics and Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands; 3) Feinstein Institute for Medical Research, Manhasset, NY.

Background. Rheumatoid arthritis (RA) is a genetically complex autoimmune disease causing severe disability through joint destruction and deformity. Alleles of the *HLA-DRB1* locus in the major histocompatibility complex (MHC) have long been known to increase risk of RA; recent genome-wide association studies have resulted in over 30 established loci associated with RA risk. However, effects of these loci are small and much of the heritability of RA is not explained. Isolated populations offer a unique opportunity for the study of genetic diseases, as disease-causing alleles may be enriched in such populations; furthermore there is a much more genetically homogeneous background than in a broader population. Together these may allow associations to be observed which would be more difficult to detect in a traditional genetic association study. **Methods and Results.** We performed Illumina 660W genotyping on 201 RA cases and 100 healthy controls from a genetically-isolated Dutch population, and utilized Illumina 317K genotyping on another 500 healthy controls from the isolate. After strict quality control, SNPs were imputed up to the HapMap2 using MACH, and SNPs retained with quality score > 0.8 and RSQ > 0.3. We are currently testing for association using the software ProABEL in order to account for imputed genotype probabilities and to adjust for relatedness in the population. In spite of the relatively small size of this study, 58 SNPs in the MHC reached genome-wide significance ($p < 5x10^{-8}$), with the top hit as expected in the *HLA-DRB1-HLA-DQA1* region; we are investigating an additional 169 signals genome-wide with $p<5x10^{-8}$. New SNP associations will be investigated in replication cohorts. Also, any regions with multiple associations obtained in the initial association screen will be tested for independence using conditional analysis methods such as multivariate logistic regression and log-ratio testing of haplotypes. **Conclusions.** Previously-unknown loci associated with RA may be discovered using this isolated population, as well as confirmation of previously unreplicated loci.

425T

Caucasian and Asian specific rheumatoid arthritis risk loci identified by GWAS and meta-analysis show limited replication and apparent allelic heterogeneity in north Indians. *B.K. Thelma[1], P. Prasad[1], R. Gupta[2, 3], A. Kumar[2, 4], R.C. Juyal[5].* 1) Dept Genetics, Univ Delhi, South Campus, New Delhi, India; 2) ex-All India Institute Of Medical Sciences, New Delhi, India; 3) Division Of Rheumatology & Clinical Immunology, Medanta Bone & Joint Institute, Medanta The Medicity, Gurgaon, India; 4) Department Of Rheumatology, Fortis Flt. Lt. Rajan Dhall Hospital, New Delhi, India; 5) National Institute Of Immunology, New Delhi, India.

Several genes/loci have shown to be consistently associated with rheumatoid arthritis (RA) in European and Asian populations based on Genome-wide association studies and meta-analysis. To evaluate the transferability status of these findings to an ethnically diverse north Indian population, we performed a replication analysis. We investigated the association of 47 single-nucleotide polymorphisms (SNPs) at 43 of these genes/loci with RA in a north Indian cohort comprising 983 RA cases and 1007 age and gender matched controls. Genotyping was done using Infinium human 660w-quad. Association analysis by chi-square test implemented in plink was carried out in two steps. Firstly, association of the index or surrogate SNP (r2 >0.8, calculated from reference GIH Hap-Map population) was tested. In the second step, evidence for allelic/locus heterogeneity at aforementioned genes/loci was assessed for by testing additional flanking SNPs in linkage equilibrium with index/surrogate marker. Of the 44 European specific index SNPs, neither index nor surrogate SNPs were present for nine SNPs in the genotyping array used in the study. Of the remaining 35, associations were replicated at seven genes namely rs1217407 (p=3×10-3) in PTPN22; rs13119723 (p=0.008) in IL2-21; rs660895 (2.56×10-5); rs6457617 (p= 1.6×10-09); rs13192471 (p=6.7×10-16) in HLA-DRB1; rs9321637 (p=0.046) in TNFA1P3; rs13293020 (p=0.01) in CCL21; rs2104286 (p=1.9×10-4) in IL2RA and rs2793108 (p=0.006) in ZEB1 with SNPs in HLA-DRB1 and IL2RA withstanding Bonferroni correction. Of the three Asian specific loci tested, rs2977227 in PADI4 showed modest association (p<0.02). Further, of the 140 flanking SNPs (in LE with index/surrogate variant) tested, we observed association at five additional genes namely PTPRC, AFF3, CD28, CTLA4, and IL2RB. This study indicates limited replication of European and Asian specific index SNPs and apparent allelic/locus heterogeneity in RA etiology among the genetically distinct north Indians warranting independent GWAS in this population. However, replicated associations of HLA-DRB1, PTPN22 (which together are known to explain around 50% of the heritable risk to RA) and IL2RA suggest that cross-ethnicity fine mapping of such genes/loci is apposite for identification of the causal variants.

426T

Genome-wide association study for biliary atresia in a Caucasian population. *E.A. Tsai[1,3], B.A. Haber[3], H.C. Lin[2,3], N.B. Spinner[3], M. Devoto[4,5].* 1) Genomics and Computational Biology, University of Pennsylvania SOM, Philadelphia, PA; 2) Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia, Philadelphia, PA; 3) Division of Gastroenterology, Hepatology and Nutrition, The Children's Hospital of Philadelphia, Philadelphia, PA; 4) Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA; 5) Department of Pediatrics, University of Pennsylvania SOM, Philadelphia, PA.

Biliary atresia (BA) is a rare liver disease of neonates that accounts for 50% of all pediatric liver transplantations in the United States and has a worldwide incidence of 1:12,000. The etiology of BA is unknown. It is hypothesized that BA may be caused by a genetic predisposition, an environmental exposure (virus or toxin) or a combination of factors. A previous genome-wide association study (GWAS) of Chinese individuals (324 cases, 481 controls) identified a potential susceptibility locus within 10q24.2. To test the hypothesis that there is a genetic susceptibility to BA, we carried out a pilot GWAS on a Caucasian population of 171 cases and 1,630 controls to look for loci that confer susceptibility to BA. Samples were genotyped on the Illumina HumanHap 550v3 and 610 Quad BeadArrays. Our BA population was identified by the NIH funded Childhood Liver Disease Research and Education Network (ChiLDREN). While our sample size is small, we were unable to replicate the GWAS signal on 10q24.2. In our study, there was only one signal that passed genome-wide significance (p=1.0E-23). This signal maps within 4q35.1 in a gene desert. We hypothesize that there are genomic susceptibility loci that do not achieve genome-level significance due to limited sample size. To investigate SNPs with a suggestive p-value that did not reach genome-wide significance, we annotated the intragenic SNPs with p<0.001 with their corresponding gene and examined this 241 gene list. From the GNF Gene Expression Atlas 2 dataset, we found that 54/241 of these genes are upregulated in fetal liver. We then asked which transcription factors regulate these genes and found that genes regulated by each of NKX6.1, FOXD3, HNF3B, FOXF2, LHX3, TBP and CEBPA were enriched over twofold. Four of the seven TFs are important in the regulation of genes in the liver. FOXD3 has been shown to be an important TF that drives embryonic stem cell development into endoderm and serves as a regulator of endodermal-specific promoter expression. Moreover, a microarray analysis study has shown that HNF3B, LHX3 and FOXF2 target genes were downregulated in zebrafish with a BA phenotype. Although there were no genome-wide significant GWAS signals in our cohort, the enrichment of genes associated with liver-specific TFs among our top hits shows that a replication in a larger cohort may uncover a genetic susceptibility to BA. This work is currently underway.

427T

Genetic variants associated with adult height cluster in genomic loci. *A.R. Wood[1], J. Yang[2], O.L. Holmen[3], E. Ingelsson[4], M.N. Weedon[1], S.I. Berndt[5], T. Esko[6], A.V. Smith[7,8], D. Shungin[9], E. Albrecht[10], D. Anderson[11], J.L. Bragg-Gresham[12], T. Fall[4], R.M. Fraser[13], M.E. Goddard[2], L.W. Jones[14], S. Kanoni[15,16], M. Kleber[17], K. Kristiansson[18], J. Luan[19], C.D. Palmer[20,21], L. Yengo[22], K. Hveem[3], P.M. Visscher[2], T.M. Frayling[1], J.N. Hirschhorn[23], GIANT Consortium.* 1) Genetics of Complex Traits, Peninsula College of Medicine and Dentistry, University of Exeter, Exeter, UK; 2) Queensland Institute of Medical Research, Royal Brisbane Hospital, Brisbane, Australia; 3) NTNU, Trondheim, Norway; 4) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; 5) Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA; 6) Estonian Genome Center and Center of Translational Genomics, University of Tartu, Estonia; 7) Icelandic Heart Association, Kopavogur, Iceland; 8) University of Iceland, Iceland; 9) Department of Public Health and Clinical Medicine and Institute of Odontology, Umeå University, Sweden; 10) Institute of Genetic Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany; 11) Telethon Institute for Child Health Research, West Perth, Australia; 12) Department of Biostatistics, Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan, USA; 13) Public Health Sciences section Centre for Population Health Sciences, The University of Edinburgh Medical School, Edinburgh, UK; 14) HudsonAlpha Institute for Biotechnology, Huntsville, AL, USA; 15) Department of Nutrition-Dietetics, Harokopio University, Athens, Greece; 16) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK; 17) Synlab Center of Laboratory Diagnostics Heidelberg, Germany; 18) National Institute for Health and Welfare, Helsinki, Finland; 19) MRC Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK; 20) Divisions of Genetics and Endocrinology and Program in Genomics, Children's Hospital, Boston, MA, USA; 21) Metabolism Initiative and Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, USA; 22) CNRS, Lille Pasteur Institute, Lille Nord de France University, Lille, France; 23) Department of Genetics, Harvard Medical School, Boston, MA, USA.

Genome wide association studies of adult height identified 180 associated loci that together explain ~12% of heritability, are enriched for functional variants and highlight genes relevant to growth. The results also suggested the presence of multiple signals at single loci (allelic heterogeneity). Testing of allelic heterogeneity has not been extensively evaluated in large consortia. We use new association data to identify additional height loci and to test for allelic heterogeneity using summary association statistics.

Within the GIANT consortium, we analyzed the metabochip genotyping array in an independent set of 71,838 individuals. This array includes 1050 SNPs representing ~300 of the loci most strongly associated with height from a previous GWAS of 133,000 individuals. We combined association statistics with those from the previous GWAS and tested for the presence of multiple signals at individual loci using an approximate conditional and joint multiple SNP regression analysis. This method is an alternative to individual-study conditional analyses and uses summary-level statistics from the meta-analysis and individual-level genotype data from a reference sample. We validated the approximate conditional analysis using 400 individuals from each of the short and tall 1% tails of height from the HUNT population of ~50,000 individuals. We added SNPs from the same loci as independent variables in a multivariable test, and tall or short status as the dependent variable.

Analyzing the 71,838 individuals in GIANT, we identified 80 variants associated with height at p<5x10-8 that were independent (based on an r2 <0.025) of previously identified signals. Of 123 independent signals with joint p-values < 5e-8 by a stepwise model selection procedure, 21 loci showed evidence of at least two independent signals. Of these 21 loci, LD between primary and secondary association signals ranged from r2 <0.01 to 0.54, eight secondary signals were validated at p<0.05 in a multivariable model comparing the 1% shortest to 1% tallest individuals in the HUNT study.

In conclusion, detailed analysis of height loci in existing and extended sample sizes increased the number of loci identified and revealed multiple signals in known loci.

428T

Using genome-wide SNP data to estimate the additive genetic variation caused by common and rare causal variants. *M.C. Keller[1], T. de Candia[1], J. Yang[2], M.E. Goddard[3,4], P.M. Visscher[2].* 1) Department of Psychology, Univ Colorado, Boulder, Boulder, CO; 2) Genetic Epidemiology, Molecular Epidemiology and Queensland Statistical Genetics Laboratories, Queensland Institute of Medical Research, Brisbane, Australia; 3) Faculty of Land and Food Resources, University of Melbourne, Victoria, Australia; 4) Department of Primary Industries, Victoria, Australia.

The relative importance of rare versus common causal variants has long been an issue of debate in the field of human genetics, but to date there has been little empirical evidence that could shed light on this issue. Recently, a method was developed to estimate the amount of additive genetic variation in a trait that is explained by considering all genome-wide SNPs simultaneously (Yang et al, 2010). Because rare variants (MAF < .01) are not highly correlated with (common) marker SNPs, we show that most of the additive genetic variation detected using this method must be due to common (MAF > .01) causal variants (see also Wray, Purcell, & Visscher, 2011). We then introduce a new method of estimating a trait's additive genetic variation that uses haplotypic information derived from genome-wide SNP data. This method can reliably detect additive genetic variation caused by moderately rare (.01 < MAF < .001) as well as common causal variants, although variation caused by extremely rare causal variants (MAF < .001) remains mostly undetectable. By combining information from both the Yang et al (2010) approach and the current approach, these genome-wide methods of estimating additive genetic variation are among the first to provide direct empirical evidence on traits' allelic spectra.

429T

Is microsatellite polymorphism an important regulator of human phenotype? *A.T. Bagshaw[1], S. Sawaya[2], N. Gemmell[2], D.M. Fergusson[3], L.J. Horwood[3], P.R. Joyce[3], M.A. Kennedy[1].* 1) Dept Pathology, Univ Otago, Christchurch, New Zealand; 2) Dept Anatomy and Structural Biology, Univ Otago, Dunedin, New Zealand; 3) Dept Psychological Medicine, Univ Otago, Christchurch, New Zealand.

Short tandem repeat arrays, also known as microsatellites, are common in promoters and may be underestimated as regulators of gene expression. Array length polymorphisms of these elements have been linked to variation in several human phenotypes, and evidence from model organisms shows that many microsatellites serve as mechanisms for rapid evolutionary adaptation, for which they are suited due to their high mutation rate several orders of magnitude greater than single nucleotide substitution. Microsatellite length changes are not detected by standard genome-wide SNP or CNV arrays, so this source of genetic variation remains under-studied [1]. We have selected 35 microsatellites from human gene promoters based on novel measures of conservation of the microsatellites among mammalian species [2] and previous evidence suggesting or directly measuring association of the genes with particular phenotypes. We will report association data of microsatellite genotypes with novelty seeking and other specific heritable components of personality measured by the Temperament and Character Inventory (TCI) questionnaire [3], and also human height and body size, in several cohorts (more than 1000 individuals). 1. Hannan, A.J., Tandem repeat polymorphisms: modulators of disease susceptibility and candidates for `missing heritability'. Trends Genet, 2010. 26(2): p. 59-65. 2. Sawaya, S., et al., , Measuring Microsatellite Conservation in Mammalian Evolution with a Phylogenetic Birth-Death Model. Submitted to Genome Research. 3. Cloninger, C., D. Svrakic, and T. Przybeck, A psychobiological model of temperament and character. Arch Gen Psychiatry, 1993. 50(12): p. 975-990.

430T

Phenome-wide studies of SNPs from GWAS in a broadly phenotyped population. *N. Eriksson, J.Y. Tung, D.A. Hinds, C.B. Do, A.K. Kiefer, B.T. Naughton, J.L. Mountain.* 23andMe, Inc., Mountain View, CA.

In many cases, SNPs associated with one trait have been found to be associated with others. This apparent pleiotropy may be due to shared etiology, correlation between the traits, or different causal variants tagged by one SNP. Here we present the results of a systematic search for pleiotropic effects using a broadly phenotyped population of over 65,000 individuals. Participants were drawn from the customer base of 23andMe, Inc. and provided self-reported information on subsets of the approximately 1000 phenotypes, including disease diagnoses, morphological traits, personality tests, and much more. We tested several thousand SNPs (limited to associations found via GWAS as well as rare variants associated with Mendelian disease) against the database of phenotypes. Our approach leads to a number of interesting hypotheses. For example, we find that an HLA SNP originally reported with ulcerative colitis (rs2395185) is associated with type 2 diabetes in our data. SNPs originally associated with tooth development near EDA (rs5936487 and rs4844096) are also associated with other morphological traits, including unibrow, attached earlobes, and lower back hair. The well-known association between FTO and obesity also correlates in our data with choice of sweet versus salty foods. We see evidence for interaction between FTO genotype and food choice with obesity. An association with Crohn's disease near a cluster of interleukin genes (rs2188962) is also linked to severity of mosquito bite reaction in our data. As a final example, we show that a SNP near TERT originally reported with glioma is also associated with skin tags (benign skin growths). Our findings also include many well known facts and novel negative findings. For example, SNPs associated with lung cancer and esophageal cancer influence smoking and drinking frequency in our data. On the other hand, we show that ACTN3 null alleles are not associated with sports choice (or other of our phenotypes) in the general population. Widely studied polymorphisms near DRD2 (rs1800497) and COMT (rs4680) are not significantly associated with any phenotypes. These results are based on a cutoff of 1e-6 for significance and are computed in a group of unrelated individuals of European ancestry controlling for sex, age, and projections onto five principal components. A false-discovery rate analysis yields an estimated false-discovery rate of about 0.025 for these results.

431T

Association study between telomere length and Age-Related Hearing Impairment in 3527 Caucasian individuals and 663 Japanese individuals. *E. Fransen[1,2], S. Bonneux[1], N. Suzuki[3], S.-I. Usami[3], G. Van Camp[1].* 1) Center for Medical Genetics, University of Antwerp, Antwerp, Belgium; 2) StatUa Center for Statistics, University of Antwerp, Antwerp, Belgium; 3) 3Department of Otorhinolaryngology, Shinshu University School of Medicine, Matsumoto, Japan.

Several reports suggest that individuals with shorter telomere length are more susceptible to age-related disorders. In somatic cells, telomere length declines with age contributing to loss of cells with age when telomeres reach a critical minimal length. Average telomere length was shown to be a heritable trait (Slagboom et al., 1994). Age-Related Hearing Impairment (ARHI) is the most common sensory disorder and the most important cause of hearing loss in the elderly, with a negative impact on the quality of life. It is a complex disease influenced by genetic as well as environmental factors. Here we test whether a shorter average telomere length leads to increase of ARHI susceptibility. In 3527 European and 663 Japanese individuals, we determined the telomere length in white blood cells using a monochrome multiplex quantitative PCR protocol (described by Cawthon, 2009). We performed association testing between the telomere length and the 3 Principal Components of the ARHI phenotype (Huyghe et al., 2008). In addition, we tested this association adjusting for environmental factors that were previously implicated in telomere shortening. The most important of these factors include smoking and BMI. Slagboom et al. (1994), Am J Hum Genet. 55:876-882. Cawthon (2009), Nucleic Acids Rev. 37:e21. Huyghe et al. (2008), Am J Hum Genet. 83:401-407.

432T

SimRare: A program to generate and analyze sequence-based data for association studies of quantitative and qualitative traits. *B. Li[1,2], G. Wang[1,2], S. M. Leal[1,2].* 1) Baylor College of Medicine, Houston, TX; 2) Rice University, Houston, TX.

Currently there is great interest in detecting complex trait rare variant associations using next generation sequence data. A number of methods have been developed to detect rare variant associations. On a monthly basis new methods are being developed for which the power and type I error need to be evaluated. In order to fairly compare rare variant methods it is necessary to generate data using realistic population demographic and phenotype models. Currently it is difficult to compare rare variant association methods because there is no standard to generate data and often the comparisons are biased. SimRare generates variant data for “gene” regions using forward-time simulation which incorporates population demographic models for Europeans or Africans. It is possible to generate both case-control and quantitative traits (random selection or selection on quantitative trait values). The phenotypic effects of variants can be detrimental, protective or non-causal. For causal variants the effect size can be determined by frequency or purifying selection coefficients. It is possible to model confounders which can either be environmental or genetic, e.g. population substructure. Additionally gene x environment and gene x gene interactions can be modeled. SimRare has a user friendly interface which allows for easy entry of genetic and phenotypic parameters. Additionally to evaluate novel association methods R libraries can be imported into SimRare or conversely the simulated data can be written to external files. SimRare has built-in functions to evaluate the performance for 15 currently available rare variant association methods. In addition to be able to evaluate type I error and power it is also possible to use SimRare to evaluate computational efficiency, control of confounders, and ability to detect interactions. Using SimRare we evaluated 15 rare variant association methods. It is demonstrated that there is not a single most powerful method and most rare variant association methods are not robust to confounders. Additionally due to the computational speed some methods are more advantageous to use especially for the analysis of exome or whole genome sequence data.

433T

Associated alleles at the CAV1/CAV2 locus in primary open angle glaucoma. *C. Pang[1], M. Zhang[2], C. Tham[1], L. Chen[1], P. Tam[1], D. Lam[1].* 1) Ophthalmology & Visual Sciences, The Chinese University of Hong Kong, Hong Kong, HKSAR, China; 2) Joint Shantou International Eye Center of Shantou University and the Chinese University of Hong Kong, Shantou, China.

Purpose: To study multiple CAV1/CAV2 SNPs in primary open angle glaucoma (POAG). Methods: We genotyped six candidate SNPs, rs926201, rs6975771, rs4730742, rs4236601, rs959173 and rs6466587, by TaqMan technology in 833 Chinese study subjects of two cohorts. The Hong Kong cohort contained 185 patients with POAG and 248 controls, and a Shantou cohort of 102 patients and 298 controls. Results: When the two cohorts were combined, rs4236601 was present at low frequencies (3.1% in POAG and 0.73% in controls). The higher-risk genotype AG was significantly associated with increased risk of glaucoma (P=0.02, OR=4.26) and the association was consistent in individual cohorts. In contrast, rs6975771 and rs959173 conferred milder protection for glaucoma (OR=0.65 and 0.61 respectively). We identified a risk haplotype G-G-A-T and a protective haplotype A-T-G-C defined by rs6975771, rs4730742, rs4236601 and rs959173, with their risk and protective alleles occurring in respective haplotypes. Conclusions: We have confirmed the association of rs4236601 with POAG. Risk and protective alleles in different haplotypes of CAV1 and CAV2 were also identified.

434T

Exome Sequencing versus Whole-Genome Genotyping: Lessons from Population Genomics of High-Altitude Adaptations in Tibetans. *S. Xu[1], L. Jin[2].* 1) Chinese Academy of Sciences Key Laboratory of Computational Biology, Chinese Academy of Sciences and Max Planck Society (CAS-MPG) Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shangh; 2) State Key Laboratory of Genetic Engineering and Ministry of Education (MOE) Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai 200433, China.

Although the cost and the time barrier of next-generation sequencing (NGS) are expected to be constantly reduced, whole-genome sequencing (WGS) of a large number of samples is currently not practical for many researchers. Alternatively, genomic capture sequencing technologies such as exome sequencing will be cost-effective approaches for years to come. On the other hand, whole-genome genotyping (WGG), a type of high-throughput technologies to rapidly determine individual genotypes at numerous loci, could still serve as a long-term routine approach for genome-wide complex trait mapping or population genetic studies. In this context, we conducted comparative analyses of the data from five recent genome-wide studies on high-altitude adaptation (HAA) in Tibetans which based on either exome sequencing or whole-genome genotyping, to explore the the pros and cons of the two strategies. The results indicated that exome sequencing did not necessarily take advantage over whole-genome genotyping approach. On the contrary, the study based on exome sequencing almost missed the top HAA signals which were identified by most of the other studies based on WGG. Our analysis also showed that evolutionary and population genetic parameters could be biased estimated based on exome sequencing data. In addition, allele frequencies of some critical loci were also significantly biased estimated from exome sequencing data, which were probably resulted from uncertainty in genotype calls or data quality per se. By considering all aspects, we suggest a two-stage study design with mixed approaches of WGG and targeted sequencing in future genome-wide studies of local adaptation and disease mapping.

435T

Dual genetic structure of the Japanese population based on autosomal SNPs and haplotypes. *Y. Yamaguchi-Kabata[1], T. Tsunoda[1], N. Kumasaka[2], A. Takahashi[2], N. Hosono[1], M. Kubo[1], Y. Nakamura[1,3], N. Kamatani[1].* 1) Ctr Genomic Med, RIKEN, Yokohama, Japan; 2) Ctr Genomic Med, RIKEN, Tokyo, Japan; 3) Inst Med Sci, Univ Tokyo, Tokyo, Japan.

Although the Japanese population has a rather low genetic diversity, it appears to have a “dual structure” arising from at least two major migrations. We recently confirmed the presence of this dual structure through principal component analysis of genome-wide SNP genotypes. Understanding the genetic differences between the two main clusters (the Hondo and Ryukyu clusters) requires a further genome-wide analysis based on a dense SNP set and comparison of haplotype frequencies. In the present study, we determined haplotypes for the Hondo cluster of the Japanese population by detecting SNP homozygotes with 388,591 autosomal SNPs from 18,379 individuals and estimated the haplotype frequencies. Haplotypes for the Ryukyu cluster were inferred by a statistical approach using the genotype data from 504 individuals. We then compared the haplotype frequencies between the Hondo and Ryukyu clusters. In most genomic regions, the haplotype frequencies in the Hondo and Ryukyu clusters were very similar. However, in addition to the HLA region on chromosome 6, other genomic regions (chromosomes 3, 4, 5, 7, 10 and 12) showed dissimilarities in haplotype frequency. These regions were enriched for genes involved in immune system, cell-cell adhesion and intracellular signaling cascade. These differentiated genomic regions between the Hondo and Ryukyu clusters likely contain genes responsible for morphological or physiological differences between the two groups.

436T

Impact of human population expansion on the load of rare and deleterious variants, and consequences for association studies. *A. Clark[1], E. Gazave[1], A. Coventry[1], E. Boerwinkle[2], C.F. Sing[3], A. Keinan[1].* 1) Dept Molec Biol & Gen, Cornell Univ, Ithaca, NY; 2) UT Houston Health Science Center, Houston, TX; 3) Univeristy of Michigan, Ann Arbor, MI.

It has long been appreciated that the human global population has been growing rapidly, from fewer than one million people with the advent of agriculture ~10,000 years ago to ~7 billion today. This 7000-fold growth in fewer than 400 generations can be divided into two epochs, with moderate exponential growth throughout most of this period followed by explosive, super-exponential growth starting fewer than 100 generations ago. This extraordinary situation challenges nearly every aspect of theoretical population genetics as it implies a massive departure from equilibrium. In particular, this recent rapid growth has generated a load of rare variation that is due to recent mutations, which are likely to play an important role in the genetic burden of complex disease. We modeled the site frequency spectrum of mutations arising in a population with accelerating growth, and show that only sample sizes in the thousands can accurately represent very recent mutations. The use of such large sample sizes is therefore necessary but has some consequences that need to be taken into consideration. First, we observe that the larger the sample size, the higher the proportion of singletons, and the stronger the deviation from the classical constant population size model, which is particularly important given the increasing sample size of genome-wide association studies. As a consequence, we observed that in each newly sequenced genome, a relatively large fraction of observed mutations are newly discovered mutations that are private to individuals. As the sample size grows to exceed the historical effective population size other aberrations occur. In this case, genealogies are expected to have multiple coalescent events at the tips, which leads to haplotype and LD patterns that differ from standard theory, and may require special consideration in the search of rare disease-causing variants. Finally, simulations designed to match observed human expansion over the last few hundred generations allowed us to test the expected accumulation of rare variants with rapid population expansion and the inflated individual load of deleterious mutations in this non-equilibrium scenario. Our simulations show how recent explosive growth of the human population can have a critical impact on the genetic architecture of complex disease and on the means by which genotype-phenotype association can best be detected.

437T

A coalescent model for genotype imputation using large reference panels. *E.M. Jewett[1], M. Zawistowski[2, 3], N.A. Rosenberg[4, 1, 5], S. Zöllner[2, 3, 6, 7].* 1) Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI 48109, USA; 2) Department of Biostatistics, University of Michigan, Ann Arbor, MI 48109, USA; 3) Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan 48109, USA; 4) Department of Human Genetics, University of Michigan, Ann Arbor, Michigan 48109, USA; 5) Life Sciences Institute, University of Michigan, Ann Arbor, Michigan 48109, USA; 6) Bioinformatics Program, University of Michigan, Ann Arbor, Michigan 48109, USA; 7) Department of Psychiatry, University of Michigan, Ann Arbor, Michigan 48109, USA.

Genotype imputation is the estimation of genotypes at untyped markers on a chromosome. Several empirical studies based on simulations and on real data have investigated features of reference panels that affect their suitability for imputation. Here, we present a theory-based approach to genotype imputation using a coalescent model to investigate how imputation accuracy changes as a function of parameters such as reference panel size and the level of divergence between target and reference populations. We find that a small internal reference panel (e.g., 100 haplotypes) sampled from the same population as the target sequences will, on average, yield higher imputation accuarcy than a large external reference panel (e.g., >1,000 haplotypes) sampled from a different population, even if the level of divergence between the two populations is small. However, the overall improvement in accuracy obtained by using such an internal reference panel is typically also small. In addition, we find that sampling many (e.g., >1,000) haplotypes from either an internal or external reference panel produces only small gains in imputation accuracy over smaller panels (e.g., <100 haplotypes). The model we develop and the distributions we derive can be used to perform detailed analyses of factors that affect imputation accuracy, and to improve the selection of optimal choices of reference panels.

438T

Allele surfing and selection evidenced from a spatial analysis of human genealogies in Quebec. *C. Moreau[1], C. Bherer[1], H. Vézina[2], M. Jomphe[2], D. Labuda[1,3], L. Excoffier[1,4,5].* 1) Research Center, Ste-Justine Hospital, University of Montreal, Montreal, PQ, Canada; 2) BALSAC Project, University of Quebec at Chicoutimi, Chicoutimi, PQ, Canada; 3) Pediatrics department, University of Montreal, Montreal, PQ, Canada; 4) CMPG, Intitute of Ecology and Evolution, University of Berne, Baltzerstrasse 6, 3012 Berne, Switzerland; 5) Institute of Bioinformatics, 1015 Lausanne, Switzerland.

Records of human genealogies offer a unique possibility to study the genetic consequences of recent demographic processes and to check predictions of theoretical models. Extensive genealogies are available for the whole population of Charlevoix-Saguenay-Lac-St-Jean (CSLSJ), a region of North-Eastern Quebec recently colonized in a stepping-stone manner in the last three centuries. This large pedigree including the genealogical relationships among more than one million individuals born over three centuries appears ideal to study the genetic impact of a documented range expansions in humans. The recorded time and location of the marriages of all the individuals and their children allowed us to precisely reconstruct the spatial and temporal dynamics of the range expansion and to distinguish demographic processes happening on the wave front, defined as newly opened parishes. Overall, we find that individuals reproducing at the wave front had a higher fertility than the other individuals, corresponding to a 9-16% selective advantage of reproducing at the front, depending on the regions. This resulted in ancestors reproducing at the wave front leaving up to 2.4 fold larger genetic contribution to the present CSLSJ population than ancestors who did not reproduce on the front. We also find that the ancestors of the SLSJ population reproduced significantly more at the front than expected, whereas the reverse situation is found in Charlevoix, the oldest settlement of CSLSJ. These results are compatible with the allelic surfing phenomenon predicting an overrepresentation of genes originating on the wave front in newly colonized territories. It is also the first documented evidence in humans of a selective advantage to be on an expanding wave front, and the implications of this result will be discussed in the context of recent human evolution. (Supported by Réseau de Médecine Génétique Appliquée of Fonds de Recherche en Santé du Québec).

439T

Saudi NGHA biobank; designed study as a longitudinal investigation of constitutional and environmental factors influencing genetics disease in Saudi Arabia. *I. Alabdulkareem[1], M. Albalwi[1,2], M. Aljumah[1].* 1) KAIMRC, National Guard Health Affairs, Riyadh, Riyadh, Saudi Arabia; 2) Department of Pathology and Laboratory Medicine. King Abdulaziz Medical City, Riyadh, Saudi Arabia.

Biospecimens storage is one of the modern practices to secure high standard research materials available for investigators worldwide. Saudi Biobanking (SB) is designed study as a longitudinal investigation of constitutional and environmental factors influencing illness in Saudi Arabia. The SB objective is to implementation the highest standards of biological banking as it is vital towards perform genomic/proteomics databases. These databases will increase the quality of health care by creating a linking them life style, environmental factors and clinical practice. The SB is 4 phases plan that as follow; DNA/RNA banking, blood components/urine/tissues and bone/infectious banking respectively. SB team is targeting 200,000 individuals for families and diseased subjects for fluid and solid samples while DNA/RNA reservoir capacity is 1.8Million matrix tubes for genetics diseases that seen in day to day clinical practice. Phase one is already lunched by banking DNA using a complete automated DNA banking that carried out a complete robotic extraction of DNA from blood followed by banking the samples at -20C°. Retrieving the sample(s) is also auto programmed by the LIMS system. Additional to that, each subject's DNA is extracted manually and sent for semi automated dry storage as a back up.

440T

Secretor Genotype (FUT2 gene, rs601338) Is Strongly Associated with the Composition of Bifidobacteria in the Human Intestine. *N. Alakulppi, P. Wacklin, H. Mäkivuokko, J. Nikkilä, H. Tenkanen, P. Sistonen, J. Räbinä, J. Partanen, K. Aranko, J. Mättö.* Finnish Red Cross Blood Service, Helsinki, Finland.

Intestinal microbiota plays an important role in human health, and its composition is determined by several factors, such as diet and host genotype. However, thus far it has remained unknown which host genes determine the microbiota composition. We studied the diversity and abundance of dominant bacteria and bifidobacteria from the faecal samples of 71 healthy individuals. In this cohort, 14 were non-secretor (NS) individuals and the remainders were secretors (S). The secretor status is defined by the expression of the ABH and Lewis histo-blood group antigens in the intestinal mucus and other secretions. It is determined by fucosyltransferase 2 enzyme, encoded by the FUT2 gene. Non-functional enzyme resulting from a nonsense mutation (rs601338) in the FUT2 gene leads to the NS phenotype. To verify that rs601338 defines the NS-S phenotypes, 255 healthy Finnish individuals were sequenced for the FUT2 coding exon. All individuals had the same serological phenotype deducted from the rs601338 genotype. Compared with HapMap, the same strongest LDs were found between SNPs rs601338 and rs492602 or rs681343. A total of 1040 genotypes from samples representing the whole Finland showed significant differencies between the counties (NS rs601338 frequency min - max = 3 - 30%, mean 15%). PCR-DGGE and qPCR methods were applied for the intestinal microbiota analysis. Principal component analysis of bifidobacterial DGGE profiles showed that the 14 samples of NS individuals formed a separate cluster within the 57 S samples. Moreover, bifidobacterial diversity ($p<0.0001$), richness ($p<0.0003$), and abundance ($p<0.05$) were significantly reduced in the samples from the NS individuals as compared with those from the S individuals. In contrast to bifidobacteria, several bacterial genotypes were more common and the richness ($p<0.04$) of dominant bacteria as detected by PCR-DGGE was higher in the NS individuals than in the S individuals. We showed that the diversity and composition of the human bifidobacterial population is strongly associated with the histo-blood group ABH S/NS status, which consequently appears to be one of the host genetic determinants for the composition of the intestinal microbiota. This association can be explained by the difference between the S and NS individuals in their expression of ABH and Lewis glycan epitopes in the mucosa.

441T

Facts related to the collection of biological samples in the National Health Examination Survey - Portuguese Component of the European Health Examination Survey. *M. Barreto[1], V. Francisco[1], P. Rasteiro[2], E. Sousa[2], A. Vicente[1], M. Bourbon[1], A. Fernandes[2], A. Beleza[2], F. Mendonça[3], A. Gil[1], C. Matias Dias[1].* 1) Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal; 2) Laboratório de Saúde Pública Dra. Laura Ayres, Faro, Portugal; 3) Administração Regional de Saúde do Algarve, Faro, Portugal.

The objective of the National Health Examination Survey (NHES), which corresponds to the Portuguese component of the European Health Examination Survey (EHES), is to collect health data, related risk factors and biological samples of the Portuguese population, using the EHES recommended methodology. These surveys involve an interview, clinical and physical measurements and blood collection. In this context, we herein describe the pilot study performed in S. Brás de Alportel in the Algarve region. For this pilot study, we have recruited 221 individuals (95 males and 126 females), between 25 and 91 years old, who were enrolled in the Health Centre of S. Brás de Alportel (Algarve). For each participant, we have collected 16.5 ml of total blood, in five different Vacutainer® tubes, which was later processed into serum, plasma and DNA. We have performed several biochemical analyses(total cholesterol, LDL, HDL, glucose, tryglicerides, creatinine, ALT, AST, . -GT, CRP and iron) and a complete blood count. From the 221 participants in this pilot study, we were able to collect blood to 219 (99.5%). To 185 of these (84.5%) we were able to collect the total amount of blood. The biochemical analyses were performed in all the samples. The total blood count was performed in 103 samples (47%) due to transport constraints. We have also collected DNA from 210 participants (95.9%). We have created a biobank comprising 1847 serum aliquots and 959 plasma aliquots, which have been stored at -80°C and 210 DNA aliquots which have been stored at 4°C. In conclusion, during this study, we have optimized the logistics and procedures to perform the large scale study for the NHES and EHES. In addition, we have created a biobank comprising detailed questionnaire data, physical and clinical data and biological samples from a representative sample of S. Brás de Alportel in Algarve, Portugal. This biobank will allow us to perform future studies, including the determination of the prevalence of gene variants of public health interest, the characterization of gene-environment interactions in the development of chronic diseases and the genetic structure of the Portuguese population. The success rate, the quality of the data and of the biological samples was high and comparable to similar studies.

442T

Complexities in geneticists' views on "race" crossing: No evidence for change on "political" grounds. *E.B. Hook.* School of Public Health, Univ California, Berkeley, CA. 94720-7360 USA.

Virtually all human geneticists today would not view "race" crossing as "dysgenic", in fact the opposite because of diminished autosomal recessive disorders in offspring. But in the 1920s a major concern was that segregation and recombination might break up any "adaptive gene complexes" (AGC) in the genome. Presumably any AGC within "races"; had evolved in response to selective pressures, so outcrossing would be deleterious. Because of this theoretical objection, in the absence of empirical data many US and UK geneticists in the 1920s, ostensibly on "social policy" grounds condemned strongly "race crossing". In the late 1930s, 1940s and 1950s however, US and UK geneticists expressed opposition to this blanket condemnation. W.B. Provine has claimed that this change was based not on any new evidence but, rather, because of revulsion to Nazi doctrines on race biology and their implications. Geneticists changed their minds on this matter he claimed, in essence, to fit their political viewpoints (Science 1973 182:790-6). I queried five eminent geneticists established professionally before World War 2 (Dobzhansky, Dunn, Huxley, Stern, and Wright), as well as four who were graduate students (Crow, Neel, Reed and Steinberg) about the claims. None knew of any who changed their minds on effects of race crossing in response to Nazi views or any other reason. Huxley reiterated an earlier view that "wide" crosses could have adverse effect. Curt Stern, who had fled Nazi Germany, in all editions of his textbook, (1949, 1960, 1973) took an agnostic position on race crossing, and referred to adverse outcomes resulting from Rh incompatibility if races differed in blood group proportions. Moreover, the theoretical issue of possible dysgenic effect was still regarded as an open one in the 1960s as exemplified by investigations in Hawaii (Morton et al. 1967). In summary, there is no evidence anyone changed his/her mind about race crossing for political reasons. Those who strongly opposed race crossing died or tended to grew silent out of recognition of affinity with Nazi views; others appeared who spoke out against extremist views on race crossing. Provine wrote when political and social implications of human genetics and race were emerging as part of major public debates about many policies. The notion that the zeitgeist changed geneticists views appeared popular to sociologists of scientific knowledge, but distorts a complex history of genetics.

443T

Tracing the population origin of non-European chromosomal segments identified by the method of rare heterozygotes and homozygotes (RHH) in admixed subjects of European Caucasian descent. *R. McGinnis[1], W. McLaren[2].* 1) Wellcome Trust Sanger Institute, Cambridge, United Kingdom; 2) European Bioinformatics Institute, Cambridge, United Kingdom.

By rapid software analysis of GWAS SNP genotypes, the method of rare heterozygotes and homozygotes (RHH) can visualize genome mosaicism in admixed European Caucasians with African or Asian ancestry (McGinnis et al, Human Molecular Genetics 19:2539-2553, 2010). This raises the methodological issue of determining the exact non-European population from which a visualized chromosomal segment of admixture arises. This determination is complicated since typically only one of the subject's two extended haplotypes spanning the segment derives from the non-European population. To identify segment origin in admixed Caucasians of the 1958 British Birth Cohort (58BBC) genotyped on the Illumina 550K (Illm550K) chip, we pursued several strategies. To date, the most promising has been to derive the "European" haplotype spanning a subject's admixed segment by "zeroing" the subject's heterozygous Illm550K SNPs in the segment and imputing the zeroed genotypes using (a) the subject's homozygous Illm550K SNPs plus (b) genotypes at all Illm550K SNPs in the segment from ~1500 unadmixed 58BBC subjects. The resulting combination of imputed and called homozygous genotypes define the European haplotype which in turn yields the non-European haplotype by subtracting the imputed allele from each zeroed heterozygote. In this way, we derived a non-European haplotype from ten 58BBC subjects with RHH-identified African or Asian admixture across a 15 megabase (Mb) region on chromosome 1 which contained ~3300 Illm550K SNPs; and we similarly derived the 15 Mb non-European haplotype from multiple simulated admixed Caucasian subjects (who were simulated by pairing a phased HapMap 3 European [CEU] chromosome with a phased chromosome 1 from a HapMap3 non-European [CHB, YRI, LWK, or GIH]). We applied principle components-multidimensional scaling (PC-MDS) analysis to compare each derived non-European haplotype with haplotypes or genotypes of 51 populations in the Human Genome Diversity Panel (HGDP) typed by Illm550K. We will illustrate our results - which achieved partial success: each non-European haplotype derived from a simulated subject clustered with the correct subset of HGDP populations and we were able to trace each 15 Mb segment from the ten admixed 58BBC subjects to a subset of Subsahara African or Asian HGDP populations. However the approach currently lacks sufficient resolution to precisely trace a 15 Mb segment to a single HGDP population.

444T

Killer cell Immunoglobulin like Receptors distribution in healthy Brazilian Caucasian Kidney donors. *M.M. Moraes[1], R. Benvenutti[2], C. Gomes[2], C. Pozzi[2], F.L.C. Contieri[2], M.G. Bicalho[1].* 1) Departamento de Genética, Universidade Federal do Paraná, Curitiba, Paraná, Brazil; 2) Unidade de Transplante Renal do Hospital Universitário Evangélico, Faculdade Evangélica do Paraná, Curitiba, Paraná, Brazil.

Killer-cell Immunoglobulin like Receptors (KIRs) are Natural Killer (NK) cell surface proteins that play an important role modulating the cytotoxicity of these innate immune system cells. The balance of activating and inhibitory signals from HLA class I on target cells and KIR interactions results in induction or inhibition of NK-mediated target cell lysis. These receptor interactions were recently studied in kidney transplant patients where patient's NK cells may recognize donor cells that do not express specific ligands and thus avoid cell lysis. These KIR genes can be present or absent into individual repertory and thus exhibit different combinations of 14 genes (8 functional genes encoding inhibitory receptors and 6 genes encoding activator receptors) and 2 pseudogenes setting genotypes. KIR frequencies and genotypes data may contribute to a better-understanding of these genes evolution and the mechanisms involved in NK-mediated cytotoxicity. In this study, KIR genotyping was carried out using the KIR SSO Genotyping Test (One Lambda Inc.) according to manufacturer's instructions and PCR-SSOP from peripheral blood leucocytes of 93 Caucasian unrelated kidney donors from HUEC Hospital (Paraná - Brazil). Results and analysis were produced with HLA Visual® (One Lambda Inc.) software package. We compare KIR gene carrier frequency (rxc Contingency Tables) from our sample with frequency data from other Caucasian populations (Brazilian and European) described in the literature. Gene frequencies found in the present study were: KIR3DL3(1.00), KIR2DS2(0.53), KIR2DL2(0.45), KIR2DL3(0.90), KIR2DP1(1.00), KIR2DL1(0.99), KIR3DP1(1.00), KIR2DL4(1.00), KIR3DL1(0.94), KIR3DS1(0.41), KIR2DL5(0.55), KIR2DS3(0.37), KIR2DS5(0.22), KIR2DS1(0.41), KIR2DS4(0.82) and KIR3DL2(1.00). These frequencies are very similar to frequencies found in another Brazilian population from a different region, as well as for the French and British Caucasian populations. 39 different genotypes were identified and the most frequently observed were KG-01-1001111110000011 (0.33), KG-02-1111111110000011 (0.06) and KG-03-1111111110110011 (0.05). The differences found in gene frequencies and genotypes may reflect the contribution of two other important components (Africans and Amerindians) of the Brazilian population. Knowledge of the immune systems genetic background of a population may help in understanding the mechanisms mediated by KIR involved in innate immune response of NK cell.

445T

Genetic ancestry and population structure of geographically separated African American populations. *M.C.Y. Ng[1], S. Sajuthi[2], A. Cupples[3], J. Divers[4], J. Dupuis[3], M. Fornage[5], L. Kao[6], E. Larkin[7], M. Li[8], C.T. Liu[3], S. Musani[9], J. Mychaleckyj[10], G. Papanicolaou[11], A. Reiner[12], D. Siscovick[12], X. Zhu[7], B. Freedman[13], J. Wilson[9], D.W. Bowden[1].* 1) Center for Genomics and Personalized Medicine Research, Wake Forest University School of Medicine, Winston-Salem, NC; 2) Program in Molecular Genetics and Genomics, Wake Forest University School of Medicine, Winston-Salem, NC; 3) Department of Biostatistics, Boston University School of Public Health, Boston, MA; 4) Department of Biostatistical Sciences, Wake Forest University School of Medicine, Winston-Salem, NC; 5) Research Center for Human Genetics, The University of Texas Health Science Center at Houston, Houston, TX; 6) Department of Epidemiology, Johns Hopkins School of Medicine, Baltimore, MD; 7) Center for Clinical Investigation, Case Western Reserve University, Cleveland, OH; 8) Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, PA; 9) University of Mississippi Medical Center, Jackson, MS; 10) Public Health Sciences, University of Virginia School of Medicine, Charlottesville, VA; 11) NHLBI, Bethesda, MD; 12) Department of Medicine and Epidemiology, University of Washington, Seattle, WA; 13) Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC.

African Americans (AA) are a recently admixed population formed from their African and European ancestral populations. It is unclear whether AA are genetically homogeneous between US geographic regions. This has implications in study design such as the selection of appropriate reference haplotypes for imputation and controlling population structure in association studies. This study examined the African ancestry and population structure of 8130 unrelated AA from six studies in the Candidate Gene Association Resource (ARIC, CARDIA, CFS, JHS, MESA) and a Wake Forest Health Sciences sample. We analyzed 86472 autosomal uncorrelated SNPs genotyped using Affy 6.0 platform in AA from the six studies as well as 578 HapMap samples of Africans (YRI, LWK, MKK), Europeans (CEU) and AA (African American Southwest, ASW). Estimation of ancestry proportions in AA including ASW using FRAPPE revealed a wide range (0-100%) of African ancestry within each study, with mean African ancestry of 74-84% across studies. The allele frequencies were highly correlated among AA ($0.85 < r < 0.99$). F_{ST} and principal component analyses also revealed little population structure among AA in different studies ($0 < F_{ST} < 0.001$), and AA were genetically more close to YRI than LWK and MKK (F_{ST} to YRI=0.004-0.006, LWK=0.007-0.008, MKK=0.013-0.014). Our results are consistent with the history of West Africa origin of AA. While there is substantial variation of African ancestry within studies, the population structures of each sample, including ASW, are similar to other AA recruited at different geographic areas. Geographically different AA samples are statistically indistinguishable. These results suggest the generalizability of ASW to represent the general AA population in US.

446T

Human Adaptation and Evolution in Response to Helminth Parasites. *B. Sadler.* Arizona State University Tempe, AZ.

The gut of humans, and indeed all vertebrates, is colonized by other species. There are three main categories of organisms that can live, grow, and reproduce within the human gut for extended periods of time. These are 1)helminths, multicellular eukaryotic worms, 2)protozoan parasites, and 3)gut bacteria that typically make up what is referred to as our microbiome. A variety of observations suggest that humans have been coevolving with helminths for an extended period of time. For example, evidence for the presence of human-specific helminths in ancient feces indicates that humans have been parsitized by helminthes for tens of thousands of years. The presence of helminths in our closest living relatives, non-human primates adds additional support for an evolutionarily old relationship between humans and helminths. Finally, isolated indigenous groups, who are the closest living examples of our ancestral hunter-gatherer state, have a high prevalence and incidence of helminth infections. Together, these observations suggest that helminth infection was common in past human environments. Parasitism by helminths decreases host fitness in a number of ways. For example, helminth infection can impact host fertility and reproduction, stunt host growth, increase visibility to predators, impair gastrointestinal function, and suppress the immune system. Therefore, adaptive responses to these selective pressures leading to evolutionary changes should exist. This poster will discuss 1)the role of helminths in directing vertebrate host adaptation and evolution, 2)current evidence of various types of host adaptations including behavioral, anatomical and metabolic, as well as evidence of immunological evolution in response to helminths, and 3)specific immunological pathways and genetic variants that have been implicated in human susceptibility to helminth infection. These lines of evidence support the hypotheses that 1)helminths have had a profound effect on our own evolutionary trajectory, especially with regard to immune response, 2)the fact that phylogenetically diverse species response similarly to helminths says something about the antiquity of this host/parasite relationship, and 3)heritability of certain immunological genetic susceptibility variants explains some of the variance in susceptibility within and between populations, but that helminth species-specific resistance variants may be difficult to identify, so other types of candidate genes may be helpful.

447T

Integrating population diversity, conservation, and epigenomic information at regulatory elements, noncoding transcripts, and disease-associated regions. *L.D. Ward[1,2], R.C. Altshuler[1,2], P. Kheradpour[1,2], J. Ernst[1,2], O. Zuk[2], M. Garber[2], K. Lindblad-Toh[2,3], E. Birney[4], M. Kellis[1,2], The ENCODE Consortium.* 1) CSAIL, Massachusetts Institute of Technology, Cambridge, MA; 2) The Broad Institute of MIT and Harvard, Cambridge, MA; 3) Departmentof Medical Chemistry and Microbiology, Uppsala University, Uppsala, Sweden; 4) European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK.

In previous work, we used the ChromHMM method to identify enhancers in nine human cell lines from ENCODE histone modification data, and discovered motifs associated with the cell-type specificity of enhancers (Ernst *et al.*, 2011). These enhancer maps and putative regulatory motif instances allow us to generate hypotheses of causal SNPs in strong LD with variants associated with phenotypes by GWAS. We have now extended this analysis to bring in conservation data from low-coverage sequencing of 29 mammals, using the SiPhy method (Garber *et al.*, 2009) to identify constrained elements at unprecedented resolution. In particular, we were interested in cases where the lead SNP reported by an association study did not lie in a conserved element, but a strongly-linked variant did. We find several compelling examples: (1) a tooth development-associated variant rs6504340 located in an unconserved region between the developmental genes *HOXB1* and *HOXB2* (Pillas *et al.*, 2010) is strongly linked to another intergenic variant, rs8073963, which disrupts a deeply-conserved instance of a motif recognized by Forkhead family proteins and is predicted to be a strong enhancer in K562 cells; (2) lead SNP rs10948197 associated with height in Koreans (Kim *et al.*, 2009) is in an unconserved region but is linked to rs3799976 in a conserved element and disrupts a Forkhead family motif; (3) a height-associated variant discovered in two studies, rs10906982 (Weedon *et al.*, 2008; Liu *et al.*, 2010), does not lie in a conserved element but is strongly linked to a variant in a conserved element, rs7183263, which lies in a motif recognized by homeodomain proteins and is a strong enhancer in skeletal muscle myoblast (HSMM) cells. We also studied the pattern of conservation and population variation associated with regulatory elements, by combining heterozygosity values from the 1000 Genomes Project with transcription factor binding data and ChromHMM chromatin states from ENCODE. We find a signal of decreased heterozygosity, evidence of selection in the human population, at intergenic, TSS-distal motif instances bound by their regulators, as well as strong enhancers identified by histone modifications; this constraint is also seen across mammals. In addition, at novel intronic and intergenic transcripts identified by ENCODE (and currently absent from the GENCODE annotation), we consistently find decreased heterozygosity among humans.

448T

***EPAS1* and *EGLN1* associations with High Altitude Sickness in Han and Tibetan Chinese at the Qinghai-Tibetan Plateau.** *N. Buroker[1], X-H. Ning[1], Z-N. Zhou[2], K. Li[3], W-J. Cen[3], X-F. Wu[2], W-Z. Zhu[2], C.R. Scott[1], S-H. Chen[1].* 1) Pediatrics, 356320, University of Washington, Seattle, WA; 2) Laboratory of Hypoxia Physiology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, China; 3) Lhasa People Hospital, Tibet, China.

Acute (AMS) and chronic (CMS) mountain sicknesses are illnesses that occur among lowlander Han Chinese visiting or highlander Tibetan Chinese inhabiting high altitude environments, respectively. Both diseases are influenced by genetics. Consequently, some individuals are genetically less fit than others when stressed by an extreme high-altitude environment. The *EPAS1* [ch2: 46441523 (hg18], *EGLN1* (rs480902) and (rs516651) SNPs were studied in association with high altitude illness among Tibetan residents and Han Chinese visitors at the Qinghai-Tibetan plateau. Direct sequencing was used to identify individual genotypes for the three SNPs. Age was found to be significantly associated with the *EPAS1* SNP in the CMS Tibetan patients while HR and SaO2 were found to be significantly associated with the *EGLN1* (rs480902) SNP in the AMS Han patients went compared to Tibetan and Han control study groups. The CMS patients were found to diverge significantly for the *EPAS1* SNP from their Tibetan control group as measured by genetic distance (0.123) indicating directional selection of the *EPAS*-G allele with age and illness. The *EGLN1* (rs480902) and (rs516651) SNPs are found in NFE2L1 and PPAR transcriptional factor response elements, respectively. The *EGLN1* (rs480902) SNP has a significant correlation with HR and SaO2 in AMS patients. In conclusion, AMS and CMS were found to be significantly associated with the *EPAS1* and *EGLN1* SNPs indicating these mutations have an affect on both illnesses.

449T

The landscape of recombination in African Americans. *A.G. Hinch[1], A. Tandon[2,4], N. Patterson[2], Y. Song[3], N. Rohland[2], C.D. Palmer[2], G.K. Chen[5], K. Wang[6], S.G. Buxbaum[7], . AABCC[8], . AALCC[9], . AAPCC[10], . CARe[11],. CHOP[12], S. Redline[13], J.N. Hirschhorn[2], B.E. Henderson[5], H.A. Taylor, Jr[7], A.L. Price[14], H. Hakonarson[6], S.J. Chanock[15], C.A. Haiman[5], J.G. Wilson[16], D. Reich[2,4], S.R. Myers[1,3].* 1) WTCHG, Oxford University, Oxford, UK; 2) Broad Institute, Cambridge, MA; 3) Dept of Statistics, Oxford University, Oxford, UK; 4) Dept of Genetics, Harvard Medical School, Boston, MA; 5) Keck School of Medicine, University of Southern California, Los Angeles, CA; 6) Children's Hospital of Philadelphia, Philadelphia, PA; 7) Jackson State University, Jackson, MS; 8) African American Breast Cancer Consortium; 9) African American Lung Cancer Consortium; 10) African American Prostate Cancer Consortium; 11) Candidate Gene Association Resource Study; 12) Children's Hospital of Philadelphia; 13) Brigham and Women's Hospital, Boston, MA; 14) Harvard School of Public Health, Boston, MA; 15) National Cancer Institute, Bethesda, MD; 16) University of Mississippi Medical Center, Jackson, MS.

Recombination, along with mutation, is the ultimate source of variation in populations. A key tool for understanding recombination is a “genetic map” measuring the probability of crossover events at each position in the genome. We leverage the recent mixture of people of African and European ancestry in the Americas to build a map based on about 2.1 million crossovers in 30,000 unrelated African Americans. At intervals of more than three megabases it is nearly identical to one built in Europeans . At finer scales it differs significantly, with 2,454 recombination hotspots that are active in people of West African ancestry but nearly inactive in Europeans. The probability of a crossover at these hotspots is almost fully controlled by the alleles an individual carries at PRDM9 (P<10-245). We identify a 17 base pair DNA sequence motif that is enriched in these hotspots, and is an excellent match to the predicted binding target of African-enriched alleles of PRDM9.

450T

Ancestry informative marker set for Han Chinese population. *H.Q. Qu[1], Q. Li[2], J.B. McCormick[1], S. Xu[3], M. Xiong[4], J. Qian[5], L. Jin[3,5,6].* 1) Division of Epidemiology, Human Genetics and Environmental Sciences, The University of Texas School of Public Health, Brownsville, Texas 78520, USA; 2) Endocrine Genetics Lab, The McGill University Health Center (Montreal Children's Hospital), Montréal, Québec H3Z2Z3, Canada; 3) Chinese Academy of Sciences and Max Planck Society (CAS-MPG) Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, P.R. China; 4) Division of Epidemiology, Human Genetics and Environmental Sciences, The University of Texas School of Public Health, Houston, Texas 77030, USA; 5) State Key Laboratory of Genetic Engineering and Ministry of Education (MOE) Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai 200433, P.R. China; 6) Key Laboratory of Computational Biology, CAS-MPG Partner Institute for Computational Biology, Chinese Academy of Sciences, Shanghai 200031, P.R. China.

Han Chinese accounts for 91.51% of Chinese population, or ~1.226 billion people. Chronic diseases including cancer, vascular disease, and infectious diseases, are the three leading causes of death in this population (He et al, 2005). Genetic association study (GAS) is a critical approach to understand molecular mechanisms and population-specific risk of these diseases, that can lead to the development of effective interventions at an individual or population level. Currently, a major issue of genetic association study is the confounding effect of population stratification. Our recent study (Li Jin group) identified obvious genetic heterogeneity between northern Han Chinese (N-Han) and southern Han Chinese (S-Han), historically divided by the natural barrier Yangtze river (Xu et al, 2009). This study highlighted the importance of the correction for population stratification in GASs on the Han Chinese population. The structured association method represented by Eigenstrat (Price et al, 2006) has been extensively demonstrated to be an effective approach. To minimize the genotyping cost of structured association studies, we developed a set of ancestry informative markers for genetic studies of Han Chinese populations. A sample of 308 Han Chinese individuals with 158,015 autosome SNPs genotyped from different regions in China (Xu et al, 2009) was analyzed. As shown by principle component analysis (PCA), N-Han and S-Han individuals formed two obviously distinct clusters by the first principle component (PC). 261 individuals were unambiguously distinguishable without obvious admixture. In these 261 individuals, ancestry information content I_n of each autosome SNP was calculated using the infocalc program (Rosenberg et al, 2003). Across 22 autosomes, an initial set of AIMs including 5,001 SNPs were selected by choosing one SNP marker with the largest I_n in each 500kb window. By a stepwise procedure, we decreased the number of AIMs by removing AIMs with the smallest I_n, and assessed the classification effects of PCAs by the maximum Matthews correlation coefficient (MCC). The classification effect was compromised significantly when less than 30 AIMs were used. We recommend at least the top 30 AIMs should be used in any structured association study on the Han-Chinese population. Robust correction for population stratification can be achieved if the top 150 AIMs are used, which can differentiate N-Han and S-Han unambiguously.

451T

Spinal Muscular Atrophy: Improved Detection of Silent (2+0) Carriers by Identification of SMN1 Founder Alleles. *M. Luo, L. Liu, I. Peter, S. Scott, C. Eversley, R. Kornreich, R.J. Desnick, L. Edelmann.* Dept Gen & Genomic Sci, Mount Sinai Sch Med, New York, NY.

Spinal muscular atrophy (SMA) is one of the most common and severe autosomal recessive diseases with a carrier frequency of 1 in 35 to 1 in 117, depending on ethnicity. SMA results primarily from *SMN1* gene copy number loss on chromosome 5q13 by either *SMN1* deletion or gene conversion with the highly homologous *SMN2*. Carrier screening is therefore performed by dosage sensitive methods; however, the detectability varies from 71-94% due to the inability to identify silent (2+0) SMA carriers with two copies of *SMN1* on one chromosome 5 and zero copies on the other. Since identification of deletion/duplication founder alleles might provide a general approach to identify silent carriers and improve carrier detection in various ethnic/racial groups, the occurrence of founder alleles in the Ashkenazi Jewish (AJ) population was investigated. Carrier screening of 692 healthy AJ adults by multiplex ligation-dependent probe amplification (MLPA) identified 1 in 46 (2.2%) deletion (1+0) carriers and 1 in 7 duplication (2+1) individuals, indicating that the detectability in this population using only *SMN1* gene dosage is ~90%. Microsatellite analyses using markers flanking the SMA locus identified two deletion and one major duplication founder haplotypes. Importantly, two tightly-linked polymorphisms in *SMN1*, g.27134T>G in intron 7 and g.27706_27707delAT in exon 8, were detected on the major AJ duplication allele, but not in 351 AJ individuals with two *SMN1* copies, making the haplotype highly specific for duplication alleles and effectively increasing the accuracy of carrier detection from 90 to 94% in this population. Surprisingly, these two linked polymorphisms were also identified on duplication alleles from other tested populations including African Americans, Hispanics and Asians. The finding that this duplication allele is shared by several ethnic groups suggests that it represents an ancient allele. Moreover, its identification among individuals who screen negative by dosage sensitive methods will improve SMA carrier detection and decrease the current residual risk that is due to the inability to detect silent (2+0) SMA carriers in different ethnic groups.

452T

Low depth, whole genome sequencing of Dai population isolate demonstrates superiority over use of whole genome genotyping arrays in uncovering population structure, demographic history and selective pressures in non-European populations. *L.J.M. Coin[1,2], R.Y. Chen[1,3], F. Zhang[1,4], Y.R. Li[1], C. Yu[1], R.N. Gutenkunst[5], Y. Wang[6], R. Nielsen[1,6], W.W. Wu[1].* 1) BGI-Shenzhen, Shenzhen, China; 2) Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, St Mary's Hospital, London, UK; 3) Wuhan University, Wuhan, China; 4) South China University of Technology, Guangzhou, China; 5) Theoretical Biology and Biophysics and Center for Nonlinear Studies, Los Alamos National Laboratory, Los Alamos, New Mexico, USA; 6) Departments of Integrative Biology and Statistics, UC Berkeley, Berkeley CA, USA.

The development of whole genome sequencing (WGS) technology provides an opportunity to overcome the limitations of previous approaches for calculating population genetic parameters. In particular, genome-wide inference using SNP genotyping arrays is largely based on a limited set of variants tagging common variation in a reference cohort, rather than all variation actually present in the study samples, which may limit resolution in the study of non-European populations. In this project, we sought to compare inferences made from genotyping arrays with those from low coverage WGS data in order to assess biases present in either approach and to demonstrate the increased power and resolution available from low coverage sequence data. We generated genotyping as well as low-coverage (4x) WGS data on 80 samples from the Dai population, one of the minority groups in Yunnan province, South China, whose demographic history has yet to be resolved. WGS genotype calls were generated using the Broad Institute pipeline incorporating allele-specific read counts and subsequent refinement via imputation with Beagle. Indel genotypes were obtained from Dindel. Genotype concordance between WGS and array derived genotypes was high (98%) after imputation. We identified 2,889,232 novel SNPs. 31.2%, 35.8% of all Dai SNPs were tagged with r^2 > 50% by the Illumina 610k, and Illumina Omni `China' 1M chip respectively, compared to 31.2%, 37.3% in Han Chinese, and 39.4%, 43.0% in CEPH European samples, indicating bias towards European genomes in array design. WGS identified 3964 SNPs with allele frequency differences between Dai and Han greater than 80%, of which 90 were fixed differences. Only 5 of these fixed differences were visible to the array, indicating a vastly improved ability to identify putative sites of positive selection with WGS data. Using WGS data we provided finer resolution of the internal structure of the Dai population than with array data, obtaining more IBD segments, and resolving relatedness within the sample more accurately. We also obtained more precise estimates of population genetic parameters relating to the history of Dai, and performed an analysis of the Y chromosome not possible with a 610k Illumina array. Our results suggest Dai originated in central China prior to migrating southwest, leading to greater migration and higher gene flow between Dai and Cambodia.

453T

Host genetic variants and Epstein-Barr viral load in the HapMap lymphoblastoid cell lines. *C.J. Houldcroft, A. Gall, A.L. Palser, S.J. Watson, P. Kellam.* Virus Genomics, Wellcome Trust Sanger Institute, Cambridge, Cambs, United Kingdom.

Statement of purpose The HapMap collection of lymphoblastoid cell lines (LCL) is a widely used resource for studying human genetic variation and phenotypic traits. The LCLs were derived by immortalising human B cells with the B95.8 strain of Epstein-Barr virus (EBV), a human gammaherpes virus. Several studies have demonstrated that EBV viral load within LCLs, including the HapMap cell lines, is a variable trait and this potentially contributes to the variance in some cellular phenotypes. Gammaherpesviruses can change between two major viral gene expression programmes (latency and lytic replication) and this effects host gene expression. This study absolutely quantifies the EBV load within a subset of the HapMap CEU panel, assesses whether any of the LCLs show evidence of a change to the lytic replication cycle of EBV, and considers to what extent the variation in EBV viral load between cell lines may be under control by host genetic variants. It also examines if there is any EBV sequence diversity within a subset of the HapMap CEU and YRI samples in 1000 Genomes. Methods Quantitative polymerase chain reaction was used to absolutely quantify viral load in a panel of HapMap CEU individuals, normalised to GAPDH. Relative quantification of EBV lytic gene transcript BZLF1 was also performed by qPCR. Western blotting assayed lytic viral protein expression in a subset of cell lines. Low-coverage Illumina sequencing of HapMap samples from the pilot and phase 1 of the 1000 Genomes data was used to assay sequence diversity within the EBV DNA content of the HapMap samples. EBV genome sequences were assembled with Bowtie. Results Viral load in the HapMap LCLs varied from 2 to 350 copies/cell (normalised to GAPDH content). The average EBV load was 77 copies/cell. LCLs with high viral loads demonstrated 2 log fold greater expression of the lytic EBV BZLF1 mRNA than in a defined latent cell line. LCLs with the highest EBV loads also exhibited viral lytic protein expression by western blot. Assembly of the EBV genome sequences of 66 CEU and YRI HapMap samples revealed no variation in the EBV genomes present in the cell lines. Future work will explore host and viral factors controlling EBV viral load within the HapMap samples. The effects of Epstein-Barr virus immortalisation of lymphoblastoid cell lines should also be considered as a source of phenotypic variation in assays.

454T

Exome Sequencing in an Isolate Population. *A. Ramachandran[1], E. Sehayek[2], J. Breslow[3], J. Friedman[4], R. Lifton[5], I. Pe'er[1].* 1) Computer Science, Columbia University, New York City, NY; 2) Genomic Medicine Institute, Cleveland Clinic, Cleveland, OH; 3) Laboratory of Biochemical Genetics and Metabolism, Rockefeller University, New York City, NY; 4) Laboratory of Molecular Genetics, Rockefeller University, New York City, NY; 5) Department of Genetics, Yale University, New Havan, CT.

Background: Small, isolate populations have been previously used in rare variant studies. This study examines the population of Kosrae in Micronesia. Since the introduction of a western diet in the late 1980s, there has been an increased incidence of metabolic diseases on the island. Several forays to the island have provided us with samples from 3000 individuals and measurements of about 30 phenotypes. Due to the high relatedness among the individuals of the island, a few dozen individuals are highly representative of the entire population of 7000. Previous studies have examined the genotypic and haplotypic data present on the island. Previous studies have identified or replicated dozens of associated SNPs and haplotypes across multiple phenotypes in this data. **Methods**: In this study, we use exome sequencing to identify potential causal variants. A pilot sample of 10 individuals chosen from extremes in BMI have been sequenced. Analysis of this pilot sample has shown several potential variants associated with extreme BMI. Some of those individuals are carriers for associated variants found using in the GWAS analysis. This allows us to examine the local regions of previously associated loci for potential causal variants. Due to the high relatedness of the population, we will impute the variants for the remaining population from the 10 sequenced exomes. Using the imputed variants, we can examine potential causal variants in greater detail. **Results**: The analysis was carried out for previously associated phenotypes with carriers in the 10 samples. Among these, several carriers of a variant associated with Campesterol, a plasma plant sterol, indicate the presence of a causal variant at the associate locus. Campesterol levels have been previously associated in Kosrae to two independent causal variants (p-value = 5E-39 and 3E-31) at the ABCG5/G8 locus. We report further refining the residual association signal, detecting an additional association signal at that locus (p-value = 4.87E-08). Exome sequencing identified a novel proximal missense variant in only carriers of the associated allele. **Conclusion**: We find that a combination of exome sequencing and GWAS data can find interpretable, likely causal, associated variants.

455T

Derived SNP allele are more frequently used as a risk-associated variants in common human diseases. *O. Gorlova, J. Ying, C. Amos, M. Spitz, I. Gorlov.* Univ Texas MD Anderson CA Ctr, Houston, TX.

The results of more than 200 genome-wide association studies (GWAS) have been published to date. We used the GWAS data to address a question whether ancestral and derived (mutant) alleles are used as risk alleles randomly, which is important for understanding evolution of the genetic control of common human diseases. We found that rarer alleles are more likely to be risk variant and common alleles to be protective. When we have analyzed ancestral and derived alleles separately, 0.96±0.01 of rare (0-0.1) derived alleles were risk variants compared to only 0.67±0.04 of rare ancestral alleles being risk alleles. Among minor alleles, mean proportion of the risk variants was 0.84±0.05 for derived and 0.63±0.02 for ancestral alleles. The mean proportion of the risk variants among derived minor alleles was 0.79±0.06 and for ancestral variants only 0.41±0.05 for early onset diseases (onset< age 30), while the corresponding proportions for the late onset diseases were 0.78±0.07 and 0.62±0.05. Thus early onset diseases are likely to have a rare derived variant as a risk allele. For the late onset diseases risk alleles tend to be more uniform both in terms of population frequency and of the ancestral versus derived status. We also examined whether the population frequency and derived status are independent predictors of the risk variant status. Low frequency but not the derived status was a predictor of being the risk variant overall, but both were predictors in early onset diseases. Mutations causing early onset diseases are likely subjected to negative selection. However such mutations can reach substantial population frequency because of the effects of the random factors like genetic drift, founder and bottle neck effects. For the late onset diseases, the negative selection does not affect allelic frequency. One can expect that mutations causing late onset diseases are evolutionary neutral, have stochastic dynamics and may completely replace ancestral allele leading to the situation when the risk allele is the ancestral (common) and protective allele is the derived (rare). This can explain why in the late onset diseases risk alleles are more random both in terms of population frequency and ancestral/derived status.

456T

Studies on different samples of western Amazon populations converge to a genetic association between a specific region of Human chromosome 4 and malaria infection. *J. Pescarini[1,2,4], A. La Luna[1,4], R.M.G. Ferreira[3,4], L.C. Pereira[1,4], C.E.M. Kawamata[1,4], F.A.B. Santos[1,4], L.M.A. Camargo[1,4], H. Krieger[1,4], L.M. Garrido[1,4].* 1) Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil; 2) Instituto de Biologia Molecular do Paraná, Paraná, Brazil; 3) Fundaçao Oswaldo Cruz, Rondônia, Brazil; 4) Instituto Nacional de Genética Médica Populacional (INAGEMP), São Paulo, Brazil.

According to the WHO there are over 250 million new cases of Malaria each year. In 2008, Brazil was responsible for over 300 thousand cases of this disease. Most of these take place in the Amazon region. Several studies on the existence of genetic factors associated to resistance/susceptibility to several infectious diseases were reported in the past years. These findings can help to the understanding, treatment, prevention and eventually cure of these infections. Recent studies (Ferreira et al, 2008) in a western riverine Amazon population using a STR panel, postulated the evidence of linkage between the number of past malaria episodes reported by individuals and a specific region located in the short arm of chromosome 4. In order to test this hypothesis, a new study was done on a independent sample from the Municipality of Monte Negro (10º15'S, 63º18'W). From an original sample of 925 individuals, 96 individuals corresponding to both tails of the normal distribution of reported malaria episodes of malaria were selected. The number of episodes was corrected by sex and age. Cases were considered those who have shown one standard deviation above the mean and controls were those who have shown one s.d. below the mean. None of the selected individuals have relatives within the sample. Also cases and controls did not differ on their ethnic composition. Available data from a GWAS of the selected individuals using the Affymetrix GeneChip Human Mapping 250K Nsp Array were used in this analysis in order to test those previous findings. The association was studied with PLink Package using the following criteria for SNP inclusion: Belonging to chromosome 4; MAF of 1%; 100% of SNP call rate and Hardy-Weinberg equilibrium > 0.001. Twenty five SNPs satisfied these criteria and were included in an association analysis. The SNP rs17527389 (X^2=12.22; P=4.724x10^{-4}) showed a significant association with reported episodes of malaria infection after Bonferroni correction (P<0.012). This SNP is located in the intron region of APBB2/FE65L1 gene. There are evidences that the protein encoded by this gene bind amyloid beta precursor protein (APP) and also suggests a role in gene expression, cell cycle regulation and apoptosis. It should be stressed that this association matches with previous results using different techniques and in an independent sample, indicating that this region includes genes with significant effects on malaria infection.

457T

Bayesian inference of genealogy from population genomic data by the spatial Markov coalescent with recombination. *C. Zheng, E.A. Thompson.* Department of Statistics, University of Washington, Seattle, WA., USA.

The population based association studies have recently become popular to uncover the genetic architecture of complex traits. Under the widely used assumption of selectively neutral mutations on complex traits, inferring genealogies from population genomic data can be separated from the further gene mapping. Unfortunately, inference of genealogies for the Wright-Fisher population under the coalescent with recombination model has been shown computationally prohibitive mainly because the number of historical recombination events exponentially increases with the length of chromosomes. We present a Bayesian framework to infer genealogical trees from single-nucleotide polymorphism (SNP) data by using the spatial Markov coalescent with recombination, where the genealogical tree at a nucleotide site is assumed to be dependent only on the tree at the previous site. The Markov assumption results in high computational efficiency, linearly scaling-up with the length of chromosomes. A novelty of our framework is that it accounts for the typing errors of SNP data that are unavoidable for large data size. We use Markov chain Monte Carlo to sample from the posterior distribution of genealogical trees, together with the recombination rate and the type error. We evaluate the model performance by simulating an ancestral recombination graph (ARG) based on the coalescent with recombination, simulating SNP data from the ARG, and analyzing them in the Bayesian framework. The results show that the true marginal genealogical trees defined by the ARG are successfully estimated with uncertainties. The Bayesian formwork can analyze SNP data with length of chromosomes ~1 megabases, one order of magnitude larger than the previous full likelihood approach based on the coalescent with recombination. Thus, it opens the possibility of making use of the posterior distribution of genealogical trees along chromosomes in the fine-scale mapping of complex traits.

458T

Genome-wide structure patterns of Native American and Admixed populations across South America inferred from dense SNP array data. *P.A. Ortiz-Tello[1], A. Moreno-Estrada[1], C.R. Gignoux[2], D. Yang-Yao[3], P.J. Norman[4], E. Sanchez-Rodriguez[5,6], J.K. Byrnes[1], F. Zakharia[1], S. Gravel[1], M. Jobin[7], C. Eng[2], S. Huntsman[2], K. Sandoval[1], Z. Layrisse[8], G. Bedoya[9], P. Parham[3], A. Ruiz-Linares[10], D. Luiselli[3], D. Pettener[3], M. Alarcon-Riquelme[5,6], E. González Burchard[2], C.D. Bustamante[1].* 1) Department of Genetics, Stanford University School of Medicine, Stanford, CA; 2) Institute for Human Genetics, University of California San Francisco, CA; 3) Dipartimento di Biologia Evoluzionistica Sperimentale, Università di Bologna, Bologna, Italy; 4) Department of Structural Biology, Stanford Unviersity School of Medicine, Stanford, CA; 5) Centre for Genomics and Oncological Research (GENYO), University of Granada, Granada, Spain; 6) Oklahoma Medical Research Foundation, Oklahoma City, OK; 7) Department of Anthropology, Stanford University, Stanford, CA; 8) Center of Experimental Medicine “Miguel Layrisse”, Venezuelan Research Institute (IVIC), Caracas, Venezuela; 9) Laboratorio de Genética Molecular, Universidad de Antioquia, Medellín, Colombia; 10) Department of Genetics, Evolution and Environment, University College London, UK.

South Amerindians comprise a diverse population with distinct cultures and linguistic families. A better understanding of the genetic diversity and substructure within Amerindian populations in South America is necessary when analyzing complex health disorders and in understanding the genomic and demographic factors shaping the genome of admixed individuals living in the Americas. In order to shed light on the fine-scale patterns of genetic structure among South Amerindian populations we have genotyped nearly 1 Million SNPs (Affy 6.0 array) on 126 individuals from six different indigenous groups both from the Andean region and the Amazon Basin, combined with publicly available Native American SNP data from Brazil, Colombia, Bolivia and Peru. Using both PCA and clustering algorithms, we have found significant substructure within the Amerindian populations. In particular, each population isolate from Brazil and Venezuela cluster separately from the rest, while populations from Colombia, Peru and Bolivia show varying proportions of a shared genetic component, suggesting some degree of genetic continuity across the Andean region. We have also performed IBD and haplotype sharing analyses as well as *FST* calculations to further evaluate population differentiation. In addition, we calculated runs of homozygosity among individuals and applied a rejection-based algorithm to estimate effective population size and bottleneck timing for each population. We demonstrate that Amerindian populations are clearly dominated by bottlenecks but their severity varies substantially among regions, suggesting differential contributions to present day admixed populations across the Americas. In order to evaluate this, we have intersected our genotypes with SNP array data from 964 admixed individuals from Colombia, Peru, Chile, and Argentina genotyped as part of a larger collaborative effort as well as part of the 1000 Genomes project. We applied a novel PCA-based method for local ancestry estimation (PCADMIX) based on phased haplotypes and allowing for multiple K admixture models. Our results show that the Native American component of the different Hispanic/Latino populations varies across geographic regions, making the characterization of their genetic structure an essential step to inform present and future GWAS in the Americas.

459T

Association between high myopia-associated genetic polymorphisms and ocular biometric parameters in middle-aged and elderly people. *J.H. Chen[1,2], S. Huang[1], Y. Zheng[1], H. Chen[1,2], P.O.S Tam[2], D.S.C Lam[2], M. Zhang[1], C.P. Pang[2].* 1) Joint Shantou International Eye Center, Shantou University/The Chinese University of Hong Kong, Shantou, Guangdong, China; 2) Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong, China.

Purpose: Myopia is a common visual impairment. One of the notable features of myopia is the elongation of the axial length in eye balls. Recent studies showed that the genetic variants of gap junction protein delta 2 (*GJD2*), insulin-like growth factor-1 (*IGF-1*) and hepatocyte growth factor (*HGF*) were associated with high myopia. In this study, we investigated single nucleotide polymorphisms (SNPs) of these genes in the ocular axial length and other ocular biometric parameters in Chinese middle-aged and elderly people in Nan'ao Island in southeast China. **Methods:** A cohort of 459 subjects age／ 45 years old was recruited. The axial length and anterior chamber depth, lens thickness, and vitreous chamber depth were measured by A-scan ultrasound biometry. SNP rs634990 near *GJD2*, rs6214 in *IGF-1* and rs3735520 in *HGF* were genotyped using Taqman assay. The results were analysed by multivariate linear regression implemented by the R statistical language, controlling age and sex. **Results:** All 3 SNPs showed high minor allele frequencies, 42.7% to 49.0%. None of them deviated from HWE (*P*-values > 0.05). Associations between the 3 SNPs and the axial length or vitreous depth were not significant (all *P*-values > 0.05). However, SNP rs6214 in *IGF-1* was associated with lens thickness in an additive model (*P*-value = 0.021), estimated regression coefficient 0.06±0.026. Analysis of the dominant and recessive models gave similar results (*P*-values = 0.061 and 0.053 respectively). Association with lens thickness was not found in the other two SNPs. *HGF* SNP rs3735520 showed marginally significant association with anterior chamber depth (*P*-value = 0.078) in a dominant model, while no association was found in the other two SNPs. **Conclusions:** No association of *GJD2* and *IGF-1*, *HGF* with the axial length was found. However, the associations between *IGF-1* and lens thickness, and between *HGF* and anterior chamber depth, suggest a biological role of these growth factors in the elongation of the eye ball.

460T

The polymorphisms of the APOBEC3H gene in the Pumwani sex worker cohort and the associations with the susceptibility/resistance to HIV-1. *M. Luo[1,2], S. Wang[1], D. Tang[1], J. Sainsbury[1,2], P. Lacap[1], J. Kimani[2,3], C. Wachihi[3], M. Kimani[3], F. Plummer[1,2].* 1) HIV and Human Genetics, National Microbiology Laboratory, Winnipeg, MB, Canada; 2) Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada; 3) University of Nairobi, Nairobi, Kenya.

Background: Human APOBEC3H has the capability to interfere with the replication of HIV-1 by mutating the negative strand of the viral DNA after reverse transcription. Studies have shown that single nucleotide polymorphisms (SNPs) and alternative splicing influence the APOBEC3H's anti-HIV activity and its interaction with HIV-1 viral protein Vif. Objective: To examine whether SNPs in the APOBEC3H gene play a role in the resistance to HIV-1 infection observed in a subgroup of women in the Pumwani sex worker cohort (PSWC). Methods: The region between exon 2 and intron 4 were amplified, sequenced, and genotyped from genomic DNA samples of women (n=1029) enrolled in the PSWC. Genotyping results were statistically analyzed using SPSS-13.0, and linkage disequilibrium and haplotype frequencies were analyzed using Helixtree®. The influence of SNPs on alternative splicing and exonic splicing enhancers (ESE) were analyzed in-silico using Alamut v1.54. Results: We identified several novel SNPs in the analyzed region and a novel SNP(+321) C>T (F107F) was enriched in the HIV-1 infected group (P=0.022,odd ratio:0.288, 95% CI:0.094-0.888) and associated with faster seroconversion (P=0.000638, Log Rank:11.66). SNP rs77993299 (+315) C>T(G105G) was enriched in the HIV resistant women (P=0.005, odds ratio:2.662, 95%CI:1.315-5.388) and showed a trend towards slower seroconversion (P=0.336). In-silico analysis predicted SNP(+321)C>T to abolish an ESE site while SNP rs77993299 had no effect. The SNPs were predicted to not have any effects on splicing. Conclusion: SNP rs77993299 and novel SNP(+321)C>T are associated with HIV-1 resistance and susceptibility, respectively. ESEs are binding sites for proteins that recruit splicing machinery an/or antagonize nearby silencer elements. The abolishment of an ESE by SNP+321C>T may decrease splicing accuracy or efficiency of APBOBEC3H, may explain its association with HIV-1 infection.

461T

Haplotypes from the SLC45A2 gene are associated with the presence of freckles and eye, hair and skin pigmentation in Brazil. *C.T. Mendes-Junior[1], N.C.A. Fracasso[2], E.S. Andrade[2], C.C.F. Andrade[2], L.R. Zanão[1], M.S. Silva[1], L.A. Marano[2], C.E.V. Wiezel[2], E.A. Donadi[3], A.L. Simões[2].* 1) Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, 14040-901, Ribeirão Preto-SP, Brazil; 2) Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, 14049-900, Ribeirão Preto-SP, Brazil; 3) Divisão de Imunologia Clínica, Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, 14048-900, Ribeirão Preto-SP, Brazil.

The SLC45A2 (solute carrier family 45, member 2) gene (5p13.2) encodes the Membrane-Associated Transporter Protein (MATP), which mediates melanin synthesis by tyrosinase trafficking and/or proton transportation to melanosomes. Two coding SNPs (E272K and L374F) have been associated with variation in eye, hair and skin pigmentation. Yuasa *et al*. (*Ann Hum Genet* 2006:802-811) studied a set of 12 SNPs spanning more than 38kb within SLC45A2 and observed different haplotype distributions in Germans, Japaneses and Sub-Saharan Africans. In spite of the small sample sizes, 84.4% of the 32 haplotypes were found in single populations. However, many of these haplotypes may be found at the Brazilian population, which is the result of intensive inter-ethnic crossings. Since the determination of eye, hair and skin pigmentation of unknown samples found in crime scenes would be of great value for forensic caseworks, the present study aimed at evaluating the influence of SLC45A2 haplotypes in the determination of such pigmentation traits in a highly admixed population sample. To achieve this goal, 12 SLC45A2 SNPs (Yuasa *et al*., 2006) were evaluated in 150 unrelated individuals from the Ribeirão Preto area, located at the Northwestern region of the São Paulo State, Southeastern Brazil. SNPs were genotyped by PCR-RFLP or Allele-Specific PCR, followed by Polyacrylamide Gel Electrophoresis. Haplotypes of each individual were inferred by two independent computational methods: PHASE and PL-EM. These methods presented same results for 139 (92.7%) individuals, with average probabilities of 0.9589 and 0.9836, respectively. Considering these 139 individuals, 23 different (10 new) haplotypes were identified. Seven haplotypes presented at least one association with a pigmentation feature, and skin color was influenced by five of them. Haplotype *hp9*, for instance, was associated with the presence of blond/red hair, pale skin and freckles, and also with absence of dark eyes, hair and skin. All haplotypes significantly associated with dark or light pigmentation features harbor the 374L and 374F alleles, respectively. However, it is noteworthy that the remaining set of haplotypes harboring the 374F allele is more frequent among individuals with intermediate than light pigmentation. These results reinforce the relevance of SNP L374F in human pigmentation, but also suggest an important role played by its surrounding variation. FINANCIAL SUPPORT: CNPq/Brazil (Grant 478843/2009-7) and CAPES.

462T

Optimal Algorithm for Haplotype Phasing with Genome-sequencing and Imputation. *D. He, B. Han, E. Eskin.* UCLA, Los Angeles, CA, 90095.

Haplotype phasing is an important step for analyzing genetic variations in the human genome in that haplotypes contain all the alleles in the human genome. There are typically two types of haplotype phasing methods: (1) Haplotype imputation using Hapmap on genotypes obtained from microarray; (2) Haplotype assembly based on reads obtained via sequencing techniques such as Sanger sequencing and next-generation sequencing. Haplotype imputation requires very low coverage but it often can only impute common alleles in the haplotypes. Haplotype assembly can assemble both common and rare alleles but it requires very high sequencing coverage and it is usually not able to fully reconstruct the haplotypes. In this work, we combine the two methods and develop an optimal algorithm to impute haplotypes based on sequencing reads. We show our method only requires low sequencing coverage and in the mean time it is able to reconstruct both common and rare alleles accurately.

463T

LD patterns in dense variation data reveal information about the history of human populations worldwide. *S. Myers[1,2], G. Hellenthal[2], D. Lawson[3], G. Busby[4], S. Leslie[5], B. Winney[5], P. Donnelly[2], W. Bodmer[5], The. POBI Consortium[1,2,5], C. Capelli[4], D. Falush[6].* 1) Dept of Statistics, Oxford University, United Kingdom; 2) Wellcome Trust Centre for Human Genetics, Oxford University, United Kingdom; 3) Department of Mathematics, University of Bristol, United Kingdom; 4) Dept of Zoology, Oxford University, United Kingdom; 5) Cancer and Immunogenetics Group, Department of Clinical Pharmocology, Oxford University, United Kingdom; 6) Max Planck Institute for Evolutionary Anthropology, Department of Evolutionary Genetics, Leipzig.

A detailed understanding of population structure in genetic data is vital in many applications, including population genetic analyses and disease gene mapping, and relates directly to human history. However, there are still few methods that directly utilize information contained in the haplotypic structure of modern dense, genome-wide variation datasets. We have developed a set of new approaches, founded on a model first introduced by Li and Stephens, which fully use this powerful information, and are able to identify the underlying structure in large datasets sampling 50 or more populations. Our methods utilize both Bayesian model-based clustering and principal component analyses, and by using LD information effectively, consistently outperform existing approaches in both simulated and real data. This allows us to infer ancestry with unprecedented geographical precision, in turn enabling us to characterize the populations involved in ancient admixture events and, critically, to precisely date such events. We applied our new techniques to combined data for 30 European populations sampled by us, or publicly available, and the worldwide HGDP data. We find almost all human populations have been influenced by mixture with other groups, with the Bantu expansion, the Mongol empire and the Arab slave trade leaving particularly widespread genetic signatures, and many more local events, for example North African (Moroccan) admixture into the Spanish that we date to 834-1394AD. Dates of admixture events between European groups and groups from North Africa and the Middle East, seen in multiple Mediterranean countries, vary between 800 and 1700 years ago, while Greece, Croatia and other Balkan states show signals of admixture consistent with Slavic migration from the north, which we date to 600-1000AD. At the finest scale, we are able to study admixture patterns in data gathered by a project (POBI) examining people within the British Isles. Our approaches reveal genetic differences between individuals from different UK counties, and show that the current UK genetic landscape was formed by a series of events in the millennium following the fall of the Roman Empire.

464T

A large-scale, multi-racial replication study identifies novel systemic lupus erythematosus (SLE) susceptibility loci at IRF8, TMEM39A, and IKZF3/ZPBP2. *C.J. Lessard[1,2], I. Adrianto[1], J.A. Ice[1], J.A. Kelly[1], H. Li[1,2], G.B. Wiley[1], A. Rasmussen[1], M.E. Alarcon-Riquelme[1,3], J.M. Anaya[4], S.C. Bae[5], E.E. Brown[6], C.O. Jacob[7], J.A. James[1,8], J. Martin[9], T.B. Niewold[10], B.A. Pons-Estel[11], B.P. Tsao[12], T.J. Vyse[13], J.B. Harley[14,15], E.K. Wakeland[16], K.M. Kaufman[1,2,17], C.G. Montgomery[1], C.D. Langefeld[18], P.M. Gaffney[1,2], K.L. Moser[1,2].* 1) Arthritis & Clinical Immunology, OMRF, Oklahoma City, OK; 2) Department of Pathology, University of Oklahoma Health Sciences Center; 3) Centro de Genómica e Investigaciones Oncológicas (GENyO), Granada, Spain; for the BIOLUPUS Network; 4) Center for Autoimmune Diseases Research (CREA), Universidad del Rosario, Bogota, Columbia; 5) Department of Rheumatology, The Hospital for Rheumatic Diseases, Hanyang University Medical Center, Seoul, Republic of Korea; 6) University of Alabama at Birmingham, Department of Epidemiology; for PROFILE; 7) Department of Medicine, University of Southern California; 8) Department of Medicine, University of Oklahoma Health Sciences Center; 9) Instituto de Parasitología y Biomedicina López-Neyra, Consejo Superior de Investigaciones Científicas, Granada, Spain; 10) Section of Rheumatology and Gwen Knapp Center for Lupus and Immunology Research, University of Chicago; 11) Sanatorio Parque, Rosario, Argentina; 12) Division of Rheumatology, Department of Medicine, David Geffen School of Medicine, University of California; 13) Academic Department of Rheumatology, King's College London, London, England; 14) Cincinnati Children's Hospital Medical Center; 15) US Department of Veterans Affairs Medical Center, Cincinnati; 16) Department of Immunology, University of Texas Southwestern Medical Center at Dallas; 17) US Department of Veterans Affairs Medical Center, Oklahoma City; 18) Department of Biostatical Sciences, Wake Forest University Health Sciences.

SLE is a chronic, heterogeneous autoimmune disorder characterized by inflammation, loss of tolerance to self-antigens, and dysregulated interferon responses. In this study, we sought to replicate loci with suggestive association with SLE from a previously published genome-wide association scan. Genotyping was performed using Illumina iSelect technology. Stringent quality control measures were applied and genetic outliers determined using principal component (PC) analysis. Only SNPs with minor allele frequencies >1% were considered. After quality control filtering, 3562 SLE cases and 3491 controls of European ancestry (EA), 1527 cases and 1811 controls of African-American (AA) descent, and 1265 cases and 1260 controls of Asian (AS) origin were included in the replication analysis. SNP-SLE association was tested using logistic regression under an additive genetic model in PLINK while adjusting for the first three PCs and gender. Meta-analysis was performed using METAL. Three regions exceeded genome-wide significance (GWS) after the trans-racial replication study. One region that replicated in EA, AA, and AS subjects was located at 11q24.1 outside the 3'UTR region of interferon regulatory factor 8 (IRF8; Pmeta=4.97x10E-10). Approximately 300 additional SNPs were genotyped in the region of IRF8. Two risk haplotypes were observed within the EA subjects (P=3.85x10E-7 and P=7.99x10E-10). Logistic regression conditioning on the most significant SNP in each haplotype indicated that these effects were independent. The other two loci included a coding SNP in TMEM39A (transmembrane protein 39A; Pmeta=4.16x10E-9), and an intra-genic SNP at 17q21 between the genes IKZF3/ZBP2 in a shared promoter region (Pmeta=7.41x10E-9). All three regions have been implicated in other autoimmune diseases. In addition, 13 other loci were replicated in EA but did not exceed GWS with 5x10E-8 < P < 9.99x10E-5 (CFHR1, CADM2, LOC730109/IL12a, LPP, LOC63920, SLU7, ADATMTSL1, C10orf64, SEN3, OR8D4, FAM19A2, STXBP6, and TMCO5). IRF8 is a transcription factor involved in the regulation of the interferon pathway, which has been widely reported to be dysregulated in SLE and other autoimmune diseases. No biologically functional relevance has been published for TMEM39A. IKZF3 is an important transcription factor regulating B lymphocyte proliferation and differentiation. Thus, this study identified 3 novel SLE risk loci exceeding GWS and replicated 13 additional loci suggestive of association.

465T

Differential Expression of CD8 Variants Amongst Infected and Uninfected Men Enrolled in the Pittsburgh Center of the Multicenter AIDS Cohort Study. *R.S. Bosko Marino, L. Kingsley, J. Martinson, Multicenter AIDS Cohort Study.* Infectious Diseases & Micro, Grad School of Public Health, Univ Pittsburgh, Pittsburgh, PA.

HIV infection is known to devastate an individual's immune system but its effects on host gene expression are still only partially understood. To address this, we have begun a study of whole transcriptome variation in a cohort of men who have sex with men (MSM), including both HIV+ and HIV- participants. For this study we have recruited 478 men from the Pitt Men's Study, a participating center of the Multicenter AIDS Cohort Study (MACS). RNA was extracted from whole blood collected into PAXgene tubes at the most recent clinic visit. Following globin reduction, these RNA samples were used to synthesize cDNA for whole transcriptome expression analysis, using Illumina HT-12 arrays, at the University of Pittsburgh Genomics and Proteomics Core Laboratories. Bioconductor R modules (lumi, limma, GOstats, gplots) were used to analyze the array data. Preliminary results have identified 6 transcripts with significant differences in expression between a group of HIV- men (n=50) and HIV+ men (n=38), with adjusted p-values <0.05. The majority of these are transcript variants of the (and) chains of the T-cell surface antigen CD8, with the minority being a transcript for the predicted gene PATL2. Additional cluster analysis showed subgroups of shared expression patterns in the HIV+ and HIV- groups. In general, CD8 expression was lower in HIV infected individuals than in those uninfected with the virus. CD8 is expressed on the surface of cytotoxic T cells, which attack and destroy virus-infected cells, but it is not yet clear if these differences seen in CD8 expression represent a consequence of HIV infection or an innate characteristic of the host that may in fact influence HIV infection itself. All of the HIV+ men in this study are receiving antiviral therapy, and have low viral loads as a consequence, suggesting that their current state of infection is not the main determinant of their levels of CD8 expression. The current results of this study suggest a relationship between HIV infection and CD8 expression levels, and demonstrate that RNA samples extracted from whole blood are a viable source material for the study of the host response to infection.

466T

Polymorphisms in IL22RA2 are associated with aggravation of severe hepatic fibrosis caused by Schistosoma japonicum and S.mansoni. *A. Dessein[1,2,3], M. Sertorio[1,3], X. Hou[6], J. Li[6], X. Luo[6], M. Abdelwahed[4], A. Ahmed Hamdoun[4], H. He[6], S.A Abdelmaboud[4], J. Zhou[6], A. Monis[4], A. Varoquaux[1,2,3], N. Eldin Elwali[4], L. Argiro[1,3], Y. Lee[5,6].* 1) INSERM, U906, Marseille, F-13385, France; 2) Assistance Publique, Hôpitaux de Marseille, Marseille F-13005; 3) Université de la Méditerrannée, Marseille F-13385; 4) Institute of Nuclear medicine, Wad Medani. Gezira Sudan; 5) Queensland Institute of Medical Research, PO Royal Brisbane Hospital, Queensland 4029, Australia; 6) Hunan Institute of Parasitic Diseases; Hua-Ban Qiao Road Yueyang Hunan 414000 China.

M.Sertorio and A.Dessein are first authors. **Rationale** : Schistosomes cause severe hepatosplenic disease in humans accounting for 300.000 deaths every year. Schistosome eggs induce hepatic inflammation and extended hepatic fibrosis (HF) in 5 percent of infected subjects. HF leads to Portal Hypertension (PH) that contributes to splenomegaly which in turn aggravates PH, varices, bleedings and ascites. We have shown HF is controlled by a major locus on 6q23 and SNPs in CCN2 (CTGF)at this locus aggravate HF (J.Exp.Med. 206,2321-2328). We have observed IL-22 is increased in schistosome-infected subjects. Others reported IL-22 protects against hepatic damage and IL22RA2, also located on 6q23, encodes a soluble form of the IL-22 receptor that competes for the binding of IL-22 to its receptor. These findings prompted us to evaluate whether SNPs in IL22RA2 could aggravate HF. **Methods**: Case control studies were performed on 3 independent samples of 404 Chinese farmers (S.japonicum, sample 1) 279 Chinese fishermen (sample 2), 167 Sudanese farmers (S.mansoni, sample 3) all living in endemic regions. HF was evaluated using echography by at least two observers for each sample. All Tag SNPs in IL22RA2 (MAF > 10 percent;) were tested on both Chinese samples. To rule out that SNPs in linkage disequilibrium (LD) with the tested SNPs could account for the observed associations, we evaluated SNPs in the 500 Kb regions in 3' and 5' of IL22RA2. **Results**: We found that two SNPs in IL22RA2 in two different correlation bins were associated with HF in sample 1: SNP1: p=0.03, OR= 1.6 (1.1-2.5) and SNP2: p=0.008, OR=6.8 (1.4-33). The independent associations of these SNPs with HF were confirmed by multivariate analysis. No SNPs in LD with these SNPs could account for this result. We then tested these SNPs in sample 2 and obtained SNP1: p=0.02, OR=2.3(CI=1.1-4.7); SNP2: p=0.4. Finally, we evaluated these SNPs in sample 3 and obtained SNP1: p=0.02, OR=3 (1.2-7.6). SNP2: p=0.04, OR=6.2 (1.1-35.1). Finally, both SNPs were associated with HF in a Brazilian sample (not shown). **Conclusion**: Two SNPs in IL22RA2 are independently associated with severe HF in subjects infected with the two schistosome strains of medical importance. This is, at our knowledge, the first report of the association of SNPs in IL22RA2 with human tissue fibrosis. This indicates that IL-22 plays a crucial role in the control of HF in schistosomiasis and possibly in other chronic liver inflammations(HCV,HBV).

467T

Fine-mapping of 13q14 locus associated with susceptibility to leprosy and Crohn's disease. *F. Takeuchi.* Pathogen Genomics Center, National Institute of Infectious Diseases, Tokyo, Japan.

Although leprosy and Crohn's disease are diseases with distinct symptoms, the two diseases share many susceptibility loci found in genome-wide association studies (Zhang et al. *N Engl J Med*, 2009, 361:2609; Barrett et al. *Nature Genetics*, 2008, 8:955). One locus at 13q14 includes two genes, *CCDC122* and *C13orf31*, whose functions are both unknown. Aiming to identify the causal variant and the causal gene in the locus, we studied the association of single nucleotide polymorphism markers and haplotype classes. For testing the genotype-phenotype association, we combined previous studies on Crohn's disease, leprosy and related diseases, such as tuberculosis. Haplotype classes and frequency in various ethnicities were assessed using the HapMap and Human Genome Diversity Panel data. Utilizing the 1000 genomes data, we narrowed down the list of possible causal variants. We found that the locus could include more than one causal variant. This study shows the possibility of fine-mapping by combining multiple diseases that partially share common etiology.

468T

The impact of linkage disequilibrium on inferring coancestry in populations. *E.A. Thompson, M.D. Brown, C.G. Glazner, C. Zheng.* Department of Statistics, University of Washington, Seattle, WA.

In both pedigree-based linkage studies and in population-based association studies there has been much interest in the use of modern dense genetic marker data to infer segments of gene identity by descent (ibd) among individuals not known to be related, in order to increase power and resolution in localizing genes affecting complex traits. The ability to make such inferences derives from the fact that ibd segments in remote relatives are generally rare but not short, being typically an order of magnitude longer that the extent of linkage disequilibrium (LD). However, LD remains a major confounding factor, since LD is itself a reflection of coancestry at the population level. We have developed a hidden Markov model (HMM) approach to detection of ibd in populations. To evaluate performance, we simulated descent of genome in a population of 7000 individuals over 200 generations, and determined the realized ibd in pairs of individuals in this population. For the current study, our data consist of 500 pairs of individuals, sampled from the final generation of the simulated population. We evaluate our approach through the ability of the HMM to detect the realized segments of ibd among either the four haplotypes or the two genotypes in these 500 pairs of individuals. To study the impact of LD, we developed a simulation approach which first uses BEAGLE to fit an LD model to 1917 haplotypes from a real data study with high LD. These haplotypes are observed for 6913 markers over 140cM. From the simulated population descent and the fitted BEAGLE model, we generated realistic dense marker data for the same simulated individuals for the same set of markers but at five levels of LD. Although our HMM ignores LD, it was quite successful in detecting segments as small as 1 Mbp, among the four chromosomes of pairs of individuals, using either phased haplotypic or unphased genotypic data. Using the same calling threshold, as LD was decreased, the percentage of markers for which the correct ibd state was called increased from 50% to 97%, and the percentage failing to reach the threshold decreased from 33% to 2%. Generally, haplotypic data provided somewhat better performance than genotypic data. At all LD levels, the rates of failing to detect true ibd were higher for genotypic data. However, at the highest LD levels, the false-positive rate was higher for haplotypic data, showing the clear impact of LD on the haplotypic similarity of non-ibd segments of genome.

469T

Linkage disequilibrium decay and past population history in the human genome. *L. Park.* Natural Science Research Inst., Yonsei University, Seoul, Korea.

The fluctuation of population size has not been well studied in the previous studies of theoretical linkage disequilibrium (LD) expectation. In this study, a theoretical prediction of LD decay was derived, based primarily on the population sampling procedure, which accounted for the effects of changes of effective population sizes. The theoretical predictions and the simulated values were in excellent agreement. The equation that was derived was used to estimate effective population size (N_e) assuming a constant N_e and LD at equilibrium. These N_e estimates implied the past changes of N_e for a certain number of generations till equilibrium, which differed depending on recombination rate. When applying to human genome data, an excellent correspondence with the recent growth of human population was obtained. Specifically, four populations with three African and one Mexican ancestries showed the population growth significantly less than that of other populations. The inferred past population history of each population showed a good correspondence with historical studies, proving that the genome data could be an objective source for the population history. During the examination of overall LD decay in the human genome, in all populations, a selection pressure was observed on chromosome 14, the gephyrin gene.

470T

Population substructure effect in a case-control study of Multiple Sclerosis (MS) in Bogota - Colombia. *W.A. Cardenas[1], R. Pereira[2], M.C. Lattig[1], H. Groot[1], J. Toro[3], A. Amorim[2,4], L. Gusmão[2].* 1) Laboratorio de Genética Humana, Facultad de Ciencias Universidad de los Andes, Bogotá, Colombia; 2) IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal; 3) Unidad de Neurología, Hospital Universitario, Fundación Santa Fe de Bogotá, Facultad de Medicina-Universidad de Los Andes, Bogotá, Colombia; 4) Faculty of Sciences of the University of Porto, Porto, Portugal.

Latin American populations are known to have a complex genetic structure indicative of recent admixture events between European (EUR), Sub-Saharan African (AFR), and Amerindian/Native American (AMR) ancestral populations. These population substructures are known to lead to spurious associations in case-control research studies since allele frequency differences among subpopulations can result in frequent false-positive results. To take into account the effects of population stratification in a previous association study in Multiple Sclerosis (MS), we used 46 multiplex Ancestry Informative INDELs to determine the ethnic ancestry of 173 individuals (52 MS patients and 121 healthy controls) from the heterogeneous population of Colombia's capital - Bogota. Genotypes were evaluated using the STRUCTURE software to discriminate among the three major subpopulations characteristic of our population sample. The ancestry percentage for this population was established as: 55.6% EUR, 11.2% AFR and 33.2% AMR. Exact test of sample differentiation based on the ancestry percentage distribution in patients and controls [(EUR) 52.9% - 56.7%; (AFR) 12% - 10.8%, and (AMR) 35.1% - 32.4%, respectively] did not reveal significant differences (non-differentiation exact P value: 0.89525±0.0058) arguing that previous associations among mtDNA haplogroups, the Fok1 polymorphism in the vitamin D receptor (VDR) gene and HLA-DRB1 allele seem to be real effects in MS susceptibility, since population substructure is not affecting the results. Additionally, the use of this multiplex of 46 Ancestry Informative INDELs was able to discriminate the three ancestral subpopulations indicating that they are good markers to determine individual and population ancestry percentages in our population and are, therefore, good markers to be used in future studies examining population genetic substructure in complex disease association studies.

471T

Meta-analysis of genome-wide ancestry on over 7,000 African-American and Hispanic/Latino individuals identifies novel asthma-associated genetic loci. *C.R. Gignoux[1], D.G. Torgerson[1], J.L. Galanter[1], L.A. Roth[1], R.D. Hernandez[1], S. Sen[1], R. Mathias[2], K.C. Barnes[2], E. González Burchard[1], the EVE consortium.* 1) UCSF, San Francisco, CA; 2) Johns Hopkins University, Baltimore, MD.

Genome-Wide Association Studies have contributed new insights to the study of complex diseases. However, they do not capture all heritability of a trait and have had limited findings in ethnic minority populations. We leveraged the power of a diverse set of 9 studies in the EVE asthma genetics consortium: four African-American and five Hispanic/Latino studies, to look for additional loci associated with asthma. Following the initial GWAS meta-analysis we performed a different test to harness the intrinsic admixture in these populations: genome-wide ancestry association testing (or admixture mapping) for differences in ancestry, rather than genotype, at each locus on the genome. Ancestry association in theory can have more power than GWAS due to better coverage in admixed populations and fewer effective comparisons. It may also identify candidate regions more likely to contain population-specific rare variants.

We first estimated ancestry at each locus in the genome for each study followed by interpolation across SNPs based on the block-like nature of local ancestry. Our final dataset included over 2 million sites across the autosomes in 3,106 African Americans and 3,902 Latinos. We performed meta-analyses (both ethnic-specific and combined) using weighted p-values, and for our top hits we implemented a mixed effects model to account for ethnic differences.

We found strong evidence for associations between African ancestry and asthma, with two significant hits in 4p12 and 6q14 ($p < 10^{-8}$). In addition, we found suggestive associations with the other two ancestries: 7q31 for Native American ancestry ($p \sim 7 \times 10^{-6}$), and 1q42 ($p \sim 10^{-7}$) for European ancestry. Combining African-American and Latino studies narrows our peaks to small regions of the genome. Our most significant association for ancestry on 4p12 had a combined OR of 1.49 (1.25-1.78) for African ancestry in African Americans with no evidence of heterogeneity across all studies ($I^2 \sim 0$) including an Afro-Caribbean population from Barbados. This region also has biological plausibility as it contains *GABRA2*, known to be induced in the airway epithelium of individuals with asthma.

To our knowledge this study is the first meta-analysis of genome-wide ancestry association. We highlight the importance of studying diverse ethnicities for complex disease and demonstrate the hidden potential of available genome-wide data for additional disease mapping strategies.

472T

Association of Mitochondrial DNA Sequence with Longevity in Turkish Population. *H.H. Aydin, O. Guney, H. Ak Celik.* Dept. of Medical Biochemistry, School of Medicine, Ege University, Bornova, Izmir, Turkey.

Aging is a multiple factor related process, associated with progressive decrease in physiological functions, lower survival and decreased ability for reproduction as the age increases, and ultimately resulting in death. One of the widely recognized mechanisms for aging is mitochondrial mutations. Maternally inherited mitochondrial genome, lacking genome recombination and having inadequate repair mechanisms, is more vulnerable to mutations as the mitochondria itself is a source for free radicals. Mitochondrial DNA variations, resulted from either inherited mutations or somatic mutations, may affect life expectancy by altering respiratory chain and free radical formation. Individuals older than 90 (n=25) and middle-aged individuals having no chronic disease (n=25), were included to our study as +90 group and control group respectively. Mitochondrial DNA sequence analysis for both groups was carried out with Affymetrix microarray system. From this sequence data polymorphic variants were detected by using rCRF sequence as a reference and haplogroup clusters to which individuals belong were indentified accordingly. Polymorphic variant and haplogroup cluster differences between +90 group and control group were revealed and statistically evaluated. Frequency of C150T polymorphism, which was previously identified as a longevity region by 3 similar studies, was found to be higher in +90 group compared to control group. T16304C polymorphism was found to be at a higher frequency in control group compared to +90 group. It is considered that this variant may have a negative effect on longevity. Data obtained from haplogroups suggest that H, HV, R and N are more common haplogroups in Turkish population. No significant difference was detected between +90 group and control group regarding to the haplogroup status. This study is the first of its kind conducted in Turkish population. Mitochondrial sequence data and haplogroup data of Turkish population will be a source for future studies.

473T

Mitochondrial DNA in Myanmar: insights into the peopling of South-East Asia. *J. Horst[1], M. Summerer[2], D. Horst[3], G. Erhart[2], B. Horst[4], A. Manhart[2], T. Sanguansermsri[2], F. Kronenberg[2], A. Kloss-Brandstätter[2].* 1) Institut für Humangenetik, Universität Münster, Germany; 2) Division of Genetic Epidemiology, Innsbruck Medical University, Innsbruck, Austria; 3) Pathologisches Institut, Ludwig-Maximilians-Universität, Münster, Germany; 4) Department of Dermatology, Section of Dermatopathology, Columbia University, New York, NY.

BACKGROUND: The Union Myanmar (Burma) is the largest country in Southeast Asia, with an estimated population of around 56 million people. As a consequence of its history, Myanmar is inhabited by various ethnic groups, amongst them the Tibeto-Burman Bamar represent 68% of the population. Originating from present-day Yunnan (China) they migrated to Ayeyarwady valley in the 7th century AD replacing and absorbing the Pyu and the Mon. Until the British controlled Burma between 1886 and 1948, Burma was ruled by changing kingdoms and dynasties. We analyzed the entire mtDNA control region of 327 unrelated donors from Myanmar according to highest forensic quality standards. To refine haplogroup information, especially in the complex haplogroup M, 43 selected lineages were subjected to complete mitochondrial genome sequencing. In order to describe the mtDNA setup of Myanmar more thoroughly, the data were subjected to population genetic comparisons with other Southeast and East Asian groups.

RESULTS: In general, the Myanmar mtDNA profiles were characteristic for Southeast Asian populations in terms of haplotype composition and genetic structure, however, genetic comparisons with surrounding populations revealed genetic differentiation. The Myanmar sample exhibited pronounced mtDNA diversity, reflecting the huge number of ethnic groups. We detected 8 new so far un-described mtDNA lineages represented by 13 haplotypes. No traces of European contribution to the gene pool were detected.

CONCLUSIONS: In this study, we portray the great mtDNA variety of Myanmar for the first time. Our findings will contribute to clarify the migration history of the region. They encourage setting up regional and subpopulation databases, especially for forensic applications.

474T

Diversity of the 3' untranslated region of the *HLA-G* gene in Amerindians from the Brazilian Amazon and the influence of Natural Selection. *N.F. Cagnin[1], C.T. Mendes-Junior[2], E.C. Castelli[3], E.A. Donadi[4], A.L. Simões[1].* 1) Department of Genetics, FMRP, University of São Paulo, USP, Ribeirão Preto, São Paulo, Brazil; 2) Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Brazil; 3) Department of General Biology, Biological Sciences Institute, Federal University of Goiás, Goiânia, Brazil; 4) Division of Clinical Immunology, Department of Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, Brazil.

The *HLA-G* gene, primarily expressed on trophoblast cells at the maternal-fetal interface, plays a key role in the modulation of the maternal immune system during pregnancy, enabling foetuses to survive unharmed in a genetically foreign host during successful pregnancies. This fact has triggered interest on aspects of population dynamics of this gene, especially the regulatory regions, such as the 3' untranslated region (3'UTR) that would be related to different levels of gene expression due to post-transcriptional mechanisms. The involvement of the 3'UTR in the gene expression patterns suggests that this region is under influence of natural selection, which can be best evaluated in isolated populations such as Amerindians from the central Brazilian Amazon. Evidence of balancing selection at *locus* 14bp insertion/deletion located in the *HLA-G* 3'UTR, has already been found, but analysis of the effect of natural selection on the haplotypes formed by this and other polymorphic *loci* of the 3'UTR has not been reported. So, this study aimed to determine the frequencies of haplotypes at the *HLA-G* 3'UTR in Amerindians from the Brazilian Amazon, and verify the existence of selective pressure on this gene region. For this, we sequenced the 3'UTR of the *HLA-G* in 144 Amerindians from villages belonging to five Brazilian Amazon's tribes: Bom Jardim, Campo Alegre, Nova Itália and Marajá, villages of *Tikúna* tribe, Sete Estrelas and Morada Nova of *Katukina*, Cana Brava and Paredão of *Kaxináwa*, Vida Nova of *Marúbo* and a *Yaminawa* village. We found a total of seven polymorphic *loci*, the 14bp insertion/deletion and SNPs at the positions +3010G/C, +3027A/C, +3035C/T, +3142G/C, +3187G/A, +3196G/C. Departures from Hardy-Weinberg equilibrium weren't found. These polymorphic *loci* defined seven haplotypes, but only two were found in all villages. The haplotype frequencies varied a lot between villages and tribes. Departure from selective neutrality was tested by two different tests, Tajima's D and Ewens-Watterson's neutrality tests, and both revealed evidence of balancing selection. Statistically significant values were found in *Katukina*, Morada Nova, *Marúbo*, and the whole Amerindian sample by Tajima's D test and in *Yaminawa* and Campo Alegre by Ewens-Watterson's test. Seven other marginally significant values ($p \leq 0.1$) were observed. In conclusion, the neutrality tests suggest great possibilities of balancing selection acting in the 3'UTR of the *HLA-G* gene.Financial support: CNPq.

475T

Search for selection in coding regions surrounding large insertions and deletions between humans and four primate species. *W. Guiblet[1], K. Zhao[3], A.L. Roca[3], J.C. Martinez-Cruzado[1], S. Massey[2], T.K. Oleksyk[1].* 1) Department of Biology, University of Puerto Rico at Mayagüez, Mayagüez, PR 00680; 2) Department of Biology, University of Puerto Rico at RÍo Piedras, San Juan, PR 00931; 3) Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA.

Genes are highly conserved sequences and usually show very few differences between closely related species, such as humans and primates. In this study, we are focusing on >10 bp insertions and deletions (indels) found when comparing humans, chimpanzees, gorillas, orangutans and rhesus macaque genome sequences with the purpose of testing indel flanking regions for selection. From 33,479 indels identified by comparing reference genomes pairwise, we chose 186 within coding sequences with a potential high-impact on protein sequence. These fragments have been validated in laboratory by PCR and electrophoresis to distinguish real features from computational artifacts, and further tested for the presence of polymorphism in multiple human populations. First, we used five different algorithms (PAML package) to produce over 7,000 pairwise Ka/Ks values. Among the 186 indel regions, eight fragments showed significant Ka/Ks ratios between human and at least one primate sequence. Next, we searched if these features are found within selective sweep regions demonstrating diminished multilocus heterozygosity and high divergence (FST) by comparing genotype data between populations of the Human Genome Data Panel (HGDP). Finally, indels were further interrogated for recent signatures of selection in human populations by evaluating extended haplotype homozygosity around ancestral and derived alleles in the polymorphic loci. The significance of our approach was evaluated by the resampling method, where exactly the same procedures and tests have been performed with a 10x dataset of randomly created indels matched by size distributed across the reference genome.

476T

Intra-European allele frequency differences at height-associated SNPs suggest widespread selection on standing variation. *J.N. Hirschhorn[1,2,3], M.C. Turchin[1,2], C.W.K. Chiang[1,2], C.D. Palmer[1,2], S. Sankararaman[2], D.E. Reich[2,3], GIANT consortium.* 1) Endocrinology and Genetics, Children's Hospital, Boston, MA; 2) Dept of Genetics, Harvard Medical School, Boston, MA; 3) Broad Institute, Cambridge, MA.

Individuals of Northern European ancestry are on average taller than those of Southern European ancestry. It is unknown whether this phenotypic difference is driven by genetic differences, and if so, whether due to natural selection or drift. As human height is polygenic, partly influenced by common, ancient genetic variation, a recent change in height could occur through effects on pre-existing variation at a large number of loci in the genome. Signals of such polygenic adaptation have been difficult to detect, but, given genome-wide data on the effects of common variants on height, we hypothesized that we might be able to observe a signal as a systematic increase in allele frequency (AF) of the height-increasing allele in a Northern European (NEur) compared with a Southern European (SEur) population. We examined height-associated SNPs for which we had access to directly measured AF data in >200 individuals from NEur and SEur, focusing first on 180 loci previously identified by the GIANT consortium . The height-increasing alleles at these loci had significantly higher AF in NEur than in SEur (average AF NEur-SEur = 0.012, $p<4.3\times10^{-4}$, $p<0.0056$ compared with random frequency-matched SNPs). The increased AF in NEur of height-increasing alleles persisted through much of the genome, suggesting that many more height-associated variants beyond these 180 loci contribute to the height difference between NEur and SEur. The alleles with higher AF in NEur were, as a group, associated with increased height in a family-based sample of 1,762 sibships, so stratification does not account for our results. We modeled the combined effects of drift and selection, and the genome-wide AF data were much more consistent with a model incorporating selection than with a model of genetic drift alone (likelihood ratio $p<10^{-100}$). Our results also suggest that individual selection coefficients might be in the order of 10^{-5}. In summary, our study indicates that the height gradient seen in Europe is at least partially attributable to AF differences at height-associated variants, including potentially many common variants with quite small effects, and is not likely due to the effects of random drift. This finding suggests that there may have been selection in Europe on many variants associated with height, representing one of the first examples of widespread selection on standing genetic variation that underlies a human polygenic trait.

477T

Shared signatures of selection in the genomes of chimpanzees and humans. *E.M. Leffler[1], S. Pfeifer[2], L. Ségurel[1], A. Auton[3], O. Venn[3], R. Bontrop[4], R. Bowden[2], J. Broxholme[3], A. Fledel-Alon[1], R. Hernandez[5], P. Humburg[3], Z. Iqbal[3], G. Lunter[3], J. Maller[3], S.C. Melton[1], T. Street[2], A. Venkat[1], S. Myers[2], P. Donnelly[2,3], G. McVean[2,3], M. Przeworski[1,6].* 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Department of Statistics, University of Oxford, Oxford, UK; 3) The Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; 4) Biomedical Primate Research Centre, Rijswijk, The Netherlands; 5) Department of Bioengineering and Therapeutic Sciences, University of California - San Francisco, San Francisco, CA; 6) Department of Ecology and Evolution, University of Chicago, Chicago, IL.

Since the chimpanzee and human lineages split 5-7 million years ago, genetic changes on each lineage have led to differences between the two species today. The proportion of changes that were adaptive, the modes of selection that were important, and how these differ for humans and chimpanzees are of great interest for understanding how modern humans and our closest relatives evolved. Until recently, data for comparative, genome-wide population analyses was limited, particularly for chimpanzee. We have sequenced 10 western chimpanzees (Pan troglodytes verus) to 8-10X coverage as part of the PanMap project and combined these with 1000 Genomes low coverage data to assess evidence and identify candidates for positive selection in both humans and chimpanzees. We used a McDonald-Kreitman based approach to test whether similar groups of genes show evidence for high rates of non-synonymous substitution on both lineages. We also used the joint polymorphism data to look for long-term balancing selection, of which there are currently very few known examples. To this end, we focused on single nucleotide polymorphisms (SNPs) present in both species. We find that approximately 0.8% of the chimpanzee SNPs are shared, in excess of what is expected by chance after accounting for variation in the mutation rate. Moreover, we identify specific regions with several shared polymorphic sites in linkage disequilibrium, suggestive of long-term balancing selection.

478T

Population specific susceptibility to Crohn's disease and signals of natural selection on standing variation. *S. Nakagome[1], S. Mano[1,2], L. Kozlowski[3], J.M. Bujnicki[3,4], H. Shibata[5], Y. Fukumaki[5], J.R. Kidd[6], K.K. Kidd[6], S. Kawamura[7], H. Oota[8].* 1) The Institute of Statistical Mathematics, Tokyo, Japan; 2) Japan Sciece and Technology Agency, Saitama, Japan; 3) International Institute of Molecular and Cell Biology in Warsaw, Warsaw, Poland; 4) Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland; 5) Research Center for Genetic Information, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; 6) Yale Uiversity School of Medicine, New Haven, CT; 7) Graduate School of Frontier Sciences, University of Tokyo, Chiba, Japan; 8) Kitasato University School of Medicine, Kanagawa, Japan.

It is generally thought that population specific susceptibility of complex diseases is attributed to geographic differences in the risk allele frequency. The risk alleles are widespread in diverse populations, which can be primarily explained by random genetic drift through demographic history, and rarely by natural selection. Elucidating the evolutionary history of complex disease alleles is, therefore, of fundamental importance for understanding the ethnic-differences in the disease susceptibility. We focus on Crohn's disease (CD) as a model of evolutionary study of complex disease alleles. Recent genome-wide association studies and classical linkage analyses have identified more than 70 susceptible genomic regions to CD in Europeans, while few of them have been reproduced in non-European populations. Our population genetics analysis on the eight European-specific susceptibility genes (*NOD2*, *IL23R*, *TNFSF15*, *ATG16L1*, *SLC22A4*, *SLC22A5*, *IRGM*, 10q21) using the HapMap data shows that, only in the *NOD2* locus, the CD-risk alleles are linked with a specific haplotype to CEU whose frequency is significantly higher compared with those of the entire genome regions. We subsequently examined nine global populations including 192 Africans, 268 Europeans, and 95 East Asians, and found that the *NOD2*-risk alleles inducing serious conformational and functional changes spread over Europeans. The risk alleles hitchhiked with a high-frequency haplotype (H1) exclusive to Europeans, and the time to most recent common ancestor (TMRCA) of the H1 predated the human dispersal out of Africa. Further, the H1 is likely to have experienced negative selection: the haplotype has almost become extinct in Africans and has not been affected by historical European expansion. Nevertheless, the H1 has survived only in European populations, suggesting the haplotype is advantageous to the populations. Here we propose a hypothesis that the CD-risk alleles have been maintained by natural selection from the standing variation whereby the deleterious haplotype of *NOD2* can become advantageous in diploid individuals because of heterozygote advantage and/or inter-genic interactions.

479T

Metabolic insight into mechanisms of high-altitude adaptation in Tibetans. *T.S. Simonson[1], R.L. Ge[2], R.C. Cooksey[1], C.D. Huff[2], T.N. Wuran[1], J. Xing[2], G. Qin[1], D.J. Witherspoon[2], B. ZhengZhong[1], J.T. Prchal[1], D.M. McClain[1], L.B. Jorde[1].* 1) Departments of Human Genetics, Hematology, and the Division of Endocrinology, Metabolism, and Diabetes, University of Utah, Salt Lake City, UT; 2) Qinghai University Medical School, Xining, Qinghai, People's Republic of China.

Recent studies have used various different analytical methods to identify genes targeted by natural selection in high-altitude populations located throughout the Tibetan Plateau. Despite differences in analytic strategies and population samples, several of the same hypoxia-related genes were identified in multiple studies. The mechanisms that afford adaptation to high-altitude hypoxia, however, remain unclear. By employing the same analytic strategies (iHS and XP-EHH statistics) used in our previous study of Tibetan high-altitude adaptation, we have identified common targets of natural selection in a second linguistically distinct Tibetan population from a different region of the Tibetan Plateau. Our analysis provides evidence for positive selection in each population at the $p < 0.01$ significance level for *EPAS1*, *EGLN1*, *HMOX2*, and *CYP17A1*. Three genes have previously been shown to be associated hemoglobin (Hb) levels in Tibetans (*EPAS1*, *EGLN1*, and *PPARA*), although it is unclear whether these associations reflect direct selection on Hb level or are consequences of selection on other advantageous traits. We considered these replicated selection targets for analysis with additional, potentially adaptive metabolic phenotypes that may provide insight into mechanisms of high-altitude adaptation. Considering the strong metabolic demands imposed by hypoxia, we hypothesized that a shift in fuel preference from fatty acid oxidation to glucose oxidation and glycolysis would provide adaptation to decreased oxygen availability at high altitude. Measurements of serum metabolites collected from these Tibetan groups are consistent with this hypothesis: the previously identified *EPAS1* and *PPARA* haplotypes are associated with increased serum lactate ($p = 0.0007$) and triglyceride ($p < 0.04$) levels, respectively. Although further studies are required to assess the molecular mechanisms underlying these patterns, these changes suggest that genetic adaptation to high altitude involves alteration in energy utilization pathways.

480T

Accelerated evolution of brain specific microRNAs in Homo sapiens. *S. Chakraborty[1], MM. Ali[1], R. Chakravorty[2].* 1) Department of Biochem and Molelular Biology, University of Dhaka, Dhaka-1000, Bangladesh; 2) Department of EEE, University of Melbourne, National ICT Australia, Victoria 3010, Australia.

The hallmark of human evolution encompasses the dramatic increase in brain size and complexity. This prominent phenotypic evolution of human brain has been correlated with the accelerated positive selection of the genes linked to nervous system development. In this study we focused on the evolution the microRNAs-the widespread post transcriptional regulators of gene expression that are defined by their unique biogenesis, which involves their precise excision from the stem of a fold-back precursor. The intricate interplays of mature miRNAs and their target genes are indispensable in human brain development. This study involves systematic mining of various miRNA databases, analysis of gene expression profiles and sequence divergence of miRNAs with known conserved functions across primates and rodents. Specifically we studied the evolutionary patterns at different functional regions of a large number of precursor miRNA sequences, extracted from the miRBase. Our analysis revealed primate brain specific miRNAs bear traces of relatively higher rate of evolution compared to rodents while among the primates the rapid evolutionary pattern is salient in the case of human compared to other primates. Moreover our findings indicated that brain specific pre-miRNA in primates have evolved at a faster rate than those that are not expressed in brain. These results suggest the distinct patterns of sequence divergence that may exist within the human brain specific precursor miRNA sequences, possibly reflecting the evolution of structural determinants involved in miRNA processing and expression in human brain. These results also signifies the co-evolution of miRNAs and their brain specific target genes since the genes involved in the brain development have also undergone an accelerated evolution and serve as targets of miRNAs.

481T

Association of the European Lactase Persistence variant (LCT-13910 C>T polymorphism) with obesity in the Canary Islands by Mendelian randomization. *R. ÁLMON[1], E.E. AlVAREZ-LEON[2], LI. SERRA-MAJEM[2].* 1) Örebro University, Family Medicine Research Centre, School of Health and Medical Sciences, Örebro, Sweden; 2) Preventive Medicine Service. Canary Health Service; and Department of Clinical Sciences, Faculty of Health Sciences, University of Las Palmas de Gran Canaria. Canary Islands, Spain.

European lactose tolerance genotype (LCT -13910 C>T) has been positively associated to body mass indexes (BMI) in a meta-analysis of 31,720 individuals of northern and middle European descent. A strong association of LP (lactase persistence) with BMI and obesity has also been traced in a Spanish Mediterranean population. The T-allele of the LCT-13910 C>T has been subject to a strong positive selection in recent history enabling an unrestricted diet concerning milk and milk products, and stands proxy for the lifetime exposure pattern, milk intake, that may increase the susceptibility to develop obesity. The aim of this study was to analyze a potential association of LP compared to LNP (lactase non-persistence) with BMI (kg/m2) in inhabitants of the Canary Islands in Spain using Mendelian randomization (MR). A representative, random sample of adults belonging to the Canary Islands Nutrition Survey (ENCA) in Spain, aged 18-75 years (n = 551), was genotyped for the LCT - 13910 C>T polymorphism. Milk consumption was assessed by a validated questionnaire. Anthropometric variables were directly measured. LP individuals were significantly more obese than LNP subjects ($\chi 2=10.59$; $p<0.005$). LP showed in a multivariate linear regression analysis a positive association to BMI compared to LNP, () =0.96; 95% CI: 0.08-1.85, $p<0.033$). In a multinomial logistic regression analysis normal range weight LP subjects showed an odds ratio (OR) for obesity of 2.41; 95%CI 1.39-418, ($p=0.002$) compared to LNP. Overweight LP subjects showed an OR of 2.32; 95%CI 1.39-3.88; ($p=0.001$) to develop obesity compared to LNP. The T-13910 of the allele LCT-13910 C>T polymorphism is positively associated to BMI. LP is positively associated with obesity in this population. In nutritionally replete countries and high average life expectancy LP status might increase the susceptibility to develop obesity.

482T

Differential selection pressures drive allelic variation of the *Toll-like receptor-2* (*TLR2*) locus in two closely related genus *Macaca* species, *M. mulatta* and *M. fuscata*. *M. Yasunami[1], A. Takaki[1], A. Yamazaki[1], T. Maekawa[1], H. Shibata[1], K. Hirayama[1], A. Kimura[2], H. Hirai[3].* 1) Inst Trop Med, Nagasaki Univ, Nagasaki, Japan; 2) Med Res Inst, Tokyo Med Den Univ, Tokyo, Japan; 3) Primate Res Inst, Kyoto Univ, Inuyama, Japan.

Macaca species of old world monkeys share the last common ancestor of 25 million years ago (Mya) with human and hominoids, and have been widely used for biomedical research as good model organisms mimicking humans in many aspects. Divergent speciation of different genus *Macaca* species with distinct geographic distribution in Asia is estimated at 0.9 - 2.5 Mya, which is similar to the settlement of modern human populations out of Africa. *Toll-like receptor 2* (*TLR2*) plays an important role in the recognition of molecular pattern derived from a variety of pathogenic microbes, such as bacterial lipopeptides, and polymorphism of human *TLR2* locus may explain individual susceptibility to certain infectious diseases to some extent. In the present study, we compared polymorphisms of *TLR2* locus in two closely related old world monkey species, rhesus monkey (*Macaca mulatta, Mamu*) and Japanese monkey (*M. fuscata, Mafu*). By nucleotide sequencing of the coding exon of *TLR2* gene from 21 *Mamu* and 35 *Mafu* individuals, we could assign 17 haplotype combinations of 17 coding SNPs of ten non-synonymous and seven synonymous substitutions. A non-synonymous substitution at codon position 326 appeared to be differentially fixed in each species, asparagine for *Mamu* whereas tyrosine for *Mafu*, and may contribute to certain functional properties which were predicted by computational chemistry, because it locates in the region contributing to ligand binding and interaction with dimerization partner of TLR2-TLR1 heterodimeric complex. Although *TLR2* alleles have diverged to similar extent in both species, they have evolved in significantly different ways; *TLR2* of *Mafu* has undergone purifying selection, while the membrane-proximal part of the extracellular domain of *Mamu TLR2* exhibits higher rates of non-synonymous substitutions, indicating a trace of Darwinian positive selection.

483T

Genomic Variation in a Brazilian Population. *G. Ananina[1], J.P.C. Vasconcellos[2], R. Vicentini[1], F. Menaa[1], F.F. Costa[3], M.B. de Melo[1].* 1) Center for Molecular Biology and Genetic Engineering (CBMEG), UNICAMP, Campinas, São Paulo, Brazil; 2) Department of Ophtalmology and Otorhinolaryngology (HEMOCENTRO), UNICAMP, Campinas, São Paulo, Brazil; 3) Department of Hematology and Hemotherapy (HEMOCENTRO), UNICAMP, Campinas, São, Paulo, Brazil.

Genomic studies on the population level have both theoretic and applied importance. They may contribute to our knowledge on anthropological and genetic history of a population, and, may also be crucial for pharmacological and public health applications. Genetic studies using microsatellites, mtDNA and some nuclear markers appoint that Brazilian population is highly admixed and this admixture occurred quite recently. Altogether, studies of Brazilian population on the genomic level are at the very beginning. We present genomewide analysis of the first cohort of 22 Brazilian individuals (BR) from the São Paulo State. Recruitment methods and blood collection were approved by the Ethic Committee of FCM-UNICAMP (Campinas, SP, Brazil). Genotyping was done with Affymetrix® Genome-Wide Human SNP 6.0 Array (Affymetrix Inc., CA, USA) following manufacturer's recommendations. For SNPs markers, genotype calls were obtained using the birdseed algorithm (v2.0) implemented in the Affymetrix Genotyping Console software. Samples passing the recommended control values (contrast QC > 0.4; median absolute intensity pairwise differences MAPD < 0.3) were analyzed for CNV. Toronto CNV map was used as the default map for comparison. Simultaneously, the data of three populations of HapMap270 Project (CEU, YRI, JPT, 20 unrelated individuals of each) were processed. Population stratification was accessed by statistical methods implemented in EIGENSOFT (v 3.0) software package. F_{ST} values of pairwise population differentiation, based on SNPs markers, were: 0.021 (BR-CEU); 0.096 (BR-YRI); 0.101 (BR-JPT). Mean number of autossomic copy number variants (CNVs) longer than 1kb was about 97 per individual. The overall mean size of the losses was 26.6 kbp, ranging from 1 to 3,144 kpb, while the overall mean size of the gains was about 86 kbp (1-23,536 kbp). In our samples, we found one variant (520kbp) not present in the reference map; but has been found in one individual of Yorubian (African) origin (McKernan et al. 2009). Furthermore, we observed 25 de novo CNVs ranging from 100 to 500 kbp (6 gains, 6 losses) and 142 smaller variants (50-100 kpb; 105 gains, 37 losses). Actually, we are increasing the sample size, the number of worldwide populations, and validating the found CNVs. We expect that our results will contribute to the better knowledge of genetic variation in the Brazilian population, one of the biggest on the South American continent. **Financial support: CNPq, FAPESP**.

484T

Mitochondrial DNA (mtDNA) heterogeneity within and among East African Bantu ethnic groups and their complex evolutionary histories. *K. Batai[1], C.M. Kusimba[2], E. Leenheer[1], S.R. Williams[1].* 1) Department of Anthropology, University of Illinois at Chicago, Chicago, IL; 2) Department of Anthropology, Field Museum, Chicago, IL.

Many linguists, historians, and archaeologists believe that Bantu-speaking farmers bearing iron technology carried their language with them as they spread relatively rapidly from central Africa throughout sub-Saharan Africa around 2,000-3,000 years ago. The genetic homogeneity found among Bantu-speaking people today is one of the arguments used to support a rapid expansion model. The nature of interactions between Bantu migrants and other people already living in these areas, however, is still debated.

The goal of this study was to examine mtDNA variation in two Bantu ethnic groups, the Taita and Mijikenda, on the northeastern periphery of the Bantu expansion in east Africa where the greatest genetic, cultural, and linguistic variation exists, in order to examine the nature of Bantu and non-Bantu interactions. We sequenced mtDNA HVRI of 474 individuals from three Taita and nine Mijikenda tribes and compared them with the mtDNA variation in other Bantu and non-Bantu populations.

Our results confirm that the east African Bantu populations are comparatively more heterogeneous than other Bantu populations. The east African Bantus spread between non-Bantu east Africans and the central African Bantus on the multidimensional scaling plot. When the Mijikenda and the Taita were compared with each other, we observed a different pattern. Although Bantu-speaking populations are generally genetically less diverse than other sub-Saharan populations, the Taita are genetically very diverse. They show little internal differentiation and have mtDNA variation similar to mtDNA variation observed in the Turkana, Kenyan Nilo-Saharan speakers. The Mijikenda are genetically less diverse than the Taita and show significant internal tribal differentiation. Their mtDNA variation is similar to the central African Bantu mtDNA variation. The estimated migration rates among the east African Bantus and between east African Bantus and the non-Bantu east Africans are high. These results emphasize that individual east African Bantu populations have unique histories of interactions with non-Bantu speaking populations. These findings have important implications for genetic epidemiological studies because the high levels of population differentiation in Africa may complicate association studies.

485T

Estimating Genetic Ancestry Using a 5-Population Model. *M. Bauchet, J.J. Bryan, A.B. Carter, V.L. Vance, H.Y. Chen, C.L. Mouritsen.* Research and Development, Sorenson Genomics, Salt Lake City, UT.

The estimation of an individual's genetic ancestry can be an important tool with many applications. The pharmaceutical and personal care product industries can greatly benefit from qualitatively stratifying study participants when their products may have potential varied effects on a consumer, relative to genetics and ancestry. In the field of Forensics, genetic ancestry estimations may provide additional information about DNA evidence collected at a crime scene, when little or no other evidence is available. Genetic ancestry can also be informative to professional genealogists and satisfy one's curiosity about their genetic ancestry as a hint to their ethno-geographic background. We have designed a novel methodology of estimating human genetic ancestry against a model of 5 genetically distinct, putative parental populations. The populations and the reference samples representing them are as follows: Western European (HapMap CEU, Northwest European descent residing in Utah), West Sub-Saharan African (HapMap YRI, Yoruba from Ibadan, Nigeria), East Asian (HapMap CHB from Beijing, China), Indigenous American (Compilation of samples identified as being from populations indigenous to North, Central, and South America including Maya, Pima, Karitiana, Surui, and Arawak descent), and the India Subcontinent (HapMap GIR, Gujarati Indian descent residing in Houston, TX). Our method uses 190 SNP Ancestry Informative Markers (AIMs) chosen from their scored ability to specifically differentiate between the 5 reference populations using Principal Component Analysis (PCA) as the comparative analysis tool and includes some markers identified as informative in previous genetic ancestry estimation publications. Using the program FRAPPE and uniquely designed algorithms, the method compares an unknown individual sample to at least a hundred randomly selected subsets of individuals from the reference populations. Background interference is calculated simultaneously and is used to estimate confidence intervals based on a calibration that was effected using thousands of worldwide individuals. We also evaluated hundreds of individuals of known origin different from any of the reference populations, giving us an indication of ancestry profiles for people who do not match exactly our model. We show that our method and test offer a comprehensive estimate of an individual's genetic ancestry.

486T

Changes in site frequency spectrum due to recent genealogic connections in the Quebec population. *C. Bherer[1], MH. Roy-Gagnon[1,2], L. Excoffier[1,3,4], H. Vézina[5], D. Labuda[1,6].* 1) Sainte-Justine Hospital Research Center, Université de Montréal, Montréal, Québec, Canada; 2) Dept. of Social and Preventive Medicine, Université de Montreal, QC, Canada; 3) CMPG, Institute of Ecology and Evolution, University of Bern 6, Baltzerstrasse, CH-3012 Bern, Switzerland; 4) Swiss Institute of Bioinformatics, 1015 Lausanne, Switzerland; 5) BALSAC Project, Université du Québec à Chicoutimi, QC, Canada; 6) Dept. of Pediatrics, Université de Montreal, QC, Canada.

Deep individual genealogies recapitulate the recent and potentially complex demographic history of populations. Here, we use extensive and unique information on the genealogy of the Quebec population to study the effect of recent demographic history on its gene pool by performing genetic simulations conditional on genealogical structure. By using both genomic and genealogical data, we have recently shown that the regional populations of Quebec are stratified and thus exhibit allele frequency differences. In this study, we investigate how the specific demographic history of Quebec regional populations shaped their site frequency spectra. We used a sample of 2,221 individuals representative of Quebec population, which was then partitioned into eight subsamples corresponding to specific geographic regions. These individuals belong to a 153,447 ancestors genealogy spanning on average 9.3 generations back to the immigrant founders and reconstructed using the BALSAC database. Allele dropping simulations of the full spectrum revealed no distortion of the allele frequency spectra between the founders and the regional samples, nor between regions for frequency classes larger than 2%. However, we observed a general loss of rarer alleles in contemporary samples, which was more pronounced in eastern regions. The joint site frequency spectra computed for all pairs of regions were very similar, thus suggesting that the differences between regions could be explained by frequency changes occurring at specific loci. Indeed, assuming a given allele frequency among the founders, we observed a larger variance in the extent of drift for the eastern regions of Quebec. Backward coalescent-like simulations conditioned on the genealogical structure revealed highly variable distributions of the number of founder's genes surviving in their contemporary descendants. For a given locus, a majority of the founders' alleles usually goes extinct and a small minority expands in the population. All these features might explain the elevated prevalence and regional distribution of rare Mendelian diseases in the Quebec population. Our findings have implications for better design of epidemiological studies conducted in the population of Quebec.

487T

The occurence of factor V Leiden in Roma (Gypsy) and majority population in Slovakia. *A. Bozikova[1, 2], I. Bernasovsky[1, 2], D. Gabrikova[1, 2], R. Behulova[2], I. Boronova[1, 2].* 1) Excellence Centre of Animal and Human Ecology, Faculty of Humanities and Natural Sciences, University of Prešov, Prešov, Slovakia; 2) Department of Biology, Faculty of Humanities and Natural Sciences, University of Prešov, Prešov, Slovakia.

The most common hereditary form of thrombophilia in the Caucasian population is factor V Leiden (G1691A), causing APC resistency. The prevalence of this mutation varies widely in healthy Caucasian population. The aim of our study was to determine the frequency of factor V Leiden mutation in Roma and majority population from Eastern Slovakia. We analyzed 540 asymptomatic individuals (269 Slovak majority individuals and 271 Slovak Romanies) by real-time PCR method. The occurrence of factor V Leiden in Roma population was significantly higher than in majority population, detected allele frequencies were 2.97% vs. 6.64% (p=0.0049) in majority and Roma population respectively. The allele frequency of factor V Leiden in Roma population from Eastern Slovakia is one of the highest in Europe. Our results confirm an uneven geographical and ethnic distribution of factor V Leiden.

488T

An admixture simulation program for use in validating genetic ancestry estimation systems. *J.J. Bryan, V.L. Vance, M. Bauchet, C.L. Mouritsen.* Research and Development, Sorenson Genomics, Salt Lake City, UT.

A key area of interest in studying population genetics is detection and estimation of genetic admixture in offspring from parents with mixed ancestry. Current methods used to estimate admixture vary in the type and number of genetic markers analyzed as well as the statistical algorithms used in the calculations. The goal of each method is to measure the genetic affinity of an individual to representative populations, generally reported in terms of relative percentages. It is important to determine the ability of the method to accurately estimate levels of population admixture through controlled experiments, testing as many permutations as possible. Identifying and testing subjects with known levels of admixture for specific populations can be very costly and time consuming. In reality, finding and testing samples to represent all possible admixture ratios, even between just a few populations, can be extremely difficult. To address this problem, we have designed a program wherein we may generate and control for, targeted levels of admixture between the population reference samples in order to validate systems for estimating genetic ancestry. The simulation algorithm is capable of randomly generating innumerable offspring, each with unique genotypes, selected from virtual unions of up to 8 parental (P1) individuals. This program allows one to control admixture contributions from parental populations in increments of 12.5% in the 3rd filial (F3) generation. P1 samples are randomly chosen and paired from an established parental genotype database to fulfill desired admixture ratios for a final resultant F3 generation individual. At each union in a generation, 1 allele for every bi-allelic marker in each individual is randomly selected to contribute to the next generation. By using this new program, 10,000 admixed simulations were generated from 383 real samples used in the P1 generation. The P1 samples were selected from 5 putative parental populations and were compared in a variety of combinations and ratios in increments as low as 12.5% with multiple unique offspring generated from each simulated mating. Reproducibility was tested using different P1 samples for each admixture combination and ratio. This simulation program was developed to facilitate the validation of a 190 SNP, human genetic ancestry estimation algorithm. The program allowed for well-controlled experimentation and significant savings in time and expenses.

489T

Estimating heterozygosity rates of low-coverage sequence genomes by leveraging joint spectra. *K. Bryc[1], N. Patterson[2], D. Reich[1,2].* 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Broad Institute of Harvard and MIT, Cambridge, MA.

Heterozygosity, or the fraction of nucleotides within an individual that differ between their two parental chromosomes, can be informative about population demographic history and natural selection. In practice, estimating heterozygosity from sequence data is confounded by sequencing errors and lack of power to detect SNPs. Obtaining an unbiased estimate of heterozygosity is especially difficult for ancient genomes (such as Neandertal or Denisova) where the sequences are expected to have a high error rate, or with low-coverage sequence data where there is low power to detect heterozygous SNPs.

Here, we present a method that leverages the genome-wide joint information across sequence reads from a panel of individuals, to estimate the heterozygosity of an individual of interest. We assume independence between the observed alleles of the individual at a particular locus and the combination of observed alleles in a panel of individuals, conditional on the true underlying genotype. We then apply an Expectation-Maximization (EM) algorithm to estimate the most likely distribution of counts across the unknown underlying genotypic states, from which we obtain an estimate of the proportion of loci that are heterozygous in that individual. An advantage of this method is that it returns an unbiased and accurate estimate of heterozygosity even when the individual has low sequence coverage (as low as 2X on average). Our method learns the joint spectrum directly from the sequence read data without requiring an explicit assumption of the demographic relationships among the analyzed samples. Since it can utilize panels assembled from individuals from any population or set of populations and it does not require genotype calls, our method is robust to any inaccuracies that might stem from demographic modeling and from sequence error processes.

We apply our EM method to simulated data of 1Gb of sequence at 2X, 5X, 10X, and 20X coverage, and find that our method performs well at estimating the true heterozygosity even when the sequence error rate is extreme and coverage is low. We also report results from subsampling of real data from NA12878, a deeply sequenced European American individual, and compare the estimates of heterozygosity for the low-coverage compared with the high-coverage data.

490T

Identification of a new susceptibility variant for multiple sclerosis in OAS1 by population genetics analysis. *R. Cagliani[1], M. Fumagalli[1], F.R. Guerini[2], S. Riva[1], D. Galimberti[3], G.P. Comi[3], C. Agliardi[2], E. Scarpini[3], U. Pozzoli[1], D. Forni[1], D. Caputo[4], R. Asselta[5], M. Biasin[6], E.M. Paraboschi[5], N. Bresolin[1,3], M. Clerici[7,8], M. Sironi[1].* 1) Scientific Inst IRCCS E.Medea, Bosisio Parini, Italy; 2) Laboratory of Molecular Medicine and Biotechnologies, Don C. Gnocchi Foundation ONLUS, IRCCS, Milano, Italy; 3) Dino Ferrari Centre, Department of Neurological Sciences, University of Milan, Fondazione Ca' Granda IRCCS Ospedale Maggiore Policlinico, 20122 Milano, Italy; 4) Multiple Sclerosis Unit, Don C. Gnocchi Foundation ONLUS IRCCS, S. Maria Nascente, Milano, Italy; 5) Dipartimento di Biologia e Genetica per le Scienze Mediche, Università degli Studi di Milano, Milano, Italy; 6) Chair of Immunology, DISC LITA Vialba, University of Milano, Milano, Italy; 7) Chair of Immunology, Department of Biomedical Sciences and Technologies LITA Segrate, University of Milan, 20090 Milano, Italy; 8) Fondazione Don C. Gnocchi, IRCCS, 20148 Milano, Italy.

Contrasting results have been reported concerning the association of a splice-site polymorphism (rs10774671) in OAS1 with multiple sclerosis (MS). We analysed two OAS1 regions encompassing alternatively spliced exons. While the region carrying the splice-site variant is neutrally evolving, a signature of long-standing balancing selection was observed across an alternative exon 7. Analysis of variants in this exon identified an insertion/deletion polymorphism (rs11352835, A/-) that originates predicted products with distinct C-termini. This variant is located along the major branch of the haplotype genealogy, suggesting that it may represent the selection target. A case/control study for MS indicated that rs11352835 is associated with disease susceptibility (for an allelic model with the deleted allele predisposing to MS, OR: 1.27, 95% CI: 1.072-1.513, p=0.010). No association was found between rs10774671 and MS. As the two SNPs are in linkage disequilibrium in Europeans, the previously reported association between rs10774671 and MS susceptibility might be driven by rs11352835, possibly explaining the contrasting results previously observed for the splice-site polymorphism. Thus, we describe a novel susceptibility variant for MS in OAS1 and show that population genetic analyses can be instrumental to the identification of selection targets and, consequently, of functional polymorphisms with an effect on phenotypic traits.

491T

The development of interactive maps to further describe Y and mtDNA haplogroups— A new educational tool. *H.Y. Chen, C.L. Mouritson, A.B. Carter.* Research and development, Sorenson Genomics, Salt Lake City, UT.

Background: Mitochondrial DNA and Y chromosome haplogroup maps can provide visual depictions of the geographical distributions of known haplogroups. 14 peer-reviewed publications were reviewed and a database was developed of 109 different populations each being evaluated for their proportional makeup from any of 18 primary Y haplogroups. Latitude and longitude coordinates for each population were determined from the original location of the samples used in each publication. In the database, haplogroup percentages were assigned to a relevant global geographic coordinate using the Boundary Map feature of Mapview software. Through the global coordinates the haplogroups could be populated on maps with geographical associations. Ultimately, 540 data points were used to create 26 Y haplogroup maps (18 world gradient maps, 7 continental pie maps and 1 world pie map). Utilizing the same method, a database of 96 different mtDNA populations was constructed, each being evaluated for 27 primary mtDNA haplogroups according to 23 peer-reviewed articles. This resulted in 790 data points used in the creation of 34 mtDNA haplogroup maps (27 world gradient maps, 6 sub-continental pie maps and 1 world pie map) **Validation**: Data within the newly constructed database were verified against the Haplogroup specifications defined by YCC (Y Chromosome Consortium) for Y haplogroups and PhyloTree for mtDNA haplogroups. **Map descriptions**: a) Gradient maps were developed to exhibit the approximate geographical distribution of a specific haplogroup using representative color intensity; b) Continental/sub-continental pie maps were created to show the percent makeup of each haplogroup in association with other haplogroups for a specific region; c) World pie maps were created to demonstrate the percent makeup of each haplogroup in association with other haplogroups for the entire world. **Features**: Special features were designed to allow viewers to navigate the maps without the need of returning to the home map. 1) Clicking map graphics to view corresponding gradient maps; 2) Clicking the title or space between letters allows one to observe the world pie map; 3) When viewing the world pie map, users can select continental/sub-continental areas to view continental/sub-continental pie maps. **Conclusion**: This educational tool provides 60 data-dependent maps of Y and mtDNA haplogroups with convenient navigation features.

492T

Inferring admixture proportions and recent admixture events. *D.J.M. Crouch, M.E. Weale.* King's College London, London, United Kingdom.

The identification of admixture proportions for forensic DNA samples has recently had some success in criminal investigations (Phillips et al. (2009), PLoS ONE 4: 8). If researchers were able to differentiate first generation admixture events, this might shed new light on the identity of samples. We describe a new method, based on maximum likelihood estimation, for simultaneously inferring admixture proportions in a SNP-genotyped individual, and the genetic divergence of their unobserved parents with respect to the general population, based on allele frequencies from reference groups. This is achieved by examining the reduction in homozygosity that one expects to see after first generation admixture. We use simulated individuals, under various parental admixture scenarios, to validate the method. The simulations showed that, with 80,000 independent SNPs, most parental admixtures could be accurately identified at the intercontinental level of divergence (Fst>0.1), although large effects such as pure first generation admixture can be predicted for more closely related populations (Fst>0.02). These results take into account the sampling errors in the reference allele frequencies.

493T

Genomic stability of B lymphoblastoid cell lines established from the Sonoda-Tajima Cell Collection, the collection of South American indigenous populations. *I. Danjoh, K. Saijo, M. Nagayoshi, Y. Nakamura.* Cell Engineering Division, RIKEN BioResouce Center, Tsukuba, Ibaraki, Japan.

<Objectives>

The Sonoda-Tajima Cell Collection includes peripheral blood mononuclear cell (PBMNC) samples obtained from over 3,500 individuals belonging to a range of ethnic minority groups across the world, in particular from South America. Drs. Sonoda and Tajima, a professor emeritus at Kagoshima University in Japan and the director of Aichi Cancer Center Institute in Japan, had spent nearly 30 years to collect the samples, and the collection is made all the more valuable by the fact that some of these ethnic populations have since died out, and thus it will be impossible to prepare a similar cell collection again.

The collection was donated to our institute, a public cell bank in Japan, in 2005 by Dr. Sonoda to make it available to researchers throughout the world. As the original PBMNC collection would obviously have been rapidly exhausted if used directly, we, therefore, have immortalized 533 samples with the Epstein-Barr Virus and established B lymphoblastoid cell lines (B-LCLs).

As there is continuing controversy over whether the B-LCL genome is stably maintained, we performed an array comparative genomic hybridization (array CGH) analysis to confirm the genomic stability of the cell lines.

<Methods>

We first checked karyotypes by conventional G-band staining method, and then analyzed detailed genome structures by array CGH with 2x400K microarray (Agilent). PBMNCs prepared from the same individuals were used as parental somatic cell controls for array CGH. B-LCLs at early stage (approx. passage 10) or at progressed stage (approx. passage 30) after establishment, or FACS-sorted B lymphocyte population were used as the sample for array CGH, and monitored the structural alteration of genomes during culture period of B-LCLs.

<Results and Conclusion>

There were no detectable aberrations of karyotypes analyzed by G-band staining of chromosome. Although array CGH analysis showed some alteration in B-LCLs at early stage, most of them were also detected in B lymphocyte population, which shows they were not introduced during establishment of B-LCLs. In addition, array CGH data suggested the genomic rearrangements occurred within somatic cells. In progressed passage of B-LCLs, genome structure was well-maintained but clonal expansion was observed in some cases in comparison to early stage B-LCLs.

Our data indicate the stable genome structure of B-LCLs, especially at early stage, to be used for genetic analysis.

494T

Sampling scheme as a determinant of the major axis of genetic variation in principal components analysis. *M. DeGiorgio[1], N.A. Rosenberg[1,2].* 1) Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI; 2) Department of Human Genetics and the Life Sciences Institute, University of Michigan, Ann Arbor, MI.

Principal component (PC) maps, which plot the values of a given PC estimated on the basis of allele frequency variation at the geographic sampling locations of the set of populations analyzed, have commonly been used to investigate the properties of past range expansions. Some studies have argued that the axis of greatest variation (i.e., the first PC) is parallel to the axis of expansion. In contrast, others have identified an opposite pattern in which the axis of greatest variation is perpendicular to the axis of expansion. In this study, we seek to resolve this disagreement by investigating the effect of sampling scheme on the direction of the axis of greatest variation under a two-dimensional range expansion model. From simulated datasets generated using each of two different schemes for the sampling of populations under the range expansion model, we create PC maps for the first PC. We find that depending on the sampling scheme, the axis of greatest variation can be either parallel or perpendicular to the axis of a range expansion. We provide explanations for this result in terms of within- and between-population coalescence times.

495T

Genomic insights into recent human adaptations driven by selection on standing variation. *L.S. Emery, J.M. Akey.* Dept. of Genome Sciences, University of Washington, Seattle, WA.

Recent studies have demonstrated that classic selective sweeps were relatively rare in recent human history. Instead, emergent selective pressures due to a changing environment could act on pre-existing variation. This model, called selection from standing variation, results in different genomic signatures than that of the classic selective sweep. While statistical tests to detect classic selective sweeps are well-established, it remains difficult to identify the signatures of selection from standing variation. Here we develop, test, and optimize several statistics to detect and fine-scale map recent selection acting on standing variation in human populations. We extensively validate the power and operating characteristics of these statistics thorough coalescent simulations over a broad range of demographic parameters and selective models. Finally, we apply our statistics to whole-genome sequences from the 1,000 Genomes Project and Complete Genomics data sets. These analyses provide new insights into the molecular substrates of positive selection acting on standing variation and have important implications for human health.

496T

SCN1B sequence variations in Iranian Patients with Epilepsy. *B. Sedaghatikhayat[1], M. Moghaddasi[2], M. Houshmand[5], S. Zeinali[3], S.H. Tonekaboni[4], M.S. Fallah[3], M. Mamarabadi[2], A. Ebrahimi[1].* 1) Parseh Medica Genetics Center, Tehran, Tehran, Iran; 2) Rasool Akram Hospital, Tehran University of Medical Sciences, Tehran, Iran; 3) Kawsar Human Genetics Research Center, KAWSAR Genomics & Biotech Center, Tehran, Iran; 4) Shahid Beheshti University of Medical Sciences, Tehran, Iran; 5) National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran - Karaj Highway, Tehran, Iran.

Epilepsy is a common chronic neurological disorder that is characterized by recurrent unprovoked seizures. Molecular studies of candidate genes can help us to define a correct differential diagnosis. So we studied SCN1B gene in Iranian patients with Idiopathic Epilepsy includes Febrile Seizure, Generalized Epilepsy with Febrile Seizures (GEFS+) or Dravet syndrome, diagnosed clinically, to explain genotype-phenotype correlation. Materials and Method: We screened 34 selected epileptic unrelated Iranian probands for all coding regions of SCN1B by PCR amplification and direct Sequencing. All families and probands were previously screened for SCN1A and mtDNA mutations. Results: PCR amplification of whole coding regions and splicing sites of SCN1B followed by direct sequencing revealed two novel sequence variations in patients (p.248 R>S, p.210 L>P) which did not detected in the healthy normal family members. Conclusions: According to final results it seems that these two novels SCN1B variations are not causative mutations in epileptic patients' but they can act as genetic predisposition factors in epileptic phenotypes which introduce a susceptibility especially in response to antiepileptic drugs. Key Words: Epilepsy, SCN1B, SCN1A, GEFS+.

497T

Clinical and genetic heterogeneity in autosomal dominant partial epilepsy with auditory features: Implications for genetic counseling. *F.Rossi. Torres[1], E. Bilevicius[2], R. Secolin[1], N.F. Santos[1], E. Kobayashi[3], L.A.C. Sardinha[2], F. Cendes[2], I. Lopes-Cendes[1].* 1) Department of Medical Genetics, Universidade Estadual de Campinas; 2) Department of Neurology, Universidade Estadual de Campinas; 3) Department of Neurology and Neurosurgery, McGill University.

Autosomal dominant partial epilepsy with auditory features (ADPEAF) also known as autosomal dominant lateral temporal lobe epilepsy (ADLTLE) is a monogenic form of epilepsy syndrome characterized by partial seizures with predominant auditory symptoms originating in the temporal lobe cortex. Mutations in the leucine-rich, glioma inactivated 1 gene (*LGI1*) are found in about 50% of patients with ADPEAF/ADLTLE. It has been described that patients with ADPEAF/ADLTLE do not have structural abnormalities in the brain. We assessed different families with ADPEAF/ADLTLE in order to further investigate genetic, clinical and neuroimaging findings in this disorder. We studied four families (S, MG, N and B), all with clinical features of ADPEAF/ADLTLE. Families were subjected to clinical evaluation, including neurological exam, electroencephalogram and high resolution magnetic resonance imaging (MRI). Mutation screening was performed by automatic sequencing of the entire coding region of *LGI1*, *LGI2*, *LGI3*, and *LGI4* genes. Auditory auras were identified in all families; however, there was an excess of *dejá-vù* episodes in the MG family (n = 12/21). Structural abnormalities in the left temporal lobe were identified in individuals belonging to the S family (n = 8/ 14). A point mutation in the acceptor splice site of exon 8 of *LGI1* gene (IVS7-2A> G) was identified in all affected individuals of family S. However, mutation screening in *LGI1*, *LGI2*, *LGI3*, *LGI4* genes did not identify pathogenic variations in families MG, N and B. We observed genetic and clinical heterogeneity in our group of ADPEAF/ADLTLE families. While individuals carrying the *LGI1* mutation in the S family have a clear identifiable structural abnormality in the left temporal lobe, individuals from families without *LGI1* mutations have normal MRI findings. These results suggest that temporal lobe structural abnormalities, when present, may indicate the presence of mutations in *LGI1*. In addition, we found evidence that a high frequency of *déjà vu*, as it is observed in the MG family could indicate that the presence of mutations in *LGI1* is less likely. These clinical findings may prove to be important for initial genetic counseling of families with ADPEAF/ADLTLE, especially when considering genetic testing for *LGI1*.

498T

A wide spectrum of clinical and brain MRI findings in patients with *SLC19A3* mutations. *K. Yamada[1], K. Miura[2], K. Hara[3], M. Suzuki[2], N. Nakanishi[4], T. Kumagai[2], N. Ishihara[1], Y. Yamada[1], R. Kuwano[5], S. Tsuji[6], N. Wakamatsu[1].* 1) Gen/Aichi Human Serv Ctr, Inst Dev Res, Aichi, Japan; 2) Pediat. Neurol/ Aichi Human Serv Ctr, Ctrl Hosp, Aichi, Japan; 3) Neurol/ Niigata Univ, Brain Res Inst, Niigata, Japan; 4) Perinatol/ Aichi Human Serv Ctr, Inst Dev Res, Aichi, Japan; 5) Mol Genet/Niigata Univ, Brain Res Inst, Niigata, Japan; 6) Neurol/ Univ, Tokyo, Tokyo, Japan.

We conducted on the detailed clinical, brain MRI and molecular genetic analysis of four patients in a Japanese pedigree who presented with epileptic spasms in early infancy, severe psychomotor retardation, and characteristic brain MRI findings of progressive brain atrophy and bilateral thalami and basal ganglia lesions. Genome-wide linkage analysis revealed a disease locus at chromosome 2q35-37, which enabled identification of the causative mutation in the gene *SLC19A3*. A pathogenic homozygous mutation (c.958G>C, [p.E320Q]) in *SLC19A3* was identified in all four patients and their parents were heterozygous for the mutation. SLC19A3 (solute carrier family 19, member 3) is a thiamin transporter with 12 transmembrane domains. Homozygous or compound heterozygous mutations in *SLC19A3* cause two distinct clinical phenotypes, biotin-responsive basal ganglia disease and Wernicke's-like encephalopathy. Biotin and/or thiamin are effective therapies for both diseases. However, administration of a high dose of biotin for one year improved neither the neurological symptoms nor the brain MRI findings in one patient. Our cases broaden the phenotypic spectrum of disorders associated with *SLC19A3* mutations and highlight the potential benefit of biotin and/or thiamin treatments and the need to assess the clinical efficacy of these treatments. We constructed mouse model of our patient and discuss brain phenotypes of this model.

499T

Heritability and Familiality of Personality Dimensions in the Korean Schzophrenic LD Families. *B. Lee[1,2], Y. Chung[1,2], J. Park[1,2], S. Kim[1,2], C. Kang[1,2], J. Kim[1,2], Y. Lee[1,2], E. Moon[1,2].* 1) Department of Psychiatry, Pusan National University Hospital, Busan, Korea; 2) Department of Psychiatry, Pusan National University, Busan, Korea.

Purpose: Schizophrenia is the most devastating mental illness that causes severe deterioration in social and occupational functioning. But the mystery for elucidating its causes is in line with brain's mystery. One possible mechanism for causing that syndrome may be the genetic aberrations in neurodevelopment and neurodegeneration. Categorical syndrome such as schizophrenia could be the complex of many continuous mental structure phenotypes including several personality development/degeneration dimensions. This is the study to search heritability and familiality of personality dimensions in the Korean schizophrenic LD(Linkage Disequilibrium) families. Method: We have recruited 306 probands(with schizophrenia) with their parents and siblings whenever possible. For best estimation of diagnosis, we have used medical records and a Korean version of DIGS & FIGS. We have used MMPI, SCL-90R, TCI, NEO questionnaires for measuring personality and symptomatic dimensions. Heritabilities of personality dimensions in total 738 family members were estimated using Sequential Oligogenic Linkage Analysis Routines(SOLAR). Personality dimensions in total family members were compared with those in 336 healthy unrelated controls for measuring the familialities. Genetic/environmental correlations with symptomatic dimensions for significant personality dimensions aggregated in families were investigated. Result: Four of the 10 MMPI variables, two of the 5 NEO variables, five of the 7 TCI variables were not significantly heritable and were excluded from subsequent analyses. The three groups(control, unaffected 1st degree relative, case) were found to be significantly different and with the expected order of average group scores for five of the MMPI scales, three of the NEO scales, and two of the TCI scales. Genetic/environmental correlations with symptomatic dimensions for significant personality dimensions aggregated in families will be suggested. Conclusion: Our results show that the aberrations in several personality dimensions could form the complexity of schizophrenic syndrome as a result of genetic-environment coactions or interactions in spite of some limitations(recruited family, phenotyping). These will be the base as important coefficients of so mysterious equations forming schizophrenia. But still, most areas in positional genetic variations and environmental factors as loaded variables of equations for causing that syndrome remain doubtful.

500T

Fragile X, intermediate, and premutation alleles in the Autism Genetic Resource Exchange (AGRE). *W. Brown, A. Glicksman, X. Ding, N. Ersalesi, C. Dobkin, S. Nolin.* Dept Human Genetics, NYS Inst Basic Res in DD, Staten Island, NY.

AGRE is an autism family registry and biosample resource that includes primarily multiplex families, having two or more children, that are affected by autism spectrum disorders (ASD). Family data are available online, and genetic material is available for analysis. The families were recruited by ads and via support groups by Cure Autism Now/Autism Speaks. Thus, they represent a relatively unbiased sample. ~35% of the first 480 families had genetic testing prior to entering the registry. The individuals receive confirmatory ADI-R and ADOS testing. We have screened the FMR1 locus in one proband from 1641 families. We found 7 families with fragile X (FX) present. Among these, 6 were found among the first set of 480 families and among 312 AGRE families that had had no prior genetic screening, the rate was ~1.9%. An estimate of the Raven IQ score of the autistic subjects was 80±35 with range 34-144. Thus, the AGRE sample is likely to have a higher IQ distribution than typical for FX subjects (mean ~40±25). Previous prevalence studies of FX in autistic samples range from 0-16%; with a mean of ~4%; (Feinstein 98). Our 1.9% is similar to a report of 1.6% among 123 unrelated autistic individuals (Bailey 93), but lower than the 13% we found on an earlier multicenter study of 183 individuals (Brown 86). A growing awareness of FX has likely decreased the probability of finding FX in the more recently recruited multiplex ASD families due to screening and exclusion. The 1.9% is lower than the expected 4%, perhaps due to higher IQs in AGRE subjects typical than for FX. But it confirms an association of FX and ASD. **Possible association of ASD and premutations (PRE).** Among 1447 male probands tested, there were 2 with PRE (59 & 64 CGGs) and 12 with intermediate (45-54) alleles (IA) (46, 47, 47, 47, 48, 48, 48, 49, 50, 50, 51, 54) for an IA prevalence of 0.83%. Among 194 female probands tested there were 2 with PRE (55, 59) and 7 with IA (45, 46, 48, 50, 52, 53, 54). Since females have two alleles, dividing by 2 gives an IA prevalence of 1.8% in female alleles or an overall IA prevalence of 0.9%. Our published control value for 2500 X chromosomes was 1.7% (Brown 96), and our more recent control value based on carrier screening of 9064 X chromosomes was 1.15%. Thus, there was no excess of IA or PRE among the AGRE registry ASD probands. This finding suggests ASD is **NOT** associated with IA or PRE alleles.

501T

Translational Profiling Mouse Models of Fragile X-associated Tremor/Ataxia Syndrome. *J.N. Galloway, C. Shaw, P. Yu, D.L. Nelson.* Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) is a late-onset neurodegenerative disorder primarily affecting male premutation allele carriers of Fragile X Mental Retardation 1 (*FMR1*) gene. The current molecular model for the disease suggests the clinical features of FXTAS result from elevated expression of the expanded CGG repeat in the premutation *FMR1* mRNA. Since there are rarely alterations in the level of the *FMR1* gene product, FMRP, it is likely that the expanded CGG repeat in this RNA is toxic. Indeed, models in both fly and mouse have demonstrated that expanded CGG repeats in heterologous mRNAs are sufficient to confer degeneration of neurons with similar features. This observation has led to the hypothesis that aberrant interactions between the expanded CGG sequences and RNA-binding proteins may be responsible for the pathology in FXTAS, possibly manifested in changes in regulation of RNA splicing, transport or translation. We have previously described a Purkinje neuron specific transgenic mouse model of FXTAS that exhibits many human FXTAS clinical features including the formation of ubiquitin positive intranuclear neuronal inclusions and death of Purkinje neurons along with motor coordination and motor learning deficits. Our current efforts focus on understanding changes in translational profiles of diseased Purkinje neurons in our mouse model of FXTAS. Using the Purkinje neuron specific model, developed by the Heintz group at Rockefeller University, that expresses an EGFP-tagged ribosomal protein L10a, and the translating ribosome affinity purification (TRAP) methodology to affinity purify polysomal mRNAs, we have performed comparative analysis of gene translational profiles of various age groups of our FXTAS model using microarray technology. Our results suggest that translational profiles are altered as early as 4 weeks of age by the presence of expanded CGG repeat RNA. More than 400 mRNAs are significantly elevated or decreased in abundance on polyribosomes. The profiles reflect changes in genes involved in alternative splicing, mRNA transport, miRNA metabolism, translational regulation and programmed cell death. Preliminary data indicated that one candidate, *Adcy7*, is elevated nearly 16 fold over wildtype by four weeks. Work is in progress to validate additional genes from our microarray study of 8 and 12 week models. This study will identify many new candidate genes involved in the progression of FXTAS related pathology.

502T

***NAT1* and tobacco smoke exposure in multiple sclerosis: evidence for a gene-environment interaction.** *L. Barcellos[1,2], F. Briggs[1], B. Acuna[2], L. Shen[2], P. Ramsay[1], H. Hong[1], A. Bernstein[2], C. Schaefer[2].* 1) Div Epidemiology-SPH, Univ California, Berkeley, Berkeley, CA; 2) Division of Research, Kaiser Permanente, Oakland, CA.

Multiple sclerosis (MS) is a complex and heterogeneous autoimmune disease of the central nervous system with a multifactorial etiology, involving both genetic and environmental components. While many environmental exposures have been investigated, exposure to tobacco smoke has consistently demonstrated association with increased risk of MS and disease progression. However, not all smokers develop MS, and only some individuals with MS were ever smokers. We hypothesized that host genetics contribute to metabolism of tobacco smoke constituents, and therefore to development of MS in smokers. A gene-environment (GxE) investigation was conducted to determine whether variation within *NAT1* located on chromosome 8p22 encoding the enzyme n-acetyl transferase 1 modifies the risk of MS associated with tobacco smoke exposure *NAT1* is known for its important role in tobacco smoke constituents metabolism, and variation within this locus has been well-established in several cancers. To investigate this possibility in MS, we utilized comprehensive smoke exposure and genetic data collected from ~2,000 White, non-Hispanic MS case and control members of the Kaiser Permanente (KP)Northern California Region HealthPlan. The KP MS Research Program was established to support rigorous epidemiologic investigations of both genetic and environmental risk factors in a large, population-based study sample. Tobacco smoke exposure before the age of 20 was significantly associated with greater risk of MS using logistic models adjusted for age and other risk factors including gender, history of infectious mononucleosis and *HLA-DRB1*1501* status. Tobacco smoke exposure before the age of 20 was significantly associated with greater risk of adult-onset MS (odds ratio [OR] (95% Confidence Interval)=2.0 (1.5-2.6), p=7x10-7) analyses. Six of 16 SNPs within *NAT1* significantly increased the risk of MS conferred by tobacco smoke: rs6586711: odds ratio of interaction (OR_I)=1.6, p=0.004; rs11996662: OR_I=1.7, p=0.005; rs7012951: OR_I=1.7, p=0.008; rs2188023: OR_I=1.5, p=0.02; rs3901866: OR_I=1.5, p=0.02, rs4986990: OR_I=2.6, p=0.03. Interestingly, none of the *NAT1* variants demonstrated a marginal association with MS, despite power for detection. We have identified a potential MS susceptibility locus that is important in the context of environmental exposure. Results from this study provide insight to underlying disease mechanisms in MS; our approach has strong application to additional GxE studies in MS.

503T

Gene-environment and functional classification analyses suggest novel candidate genes and processes that may be contributing to the susceptibility of adult-onset multiple sclerosis. *F. Briggs[1], B. Acuna[2], L. Shen[2], P. Ramsay[1], H. Quach[1], A. Bernstein[2], C. Schaefer[2], L. Barcellos[1,2].* 1) Sch Pub Hlth, Univ California, Berkeley, Berkeley, CA; 2) Division of Research, Kaiser Permanente, Oakland, CA.

Multiple sclerosis (MS) is an inflammatory and demyelinating autoimmune disease with complex genetic and environmental components. The search for genetic risk factors has been successful, however much of the genetic component remains unknown, particularly the identity of variants and biological pathways that modulate exposure to environmental risk factors. A large body of evidence has demonstrated, convincingly, that socioeconomic position (SEP), perhaps in childhood and adulthood, is one of the most significant predictors of chronic disease and long-term health outcomes in adults. To investigate this possibility in MS, we utilized comprehensive exposure and genome-wide association (GWA) data collected from ~2,000 White, non-Hispanic MS case and control members of the Kaiser Permanente Northern California Region HealthPlan. The Kaiser Permanente MS Research Program was established to support epidemiologic investigations of both genetic and environmental risk factors in a large, population-based study sample. Logistic models were used, and analyses were adjusted for age, and established MS risk factors (gender, history of smoking and infectious mononucleosis, and *HLA-DRB1*15* status). Several related measures of SES collected demonstrated significant associations with risk of MS, including: *low household education at age 10*: odds ratio [OR=1.3, p=0.002; *less than a college education*: OR=1.4, p=0.003; *low life-course socioeconomic position*: OR= 1.9, p= $2x10^{-5}$, and *low social mobility*: OR=1.7, p=$8x10^{-4}$. We conducted GxE analyses using SEP exposures and identified several gene candidates that distinguished low from high SEP, including *NPAS3*, p=$2x10^{-6}$; *DPP6*, p=$2x10^{-6}$; *DIP2C*, p=$3x10^{-6}$, and *KANK1*, p=$5x10^{-6}$. Functional classification tools using KEGG PATHWAY and Gene Ontology, performed pathway analyses of gene candidates (p<0.001). Genes participating in axon guidance were significantly overrepresented (p=$6x10^{-5}$; KEGG). Based on Gene Ontology databases, genes involved in cell adhesion (p=$3x10^{-6}$), MAPKK activation (p=$7x10^{-4}$), plasma membrane (p=$9x10^{-4}$), and neuron differentiation (p=$1x10^{-3}$), were overrepresented. Our results demonstrate evidence for specific biological pathways acting through gene products important to MS pathogenesis in the context of life course SEP. The combined bioinformatics approach described here to characterize genes and pathways relevant to environmental exposures in MS may shed light on mechanisms contributing to disease etiology.

504T

A study of depression and anxiety among female carriers of the *FMR1* premutation and the impact of raising a child with fragile X syndrome: evidence for moderation by the corticotrophin-releasing hormone receptor 1 gene. *J.E. Hunter[1], A. Abramowitz[2], J.F. Cubells[1,3], S.L. Sherman[1].* 1) Dept Human Genetics, Emory Univ, Atlanta, GA; 2) Dept Clinical Psychology, Emory Univ, Atlanta, GA; 3) Dept Psychiatry and Behavioral Sciences, Emory Univ, Atlanta, GA.

The *FMR1* contains a polymorphic CGG repeat in the 5'UTR of exon 1. Once unstable, this triplet repeat is capable of expansion across generations with maternal transmission. Thus women who carry a premutation, defined as an allele with 55-199 repeats, are at risk of passing on a full mutation, defined as an allele with >200 methylated repeats, to their offspring, leading to the intellectual disability disorder, fragile X syndrome (FXS). Mounting evidence suggests that some premutation carriers may be vulnerable to anxiety and depression. The goal of this study was to test the hypothesis that among females who carry a premutation, the stress of raising a child with FXS would be moderated by their endogenous cortisol activation influencing their potential for anxiety and depression outcomes. To this end, we genotyped the corticotrophin releasing hormone receptor 1 gene (*CRHR1*) among 279 female carriers of the FMR1 premutation (111 with a child with FXS, 168 without a child with FXS) who had completed self-report questionnaires assessing symptoms of depression [Centers for Epidemiological Studies Depression Scale (CESD)] and anxiety [State Trait Anxiety Inventory (STAI) and Social Phobia and Anxiety Inventory (SPAI)]. Results indicate a statistically significant interaction between *CRHR1* genotypes and the status of raising a child with FXS to predict anxiety symptoms reported on the SPAI across multiple SNPs (rs7209436, p=0.0005; rs242924, p= 0.0071; rs173365, p=0.0022). Overall, our data provide evidence that differential cortisol activation as measured by variants in the corticotrophin-releasing hormone receptor mediates levels of anxiety related to the stress of raising a child with FXS among female carriers of *FMR1* premutation alleles.

505T

A molecular mechanism underlying the interaction of serotonin transporter gene polymorphism and stress on central serotonin transmission and risk of depression. *A.J. Jasinska[1], C.A. Lowry[2], M. Burmeister[3].* 1) Psychology and Neuroscience Program, University of Michigan; 2) Integrative Physiology and Center for Neuroscience, University of Colorado-Boulder; 3) Human Genetics, Psychiatry, and Molecular and Behavioral Neuroscience Institute, University of Michigan.

The serotonin transporter gene (5-HTT)-linked polymorphic region (5-HTTLPR) is the most studied genetic variant in psychiatric neuroscience. Drawing on neuroanatomical, neurochemical, and neuroimaging studies of the central serotonin system, we propose a mechanism underlying the interaction of the 5-HTTLPR and stress on central serotonin transmission and risk of depression. These results add to our understanding of gene-environment interactions in psychiatric and behavioral genetics. The proposed mechanism has four key features. (1) Serotonergic projection neurons, which project from the dorsal raphe nucleus (DRN) to most areas of the forebrain, are regulated by serotonergic interneurons, whose axon terminals are contained within the DRN. In the absence of severe stressors, the projection neurons are tonically active, while the interneurons are silent. When a severe stressor is detected, the interneurons are activated and inhibit some projection neurons, terminating serotonin release in some projection regions. (2) The serotonin transporter (encoded by the SLC6A4 gene) is responsible for reuptake of serotonin from the extracellular space. The efficiency of serotonin reuptake in the DRN determines the duration of stressor-induced inhibition of serotonin release in forebrain regions: a lower efficiency of reuptake means a relatively longer inhibition. (3) This difference in duration alters the balance in a critical circuitry connecting the DRN, the amygdala, and the ventromedial prefrontal cortex (VMPFC), responsible for stressor reactivity and emotion regulation. Specifically, if the efficiency of serotonin reuptake is compromised, the VMPFC remains inhibited for longer and fails to inhibit serotonergic interneurons, resulting in a positive feedback loop between the amygdala and the DRN. Over time, these neural signaling pathways are strengthened due to synaptic plasticity, yielding a hyper-reactivity to stress in the amygdala and a hypo-reactivity of emotion regulation in the VMPFC. (4) Importantly, the variable efficiency of serotonin reuptake in the DRN has an impact only in the presence of a severe stressor. Thus, the effects of functional variation in the serotonin transporter gene (including, but not limited to, the 5-HTTLPR) on central serotonin transmission and risk of depression depend on stressor exposure. This dependency, we propose, is the underlying mechanism of the gene-environment interaction first observed by Caspi et al. (2003).

506T

BDNF Val66Met genotype as a moderator of early life stress response but not late life stress response and its impact on negative affectivity. *C. Lattig[1], C. Perea[1], A. Paternina[1], Y. Gomez[2].* 1) Dept Genetics, Univ de los Andes, Bogota, Colombia; 2) Dep. Psychology, Univ de los Andes, Bogota, Colombia.

Background: Gene x environment (G x E) interactions are known to predict susceptibility to disorders such as depression and anxiety. Childhood adverse experiences and number of stressful life events (SLEs) have been widely studied as environmental risk factors; however response to SLEs has not been studied. Here we present a first attempt to look at the interaction between the response to personal and academic stressful events during different life stages and the gene polymorphisms 5-HTTLPR, 5-HTTVNTR (STin2), HTR1A C(-1019)G, and BDNF Val66Met in the prediction of negative affectivity (NA). Methods: Standardized questionnaires (ST-DEP and STAI) were used to measure negative affectivity derived from depression and anxiety in a sample of 303 undergraduate students. Response to stressful events during childhood, high school and college years was evaluated together with a self-report format of personal history. Multiple logistic regression analysis was used to perform association and G x E analysis using SSPS, R and Plink statistical programs. Results: Negative affectivity is strongly associated with childhood maltreatment and stress response. Gene associations were observed between 5-HTTVNTR allele 12 and the S_12 haplotype with NA derived from both depression and anxiety high scores. The BDNF gene variant was not associated with NA derived from depression or anxiety alone, but it was associated with the comorbid presentation. A significant G x E interaction was observed between the BDNF Val66Met and stress response during childhood and college years although the risk for negative affectivity conferred by stress response during childhood was only significant among the Met allele carriers, while stress response during college years was a significant risk factor regardless of the BDNF Val66Met genotype. A significant G x E interaction was also found between the HTR1A C(-1019)G variant and childhood maltreatment. Conclusion: Altogether, our results demonstrate that the BDNF Val66Met variant moderates the effect of stress during both childhood and college years; although this effect seems to be more critical during childhood given that the risk conferred by childhood stress was restricted to the Met allele carriers. We also found that the HTR1A C(-1019)G variant moderates the effect of childhood maltreatment in our study population.

507T

Alcohol Use in the Onset of Rapid-Onset Dystonia-Parkinsonism, DYT12. *B.M. Snively[1], D.F. Hill[1], N. Boggs[1], L. Ozelius[2], K.J. Sweadner[3,4], C. Suerken[1], W.V. McCall[1], A. Brashear[1].* 1) Wake Forest School of Medicine, Winston-Salem, NC; 2) Mount Sinai School of Medicine, New York, NY; 3) Harvard Medical School, Boston, MA; 4) Massachusetts General Hospital, Boston, MA.

Rapid-Onset Dystonia-Parkinsonism (RDP), DYT12, is characterized by rapid onset and stabilization of symptoms without recovery. RDP is caused by mutation of the (3 subunit of the Na+/K+-ATPase gene (*ATP1A3*) and is dominantly inherited with reduced penetrance. RDP is unique among dystonias in that onset has been associated with triggers: life stress, physical stress, exposure to excessive heat, and alcohol use. Each of these 4 categories of triggers has been reported across multiple families. Our focus here is to describe data on alcohol use at the time of RDP onset in 20 affected individuals with one copy of either the recurrent mutation T613M (12 individuals in 3 families) or I758S found in one large family (8 individuals). *ATP1A3* variants were confirmed in patients and family members by direct sequencing; 3 were unaffected carriers. Among the RDP patients, 30% were female; average age (SD) at onset of RDP was 25 (14) years. The RDP severity score (1-4) was 1 in 15% (mild limb dystonia), 2 in 5% (affected arm and bulbar muscles with normal gait), 3 in 30% (also legs affected but walking unassisted), and 4 in 50% (severe bulbar and limb involvement and unable to walk). Among the RDP patients overall, 79% reported a trigger of some type, including 40% reporting alcohol use in the 24 hours before symptom onset. Of these, 75% (6 of 8) said they had been drunk within 24 hours prior to onset. Among the RDP patients stratified by T613M and I758S respectively, 25% and 63% reported alcohol use at the time of onset, and of these, 67% and 80% said they had been drunk. Stratifying by sex, 17% of females (1 of 6) and 50% of males (7 of 14) reported alcohol use at the time of onset; of these, the one female and 83% of males said they had been drunk within 24 hours. Differences in alcohol use by affection status were not evaluated due to limited numbers. In conclusion, the *ATP1A3* gene codes for the (3 subunit of the Na,K-ATPase protein implicated in synaptic physiology in neurons throughout the brain. The association of alcohol as a possible trigger of RDP highlights the interaction of genetic variants and environmental causes of RDP. Further study of alcohol as a trigger of RDP may suggest possible preventive measures and/or treatment of this disabling dystonia. This work is supported by NINDS grant R01NS058949 (AB).

508T

Childhood adversity increases risk for nicotine dependence and interacts with alpha-5 nicotinic acetylcholine receptor genotype specifically in males. *P. Xie[1,3], H.R. Kranzler[4], H. Zhang[2,3], D. Oslin[4], R.F. Anton[5], L.A. Farrer[6], J. Gelernter[1,2,3].* 1) Department of Genetics, Yale University, New Haven, CT; 2) Department of Psychiatry, Yale University School of Medicine, New Haven, CT; 3) VA CT Health care Center, West Haven, CT; 4) Department of Psychiatry, University of Pennsylvania and the Philadelphia VA Medical Center, Philadelphia, PA; 5) Center for Alcohol and Drug Programs, Department of Psychiatry, Medical University of South Carolina, Charleston, SC; 6) Departments of Medicine, Neurology, Ophthalmology, Genetics and Genomics, Epidemiology, and Biostatistics, Boston University Schools of Medicine and Public Health, Boston, MA.

Both genetic and environmental factors influence the risk for nicotine dependence (ND). Several large genome-wide association studies have consistently observed significant association signals in the alpha-5 nicotinic acetylcholine receptor gene (CHRNA5) with smoking behaviors [1,2,3]. Dysfunctional CHRNA5 causes an increase in nicotine intake in rodent experiments [4]. In this study, we genotyped a nonsynonymous SNP, rs16969968, in CHRNA5 for 2206 EAs (1301 men and 905 women) to investigate the presence of a sex-specific gene-by-environment interaction effect of this marker on ND risk. We observed that childhood adversity significantly increased ND risk in both sexes; the effect in women was twice that in men. Significant interactive effects of childhood adversity and rs16969968 genotype were observed in men (OR=1.79, 95% CI=1.18-2.71, P =.0049). No interaction was found in women. We conclude that the effect of childhood adversity on ND risk is moderated by in CHRNA5 in EA men, but not in EA women. [1] Liu, Tozzi, Waterworth, et al. Nat Genet 42:436-440 (2010). [2] Tobacco and Genetics Consortium. Nat Genet 42:441-447 (2010). [3] Thorgeirsson, Gudbjartsson, Surakka, et al. Nat Genet 42:448-453 (2010). [4] Fowler, Lu, Johnson, et al. Nature 471:597-601 (2011).

509T

Interaction of Personality and CHRNA5 in Cocaine Dependence and Cocaine-Induced Paranoia. *T. Zayats[1], BZ. Yang[1], H. Kranzler[2], P. Xie[1], J. Poling[1], D. Oslin[2], L. Farrerr[3], J. Gelernter[1,4].* 1) Department of Psychiatry, Yale University School of Medicine, West Haven, CT, USA; 2) Department of Psychiatry, University of Pennsylvania, and Philadelphia VAMC, Philadelphia, PA, USA; 3) Departments of Medicine, Neurology, Ophthalmology, Genetics and Genomics, Biostatistics and Epidemiology, Boston University Schools of Medicine and Public Health, Boston, MA, USA; 4) Departments of Neurobiology and Genetics, Yale University School of Medicine, New Haven, CT, USA.

Introduction and Purpose: CHRNA5 SNP rs16969968 and personality both contribute to cocaine dependence (CD) risk. However, little is known about how these risk factors act in contribution to the phenotype. We studied the interaction of personality traits and rs16969968 in relation to CD and cocaine-induced paranoia (CIP) in both European Americans (EAs) and African Americans (AAs). Materials and Methods: The sample was comprised of 1432 EA subjects (418 screened controls and 1014 cases) and 1514 AA subjects (336 screened controls and 1178 cases). All participants were assessed with Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA). The diagnosis of CD was made using DSM-IV criteria and subjects with schizophrenia or bipolar disorder were excluded. For analysis of CIP, all subjects were divided into 3 groups: (A) Controls with no CIP (418 EA and 336 AA); (B) Cases without CIP (328 EA and 364 AA) and (C) Cases with CIP (686 EA and 814 AA). Personality was assessed by the NEO-PI-R: a 240-item measure of the Five Factor Model: Extraversion, Agreeableness, Conscientiousness, Neuroticism and Openness to Experience. Analysis was based on logistic regression. All models were adjusted for sex and age. Bonferroni correction was used to account for multiple testing (independent tests only). Results: Among EAs, conscientiousness (controls mean: 50.28, SD: 11.72 and cases mean: 38.77, SD: 10.81) was the only NEO measure that revealed a significant interaction with rs16969968 on CD risk (p=0.001; OR=1.62, 95%CI: 1.23-2.12). Similarly, in EAs, conscientiousness (group A mean: 50.28, SD: 11.72; group B mean: 39.29, SD: 11.08; group C mean: 38.53, SD: 10.68) interacted with rs16969968 on risk of CIP (p=4.0e-04, OR=1.72, 95%;CI: 1.28-2.30 comparing group A versus group C; p=0.042, OR=1.45, 95%CI: 1.06-1.98 for group A versus group B). No significant interaction was observed in the AA sample. Conclusion: Conscientiousness may interact with rs16969968 in CHRNA5 in determining the risk of CD and CIP in EA subjects.

510T

Changes in expression profiles of differentiated SK-N-SH cells resulting from over-expression of *MECP2*_e1 and *MECP2*_e2. *M. Orlic-Milacic[1], L. Kaufman[1], A. Mikhailov[1], P. Gianakopoulos[1], B. Minassian[2], J. Vincent[1].* 1) Neurogenetics, CAMH, Toronto, Canada; 2) Genetics & Genome Biology, The Hospital for Sick Children, Toronto, Canada.

Rett syndrome (RTT) is a progressive neurodevelopmental disorder that affects almost exclusively females with a frequency of 1:10000 births. Features of RTT include loss of speech and motor skills, autistic-like social behavior and intellectual disability. Mutations in the *MECP2* gene are responsible for the majority of Rett syndrome cases. *MECP2* functions in transcription regulation and has two transcript variants, *MECP2*_e1 and *MECP2*_e2. There is accumulating evidence that *MECP2*_e1 is the etiologically relevant variant for RTT. In this study we aim to detect genes that are differentially regulated by two *MECP2* isoforms. For this purpose, the human neuroblastoma cell line SK-N-SH was stably infected by lentiviral vectors over-expressing *MECP2*_e1, *MECP2*_e2, or eGFP. Infected neuroblastoma cells were subsequently differentiated into neurons. RNA from these cells was used for gene expression analysis on human Agilent microarrays. There were ~800 genes with at least 3-fold change in expression level when *MECP2*_e1 was overexpressed in comparison with the eGFP control, with uncorrected p values less than 0.05. When *MECP2*_e2 was over-expressed, levels of ~230 genes changed at least 3-fold. We used quantitative RT-PCR to verify microarray results for 30 of these genes that, in addition to significant change in expression level, are known to be involved in cognitive function and neuronal development. We found significant up-regulation of several genes resulting from over-expression of the *MECP2*_e1 isoform including *NAV3* (5-fold increase), *SRPX2* (17-fold increase), *ITGA3* (5-fold increase), *NDN* (3-fold increase), *NPY1R* (6-fold increase), *NEFM* (2-fold increase), *SYN3* (2-fold increase) and *SEMA3D* (6-fold increase). Both *MECP2*_e1 and *MECP2*_e2 up-regulated *GABRA2* (18 and 3-fold increase, respectively), *KCNA1* (7 and 3-fold increase, respectively), *FOXG1* (6 and 3-fold increase, respectively), *NROB1* (8 and 4-fold respectively) and *FOXP2* (induced by over-expression of both isoforms). Down-regulation of expression in the presence of *MECP2*_e1 was seen with *CNTN4* (2-fold decrease), *UNC5C* (40% decrease) and *RPH3A* (30% decrease). Several genes, while potentially relevant to RTT, had very low levels of expression and could not be validated by this method, including *DLGAP2*, *CRH*, *GABRB1*, *GRIN2A* and *PTCHD1*. Studying differential regulation of genes by different isoforms of *MECP2* can help us to understand RTT etiology and determine the clinical relevance of specific mutations.

511T

Exploring established multiple sclerosis susceptibility loci in a Hispanic cohort. *A.H. Beecham[1], A. Hadjixenofontos[1], P.L. Whitehead[1], I. Konidari[1], D. Martinez[1], E. Lalanne[1], K. Belanger[1], L. Espinoza[1], S. Clarke[1], S. Delgado[2], J.L. Haines[3], M.A. Pericak-Vance[1], J.L. McCauley[1].* 1) John P. Hussman Institute for Human Genomics, Miller School of Medicine, University of Miami, Miami, FL, USA; 2) Multiple Sclerosis Division, Department of Neurology, Miller School of Medicine, University of Miami, Miami, FL, USA; 3) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN, USA.

Multiple sclerosis is a neuroimmunological and neurodegenerative disease. As with most genetic studies of complex disease, the primary focus of genetic studies has been on individuals of European descent. The genetics of MS is no exception to this phenomenon. While there are few epidemiological surveys in MS on individuals originating from Latin America, those that exist suggest a higher prevalence of MS than was previously believed. As the US population continues to grow in part due to immigration from this region, the proportion of individuals with MS of Hispanic/Latino ethnicity will also grow. Understanding the etiology of MS in individuals with Hispanic/Latino ethnicity is therefore an important undertaking. Moreover, the generalization of current findings to individuals of different ancestry is an important and unstudied area. As members of the International Multiple Sclerosis Genetics Consortium (IMSGC), we have access to the Immunochip panel, a custom Illumina iSelect array containing 196,524 SNPs representing loci identified in numerous autoimmune diseases. Using this panel, we have genotyped an initial 200 Hispanic MS samples (~150 cases/ ~50 controls). Our current analyses are limited to examining established MS loci (~24 loci including the well-established HLA-DRB1501) within this dataset. Association analyses, using logistic regression and covariates PCA1-2 from Eigenstrat analysis, indicate that a number of these loci (e.g. HLA-DRB1501: p= 0.004, *PTGER4*: p=0.01, *ZMIZ1*: p=0.04) replicate within our population using a modest significance threshold ($p \leq 0.05$). Of even more interest, these loci are trending with stronger effect sizes in our population compared with the expected range of effect sizes seen in studies of European populations. We acknowledge and appreciate the limited power to detect small effect sizes within known MS risk loci and moreover the small sample size at this stage. However, it is important to point out that from our own collaborative studies within the IMSGC, we find that different European populations have vastly different effect sizes for established MS loci. In this regard, and as our preliminary data might suggest, we may find significantly different effect sizes for certain loci within our Hispanic dataset.

512T

Genome-wide Association Analyses Identifies Novel Loci Associated with the Onset Age among Cases with Late-Onset Alzheimer's disease. *K.L. Hamilton-Nelson[1], A.C. Naj[1], Y.S. Park[1], R. Rajbhandary[1], G.W. Beecham[1], E.R. Martin[1], R. Mayeux[2], J.L. Haines[3], L.A. Farrer[4], G.D. Schellenberg[5], M.A. Pericak-Vance[1], The Alzheimer's Disease Genetics Consortium.* 1) John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL; 2) Taub Institute for Research on Alzheimer's Disease, Columbia University, New York, NY; 3) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 4) School of Medicine, Boston University, Boston, MA; 5) School of Medicine, University of Pennsylvania, Philadelphia, PA.

While studies of late-onset Alzheimer Disease (LOAD) have found that some risk loci, notably *APOE*, may also contribute to earlier age of onset (AAO) of LOAD, variants in newly identified LOAD risk loci such as *CLU* and *PICALM* have not been similarly examined. We examined associations of variants at ten LOAD risk loci (*APOE*, *CLU*, *PICALM*, *CR1*, *BIN1*, *CD2AP*, *EPHA1*, *ARID5B*, the *MS4A* region, *ABCA7*, and *CD33*) from two recent genome-wide association studies (GWAS) to determine if they may also contribute to earlier AAO among 9,160 LOAD cases from 14 datasets of the Alzheimer's disease Genetics Consortium (ADGC). We examined association with AAO among the variants most significantly associated with LOAD at each locus, modeling genotypic effect on AAO linearly using generalized estimating equations (GEE) with adjustment for population substructure and cohort effects.Preliminary analyses confirmed association of APOE regional variation with AAO (*APOC1*, $P=4.7\times10^{-100}$). Variants at several of the other nine LOAD risk loci also demonstrated statistically significant associations ($P<0.005$), including rs6701713 in *CR1* (P=0.0053), rs7561528 in *BIN1* (P= 0.0029), rs561655 in *PICALM* (P=0.0014), and were similarly associated after additional covariate adjustment for sex and number of *APOE* , 4 alleles. As genomic variation may contribute to severity (i.e., earlier AAO) and not necessarily risk of disease, we also examined 2,324,442 genotyped and imputed SNPs with associations of $P>10^{-5}$ from the ADGC GWAS of LOAD risk to identify previously unknown genetic contributors to AAO of LOAD. In a model adjusting for sex and dosage of the *APOE* , 4 allele, with which associations in *APOE* faded as expected, the AD candidate gene *VDR* demonstrated association with AAO near genome-wide significance ($P= 6.18\times10^{-7}$), as did variants in several other regions, including a chromosome 8q24.11 region including *RAD21* ($P=2.38\times10^{-6}$), chromosome 6q16.1 ($P= 6.17\times10^{-6}$), *CIB4* ($P=7.70\times10^{-6}$), and *ABCC4* ($P=9.25\times10^{-6}$). In AAO analyses among LOAD cases, we confirmed associations of variation near 19q13.32 (*APOE* region) with genome-wide statistical significance and observed associations with AAO at risk loci *CR1*, *BIN1*, *PICALM*, and *ABCA7*, and detected several strong associations among genes with weak or no association with risk in the ADGC, including the previous AD candidate gene *VDR*. Potential roles for these SNPs merit further investigation, including replication in other GWAS datasets.

513T

A Novel Set of Genetic Variants Associated with Autism Spectrum Disorder (ASD) Susceptibility Is Revealed by Application of the Pathway-PDT. *Y.S. Park[1], M. Schmidt[1], E.R. Martin[1], P.W. Whitehead[1], I. Konidari[1], M.L. Cuccaro[1], J.L. Haines[2], M.A. Pericak-Vance[1], R.H. Chung[1].* 1) John P. Hussman Institute of Human Genomics, University of Miami Miller School of Medicine, Miami, FL, USA; 2) Vanderbilt Center for Human Genetics Research, Vanderbilt University, Nashville, TN, USA.

Pathway analysis, jointly considering the effect of a set of genes, provides an alternative to single-locus association tests in genome-wide association studies (GWAS). This has become an attractive secondary analysis that can uncover associations missed by single-locus screens. However, most pathway analyses conducted thus far focused on unrelated cases and controls or used only P-values calculated from GWAS, which is not optimal for family-based study as LD and family structures can't be specifically modeled in the analysis. We recently developed a novel family-based pathway analysis tool, Pathway-PDT, that uses raw genotype data from nuclear families with one or more affected siblings. Pathway-PDT tests for the significance of an enriched set of genes in a pathway relative to the background genes. We applied Pathway-PDT to two independent family datasets for ASD - the Hussman Institute for Human Genomics (HIHG) dataset and the Autism Genetic Resource Exchange (AGRE) dataset. The joint datasets contained a total of 2,153 individuals with ASD and 2,847 unaffected family members from 1,444 independent families. Markers were imputed based on HapMap3 data with IMPUTE2, which resulted in 817,127 common autosomal SNPs. We tested a total of 4,312 gene sets defined by the Gene Ontology (GO) Database by their associated biological processes, cellular components and molecular functions in humans. Interestingly, the set of genes involved in the positive regulation of response to external stimulus (GO:32103) was significant in the Pathway-PDT analysis even when the p-value was adjusted for multiple testing (Pathway-PDT $P<0.0005$, FDR adjusted P=0.012, permutation adjusted P=0.0095). The gene set includes interesting subsets of genes involved in the regulation of the inflammatory response (GO:50729, Pathway-PDT $P<0.0005$) and chemotaxis (GO:50921, Pathway-PDT P = 0.0025), that have been associated with autism in other studies. Within these gene sets are several genes, such as cadherin 13 (CDH13) and protein kinase C alpha (PRKCA), that we previously reported as possibly associated with autism using the noise-reduction GWAS analysis. In conclusion, our analysis of ASD GWAS data using Pathway-PDT identified significant association for the gene set for the positive regulation of response to external stimulus. This suggests that genes in this pathway should be looked at more closely and, in particular, considered jointly for association with ASD.

514T

Fine-mapping of the MHC association in multiple sclerosis using imputation. *NA. Patsopoulos[1,2,3], PL. De Jager[2, 3], PIW. de Bakker[1, 3, 4, 5] on behalf of ANZgene, GeneMSA, IMSGC.* 1) Medicine Dept., Division of Genetics, Brigham & Women's Hospital, Harvard Medical SChool, Boston, MA; 2) Program in Translational NeuroPsychiatric Genomics, Institute for the Neurosciences, Department of Neurology, Brigham & Women's Hospital, Boston, MA; 3) Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, MA; 4) Department of Medical Genetics, Division of Biomedical Genetics, University Medical Center, Utrecht, The Netherlands; 5) Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, The Netherlands.

Background: Multiple sclerosis (MS) is a neurodegenerative inflammatory disease with a heritable component. *HLA-DRB1*15:01* has been identified as the strongest association with MS. Many studies have suggested additional independent associations in HLA class I and II genes, implicating *HLA-A*02*, *HLA-B*44*, *Cw*05/08*, and *DQB1*03/06*, but the evidence is not conclusive.

Methods: We have used a novel imputation method to impute HLA class I and II alleles and their corresponding amino acids, using 6 GWAS data sets. The method leverages a reference panel of 2,767 individuals of European descent with information for SNPs and classical alleles at *HLA-A*, *-B*, *-C*, *-DRB1*, *-DQA1*, *-DQB1*, *-DPA1* and *-DPB1*. We have imputed 4,220 cases and 7,296 controls in total. We used logistic regression to identify independent HLA associations at 4-digit resolution and to test if amino acid polymorphisms within classical HLA molecules can explain the observed associations.

Results: We successfully imputed 103 classical HLA alleles at 4-digit resolution, and 335 amino acid positions. *HLA-DRB1*15:01* was the most significant effect (OR=3.0, p=1.0e-183). Conditional on *DRB1*15:01*, we found another three DRB1 alleles that were significantly associated: **03:01* (OR=1.37, p=1.48e-12), **13:03* (OR=2.39, p=3.00e-11), **04:04* (OR=1.59, p=2.68e-07). Adjusting for the *DRB1* gene effect, we identified another independent effect in *HLA-A*02:01* (OR=0.74, p=1.26e-26). Adjusting for *DRB1* and *HLA-A*02:01*, we found *DPB1*03:01* (OR=1.43, p=5.27e-13). Collectively, these classical alleles explain up to 14% of the trait variance (Nagelkerke r2). After testing individual amino acid positions in the HLA proteins, the most association position is an amino acid (-5) in the signal peptide of *DQ*) *1* (OR=3.07, p=4.78e-180 for Leu), consistent with near-perfect LD (r^2=0.98) between Leu-5 and *DRB1*15:01*. A model based on the four best independent DR) 1 amino acid positions was not better than a model based on the four classical alleles in *DRB1*.

Conclusions: We have replicated known associations of HLA class I and II alleles in MS and identified an independent *HLA-DPB1* association.

515T

From linkage to sequencing: Using cross-family IBD sharing to refine susceptibility loci in Tourette Syndrome multi-generational families. *V. Ramensky[1], D. Yu[2], S. Service[1], C. Mathews[3], P. Heutink[5], B. Oostra[6], P. Sandor[7], C. Barr[8], R. Kurlan[9], D. Pauls[2], N. Cox[4], N. Freimer[1], J.M. Scharf[2], TSAICG (Tourette Syndrome Association International Consortium for Genetics), Bayside, NY.* 1) Center for Neurobehavioral Genetics University of California, Los Angeles, CA; 2) Psychiatric and Neurodevelopmental Genetics Unit, Center for Human Genetic Research, Massachusetts General, Hospital Harvard Medical School, Boston, MA; 3) Department of Psychiatry, University of California, San Francisco, San Francisco, CA; 4) Departments of Medicine and Human Genetics, University of Chicago, Chicago, IL; 5) VU University Medical Center, Amsterdam, Netherlands; 6) Erasmus University Medical Center, Rotterdam, Netherlands; 7) Department of Psychiatry, University Health Network and University of Toronto, Toronto, Canada; Division of Brain, Imaging, and Behaviour Systems, Toronto Western Research Institute, University Health Network, Toronto, ON, Canada; 8) Genetics and Development Division, Toronto Western Research Institute, University Health Network, Toronto, ON, Canada; Program in Neurosciences and Mental Health, The Hospital for Sick Children, Toronto, ON, Canada; 9) Atlantic Neuroscience Institute, Overlook Hospital, Summit, NJ.

The recent increased focus on rare susceptibility variants in non-Mendelian disorders has resulted in a resurgence of interest in multi-generational, phenotypically loaded families. However, new approaches are needed to optimize the selection of individual family members for sequencing and to refine previously identified linkage regions that are too large for comprehensive, targeted next-generation sequencing. Here, we present analyses of large, multi-generational families with Tourette Syndrome (TS) as an example in which high-density SNP genotyping can be used to identify shared identity-by-descent (IBD) regions across apparently unrelated individuals from different families contributing to a genome-wide significant non-parametric linkage signal. TS is a highly heritable, childhood-onset neuropsychiatric disorder that causes substantial physical and psychosocial morbidity. Prior non-parametric linkage analyses of 771 individuals (213 affecteds) from 15 large, unrelated, multi-generational TS families of European ancestry identified a genome-wide significant linkage peak on chromosome 2p (Z= 5.2, p=9.8x10-8). The region of maximal linkage spanned 22 Mb, with the 95%CI extending across the entire 2p arm. Two additional regions on chromosome 6p and 17p achieved suggestive levels of significance. We used Beagle FastIBD to perform genotype phasing and subsequent IBD detection of Illumina 610Quad genotypes in 201 individuals (136 affected, 65 unaffected) from these 15 TS families. At each marker, we calculated the differential IBD rate (DIBDR) equal to the IBD rate of affected-affected cross-family pairs minus the corresponding value for affected-unaffected or unaffected-unaffected pairs. The strongest cross-family DIBDR peaks were observed on chromosomes 2 and 17, both within the previously identified linkage critical regions, and on chromosome 6, within the 95% CI for that linkage peak. Statistical significance was assessed by permutation tests. The obtained results confirm earlier findings, dramatically narrowing the region for these linkage peaks (1-4 Mb each) and indicate that apparently unrelated TS families may share common disease haplotypes. The method highlights a useful approach for transitioning from linkage data to targeted sequencing aimed at identifying the underlying susceptibility genes for non-Mendelian disorders.

516T

A family-based association and brain expression analysis of the reading disabilities candidate gene DYX1C1. *C. Tran[1], K. Wigg[2], F. Yu[2], L. Gomez[2], B. De Souza[2], T. Cate-Carter[3], A. Pitch[3], E. Kerr[3], M. Lovett[3], L. Field[4], B. Kaplan[5], C.L. Barr[1, 2, 3, 6].* 1) Institute of Medical Science, University of Toronto, Toronto, ON, Canada; 2) Division of Genetics and Development, Toronto Western Hospital, Toronto, ON, Canada; 3) Program in Neurosciences and Mental Health, Hospital for Sick Children, Toronto, ON, Canada; 4) Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 5) Department of Pediatrics, University of Calgary, Calgary, AB, Canada; 6) Department of Psychiatry, University of Toronto, Toronto, ON, Canada.

Reading disabilities (RD) refer to a specific difficulty in learning to read despite normal intelligence and access to education. Family and twin studies have shown that RD have a significant genetic component while linkage studies have identified a susceptibility locus on chromosome 15q21. Dyslexia susceptibility 1 candidate gene 1 (DYX1C1), located in this region, has been proposed as a RD candidate gene based on a chromosomal translocation. However, subsequent association studies have yielded mixed results. This study investigated the role of DYX1C1 in RD through family based association and brain tissue expression analyses. In our sample of 441 families from Toronto, Ontario, each with at least one child exhibiting reading difficulties, 10 single nucleotide polymorphisms (SNPs) in DYX1C1 were genotyped. A replication sample of 100 sibling pair families from Calgary, Alberta was also genotyped for the 10 SNPs. Categorical, haplotype and quantitative analyses were performed using Haploview, Transmit and FBAT, respectively. To examine the association between DYX1C1 variants and expression levels, 40 post-mortem individuals were genotyped and real-time PCR was performed using tissues from the dorsolateral prefrontal cortex, caudate nucleus and hippocampus. In the categorical analysis of the initial sample, rs692691 showed a trend towards association with RD (p=0.058). However, this did not hold up after correction for multiple testing (p=0.334) and was not replicated in the additional sample. The -3A/1249G haplotype was significantly undertransmitted in the initial sample (p=0.007), differing from the haplotype results of previous studies. In the quantitative analysis, rs11629841 was associated with lower performance in expressive language (p=0.034), while rs1789126 was associated with receptive language (p=0.034) and phonological awareness (p=0.044). There was no correlation between the genotyped DYX1C1 variants and expression levels in the three brain regions. Our results do not provide strong evidence for DYX1C1 as a RD candidate gene. Although some markers showed a trend towards association, these were not the associated markers identified in previous studies. It is possible that the observed association may be a signal pointing to another susceptibility gene within the same region.

517T

Refinement of the chromosome 3p22 region of interest for bipolar affective disorder and identification of a putative candidate gene. *R. Secolin[1], L. F. B. Mella[2], M. L. Santos[1], P. Dalgalarrondo[2], C. E.M. Banzato[2], I. Lopes-Cendes[1].* 1) Medical Genetics Dept , University of Campinas - UNICAMP, Campinas, SP, Brazil; 2) Medical Psychology and Psychiatry Dept, University of Campinas - UNICAMP, Campinas, SP, Brazil.

Background: BPAD is a common psychiatric illness, with a prevalence of 0.8-2.6 % in the general population. Genetic factors are known to contribute to the etiology of BPAD. Several studies, including our own previous results, have point to a region on chromosome (ch) 3p22 as a candidate to contain a susceptibility gene for BPAD . **Objectives:** We aim to refine the region of interest on ch 3p22 by genotyping additional SNPs as well as to try to identify putative candidate genes for BPAD within this region. **Methods:** We evaluated 74 families, with a total of 411 individuals, including 96 patients who fulfilled clinical criteria for BPAD according to DSM-IV classification. We genotyped a total of 94 SNPs. Statistical power was verified by TDT POWER CALCULATOR. Genotyped data and minor allele frequency estimation were processed by JINGLEFIX program. Mendelian inconsistencies and Hardy-Weinberg equilibrium were evaluated by PEDCHECK and HAPLOVIEW softwares, respectively. Family based association analysis was performed by transmission disequilibrium test (TDT) using TDTAE software and statistical results were adjusted using Bonferroni correction. In addition, we performed gene expression analysis using peripheral blood samples from 12 probands with BPAD who had been previously genotyped for a SNP of interest. We used Kruskal-Wallis test to evaluate different gene expression among groups. **Results:** Our family sample showed statistical power higher than 80% to detect association. We found a single significant association signal for SNP rs166508 ($p_{corrected}$ = 0.0187). Since this SNP is located within an intronic region of integrin alpha 9 (*ITGA9*) gene, we evaluated *ITGA9* expression in BPAD probands showing the three rs166508 genotypes found. We observed an increased gene expression in patients with the *A/A* genotype (Kruskal-Wallis pvalue = 0.0339). **Conclusions:** We demonstrated that SNP rs166508, located within intron 15 of the *ITGA9* gene, is associated with BPAD. In addition, we detected an increased expression of *ITGA9* in patients with the *A/A* genotype, which is associated with an increased transmission of *A/A* genotype to patients in our family sample. *ITGA9* encodes a subunit of the alpha 9 integrin, an integral membrane glycoprotein which is a receptor for the nerve growth factor, neurotrophin 3 and brain-derived neurotrophic factor. Supported by FAPESP, São Paulo, Brazil.

518T

SNPs genotyping and homozygosity mapping to identify candidate loci in a consanguineous family affected by a congenital muscular dystrophy with hyperlaxity (CMDH). *M. Tetreault[1], M. Vanasse[2], B. Brais[1].* 1) Laboratoire de neurogénétique de la motricité, Centre d'Excellence en Neuromique de l'Université de Montréal, CRCHUM, Montréal, Québec, Canada; 2) Clinique des maladies neuromusculaires, Centre de réadaptation Marie-Enfant, Hôpital Sainte-Justine Hospital, Montréal, Québec, Canada.

Over the past two decades, there have been major advances in defining the genetic bases of congenital muscular dystrophies (CMD). Genetic research has allowed the identification of more than 14 genes responsible for various forms of CMDs. Despite the great progress in this field, there are still a significant percentage of cases for which the mutated gene is unknown. This is particularly the case for milder forms. We recruited a consanguineous French-Canadian family from Southwestern Quebec affected by CMDH. A previous microsatellite genome wide-scan (GWS) has linked this family to 32cM region on chromosome 3p23-21. Most obvious candidate genes have been rule out in this region. A SNPs genome scan (Illumina OmniExpress) have identified six homozygous region shared by the two affected members. Among them, a 17Mb region on chromosome 3p24.3p21.3 was identified. We believe this study combine to a resequencing approach will enable us to identify a novel gene for CMDH. The uncovering of the genetic bases of muscular dystrophies serve as the essential original building blocks on which successful new therapeutic approaches can be designed.

519T

Identification of the tRNA methyltransferase gene NSUN2 as the gene for a new form of autosomal recessive intellectual disability. *J.B. Vincent[1], M.A. Khan[2], M.A. Rafiq[1], A. Noor[1], S. Hussain[3], V. Rupp[4], A.K. Vincent[1], J. Flores[3], G.E. Ishak[5], D. Doherty[6], R. Weksberg[7], M. Ayub[8], C. Windpassinger[4], S. Ibrahim[9], M. Ansar[2].* 1) Molecular Neuropsychiatry & Development Lab, Neurogenetics Section, Centre for Addiction and Mental Health, Toronto, ON, Canada; 2) Department of Biochemistry, Quaid-I-Azam University, Islamabad, Pakistan; 3) Wellcome Trust Centre for Stem Cell Research Tennis Court Road Cambridge, CB2 1QR United Kingdom; 4) Institute of Human Genetics, Medical University of Graz, Graz, Austria;; 5) Department of Radiology, Seattle Children's Hospital, University of Washington, Seattle;; 6) Division of Genetics and Developmental Medicine, University of Washington, Seattle;; 7) The Centre for Applied Genomics & Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto;; 8) Tees, Esk and Wear Valleys NHS Foundation Trust and School for Health and Medicine, University of Durham, United Kingdom;; 9) Dept. of Paediatrics, Aga Khan University, Karachi, Pakistan.

We have used genome-wide genotyping to identify a homozygosity-by-descent locus in a consanguineous Pakistani family with three intellectual disability (ID) plus distal myopathy to a 2.6Mb region on 5p15.32-p15.31, and have identified a missense mutation, Gly679Arg, at a conserved residue within the gene NSUN2. This gene encodes a methyltransferase that catalyzes formation of 5-methylcytosine at C34 of tRNA-leu(CAA), as well as functioning in spindle assembly during mitosis as well as chromosome segregation. The Gly679 residue in orthologous proteins is highly conserved across the animal kingdom. Analysis of the Gly679Arg mutation through Myc-tagged constructs carrying the mutation and overexpressed in HeLa, COS7 and HCC1954 breast cancer cells indicates that the effect of the mutation at the cellular level appears to be the prevention of NSUN2 localizing within the nucleolus, and occasionally the nucleus also. Thus it is likely that the role of NSUN2 within the nucleolus is crucial to normal neurodevelopmental processes.

520T

Serotonin gene expression significant changes could it be evidence for psoriasis related to depression? *S. Ghasemi[1], G. Ahangari[1], H. Mortazavi[2], R. Torabi[1], M. Mohamadian[1].* 1) Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran; 2) Department of Dermatology, Razi hospital, Tehran university of medical science, Tehran, Iran.

Serotonin (5HT) plays roles as a neurotransmitter and a neuromodulator which its modulation at synapses is thought to be a major action of several classes of pharmacological antidepressants. Serotonin receptor subtypes are related to depression and also have been shown to be present in certain cells of the immune system. Since serotonin also has a proliferate effect on various types of cells and psoriasis is a hyperproliferative inflammatory disease, we hypothesized that serotonin and its receptors are related to pathogenesis of psoriasis in this way serotonin can be a mediator between psoriasis, depression and stress. The aims of this study are evaluation of 5HT3a gene expression in peripheral blood mononuclear cells (PBMC) of psoriatic patients in comparison with normal individuals by Real-time PCR and finding the probable mutations in this gene via sequencing.

The PBMC was separated from whole blood by Ficoll-hypaque. The total cellular RNA was extracted and the cDNA was synthesized. This process was followed by real-time PCR using primer pairs specific for 5HT (3a) serotonin receptor mRNA and beta-actin as internal control. Our results show that relative expression of 5HT (3a) has significant decrease in psoriasis patients with comparison of healthy individuals. We are confirming our data by gathering more samples and sequencing the RNA obtained from the patients. In conclusion, 5HT3a significant decrease or any mutations on its gene can reveal an important role of this receptor in pathogenesis of psoriasis.

521T

A comprehensive nation-wide epidemiological survey for Pelizaeus-Merzbacher disease and associated disorders in Japan. *K. Inoue[1], Y. Numata[1], T. Ohkubo[1], E. Arima[1], A. Iwaki[2], K. Kurosawa[3], J. Takanashi[4], K. Deguchi[1,5], T. Yamamoto[6], H. Osaka[7].* 1) Dept MR & BD Res, Natl Inst Neurosci, NCNP, Kodaira, Tokyo, Japan; 2) Med Inst Bioregulation, Kyushu Univ, Fukuoka, Japan; 3) Genetics, Kanagawa Children's Medical Center, Yokohama, Japan; 4) Pediatrics, Kameda Medical Center, Kamogawa, Japan; 5) Deguchi Pediatric Clinic, Ohmura, Japan; 6) TIIMS, Tokyo Women's Medical Univ, Tokyo, Japan; 7) Neurology, Kanagawa Children's Medical Center, Yokohama, Japan.

Purpose: Pelizaeus-Merzbacher disease (PMD) is the most common form of congenital hypomyelinating disorders (CHD) in the CNS, but the exact frequency of this group of diseases is currently unknown. In this study, we conducted a nation-wide epidemiological study in Japan to determine the prevalence and incidence of the CHD.

Methods: We enrolled all hospitals with pediatric neurology division and institutes for severely retarded children in Japan, the total of 918 institutions. The study was composed of primary and secondary surveys: the former was a brief questionnaire to determine the presence of patients with CHD in the past 1 year, and the latter was a detail survey to obtain clinical information of the CHD patients. This study was approved by IRB at National Institute of Neurology and Psychiatry, Japan.

Results: We identified 107 patients with CHD with detailed clinical information. The prevalence for CHD was 0.8 per 100,000 (0~20 y.o.) and the incidence was 1.6 per 100,000 live births. Male to female ratio was 5.7:1 and the average age of the patients was 11.8±9.9 y.o. The clinical diagnoses included PMD (63%) and Pelizaeus-Merzbacher-like disease (8%), while 18% remained undiagnosed. Molecular testing was performed on 75% of patients, of which 61% showed *PLP1* mutations, while 29% showed no detectable mutations. Together, the incidence of PMD with known *PLP1* mutation was at least 1.44 per 100,000 male live births. Clinically, 75% of the patients previously had nystagmus, which is currently present in 56%. Similarly, 72% had a previous history of hypotonia, of which only 54% are currently hypotonic. In contrast, spasticity was continuously present in 56% of the patients.

Discussion: Little etiological studies have been conducted for PMD and associated disorders. As far as we know, this is the first report to determine the prevalence and incidence of these disorders by a comprehensive nation-wide survey. The incidence of genetically confirmed PMD was approximately 20 times less than Duchenne muscular dystrophy. About 20% of the CHD remain undiagnosed, suggesting the presence of other disease-causing genes unveiled.

522T

A Deep Intronic Mutation In An Ataxia Telangiectasia Patient Identified By Genomic Resequencing Of The *ATM* Region. *S. Cavalieri[1], R.A. Gatti[2], A. Brusco[1,3].* 1) SCDU Medical Genetics S.Giovanni Battista Hospital, Torino, Italy; 2) UCLA School of Medicine Department of Pathology and Laboratory Medicine, Los Angeles,California; 3) Department of Genetics, Biology and Biochemistry, University of Torino, Italy.

Recent development of Next Generation Sequencing (NGS) techniques is changing the approach to search for mutations in human genetic diseases. We decided to apply NGS to study a patient affected by Ataxia Telangiectasia (A-T), in which one of the two expected mutations was not found after DHPLC, cDNA sequencing and MLPA screening. Linkage analysis showed the mutated unknown allele was maternally inherited. The entire *ATM* genomic region (160 Kb) was divided in 32 partially overlapping fragments of 4-6 kb and amplified by long-range PCR (Takara) in the patient and her mother. Fragments were quantified, pooled in equimolar amounts and sequenced by the Baseclear service using an IlluminaGAIIX platform. Raw data were analyzed by the CLC Genomics workbench software. Average coverage was 300X. Comparison between the mother and daughter sequences, allowed to identify six variants shared in heterozygosis by the two genomes and not reported in the SNP (ver 131) database. All were intronic, but only one c.1236-405C>T (coverage 670X), located in IVS11 was predicted to be pathogenic as it affected splicing (BDGP, www.fruitfly.org/seq). This mutation creates a novel donor splice site (score 0.22 wt to 1.00 mutated) in position -405 of intron 11 and as a result may activate an alternative acceptor splice site in position -619 of intron 11 (score 0.71). cDNA analysis in the patient using forward and reverse primers in exon 11 and 12, showed a normal band of 260 bp and a larger extra band of 472 bp. Sequence analysis showed that the larger band contained a “pseudoexon” of 212 bp, which used the alternative 5' and 3' splice sites at positions -619 and -405 of intron 11. This insertion results in a frameshift on the cDNA and premature stop codon. This approach based on resequencing of a genomic region is a powerful strategy to identify rare mutations that affect splicing, and it is suitable for a selected group of patients in which DHPLC or cDNA sequencing plus MLPA was unsuccessful. The identification of splicing variants is important for their possible therapeutic targeting using AMO (Antisense Morpholino Oligonucleotides).

523T

Whole Exome Sequencing in First Degree Cousin Pairs with Early Age-at-onset Bipolar Disorder. *D. Chen, N. Akula, C.J.M. Steele, L. Kassem, F.J. McMahon, The Bipolar Genome Study (BiGS).* Human Genetics Branch, National Institute of Mental Health, Intramural Research Program, National Institutes of Health, Bethesda, MD.

Background: Though bipolar disorder is highly heritable, recent estimates of total genetic variance accounted for by common variants have been low. Identification of uncommon and rare variants may increase the total genetic variance explained. Methods: In this pilot study, we identified 4 first-cousin pairs with bipolar I disorder beginning before age 16. Cases were drawn from 4 distinct families of European-ancestry ascertained by the NIMH Genetics Initiative. Exome sequencing was performed with SOLiD technology. Fragment libraries were made with 5ug genomic DNA, followed by exome capture using the Agilent SureSelect Human All Exon 50MB kit. Between 95M and 120M single-end reads were generated per individual (4 exomes/SOLiD Quad). Of these, 65M-80M reads mapped to the reference genome (hg19). Duplicated reads were removed using PICARD, and non-duplicated reads were re-calibrated using GATK. Of 82K called SNVs, ~26K were filtered out due to quality control or minor allele frequencies greater than 10% in the 1000 Genomes Database. The remaining ~56K variants were then mapped to regions of the genome shared identical-by-descent (IBD) between cousin pairs, using BEAGLE IBD. Results: The proportion of single nucleotide variants shared by cousin-pairs exceeded the proportion of the genome shared IBD by 150 to 176%. Approximately 147 variants mapped within IBD regions in all 4 cousin pairs, 100% of which could be phased. One variant, predicted to be damaging by SIFT, was found to reside on the haplotype that was actually shared IBD within that cousin pair. Conclusions: High throughput sequencing of cousins with early onset bipolar disorder reveals an excess of single nucleotide variants within shared genomic regions. Uncommon and rare variants may provide new insight into the etiology of bipolar disorder.

524T

Complete Screening of *GBA* mutations in Brazilian Parkinson's disease patients. *B.C. Guimarães[1], A.V. Santos[1], J.M. Santos[1], M. Campos Jr.[1], F.L. Santos[1], A.L.Z. Rosso[2], D.H. Nicaretta[3], J.S. Pereira[4], D.J. Silva[5], C.B. Santos-Rebouças[1], M.M.G. Pimentel[1].* 1) Genetics, State University of Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil; 2) University Hospital Clementino Fraga Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; 3) Gama Filho University, Rio de Janeiro, Brazil; 4) Faculty of Medical Sciences, Biomedical Center, State University of Rio de Janeiro, Rio de Janeiro, Brazil; 5) Neuroscience Nucleus, Hospital of Clinics, Federal University of Goias, Integrated Institute of Neuroscience, Goias, Brazil.

Parkinson's disease (PD) is a common progressive neurodegenerative movement disorder, being characterized by the selective degeneration of dopaminergic neurons within the substantia nigra pars compacta and the presence of intracellular protein inclusions called Lewy bodies in the surviving neurons. PD has a complex etiology which may involve gene-environment interactions and multiple susceptibility genes. In this context, loss-of-function mutations in the glucocerebrosidase gene (*GBA*) have been well-validated as susceptibility factors for PD. This gene is located on 1q21 and comprises 11 exons that encode the lysosomal enzyme glucocerebrosidase. The main objective of our study was to investigate if alterations in the GBA gene constitute a predisposing factor for the development of PD in the Brazilian population. For this, a Brazilian cohort of 126 unrelated PD patients (24 familial and 102 sporadic cases; mean age: 66.4 ± 11.4) were screened for *GBA* mutations by complete sequencing of the gene's exons. Three mutations and one recombinant allele, previously associated with PD, were detected (A456P, L444P, N370S and RecNciI), as well as, one newly identified variant (W378C), in a total of 8 patients (6.3%). In silico analysis on the effect of amino acid substitutions in the protein function, using the program PolyPhen-2, showed that W378C variant might have a pathogenic role. Our results, until now, have been strengthen the association between this gene and PD in the Brazilian population, in addition to supporting the hypothesis that alterations in the GBA gene represent a significant genetic susceptibility factor for the development of PD. However, it is still important to perform segregation analysis of the mutations identified in order to evaluate the penetrance of these mutations.

525T

Next-generation sequencing of known and putative susceptibility genes for schizophrenia and autism spectrum disorders to detect rare high-penetrant risk variants. *E.M. Kenny, S. Furlong, P. Cormican, C. Fahey, R. Anney, G. Donohoe, A.P. Corvin, L. Gallagher, M. Gill, D.W. Morris.* Neuropsychiatric Genetics Research Group, Dept. of Psychiatry and Institute of Molecular Medicine, Trinity College Dublin, Dublin, Ireland.

Schizophrenia (SZ) and autism spectrum disorders (ASD) are complex neurodevelopmental disorders that share certain phenotypes including cognitive deficits and some behavioural characteristics. Such similarities suggest that these disorders may share an underlying pathology and thus may share some genetic risk variants. This study involves next-generation sequencing of the exonic regions of 215 potential susceptibility genes in an Irish sample of 150 cases of ASD, 300 cases of SZ and 300 controls, in order to identify single nucleotide polymorphisms, indels and structural variants contributing to one or both disorders. A multiplex target enrichment method is used whereby DNA samples are multiplexed together using DNA indexes/barcodes and enriched for the exonic regions of these genes using the Agilent SureSelect target enrichment method. This is followed by 80bp paired-end sequencing in a single lane of an Illumina GAII. Gene selection comprised of five categories: 1) Interactors of NRXN1, 2) Interactors of DISC1, 3) Genes within the Glutamate Receptor Complexes; NMDA, mGluR5 and AMPA, 4) Cell adhesion molecules and 5) Functional and Positional Candidates. Analysis of the pilot set of samples indicates that the approach undertaken is successful with an even spread of sequence information for 24 indexed samples per lane, >8X coverage for 84% of target regions and overall SNP concordance with previous GWAS data (Affymetrix 6.0) of 99.3%. A preliminary SNP analysis of 219 SZ cases and 206 controls has identified an excess of rare (nonsense) mutations in cases. We are currently validating these findings using capillary sequencing and details of these analyses will be presented.

526T

Screening of *PARKIN* mutations in Brazilian patients with early onset Parkinson's disease. *K.V. Moura[1], M. Campos Jr.[1], F.C. Rodrigues[1], F.L. Santos[1], A.L.Z. Rosso[2], D.H. Nicaretta[3], J.S. Pereira[4], D.J. Silva[5], C.B. Santos-Rebouças[1], M.M.G. Pimentel[1].* 1) Genetics, State University of Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil; 2) University Hospital Clementino Fraga Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; 3) Gama Filho University, Rio de Janeiro, Brazil; 4) Faculty of Medical Sciences, Biomedic Center, State University of Rio de Janeiro, Rio de Janeiro, Brazil; 5) Neuroscience Nucleus, Hospital of Clinics, Federal University of Goias, Integrated Institute of Neuroscience, Goias, Brazil.

Parkinson's disease (PD) is one of the most common neurodegenerative disorders associated with aging, reaching 2% at age 70. It is a disease characterized by progressive degeneration of nigra dopaminergic neurons in the basal ganglia and the presence of cytoplasmic protein inclusions known as Lewy bodies and neurites in surviving neurons. The etiology of PD is complex, with the involvement of gene-environment interactions. Knowledge achieved in the last 13 years about the genetic basis of PD clearly shows that genetic factors play an important role in the etiology of this disorder. In the present study, we screened mutations in *PARKIN* gene, whose product acts as an E3 ubiquitin ligase and has a role in mitochondrial biogenesis, in 136 unrelated Brazilian patients with early onset PD, by direct sequencing of PCR products. The presence of sequence variants was evaluated in exons 1 to 12 of *PARKIN* gene, where were found seven missense variants: c.245C>A, c.434G>A, c.659A>G, c.1016C>T, c.1021C>T, c.719C>T (each one in a patient) and c.1310C>T (in two patients). Among the mutations that alter the amino acid sequence, four variants have never been described: c.434G>A, c.659A>G, c.1016C>T and c.1021C>T. The exon 4 c.434G>A variant was identified in a patient that is a compound heterozygote (PAR2391) who also had the polymorphism c.500G>A in the same exon of the *PARKIN* gene. The c.659A>G mutation was found in only one patient (PAR1459), a sporadic case of the disease, in a heterozygous state. We identified two new variants in *PARKIN* exon 9, the alteration c.1016C>T in a patient (PAR2384) that has a family history of PD and the mutation c.1021C>T in two patients (PAR2143 and PAR2256) that are isolated cases of the disease. In silico analysis on the effect of amino acid substitutions in the protein function, using the program PolyPhen-2, showed that between the seven missense mutations identified, c.434G>A, c.1016C>T, c.719C>T and c.1310C>T variants are probably deleterious. Our data show that *PARKIN* point mutations are common in Brazilian early onset PD patients and the frequency of missense variants observed by us (5,9%) was similar to others worldwide.

527T

Population-based association study of the Dopamine Receptor D4 (DRD4) gene in Attention Deficit Hyperactivity Disorder (ADHD) and response inhibition. *S-M. Shaheen[1], Sarah. Ickowicz[1], J. Crosbie[2], A. Paterson[1], R. Schachar[2], Bingbin. Li[1], P. Arnold[1,2].* 1) Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, ON, Canada; 2) Department of Psychiatry,The Hospital for Sick Children, Toronto, ON, Canada.

ADHD is a common neurodevelopmental disorder characterized by inattention, impulsivity, and hyperactivity that commences in childhood and often persists throughout life (Gizer and Waldman, 2009). Dopamine receptor D4 (DRD4) gene polymorphisms have previously been associated with ADHD (Kim, 2009; Congdon, 2008), but few studies have examined the specific association between DRD4 and important endophenotypes of ADHD, including the stop signal task (SST). We hypothesized that in children and adolescents, polymorphisms of the DRD4 gene including single nucleotide polymorphisms (SNPs) and a widely studied variable number tandem repeat in exon 3 (VNTRIII) are associated with inhibitory control measured through the SST. DNA samples were extracted from saliva collected from participants aged 7 to 17 at the Ontario Science Centre, who completed an SST task and self-report questionnaire. 177 samples, comprising children with the highest and lowest SST scores, were genotyped on a genome-wide array. Two DRD4 promoter SNPs, rs3758653 and rs1800955, as well as the VNTRIII polymorphism were then directly genotyped in a larger sample of 364 children (182 high SST, 182 low SST). Samples successfully genotyped by both genome-wide and single SNP or VNTR genotyping showed complete concordance between methods. Association analysis was completed with Plink (Purcell, 2007) and Haploview (Barrett, 2005). The two DRD4 SNPs were not found to be significantly associated with SST performance. There was a trend for carriers of the 7-repeat allele of the exon III VNTR to perform better on the SST (p=0.06). Small sample size (n=364) may have limited the detection power for this study. Further research should be aimed at elucidating genetic relationships between variation in the DRD4 gene, response inhibition, and ADHD. Improved understanding of the genetic antecedents of ADHD will lead to alternative and effective strategies for treatment, early identification, and prevention of this common and debilitating childhood onset psychiatric disorder.

528T

A Genome-scan for Shared Autism Spectrum Disorder and Specific Language Impairment Loci. *C.W. Bartlett[1], L. Hou[1], J.F. Flax[2,3], Z. Fermano[3], A. Hare[3], S.Y. Cheong[1], S. Buyske[4], L.M. Brzustowicz[3].* 1) Battelle Ctr Mathematical Med, Columbus Children's Res Inst, Columbus, OH; 2) Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ;; 3) Department of Genetics, Rutgers University, Rutgers University, New Brunswick, NJ; 4) Department of Statistics, Rutgers University, Rutgers University, New Brunswick, NJ.

Background: The genetic basis of autism spectrum disorders (ASDs) is still largely unknown as is possible shared etiologies of ASDs and specific language impairment (SLI), a neurodevelopmental disorder of language that is not accounted by low IQ or other cognitive impairments. In order to elucidate shared loci, a genome scan was conducted on pedigrees ascertained using a novel sampling design - pedigrees that were ascertained for both ASD and SLI probands in each pedigree. Methods: Affymetrix Axiom Array SNP genotyping provided data for both linkage and association analysis on 51 families with both ASD and SLI (+27 families without an SLI proband). The posterior probability of linkage (PPL) framework was used. It is the only implementation of a joint categorical-quantitative analysis method that allows for persons with ASD to be coded as “affected due to being beyond a unknown threshold” while all other family members are analyzed using quantitative trait values on 3 factors scores derived from performance on 22 language and literacy tests. Analysis of language and reading impairment as categorical traits was also conducted. Results: Categorical trait analysis showed large peaks for both language and reading impairment on chromosomes 15 (PPL=55%, 78 Mb, build 37) and 16 (PPL= 37%, 20Mb, build 37), respectively. These results explicitly assume ASD is etiologically the same as language or reading impairment. Analysis of the 3 factors scores did not yield large linkage peaks, though GWAS of the same data did indicate strong evidence for LD (PPLD=81%, 184 Mb, build 37) on chromosome 4. Discussion: These data provide evidence for shared etiology of ASD and SLI since a lack of genetic overlap would be expected to reduce power due to the incorrect diagnostic classification of coding both ASD and SLI affected as done here. The peak locations are not known ASD or SLI loci, but this may be expected since this unique sampling design is most powerful where other studies are least powerful. Further mapping efforts are warranted to elucidate these loci and determine their effects in ASD alone, SLI alone and jointly.

529T

Smoothed functional principal component analyses of two independent GWA studies identify 67 genes significantly associated with schizophrenia. *X. Chen[1], Y. Zhu[2], J. Chen[1], M. Xiong[2].* 1) Department of Psychiatry and Virginia Institute for Psychiatric and Behavioral Genetic, Virginia Commonwealth University, Richmond, VA 23219; 2) Department of Biostatistics, University of Texas Health Science Center, Houston, TX.

Genome wide association (GWA) analysis is a powerful tool to identify risk genes for complex diseases. However, due to the testing of hundreds of thousands of markers GWA suffers considerable power loss and many risk genes with small to modest effects cannot be discovered. In recent literature, attention has been focused to develop methods to jointly analyze multi-marker associations where a combination of genetic variants from a gene or a genomic interval is taken as a unit of association analysis. Recently, we have developed a novel statistic based on smoothed functional principal component analysis (SFPCA) for testing the association between a gene and a disease, and now we apply it to two independent schizophrenia GWA datasets (MGS_GAIN and MGS_nonGAIN) obtained from dbGaP. The MGS_GAIN dataset includes 1,135 individuals diagnosed with schizophrenia and 1,362 controls with 727,479 typed SNPs. The MGS_nonGAIN dataset includes 1,089 schizophrenia subjects and 1,273 controls with typed 696,510 SNPs. Using SFPCA, we find that there are 56 and 38 genes respectively for the MGS_GAIN and MGS_nonGAIN datasets meeting genome-wide significance after Bonferroni correction. A Meta-analysis of the two datasets yields 67 genes meeting genome-wide significance. Among them, 10 genes have been reported to be associated with schizophrenia in the literature, and 7 genes are found to be differentially expressed in schizophrenia patients in an RNA-sequencing expression study using postmortem brain samples.

530T

Analysis of SNPs with an effect on gene expression identifies spermatogenesis-associated protein 7 (SPATA7) as a potential candidate gene for schizophrenia. *J. Chen[1], L. Liu[3], K. Kendler[1,2], X. Chen[1,2].* 1) Virginia Institute for Psychiatric and Behavioral Genetics, and Department of Psychiatry;; 2) Department of Molecular and Human Genetics; Virginia Commonwealth University, Suite 390A, 800 E. Leigh Street,Richmond, VA 23298, USA; 3) Shandong Mental Health Center, Jinan, Shandong, P.R. China, 250014;.

Recently we reported that two non-synonymous SNPs (rs2401751 and rs2274736) from the PTPN21 (protein tyrosine phosphatase, non-receptor type 21) gene were associated with schizophrenia. In the follow-up studies using the publicly available SNPExpress database (http://people.chgv.lsrc.duke.edu/~dg48//SNPExpress/index.php), we found that in both brain tissue and peripheral blood mononuclear cells (PBMC), these two SNPs are cis-acting elements influencing the expression of spermatogenesis-associated protein 7 (SPATA7) gene (In brain tissue, P = 1.24×10-3 and 1.21×10-3 for rs2401751 and rs2274736; in PBMC, both markers have the same P = 2.98×10-6). Using the same database, we also found that multiple SNPs, including two different non-synonymous SNPs from the SPATA7 gene, significantly affect the expression of SPATA7. The most significant SNP is rs3179969 (P = 2.75×10-4 in brain and 2.98×10-6 in PBMC). In order to test our hypothesis that those alleles that affect gene expression (eQTL) could more likely be schizophrenia susceptibility alleles, the 2 non-synonymous SNPs (rs4904448 and rs3179969) from SPATA7 were selected for association studies based on the analyses of the expression data and linkage disequilibrium (LD). In a meta-analysis of 8 independent samples including 6 European America (EA) and 2 African America (AA) samples with a total of 10,979 subjects, we found that rs3179969 is significantly associated with schizophrenia (P = 2.39×10-5), which remains significant after Bonferroni correction. While the SNP rs4904448 did not reach experiment-wide significance in the meta-analyses of all 8 samples (P = 0.034), it showed a marginal signal in the 6 EA samples (P = 0.013). Furthermore, in a meta-analysis of 3 other independent expression datasets from different brain regions, we found that these two SNPs show consistent association with the expression of SPATA7 (P = 8.79×10-5 and 1.53×10-12 for rs4904448 and rs3179969, respectively). From these data, we conclude that at least one of the two non-synonymous SNPs from the SPATA7 gene that affect the expression of SPATA7 is significantly associated with schizophrenia. These results show that eQTL-based pre-selection for follow-up is a useful approach for identifying risk loci from a moderately sized GWAS. Further investigation of this locus is warranted.

531T

A Genome-Wide Association Study of Autism Spectrum Disorders Incorporating Assessment Items from ADI-R, ADOS, and SRS. *J.J. Connolly[1], J.T. Glessner[1], H. Hakonarson[1,2].* 1) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department: Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

Autism Spectrum Disorders (ASDs) represent a group of childhood developmental disorders defined by impaired social skills, communication deficits, and repetitive or stereotyped behaviors and interests. Efforts to understand the causes of ASDs have been hampered by the genetic complexity and heterogeneity of the disorders. A potentially productive strategy for reducing this complexity is to target endophenotypes, simpler biologically-based measures that may involve fewer genes and constitute a more homogenous sample. We present results of a genome-wide association study (GWAS) of 2,165 participants from the Autism Genetic Resource Exchange (AGRE), with the aim of identifying genes that correlate with items from the Autism Diagnostic Interview-Revised (ADI-R), Autism Diagnostic Observation Schedule (ADOS), and Social Responsiveness Scale (SRS). We identify a number of genes known to be involved in neurodevelopment that correlate significantly with assessment items. These include KCND2 (overly serious facial expressions), NOS2A (loss of motor skills), and BIN1 (association of loss with physical illness). These findings may help prioritize study design and directions for future genomic efforts.

532T

Genome-wide association analyses using cerebrospinal fluid tau and phospho-tau and Aβ_{42} levels as endophenotypes for Alzheimer 's disease. *C. Cruchaga[1, 5], J.S.K. Kauwe[6], D. McKean[6], M. Bailey[6], D. Patty[6], K. Mayo[1], S. Bertelsen[1], A. Hinrichs[1], E.R. Peskind[7,8], G. Li[7,8], J.B. Leverenz[7,8,9], D. Galasko[10], A.M. Fagan[2,4,5], D.M. Holtzman[2,3,6,5], A.M. Goate[1,2,3,4,5], Alzheimer Disease Genetic Consortium (ADGC), and the Alzheimer's Disease Neuroimaging Initiative.* 1) Psychiatry, Washington University, St Louis, MO; 2) Neurology; 3) Developmental Biology; 4) Knight Alzheimer's Disease Research Center; 5) Hope Center for Neurological Disorders; 6) Department of Biology, Brigham Young University, Provo, UT, USA; 7) Departments of Psychiatry and Behavioral Sciences; 8) Veterans Affairs Northwest Network Mental Illness Research, Education, and Clinical Center, Seattle, WA; 9) Neurology, University of Washington School of Medicine; 10) Department of Neurosciences, University of California San Diego, La Jolla, CA.

Cerebrospinal fluid (CSF) Aβ_{42}, tau and tau phosphorylated at threonine 181 (ptau) are promising biomarkers for Alzheimer Disease. In this study we have performed a genome-wide association study using CSF Aβ_{42} and ptau levels as endophenotypes to identify additional genetic factors implicated in AD pathogenesis. CSF Aβ_{42} and ptau were measured in 957 European American samples. 356 samples were enrolled in longitudinal studies at the WU-ADRC, 391 in ADNI and 210 in studies at the University of Washington. The samples were genotyped using Illumina chips. The 1000 genome data and MACH software were used to impute 6 million SNPs. The normalized CSF ptau values were tested for association using an additive model in PLINK. Age, gender, site, and principle components were included as covariates. As CSF Aβ_{42} levels are not normally distributed and absolute levels of the measurements differed between samples we analyzed each series separately. Series specific analyses were performed using linear regression with age, principle components from stratification analyses and/or APOE genotype as covariates. Empirical P-values for each SNP were generated using ten million permutations. All association analyses were performed using PLINK. Metaanalysis of the three series incorporating sample size and effect direction was performed using METAL. For CSF Aβ_{42} two SNPs in the APOE-TOMM40 gene region showed genome-wide significant association (rs2075650 $p=2.6x10^{-16}$; rs157580 $p=4x10^{-08}$). Inclusion of APOE e4 presence/absence as a covariate drastically reduces the strength of association for these SNPs indicating that these associations may be due to LD with the APOE e4 allele. These results provide further evidence that genetic variation in the APOE region is strongly associated with CSF Aβ_{42} and tau levels. For CSF ptau levels, only SNPs in the APOE-TOMM40 region showed genome-wide significant association with ptau levels (rs2075650; $p= 1x10^{-9}$). Rs2075650 still showed strong association when CSF Aβ42 levels but not APOE genotype were included as a covariate ($p= 1.34x10^{-7}$). This result may indicate that APOE genotype or other SNPs in LD could affect tau pathology independently of the Aβ pathology. We found more p-values in the range 10^{-5} to 10^{-7} than would be expected by chance for both analyses. This could indicate that true positive results have not reached genome-wide significance levels due to lack of statistical power and that a larger sample is required.

533T

Genome-Wide Study and Whole-Exome Sequencing for Dementia in the Amish. *A.C. Cummings[1], L. Liang[1], D. Velez Edwards[2], R. Laux[1], L.L. McFarland[1], L. Caywood[3], L. Reinhart-Mercer[3], D. Fuzzell[1], C. Knebusch[3], C.E. Jackson[4], W.K. Scott[3], M.A. Pericak-Vance[3], J.L. Haines[1].* 1) CHGR, Vanderbilt University, Nashville, TN; 2) Vanderbilt Epidemiology Center, Vanderbilt University Medical Center, Nashville, TN; 3) Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL; 4) Scott & White, Temple, TX.

To aid in our ability to identify late-onset Alzheimer disease (LOAD) risk genes, we are studying the genetically isolated and well-defined Amish populations of middle Ohio and northern Indiana. To date we have enrolled over 2300 Amish individuals with 135 of these having either probable or possible dementia. Through the use of the Anabaptist Genealogy Database, we have accurately defined the kinship coefficients and the family structure among our collected individuals. We performed a genome-wide study (Affymetrix Human SNP Array 6.0) and successfully genotyped 830 Amish individuals (125 with LOAD) and are following up this study with whole-exome sequencing. Following extensive QC procedures for the genome-wide SNP study, 614,957 SNPs were analyzed. Using more computationally tractable sub-pedigrees, we conducted parametric multipoint linkage analysis (Merlin) in regions in which a 2-pt lod score ⁄ 3 was calculated. Because of the relatedness of these individuals, we employed the MQLS test for association, which uses kinship coefficients to correct for relatedness. The highest multipoint peak (HLOD=6.14, (=0.39, recessive model) was calculated at 2p12. Six genes lie within the 1-LOD-unit interval, none of which have previously been associated with LOAD. The lowest MQLS p-value ($1.29x10^{-4}$) under this peak is at rs2974151 located in *CTNNA2*, catenin alpha 2 isoform 1, encoding a neuronal-specific catenin. Three additional regions had a multipoint lod score >3: 3q26 (HLOD=5.27, (=0.49, dominant model), 9q31 (HLOD=4.44, (=0.34, dominant model), and 18p11 (HLOD=4.42, (= 0.21, recessive model). These results indicate heterogeneity within the Amish population and suggest novel non-*APOE* genetic effects for LOAD with the most compelling evidence for *CTNNA2*. The best approach to exhaustively explore the coding sequence of *CTNNA2* and the other significant linkage regions is whole-exome sequencing. Therefore, using the Agilent SureSelect Human All Exon 50Mb Kit, we are sequencing ~110 AD affected and unaffected close relatives, which will allow us to also investigate the rest of the exome for LOAD variants. We will verify variants with Sanger sequencing and/or genotyping. Verified variants will also be tested for in an outbred LOAD dataset to see the extent of the effect of those variants in other populations.

534T

Genome-wide association analysis localizes a QTL influencing serum brain-derived neurotrophic factor levels in a single large extended pedigree. *V.P. Diego[1], S. Kumar[1], J.W. Kent Jr.[1], T.D. Dyer[1], S.L. Santangelo[2], D.C. Glahn[3], J.L. VandeBerg[1], J. Blangero[1], S. Williams-Blangero[1].* 1) Department of Genetics, Texas Biomedical Research Institute, San Antonio, TX 78227; 2) Psychiatric and Neurodevelopmental Genetics Unit, Center for Human Genetic Research, Massachusetts General Hospital Simches Research Building 185, Cambridge St, CPZN 6-256, Boston, MA 02114; 3) Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06511.

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, is involved in regulating neuronal survival, differentiation, and outgrowth. It is expressed in the central nervous system, and in a diversity of peripheral tissues, including primary and secondary lymphoid tissues. BDNF appears to play important roles in neurodegenerative disease, psychiatric disorders, and neuroinflammatory pathology. Recent work in mental disorders indicates that serum/plasma levels of BDNF are lowered in individuals with alcohol dependence, autism, bipolar disorder, major depression and schizophrenia. To gain a better understanding of the genetics of this complex neuropeptide, we performed the first genome-wide association (GWA) analysis of quantitative serum BDNF levels to search for causal quantitative trait loci (QTLs). Serum levels of BDNF were measured using a commercial ELISA assay on blood samples obtained from 1070 individuals of the Jirel population, a well-studied genetic isolate living in eastern Nepal. All Jirels can be assigned to a single large complex pedigree. The size and complexity of this pedigree can substantially complicate genetic analyses. However, we have recently developed a novel fast exact maximum likelihood variance component-based algorithm that provides speeds of likelihood calculations equivalent to those observed for unrelated individuals, thus making genome-wide exact analyses feasible for even the largest pedigrees. Variance component analysis (allowing for covariates such as sex, age, and population structure-related principal components of random SNPs) revealed that serum BDNF level exhibits a large highly significant heritability of 0.61 ($p = 4.6 \times 10^{-47}$) in this isolated population, documenting that BDNF levels are substantially influenced by genetic factors. GWA analysis of 513,073 SNPs identified at least one SNP showing evidence of genome-wide significant association (our empirical genome-wide significance threshold = 1.3×10^{-7}). This SNP, rs2065615 ($p = 5 \times 10^{-8}$), is located on chromosome 20q13 near C20orf85 and accounts for nearly 4% of the variance in BDNF in this sample. Another association near the KIF26B gene at 1q44 nearly reached genome-wide significance ($p = 5 \times 10^{-7}$). Our results show that serum BDNF is highly heritable and amenable to QTL localization. Given that BDNF levels appear to be altered in efficacious psychiatric treatment, identification of causal genes may lead to novel drug targets.

535T

Genome-wide Association Study of Symptomatic Dimensions of Schizophrenia. *A. Fanous[1,2], B. Zhou[3], S.E. Bergen[4], S.H. Aggen[16], R.L. Amdur[1], J. Duan[5], A.R. Sanders[5], J. Shi[3], B.J. Mowry[8], A. Olincy[9], F. Amin[10], R. Cloninger[11], J.M. Silverman[12], N.G. Buccola[13], W.F. Byerley[14], D.W. Black[15], R. Freedman[9], F. Dudbridge[6], P.A. Holmans[7], P.V. Gejman[5], K.S. Kendler[16], D.F Levinson[3].* 1) Washington VA Medical Center, 50 Irving St., NW, Washington, DC, 20422, USA; 2) Georgetown University, School of Medicine 3900 Reservoir Road NW, Medical Dental Building Washington, DC 20057, USA; 3) Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, 401 Quarry Road, Stanford, CA 94304, USA; 4) Psychiatric and Neurodevelopmental Genetics Unit, Massachusetts General Hospital, Boston, MA, 02114, USA; 5) Center for Psychiatric Genetics, NorthShore University HealthSystem Research Institute, Evanston, Illinois 60201, USA; 6) Medical Research Council-Biostatistics Unit, Institute of Public Health, Cambridge CB2 2SR, UK; 7) MRC Centre for Neuropsychiatric Genetics and Genomics, Department of Psychological Medicine and Neurology, School of Medicine, Heath Park, Cardiff CF23 6BQ, UK; 8) Queensland Centre for Mental Health Research, and Queensland Institute for Medical Research, Brisbane, Queensland 4072, Australia; 9) Department of Psychiatry, University of Colorado Denver, Aurora, Colorado 80045, USA; 10) Department of Psychiatry and Behavioral Sciences, Atlanta Veterans Affairs Medical Center, and Emory University, Atlanta, Georgia 30322, USA; 11) Department of Psychiatry, Washington University, St Louis, Missouri 63110, USA; 12) Department of Psychiatry, Mount Sinai School of Medicine, New York, New York 10029, USA; 13) School of Nursing, Louisiana State University Health Sciences Center, New Orleans, Louisiana 70112, USA; 14) Department of Psychiatry, University of California at San Francisco, San Francisco, California 94143, USA; 15) Mental Health Clinical Research Center, and Department of Psychiatry, University of Iowa Carver College of Medicine, Iowa City, Iowa 52242, USA; 16) Departments of Psychiatry, and Human Genetics, Virginia Commonwealth University, Richmond, Virginia 23298, USA.

Background: Family, twin, linkage, and association studies provide convergent evidence that variation in clinical features of schizophrenia is in part due to genetic factors. Genome-wide association studies of such phenotypes have yet to be published. However, these dimensions of the illness mediate its morbidity, including functional disability. **Methods**: Exploratory Factor Analysis (EFA) of the Lifetime Dimensions of Psychosis Scale was performed in the Molecular Genetics of Schizophrenia (MGS) sample, which had been previously genotyped using the Affymetrix 6.0 genotyping array (Shi et al., *Nature*, 2009). PLINK was used to implement a test of linear regression for allelic effects on three quantitative symptomatic dimensions in the European-American sample (N=2,445). **Results**: EFA extraction indicated three phenotypic dimensions: Positive, Negative, and Affective symptoms. No SNP was associated at a genome-wide significance level. Fourteen SNPs within 50kB of a gene were moderately associated ($P<1 \times 10^{-5}$) with one of the factors, as were 20 intergenic SNPs. Associated genes included the following: CTDP1, ADAMTS6, CXCL12, PCDH20 and ZFYVE28 (Positive); GNAL, FAM38B, HLA-DQB1, and RORA (Negative); KIF26B, NLRC5, and CTNND2 (Affective). Only one such SNP was even nominally associated with schizophrenia. **Discussion**: While no single SNP was significant genome-wide, several genes with moderate association have functions with previous evidence of involvement in schizophrenia pathogenesis. These included brain development (CXCL12, PCDH20, GNAL, and CTNND2), dopaminergic transmission (GNAL), and autoimmunity (HLA-DQB1). RORA has been implicated in autism, which has strong phenomenological overlap with negative symptoms, in both human and animal studies. CTDP1 variants cause demyelinating Charcot-Marie -Tooth Disease, and several lines of evidence support defective myelination as a pathogenic process underlying schizophrenia. Finally, some members of the ADAMTS family promote neurite extension. Therefore, the genes implicated herein should be studied more thoroughly in larger samples with the same phenotypes. This is planned in the Psychiatric GWAS Consortium. Genetic variants predisposing to the illness, and those that modify it, are likely to be distinct.

536T

Genetics of Susceptibility to Multiple Sclerosis. *G. Hellenthal, International Multiple Sclerosis Genetics Consortium and Wellcome Trust Case Control Consortium 2.* Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom.

Multiple sclerosis (MS) is the most common neurological disease affecting young adults, estimated to affect over 2 million people worldwide with peak incidence occurring in individuals aged 30-40. Previous studies have established a strong genetic component to MS susceptibility, with variation within the Major Histocompatability Complex (MHC) exerting the greatest individual effect on risk. However, there is remaining controversy over more substantive details of disease etiology, such as which alleles in the MHC are important. Furthermore while inflammatory and neurodegenerative disease mechanisms are implicated, there is debate on whether these occur in sequence, inflammation determining secondary neurodegeneration or vice versa, or exert fully independent effects through clinical course. We report the results of the largest genome-wide association study (GWAS) to date exploring MS susceptibility. In particular we test for associations using data from 475,806 Single-Nucleotide-Polymorphisms (SNPs) genotyped in 9,772 affected individuals and 17,376 healthy controls sampled from 15 populations (plus replication data from 4,218 affected individuals and 7,296 controls). Novel statistical methodologies were developed to cope with complex population structure in GWAS collections such as ours where not all case samples have controls available from the same population, a scenario that may become more routine as study sizes increase. We utilized recently developed statistical methods for imputing classical Human Leukocyte Antigen (HLA) types from SNP data to examine in detail the landscape of genetic risk in the MHC, revealing that four mutations (DRB1*15:01, HLA-A*02:01, DRB1*03:01, DRB1*13:03) with effects modelled in a simple multiplicative manner within and across loci are sufficient to account for most of the risk attributable to the MHC. Outside of the MHC, we replicate 23 of the 26 previously suggested MS susceptibility loci and report the discovery and replication of at least 29 new genetic loci. Just over a third of the identified loci overlap with regions already confirmed as associated with at least one other autoimmune disease. Using pathway analysis, we show definitively that genes located in these association regions are highly enriched for cell-mediated immune mechanisms, rather than directly implicating neurodegeneration. We conclude that the critical disease mechanisms of multiple sclerosis primarily involve immune dysregulation.

537T

Genome-wide association study for Parkinson's Disease in the mid-western US Amish. *L. Jiang[1], M.F. Davis[1], A.C. Cummings[1], D. Velez Edwards[2], R. Laux[1], L.L. McFarland[1], L. Reinhart-Mercer[3], D. Fuzzell[1], C. Knebusch[3], C.E. Jackson[4], W.K. Scott[3], M.A. Pericak-Vance[3], S.L. Lee[5], J.L. Haines[1].* 1) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Vanderbilt Epidemiology Center, Vanderbilt University Medical Center, Nashville, TN; 3) Hussman Institute for Human Genomics, University of Miami, Miller School of Medicine, Miami, FL; 4) Scott & White, Temple, TX; 5) Dartmouth College, Hanover, NH.

Parkinson's Disease (PD) is the second most common neurodegenerative disorder of adults. Previous evidence has shown that PD has a heritable component. Mutations in five known genes [(-synuclein (PARK1, PARK4), Parkin (PARK2), PINK1 (PARK6), DJ-1 (PARK7), and LRRK2 (PARK8)] cause Mendelian inheritance of PD and consensus regions from genome-wide linkage and association studies of the more common non-Mendelian forms of PD implicate MAPT and SNCA. Other genes have been associated with PD but the functional variants responsible are unknown. Genetic heterogeneity complicates the verification of likely genes and the identification of other susceptibility genes. Our approach to overcome the problem of heterogeneity is to study a population isolate, the Amish communities of Indiana and Ohio. We have enrolled over 2200 Amish individuals with 32 of these diagnosed with PD. In this dataset we see a higher average kinship coefficient among our cases (.016) compared to the rest of our dataset (.012), suggesting that PD is heritable in the Amish. We have undertaken a genome-wide association study (Affymetrix Genome-Wide Human SNP Array 6.0) and genotyped 900 Amish individuals (including all individuals with PD). Using the Anabaptist Genealogy Database, we determined the family structure connecting all individuals with PD. Following QC, 614,957 SNPs and 830 individuals were analyzed. SNPs were analyzed by linkage and association analysis. Linkage analysis was conducted using Merlin after cutting the pedigree into 10 computationally feasible sub-pedigrees using PedCut. Each sub-pedigree contained at least 3 individuals with PD. Three regions generated a hlod score⁄ 3 in 2-pt linkage analysis on chromosomes 5 and 10 under a recessive model. Association analysis was conducted using MQLS, which takes into account the relatedness of the individuals. Five SNPs reached genome-wide significance (p-value ≤ 1 × 10-7). Two SNPs are located in genes (COL13A1, TMC3) and the other SNPs are located in close proximity to one another in an intergenic region on chromosome 19. We further investigated to see if the 2-pt linkage and association results overlap. Ten SNPs are suggestively linked and associated with hlod ⁄ 2 and p-value ≤ 1 × 10-4. These data suggest that there are likely multiple loci within the Amish, likely representing a subset of all the PD loci segregating in the Caucasian population and will be followed up with multipoint linkage analysis.

538T

Genome-Wide Association Study of Treatment Refractory Schizophrenia in Han Chinese. *M. Lee[1], H. Wang[1], Y. Liou[2], Y. Chen[1], C. Hong[2], J. Wu[1].* 1) National Genotyping Ctr, IBMS, Academia Sinica, Taipei, Taiwan; 2) Department of Psychiatry , Taipei Veterans General Hospital, Taipei, Taiwan.

Purpose: Schizophrenia is a severe psychiatric disorder with a prevalence estimated to be approximately 1% in the world and 0.6% in Taiwan. Its clinical manifestations are characterized by distortion of reality, delusions, hallucinations, altered emotional reactivity, disorganized behavior, social isolation and cognitive impairment. In addition, schizophrenia is a heterogeneous disorder. Reducing the heterogenetity through subphenotyping or stratification might reduce the background in the genetic study for complex disorders. Methods: Antipsychotic medication is the major treatment for schizophrenia. However, one fifth to one third of schizophrenic patients do not respond to antipsychotic treatments. These patients with treatment refractory schizophrenia (TRS) have persistent psychotic symptoms combining with poor social/work function in spite of administering at least two trials of sufficient antipsychotic doses and adequate treatment duration. Therefore, TRS may be a distinct and homogenous subgroup of schizophrenia. To identify the genetic variants susceptible for schizophrenia, we performed the first genome-wide association study focusing on TRS, a relatively homogenous subtype of schizophrenia, in 522 Han Chinese individuals with TRS and 806 controls. Results: Four loci showed suggestive significant association with TRS were identified. These loci include: rs4699030 (P = 8.41 x 10-7) and rs230529 (P = 1.07 x 10-6) are located in the gene nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFKB1); rs10218843 (P = 6.73 x 10-6) and rs11265461 (P = 5.90 x 10-6) are adjacent to signaling lymphocytic activation molecule family member 1 (SLAMF1); rs739617 (P = 1.46 x 10-5), rs17158926 (P = 3.99 x 10-5) and rs17158930 (P = 3.08 x 10-5) cluster in dedicator of cytokinesis 4 (DOCK4); and rs13049286 (P = 1.23 x 10-5) and rs3827219 (P = 1.23 x 10-5) fall in receptor-interacting serine/threonine-protein kinase 4 (RIPK4). Five isolated SNPs with P values less than 10-5 were also identified to be associated with TRS. The -94delATTG allele (rs28362691) located in the promoter region of NFKB1 was identified by resequencing and was found to associate with TRS (P = 4.85 x 10-6). The promoter assay demonstrated that the -94delATTG allele had a significant lower promoter activity than the -94insATTG allele in the SH-SY5Y cells. This study suggests that rs28362691 in NFKB1 might be involved in the development of TRS.

539T

Meta-analysis of Parkinson disease genome-wide association studies: Identification of a novel locus, RIT2, and multiple effects within known loci. *N. Pankratz[1], G.W. Beecham[2], A.L. DeStefano[3,4], T. Dawson[5], K.F. Doheny[5], S.A. Factor[6], T.H. Hamza[7], A.Y. Hung[8,9], B.T. Hyman[8,9], A.J. Ivinson[9], D. Krainc[8,9], J.C. Latourelle[3], L.N. Clark[10], K. Marder[10], E.R. Martin[2], R. Mayeux[10], O.A. Ross[11], C.R. Scherzer[8,9,12,13], D.K. Simon[9,14], C. Tanner[15], J.M. Vance[2], Z.K. Wszolek[11], C.P. Zabetian[16], R.H. Myers[3], H. Payami[7], W.K. Scott[2], T. Foroud[1], the PD GWAS Consortium.* 1) Indiana University School of Medicine, Indianapolis, IN; 2) University of Miami Miller School of Medicine, Miami, FL; 3) Boston University School of Medicine, Boston, MA; 4) Boston University School of Public Health, Boston, MA; 5) Johns Hopkins University School of Medicine, Baltimore, MD; 6) Emory University, Atlanta, GA; 7) NY State Department of Health Wadsworth Center, Albany, NY; 8) Massachusetts General Hospital, Boston, MA; 9) Harvard Medical School, Boston, MA; 10) Columbia University School of Medicine College of Physicians and Surgeons, New York, NY; 11) The Mayo Clinic, Jacksonville, FL; 12) Partners Parkinson Center, Brigham & Women's Hospital, Boston, MA; 13) Center for Neurologic Diseases, Brigham & Women's Hospital, Boston, MA; 14) Beth Israel Deaconess Medical Center, Boston, MA; 15) Parkinson's Institute, Sunnyvale, CA; 16) VA Puget Sound Health Care System and University of Washington, Seattle, WA.

Parkinson disease (PD) is the second most common adult-onset neurodegenerative disorder worldwide. Several studies have utilized genome-wide association (GWAS) methods to identify genes contributing to PD with growing evidence for the role of SNCA, MAPT, GAK/DGKQ, and the HLA region in disease susceptibility. There is evidence that there are additional loci yet to be identified. To maximize the power to detect additional risk factors having small to modest effects on PD susceptibility, we combined data from multiple GWAS. A two stage design was used to identify SNPs associated with the risk for Parkinson disease (PD). First, we combined individual level genotypic data from five recently completed GWAS of PD (Discovery Sample: 4,238 PD cases and 4,239 controls). Following imputation, a logistic regression model was employed in each dataset to test for association with PD susceptibility and the results from each dataset were meta-analyzed. Genome-wide significance was reached for SNPs in SNCA (odds ratio (OR)= 1.37; p=9.3 x 10^{-21}), MAPT (OR=0.77; p=1.5 x 10^{-10}), GAK/DGKQ (OR= 1.35; p=8.2 x 10-9/ OR=1.35; p=2.0 x 10-9), and the HLA region (OR=0.83; p=1.2 x 10^{-8}), which have been previously established in PD susceptibility. In the second stage we genotyped 768 SNPs in an independent Replication Sample of 3,738 cases and 2,111 controls. These SNPs included 100 ancestry informative markers (AIMs) and 686 SNPs that tag the LD blocks with the most extreme p-values from the meta-analysis. The Replication Sample confirmed the previously identified associations with SNCA, MAPT, and the HLA region and confirmed association with GBA (E326K OR=1.71; p=5 x 10^{-8} Combined Sample) (N370 OR=3.08; p=7 x 10^{-5} Replication sample), which had been previously associated with PD. We also replicated a novel PD susceptibility locus, RIT2, on chromosome 18 (rs12456492; p=5 x 10^{-5} Discovery Sample; p=1.52 x 10^{-7} Replication sample; p=2 x 10^{-10} Combined Sample). Conditional analyses within each of the replicated regions identified distinct SNP associations within GBA and within SNCA, suggesting that there may be more than one risk alleles within these genes. In summary, we have identified a novel PD susceptibility locus, RIT2, replicated several previously identified loci, and identified more than one risk allele within SNCA and GBA. These results have important implications as studies are being designed to identify all potentially functional disease-associated variants.

540T

Significant Association of Coding (Missense) SNPs with Familial LOAD Based on a Functional Genome-Wide Association Screen. *A.R. Parrado[1], K. Mullin[1], B. Hooli[1], L. Gotta[1], L. Bertram[2], C. Lange[3], R.E. Tanzi[1].* 1) Genetics and Aging Research Unit, Mass General Institute for Neurodegenerative Disease (MIND), Department of Neurology, Massachusetts General Hospital, Charlestown, MA, USA; 2) Max-Planck Institute for Molecular Genetics, Berlin, Germany; 3) Department of Biostatistics, Harvard School of Public Health, Boston, MA, USA.

Late-onset Alzheimer's disease (LOAD) genome-wide association study's (GWASs) have yielded novel AD candidate genes beyond APOE including CLU, CR1, PICALM, BIN1, ABCA7, MS4A6A/MS4A4E, EPHA1, CD2AP, all of which have been replicated in independent samples (www.alzgene.org). Our recent family-based GWAS also yielded novel AD candidate genes exhibiting genome-wide association, including ATXN1, GWA14q31, and CD33, of which CD33 has been confirmed. We set out to identify coding changes and particularly missense substitutions that associate with risk for LOAD. For this purpose, we performed a GWAS on the NIMH Genetics Initiative Family Sample using the Affymetrix 20K cSNP panel. To optimize statistical power, age of onset and AD affection status were tested together, as a combined phenotype, by use of the multivariate extension of the FBAT method, FBAT-GEE. In addition to the traditional p-values we report p-values from the Liptak test statistic, which utilizes all available information. The Liptak method attains higher power levels, than the FBAT-GEE approach, by combining the Z-statistics that correspond to the p-values of the family-based test (the within family information) with the rank-based p-values for population-based analysis (the between family information). Three genes contained cSNPs that exhibited previously unreported association with LOAD at a genome-wide significance level of 0.05 (Bonferroni correction, 4x10-6). These genes included: NMS, OR51F1, and AQP9(p = 3x10-7, 3x10-6, and Liptak p = 3x10-7, respectively). Additional genes containing cSNPs with suggestive evidence of association with LOAD (Liptak p ≤ 10-5) included: ZNF214, KRT7, and KLK3. This study represents the first comprehensive genome-wide screen for cSNPs associated with LOAD. The strongest evidence for association with LOAD was observed for a missense substitution in AQP9, encoding aquaporin-9. An edema model in rat brain suggests that the regulation of AQP9 expression may play a vital role in brain homeostasis via regulation of water channel activity. Aquaporin-9 has also been suggested to regulate immunological responses to bactericidal activity indicative of a role in the innate immune system. Interestingly, other recently reported AD candidate genes, e.g. CD33 and CR1, have also been implicated to play roles in the innate immune system. Replication analysis to confirm these initial findings is planned in a large independent collection of families with over 4,000 subjects.

541T

Genetics of cortical thickness in 822 twins. *A.S. Quiggle[1,2], N.K. Hansell[1], K.A. Johnson[1,2], G.A. Blokland[1,3], K.L. McMahon[2], G.W. Montgomery[4], P.M. Thompson[5], N.G. Martin[1], G.I. de Zubicaray[3], M.J. Wright[1,3].* 1) Genetic Epidemiology, Queensland Institute of Medical Research, Brisbane, Australia; 2) Centre for Advanced Imaging, University of Queensland, Brisbane, Australia; 3) School of Psychology, University of Queensland, Brisbane, Australia; 4) Molecular Epidemiology, Queensland Institute of Medical Research, Brisbane, Australia; 5) Laboratory of Neuroimaging, UCLA School of Medicine, Los Angeles, CA.

Cortical thickness measurements have gained recent interest for being a potential brain-based endophenotype for a whole host of psychiatric illnesses and cognitive measures. Studies have shown patterns of cortical thinning to be associated with schizophrenia, major depressive-disorder, autism, Alzheimer's, and cardiovascular health. Despite previous research showing the brain to be widely heritable, little is still known about the genetic underpinnings of the cerebral cortex and about which genes are likely to be involved. We acquired 3D T1-weighted structural images of 822 twins and siblings of twins (121 MZ and 162 DZ pairs, mean age 22.7 ± 3.2 SD) using a 4 Tesla scanner, and extracted cortical thickness measures in 56 *a priori* regions-of-interest (ROIs), making our study one of the largest neuroimaging studies to date. We estimated the heritability of each ROI by performing a univariate Cholesky decomposition. We then performed the first genome-wide association study (GWAS) on cortical thickness to our knowledge (n= 704, after accounting for family structure). Variance component analysis showed cortical thickness heritability to vary widely: there was no significant genetic component of the right uncus, whereas a genetic component accounted for as much as 67% of variance in the left postcentral gyrus. Our analysis revealed substantially larger common environmental factors than previous estimates, probably due to an increase in power from a larger number of DZ twins. Although the GWAS revealed no individual single nucleotide polymorphisms (SNPs) of genome-wide significance ($P=5 \times 10^{-8}$), we found, in the medial frontal gyrus, a series of SNPs in a gene at a schizophrenia susceptibility locus that, in a gene-based association test which corrects for gene-size and linkage disequilibrium between SNPs, lay just under significance ($P=3 \times 10^{-6}$ compared to gene-based significance $P=2.8 \times 10^{-6}$). More intriguing still, the significance of these SNPs in other ROIs reflected the phenotypic correlations between these gyri and the medial frontal gyrus, making it a potential genetic marker. Results and significance levels are likely to change as our sample size increases. We are in the process of finding a replication sample in order to validate these results.

542T

Genetics of cerebral asymmetry in the caudate nucleus. *M.E. Renteria[1,3], J.L. Stein[5], K. Johnson[1], S.E. Medland[1,3], K.L. McMahon[4], G.I. de Zubicaray[4], G. Montgomery[2], P.M. Thompson[5], N.G. Martin[1,3], M.J. Wright[1,3].* 1) Genetic Epidemiology Laboratory, Queensland Institute of Medical Research, Brisbane, Queensland, Australia; 2) Molecular Epidemiology Laboratory, Queensland Institute of Medical Research, Brisbane, Queensland, Australia; 3) School of Psychology, University of Queensland, Brisbane, Queensland, Australia; 4) Center for Advanced Imaging, University of Queensland, Brisbane, Queensland, Australia; 5) Laboratory of Neuroimaging, University of California Los Angeles, Los Angeles, California, USA.

The two hemispheres of the human brain exhibit anatomical and functional asymmetries across different brain regions. These differences arise from a combination of genetic and environmental factors and are thought to underlie the functional lateralization of brain function. While there is normal individual variation in asymmetry, evidence suggests that atypical symmetry or asymmetry at particular regions might lead to either superior performance or disease. In this study, we investigate the genetics of asymmetry of the caudate nucleus, a sub-cortical structure that plays roles in learning and memory and the control of voluntary movement. We obtained whole-brain MRI scans of 526 (325 females and 201 males) Australian twins. Images were processed and volumetric measures for caudate nucleus were extracted using the FSL/FIRST suite. An asymmetry index was calculated for each individual and the OpenMX structural equation modelling package was used to estimate the heritability of the trait. We then conducted a gene-based association analysis with the Versatile Gene-based Association Study (VEGAS) method, finding a statistically significant association with a gene on chromosome 14 (p-value: 2.0e-06), which is significant at the gene-based level (significance cut-off value after multiple testing correction is 2.8e-06). Our results provide a genetic association for cerebral asymmetry.

543T

Japanese subsequent GWAS identifies strong association at a novel risk locus and MCCC1 for Parkinson's disease. *W. Satake[1], K. Yamamoto[2], Y. Nakabayashi[1], M. Kubo[3], T. Morizono[3], T. Kawaguchi[3], T. Tsunoda[3], H. Tomiyama[4], M. Yamamoto[5], N. Hattori[4], M. Murata[6], Y. Nakamura[3,7], T. Toda[1].* 1) Div Neurol/Mol Brain Sci, Kobe Univ Grad Sch Med, Japan; 2) Div Genome Analysis, Medical Instit of Bioregulation, Kyushu Univ, Fukuoka, Japan; 3) Center for Genomic Med, RIKEN, Japan; 4) Dept Neurol, Juntendo Univ Sch Med, Japan; 5) Dept Neurol, Kagawa Pref Cen Hsp, Japan; 6) Dept Neurol, Natl Center Hsp Neurol and Psy, Japan; 7) Human Genome Center, Univ Tokyo, Japan.

Parkinson's disease (PD) is one of the most common neurodegenerative diseases worldwide. We reported a genomewide association study (GWAS) in a total of 2,011 cases and 18,381 controls from the Japanese (Satake W et al, Nat Genet 2009). In that GWAS, we selected 337 most associated SNPs (P <0.00054) from genome-wide 435,470 SNPs, conducted two subsequent replication studies, and detected 4 susceptibility loci for PD consequently. In the present study, we re-analyzed our genotyping data of 988 cases in the previous GWAS using more control data in order to obtain more accurate allele frequencies in controls. We subsequently genotyped and tested association for second hit more than ~9000 SNPs. Although genotyping is still underway, we have so far genotyped 1,948 cases and 28,990 controls in total and tested for association between each SNP and PD using Cochran-Armitage trend test with 1 d.f. We identified a novel susceptibility locus on chromosome 1 with P <5 x 10^{-8}. We also detected strong association at MCCC1 on 3q27 (rs10513789, P = 1 x 10^{-7}) that was identified in a recent Caucasian meta-GWAS (IPDGC, Lancet 2011). Our results identify a new PD susceptibility locus and confirmed MCCC1 in the Asian population.

544T

Genome-wide association study of narcolepsy with cataplexy in Japanese population. *H. Toyoda[1], T. Miyagawa[1], S. Khor[1], M. Kawashima[1], M. Yamasaki[1], A. Koike[2], Y. Honda[3], M. Honda[3,4], K. Tokunaga[1].* 1) Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; 2) Central Research Laboratory, Hitachi, Ltd. Tokyo, Japan; 3) Japan Somnology Center, Neuropsychiatric Research Institute, Tokyo, Japan; 4) SLEEP control project, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan.

Narcolepsy with cataplexy is a sleep disorder characterized by excessive daytime sleepiness and cataplexy. The prevalences of narcolepsy with cataplexy have been reported to be 0.02-0.18% in Japan, US and Europe, whereas 1-2% of first-degree relatives of patients develop narcolepsy, indicating genetic predispositions influence the development of narcolepsy. Several studies have showed a strong association with a specific HLA allele. Most of the patients (88-100%) with narcolepsy-cataplexy are HLA-DQB1*0602 positive whereas only 10% of healthy people possess the HLA allele. However, HLA can explain only a part of genetic contribution to the disease. We have performed the first SNP-based genome-wide association study (GWAS) in Japanese (Miyagawa et al. Nat. Genet. 2008), and participated in international GWAS (Hallmayer et al. Nat. Genet. 2009; Kornum et al. Nat. Genet. 2011). Now, we conducted an expanded GWAS using the Affymetrix Genome-Wide Human SNP 6.0 on 425 Japanese patients with narcolepsy-cataplexy compared to 1626 Japanese healthy controls.

Extensive quality control tests were performed to ensure the integrity of the data, including sample filters (total call rates／ 95%), SNP filters (SNP call rates／ 97%, MAF／ 5%, deviations from HWE in controls (P／ 0.001)), and EIGENSTRAT method to correct for population stratification. The final post-QC discovery set comprised 409 cases and 1,562 controls, and a total of 545,140 SNPs passed the quality control criteria. The estimated inflation factor (λ) on the basis of the median $\chi 2$ test was 1.03 after the quality control. Each SNP was tested for association by allelic, dominant/recessive, genotype and trend test. HLA region were excluded from further analysis. We detected 48 SNPs in 20 different regions with P < 1.47E-05, except for HLA regions. The top SNP showed P = 6.27E-06 in trend test and P = 4.92E-06 in EIGENSTRAT-adjusted model. The candidate regions were further subjected to imputation of missing genotypes to increase SNP coverage. Imputation was performed with MACH 1.0, IMPUTE v2 and PLINK 1.07 softwares using reference haplotypes from HapMap 3 (JPT+CHB). There were no SNPs that showed markedly stronger associations than genotyped SNPs in each region, even though some SNPs were found to be suggestively significant. Replication study of the top-ranking SNPs found in the discovery phase is in progress using independent sample sets.

545T

CDH13: GWAS, brain distribution and mouse model studies support roles in addiction and ability to quit smoking. *G. Uhl[1], J. Drgonova[1], D. Walter[1], B. Ranscht[2].* 1) Dept Molec Neurobiology, NIDA/NIH, Baltimore, MD; 2) Sanford-Burnham Institute, LaJolla, Calif.

Classical human genetic studies suggest that vulnerability to dependence on addictive substances shares genetic determinants with ability to quit, and with its common comorbidities, including attention deficit/hyperactivity disorder (ADHD). Genome wide association (GWA) datasets for dependence-related phenotype and abilities to quit smoking have repeatedly identified a number of genes in the following way: they contain clusters of nearby SNPs whose case vs control differences reach nominal statistical significance. The cell adhesion molecule cadherin 13/T cadherin/H cadherin (CHD13) is identified in this way in 15 of 17 independent genome wide datasets for dependence-related phenotypes or quit success. About one in 10exp12 genes of this size would be expected to be identified this frequently by chance. In most samples, clustered association signals are found in the first several CDH13 introns. These CDH13 introns also identified in metaanalyses of ADHD GWA data. CDH 13, and most of other genes identified in the same way, is expressed in both developing and adult brain. This gene, and more than ½ of the other genes identified in the same fashion, is expressed in dopaminergic and noradrenergic neurons implicated in rewarding effects of addictive substances of a variety of different classes and in regulation of locomotion. This gene and more than ½ of other genes identified in the same fashion, is also expressed in large striatal cholinergic interneurons. Searching databases of genes subjected to cis-regulation in lymphoblastoid cell lines fail to identify CDH13; these databases identify about as many “addiction/quit success” genes as expected by chance. Levels of CDH13 mRNA expressed in postmortem cerebral cortical samples from > 100 individuals also fail to provide any strong association with CDH13 SNPs in these first introns. CDH13 KO mice reveal robust influences on mouse models of drug reward/memory, some effects on models for quit success, and differences in stimulant-induced locomotion. Variations in CDH 13 are thus strong candidates to provide polygenic contributions to development of dependence on an addictive substance and for perhaps for individual differences in abilities to quit smoking Support: NIH IRP (NIDA).

546T

Investigating the association between the glutamate system genes *SLC1A1*, *DLGAP3*, and *GRIN2B* and sub-phenotypes of obsessive compulsive disorder. *P.D. Arnold[1], B.T. Doan[2], J. Beneteau[3], S. Taillefer[4], S. Shaheen[1], S.E. Stewart[5], J.L Kennedy[6], M.A. Richter[7], OCD Mini-Collaborative.* 1) Program in Genetics and Genome Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) Faculty of Nursing, University of Toronto, Toronto, ON, Canada; 3) Department of Psychology, Simon Fraser University; 4) Department of Psychology, Ryerson University; 5) Department of Psychiatry, University of British Columbia; 6) Centre for Addiction and Mental Health, Toronto; 7) Sunnybrook Health Sciences Centre, Toronto.

Obsessive-Compulsive Disorder (OCD) is a disabling heritable neuropsychiatric condition affecting 1 to 3% of the population. The phenotype of OCD is highly heterogeneous, which may reflect underlying genetic heterogeneity. There is growing evidence for glutamate dysfunction in OCD, and three main glutamate genes have been investigated by our group and others: *SLC1A1*, *GRIN2B*, and *DLGAP3*. This study examined these three genes as candidate genes for OCD, and attempted to reduce heterogeneity by subgrouping based on gender and predominant symptoms. Subjects were 252 OCD-affected probands and their first degree relatives (N=453 total) collected in a specialized OCD clinic in Toronto and genotyped as part of a larger collaborative study. Tag single nucleotide polymorphisms (SNPs) were selected using publicly available databases (dbSNP, HapMap) and the Haploview program. Genotyping was performed on the Sequenom iPlex platform (http://www.sequenom.com). Markers with genotyping success rates less than 90%, with minor allele frequency less than 0.05, and with greater than 10 Mendelian errors were excluded. In total, 36 single nucleotide polymorphisms (SNPs) were investigated for *DLGAP3*, 32 for *SLC1A1*, and 10 for *GRIN2B*. The association between OCD diagnosis and candidate genes was tested using the Family Based Association Test (FBAT); haplotype analysis was also performed. In addition, FBAT was used to test for association when the sample was stratified by gender or by symptom type (five groups identified using factor analysis). Variants in the glutamate genes *DLGAP3* and *SLC1A1* were significantly associated with OCD diagnosis. These genes showed specific associations with male gender (p = 0.04) and with the hoarding subgroup (p=0.03), which has been identified in multiple previous studies as having distinct clinical and neurobiological characteristics. *GRIN2B* did not show a significant association with OCD diagnosis or specific sub-phenotypes. Although limited by sample size and requiring replication, these results provide further evidence that *DLGAP3* and *SLC1A1* may be associated with susceptibility to OCD, particularly in males and individuals with primary hoarding symptoms. In concordance with basic neuroscience and clinical neuroimaging studies, these results provide further support for the presence of glutamatergic neurotransmission abnormalities in OCD.

547T

The Catechol-O-methyltransferase Val158Met polymorphism and the decision making in bipolar disorder. *S.B. CAMPOS[1], D.M. MIRANDA[1], M.A. ROMANO-SILVA[1], F.S. NEVES[1], F.M. PASSOS[1, 2], L.F. MALLOY-DINIZ[1, 2], H. CORREA[1].* 1) Mental Health, Universidade Federal de Minas Gerais , Belo Horizonte, Minas Gerais, Brazil; 2) Psychology, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

Catechol-O-methyltransferase (COMT) is one of several enzymes that degrade cathelocamines. The Val158Met (rs4680) polymorphism, located in exon 4 (codon 158) of the human COMT gene, has been widely researched and associated with several cognitive and behavioral phenotypes in psychiatric disorders. In this polymorphism occurs a change of a valine for a methionine at position 158. The Met variant is associated to a reduce in the enzymatic activity. The Val homozygotes catabolizes dopamine at up four times relative to Met alleles, resulting in lower prefrontal dopamine (DA) levels, that is also associates to a poorly development on neuropsychological task. Considering the relationship between COMT and executive function, we launched an investigation into the relationship between the Val158Met polymorphism and the performance in decision making. We administered a Brazilian version of Iowa Gambling Task (IGT), a neuropsycological test, to bipolar patients to measure their levels non-planning impulsivity. After the task we extract blood samples. Our sample of 64 bipolar patients, with mean age 42,6 ± 11,4 years, was selected from the psychiatrical division, Hospital das Clínicas, Belo Horizonte, Brazil. Genomic DNA was extracted from blood using the high salt method. We analyzed COMT functional polymorphism (Val158Met) selected from the International HapMap Project. Markers used were correspondent to the polymorphisms rs4680. Genotyping was performed using a PCR-Realtime in the allelic discrimination mode (Strategene Mx3005 - MxPro) using the TaqMan Genotyping Master Mix (Applied Biosystems). After genotyping, the subjects were grouped according to the presence or absence of Met allele (Val/Val x Val/Met + Met/Met). The two groups were compared using the Mann-Whitney test and results were considered statistically significant when $p < 0.05$. We found significant differences in the measures of nonplanning impulsivity of the two groups. COMT-Val homozygote performed more poorly on IGT-Br. This difference is significantly worse than individuals in the Val/Met + Met/Met group (U=248,5; Z=-2,906).

548T

A homopolymer polymorphism in the TOMM40 gene contributes to cognitive performance in aging. *O. Chiba-Falek[1, 2, 3], J.M. McEvoy[1], C. Linnertz[1], D. Attix[2, 4], M. Kuchibhatla[5], A.M. Saunders[2, 6], M.W. Lutz[6], K.A. Welsh-Bohmer[3, 4], A.D. Roses[1, 2, 6], K.M. Hayden[3, 4].* 1) Institute for Genome Sciences & Policy, Duke University, Durham, NC 27708, USA; 2) Division of Neurology, Department of Medicine, Duke University Medical Center, Durham, NC 27710, USA; 3) Joseph and Kathleen Bryan Alzheimer's Disease Research Center, Duke University, Durham, NC 27705, USA; 4) Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC 27710, USA; 5) Department of Biostatistics and Bioinformatics, Duke University Medical Center, Durham, NC 27710, USA; 6) Deane Drug Discovery Institute, Duke University Medical Center, Durham, NC 27710, USA.

A highly polymorphic T-homopolymer was recently discovered to be associated with late onset Alzheimer's disease (LOAD) risk and age of onset. The objective of this study was to explore the effects of the polymorphic polyT tract (rs10524523, referred as `523') on cognitive performance in cognitively healthy elderly. Over 180 participants were recruited from local independent-living retirement communities. Informed consent was obtained and participants completed demographic questionnaires, a conventional paper and pencil neuropsychological battery, and the computerized Cambridge Neuropsychological Test Automated Battery (CANTAB). Saliva samples were collected for determination of the TOMM40 `523' (S, L, VL) and the APOE (, 2, 3, 4) genotypes. Comparison of the `523' genotype frequencies between participants aged 80+ to those under 80 showed no difference. Multinomial logistic regression was used to evaluate performance on the various neuropsychological tests as predictors of `523' genotypes, adjusted for age, sex, education, depression, APOE status, and mental status score. Significant differences were found in a measure of free recall with the S heterozygotes group recalling fewer correct items than the S homozygotes group. Follow-up analysis in a sample restricted to APOE, 3 homozygotes only showed significant differences between S/S and VL/VL groups; the VL/VL group performed significantly worse on measures of episodic memory (CANTAB Paired-Associate learning OR 2.84, 95% CI 1.12-8.07), attention (Trail Making Test, Part A OR 2.64, 95% CI 1.23-6.13), and executive function (CANTAB measures of intra-extradimensional set shifting OR 9.27, 95% CI 1.66-64.06). In conclusion, our results suggest important APOE -independent associations between the TOMM40 `523' polymorphism and specific cognitive domains of memory and executive control in cognitively normal aging subjects. Interestingly, these specific cognitive domains are preferentially affected in early stage AD. It will be very important to assess the association of the `523' polymorphism in other large elderly cohorts from which neuropsychological data is available in order to get a better understanding of the genetic factors that contribute to successful cognitive aging.

549T

Calcium-channels are implicated in human episodic memory: Evidence from a pathway analysis in healthy individuals. *A. Heck[1], M. Fastenrath[2], L. Gschwind[2], C. Vogler[1], S. Ackermann[1], K. Spalek[2], D. DeQuervain[2], A. Papassotiropoulos[1].* 1) University of Basel, Molecular Neuroscience, Basel, Switzerland; 2) University of Basel, Cognitive Neuroscience, Basel, Switzerland.

Genome-wide association studies (GWAS) are a potent tool for the unbiased search for single marker associations with a specific trait. By reporting the best single p-values meeting the genome-wide level of significance, GWAS ignores effects possibly acting in combination, which results in restricted insight in the biological background of a phenotype. Based on the assumption that genes exert their influence within complex functional systems rather than in isolation, prior biological knowledge is of special value and can be used to inform pathway analyses used to identify combinations of genes implicated in the trait of interest. Statistical methods originating from gene expression analysis have been modified to identify genetic pathways in GWAS data. Among these methods, gene enrichment scores analysis (GSEA) (i.e. testing the overrepresentation of test statistics of functionally related genes against the distribution of all p-values) have become increasingly important. Here we performed a GSEA for an episodic memory task in 1'166 healthy Swiss individuals. We observed 12 pathways significant at the genome level (pFDR<0.05) in the screening sample. Seven pathways replicated in a second sample, including genes involved in calcium-channel activity, cell-adhesion molecules and cation transport processes. On a single marker level of replication (identical SNP, same direction of effect) we observed associations for three SNPs within the calcium channel acticty pathway (gene ontology: GO:0005262) showing robust effects on episodic memory performance. In further analyses we will use functional imaging data and correlate an additive multilocus score of these beneficial alleles with activity in memory-related brain regions.

550T

A preliminary study of genotype-phenotype correlations for rare copy number variants in children with Obsessive-Compulsive Disorder. *B. Li[1], M. Marshall[2], A. Lionel[2], S-M. Shaheen[1], S.W. Scherer[2], G.L. Hanna[3], J.L. Kennedy[4], D.R. Rosenberg[5], P.D. Arnold[1].* 1) Genetics and Genome Biology, Sickkids Research Institute, Toronto, Ontario, Canada; 2) The Centre for Applied Genomics, Sickkids Research Institute, Toronto, Ontario, Canada; 3) Department of Psychiatry, University of Michigan, Ann Arbor, Michigan, US; 4) Psychiatric Neurogenetic Section, Centre for Addiction and Mental Health, Toronto, Ontario Canada; 5) Department of Psychiatry and Behavioral Neurosciences, Wayne State University, Detroit, Michigan, US.

Background: Obsessive-Compulsive Disorder is a common, highly heritable and genetically heterogeneous psychiatric disorder. Recent studies on the role of rare copy number variants (CNVs) have demonstrated that specific CNVs may be involved in various psychiatric disorders such as autism, schizophrenia and ADHD. However, their role in OCD is not established. This pilot study aims to identify rare CNVs in children with OCD and attempts to correlate rare CNVs with phenotypic data from these patients, including clinical information and brain imaging data. **Methods:** A total of 181 OCD patients aged 7 to 17 were genotyped on the Illumina 610K array and the intensity data was used to predict CNV regions based on two programs, PennCNV and iPattern. Rare CNVs were identified by comparing the CNV calls validated by both programs to the Database of Genomic Variants. CNVs with <1% population frequency on the database were considered rare in the general population and subjected to further annotation to determine their potential relevance to OCD. Detailed clinical information was obtained for all patients and Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) data was available for a subset of 20 patients. Based on this information, we reviewed the possible correlation between the identified rare CNVs and available phenotypic data. **Results:** A total of 387 novel or rare CNV calls were identified in our 181 patients. Six CNV regions were found to be disrupting exons in functionally relevant genes involved in neurobiological processes, including NRXN1, SLC2A3, PRODH, CAPN14, ADRA2B2, NOTCH4 and PLXNA3. A patient with a deletion in NRXN1, a gene previously implicated in autism and schizophrenia, showed very early onset of severe OCD symptoms at 4 years of age. Another patient with a deletion in SLC2A3, a neuronal glucose transporter, showed very low glutamatergic concentrations (an OCD-associated phenotype) across all examined brain regions. **Conclusion:** We identified two rare CNVs present in genes previously associated with other psychiatric disorders in our patients. Data from another set of 81 probands have been genotyped and will have been analyzed by the time of presentation (total N=262) including an additional 30 subjects with brain imaging data (total N=50). It is hoped that an increased sample size in this ongoing study will help us find more CNVs in genes relevant to the etiology of OCD and more recurrent CNV-phenotype associations.

551T

Association of multiple sclerosis risk variants of *IRF5* with cerebrospinal fluid levels of the putative prognostic marker CXCL13. *M. Lindén, M. Khademi, I. Lima Bomfim, J. Hillert, T. Olsson, I. Kockum.* Karolinska Institutet, Stockholm, Sweden.

Multiple sclerosis (MS) is a multifactorial disease where both genes and environmental factors affect susceptibility. The strongest genetic association is in the *HLA* locus but several non-*HLA* genes have been confirmed as risk genes for the disease. To predict individual patient prognosis and treatment protocol is challenging. Increased levels of the B-cell chemokine CXCL13 correlate to a worse clinical course, and may represent a prognostic marker, and any genetic correlation to CXCL13 may indicate effects on MS severity. Here, we investigate a possible correlation between CXCL13 levels in cerebrospinal fluid (CSF) and some of the non-*HLA* gene variants showing association with MS. CXCL13 levels in CSF from 420 individuals (including CIS, MS and other neurological diseases) were measured using ELISA. SNPs associated with MS were genotyped in the following genes: *CD226, CD6, CLEC16A, EVI5, IL2RA, IL7R, IL12A, IL21R, IRF5, IRF8, PTGER, RBM17, RGMA, RGS1, SH2B3, STAT3, TNFAIP3, TNFRSF1A, TYK2, VAV1*. The Wald test was used for a quantitative trait association analysis of MS risk genes against levels of CXCL13 in CSF. rs3807306 and rs4728142 in *IRF5* showed significant association with CXCL13 levels ($p<0.001$ for both). Both SNPs are included in a risk haplotype associated with MS in Spanish, Finnish and Swedish populations (Kristijansdottir et al, 2008, Lima Bomfim et al, unpublished data) and are in high LD with each other ($r^2=0.95$). Therefore, and for the sake of simplicity only results for rs3807306 will be shown here. We stratified all individuals by genotype and performed t-tests with Welch's correction to test for differences in mean CXCL13 levels in the different genotype groups. Individuals carrying the risk genotype associated with MS, rs3807306-TT, had significantly higher levels of CXCL13 compared with those carrying the rs3807306-GG genotype ($p=0.0017$). The Jonckheere-Terpstra test showed an effect of allelic dosage of the rs3807306 risk allele on CXCL13 levels (J=23421.5, p=1.10-3), where an increasing number of T alleles correlates with increasing CXCL13 mean levels. IRF5 is a transcription factor that induces antiviral and inflammatory responses. The role the MS-associated *IRF5* variants in MS pathogenesis and disease progression is not known. These results suggest that elevated levels of CXCL13 in CSF are in part determined by SNPs in *IRF5* but further research on their function and possible impact on pathways regulating CXCL13 is needed.

552T

Allelic Differences between Han Chinese and Europeans for Functional Variants in ZNF804A and Their Association With Schizophrenia. *B. Su[1], M. Li[1,2], X. Luo[1], X. Xiao[1], L. Shi[1,2], X. Liu[3], L. Yin[3], H. Diao[4].* 1) Kunming Inst Zoology, Chinese Academy Sci, Kunming, China; 2) Graduate School of Chinese Academy of Sciences, Beijing, China; 3) The Second People's Hospital of Yuxi City, Yuxi, China; 4) Yunnan Mental Health Hospital, Kunming, China.

ZNF804A is a recently identified schizophrenia risk gene by GWAS as well as subsequent replications. Although the results are consistent among studies in European populations, there have been conflicting reports in Chinese populations. We conducted both association and functional analyses to test whether ZNF804A is a risk gene for schizophrenia in Chinese populations. We recruited two independent Han Chinese case-control samples (a total of 2,207 subjects) from southwestern China. A total of 6 single nucleotide polymorphisms (SNPs) were tested including the key SNP (rs1344706) showing significant association with schizophrenia in European populations and the other 5 promoter SNPs of ZNF804A. Based on the results of the association analysis, we performed two functional assays to test the impact of the risk SNP on transcriptional factor binding affinity and promoter activity. Our results showed that rs1344706 was not associated with schizophrenia in either of the two Han Chinese samples, and this result was confirmed by meta-analysis in five Han Chinese samples. However, we identified two ZNF804A promoter SNPs significantly associated with schizophrenia in both samples, and the significance were strengthened in the combined samples (rs359895, p=1.0×10-5 and rs1021042, p=3.0×10-6), and further supported by the haplotype analysis (global p<1.0×10-8). The functional assays demonstrated that the risk SNP (rs359895) can influence the Sp1 binding affinity, resulting in a higher promoter activity of the risk allele. We propose that ZNF804A is a common risk gene for schizophrenia in world populations, and the newly identified functional SNP (rs359895) is likely a risk SNP for schizophrenia.

553T

Using Cluster Analysis to Identify Homogenous Subgroups Within the Autism Spectrum. *O.J. Veatch[1], B.L. Yaspan[1], N. Schnetz-Boutaud[1], M.A. Pericak-Vance[2], J.L. Haines[1].* 1) Ctr Human Gen, Vanderbilt Med Ctr, Nashville, TN; 2) J.P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL.

Autism Spectrum Disorders (ASD) are developmental neuropsychiatric syndromes characterized by impaired social interaction, language/communication deficits and restricted, repetitive behavioral patterns. Previous studies established a strong influence of genomic variation in the etiology of ASD. While associated genomic regions have been identified, estimated effect sizes are small and the combined evidence from numerous analyses does not explain the highly heritable nature of the disorder. Disorders diagnosed within the autism spectrum are very heterogeneous with regard to phenotypic presentation. This wide variability in clinical manifestation has been cited as an explanation for difficulties in identifying common genetic variation associated with ASD and suggests that the clinical variability may be explained in large part by underlying genetic heterogeneity. We chose to use clustering methodology to explore ASD data in an attempt to uncover highly similar genetic subgroups. Single Nucleotide Polymorphism (SNP) data from Caucasian families in the publicly available Autism Genetic Research Exchange (AGRE) dataset was previously evaluated using a pathway-based approach in the Pathway Analysis by Incorporating Structure (PARIS) program. Published results identified 17 biological pathways which met the significance threshold of $p < 0.05$. We further analyzed these pathways to identify risk SNPs driving the associations. We identified a total of 895 SNPs, following pruning for linkage disequilibrium (LD), located in pathways of interest which significantly increased ASD risk ($p < 0.05$). Individuals were scored based on presence of these risk alleles to create a genotype risk score. Clustering was initially done using solely the presence of risk alleles at each locus to identify genetically homogenous groups. In order to identify sub-phenotypes related to underlying genetic risk, we then included available Autism Diagnostic Interview-Revised (ADI-R) scores, Autism Diagnostic Observation Schedule (ADOS) scores, Vineland Adaptive Behavior Scale (VABS) scores and head circumference data in the clustering analysis. Unique clusters of ASD cases are currently being assessed. This approach to ASD gene discovery allows effective evaluation of a broad array of data and enables a more complete phenotype definition thereby increasing power to detect genetic factors influencing risk in subsets of individuals with ASD.

554T

Chromosome 9p21 is associated with frontotemporal lobar degeneration in Finland. *A.E. Renton[1], A.M. Remes[2], A.-L. Kaivorinne[2], T. Peuralinna[3], P.J. Tienari[3], B.J. Traynor[1].* 1) Neuromuscular Diseases Research Group, Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, MD; 2) Department of Clinical Medicine, Neurology, University of Oulu, Oulu, Finland; 3) Department of Neurology, Helsinki University Central Hospital and Molecular Neurology Programme, Biomedicum, University of Helsinki, Helsinki, Finland.

We recently completed a genome-wide association study (GWAS) of amyotrophic lateral sclerosis (ALS) in Finland, which identified a highly significant association peak on chromosome 9p21. All of the Finnish ALS patients associated with this locus shared a 42-SNP haplotype spanning 232 Kb, strongly suggesting a founder effect in this population. Some of the SNPs in this haplotype were also marginally associated with frontotemporal lobar degeneration (FTLD) in an independent GWAS. Furthermore, unpublished data suggest that Finland has a high rate of FTLD, and clinical, neuropathological and epidemiological studies suggest that ALS and FTLD are part of the same neurodegenerative disease spectrum. Given these observations, we hypothesized that the chromosome 9p21 locus may also be responsible for a proportion of FTLD in the Finnish population. To test this, we selected nine SNPs that tagged the original 42-SNP haplotype identified at this locus, genotyped them in 80 Finnish FTLD patients, and compared the haplotype frequency with previously-generated Finnish control data. Overall, 22.5% of these FTLD cases carried the same chromosome 9p21 risk haplotype as the ALS patients. This carrier frequency was similar to that observed previously in Finnish ALS patients. The 9p21 risk haplotype was significantly overrepresented in FTLD compared to controls ($p = 9.22\text{x}10^{-11}$, odds ratio = 7.7). Our results suggest that the chromosome 9p21 locus accounts for a high percentage of Finnish FTLD cases, and confirm that this risk haplotype can clinically present as either ALS or FTLD. It is possible that the chromosome 9p21 locus also accounts for a sizeable proportion of FTLD in other parts of Scandinavia, such as the Lund focus of cases in the south of Sweden.

555T

Genome-wide scan suggested novel Alzheimer disease susceptibility genes by factoring influence of *APOE*. *J. Buros[1], G. Jun[1,2,3], B.N. Vardarajan[1], K.L. Lunetta[3], R. Mayeux[4,5], J. Haines[6], M.A. Pericak-Vance[7], G. Schellengberg[8], L.A. Farrer[1,2,3,9,10], The Alzheimer Disease Genetics Consortium.* 1) Medicine (Biomedical Genetics), Boston University School of Medicine, Boston, MA; 2) Ophthalmology, Boston University School of Medicine, Boston, MA; 3) Biostatistics, Boston University School of Public Health, Boston, MA; 4) The Taub Institute on Alzheimer's Disease and the Aging Brain and The Gertrude H. Sergievsky Center, College of Physicians and Surgeons, Columbia University, New York, NY; 5) Epidemiology, Mailman School of Public Health, Columbia University, New York, NY; 6) Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN; 7) The John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL; 8) Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA; 9) Epidemiology, Boston University School of Public Health, Boston, MA; 10) Neurology, Boston University School of Medicine, Boston, MA.

The *APOE* , 4 allele is the most significant genetic risk factor for late-onset Alzheimer's disease (LOAD). We investigated interaction of *APOE* with other loci on a genome-wide level to identify novel LOAD susceptibility genes. A sample of 11,641 AD cases and 11,341 cognitively normal controls from the Alzheimer Disease Genetics Consortium (ADGC) were evaluated for 2,312,972 genotyped and imputed SNPs. Association of each SNP with LOAD was examined separately in subgroups of subjects with *APOE* genotypes , 3/, 3 and , 3/, 4 using logistic regression adjusting for age and sex. Top-ranked SNPs were further examined within categories of other *APOE* genotypes and by tests of interaction with , 4. All analyses were conducted within constituent ADGC datasets and the results were combined using meta-analysis. Among 10,083 , 3/, 3 subjects (37% cases), the most significant results were obtained for three previously identified AD loci (*BIN1*, *ABCA7*, and *CD33*) and one novel locus near *PTK2B* (rs17447007; P = 1 x 10^{-7}; other *APOE* genotypes: P > 0.50). Among 8,102 , 3/, 4 subjects (70% cases), SNPs in four novel genes (*PRDM12, EXOC5, METTL19* and *SMAD3*) showed strong evidence for association (P < 10^{-5}). The most significant result was observed for SNP rs12899234 near *SMAD3* (P = 8 x 10^{-7}; other *APOE* genotypes: P > 0.29). Analysis of a full model that included terms for this SNP, *APOE* and an interaction term was very significant (interaction term P = 9 x 10^{-6}). This study suggests that there are several novel genes for LOAD whose effects on LOAD risk are either independent of or through interaction with *APOE* genotype.

556T

Identification of Novel Schizophrenia Loci by Homozygosity Mapping Using DNA Microarray Analysis. *N. Kurotaki[1], H. Mishima[2], S. Ono[1], A. Imamura[1], S. Tasaki[1], T. Kukuchi[1], N. Nishida[3], K. Tokunaga[3], KI. Yoshiura[2], H. Ozawa[1].* 1) Department of Neuropsychiatry, Nagasaki University, Nagasaki, Japan; 2) Department of Human Genetics, Nagasaki University, Nagasaki, Japan; 3) Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

The recent development of high-resolution DNA microarrays, in which hundreds of thousands of single nucleotide polymorphisms (SNPs) are genotyped, enables the rapid identification of susceptibility genes for complex diseases. Clusters of these SNPs may show runs of homozygosity (ROHs) that can be analyzed for association with disease. An analysis of patients whose parents were first cousins enables the search for autozygous segments in their offspring. Schizophrenia (SCZ) is categorized as a severe chronic debilitating psychosis that affects approximately 1% of the global population. Although genetic factors are reported to contribute to the disease and multiple responsible loci have been identified from linkage analysis and case-control association studies, there have been few reproducible results to date. Some reports suggested that homozygosity mapping is a powerful tool not only for investigating single gene defects but also for rare genomic variants in complex traits. They observed homozygous deletions in patients with autistic disorders and concluded that genomic alterations might be a subset of disease-causing mutations in chromosomal regions. The increased susceptibility to SCZ observed in consanguineous families suggests that genomic recessive variations may be involved in its etiology. Considering this and other results, we hypothesized that homozygosity mapping, including identical by descent (IBD) analysis, would be a highly constructive method for identifying the loci responsible for SCZ. Here, using the Affymetrix® Genome-Wide Human SNP Array 5.0 to determine ROHs, we genotyped 9 individuals with schizophrenia (SCZ) whose parents were first cousins. We identified overlapping ROHs on chromosomes 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 16, 17, 19, 20, and 21 in at least 3 individuals. Only the locus on chromosome 5 has been reported previously. The ROHs on chromosome 5q23.3-q31.1 include the candidate genes histidine triad nucleotide binding protein 1 (HINT1) and acyl-CoA synthetase long-chain family member 6 (ACSL6). Other overlapping ROHs may contain novel rare recessive variants that affect SCZ specifically in our samples, given the highly heterozygous nature of SCZ. Analysis of patients whose parents are first cousins may provide new insights for the genetic analysis of psychiatric diseases.

557T

Association Study of Cathepsin D Gene Polymorphism with Alzheimer's Disease. *M. Noruzinia[1], A. Sayad[1], M. Zamani[2], M. Harirchian[3], A. KazemNezhad[4], E. ShahsavandAnanloo[5].* 1) Department of Medical Genetics, Tarbiat modares university, Tehran, tehran, Iran; 2) Department of Neurogenetics, Iranian Centre of Neurological Research, Tehran University of Medical Sciences, Tehran, Iran; 3) Department of Neurology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran; 4) Department of Biostatistics, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran; 5) Department of Genomic Psychiatry (DGP), Roozbeh Psychiatric Hospital.

One of the most prevalent forms of dementia is Alzheimer's disease (AD). Complex inheritance and multi-factorial patterns of late-onset Alzheimer's disease (LOAD) along with its heterogeneity are due to the presence of different AD predisposing genes with different influence on disease development among various populations. A key event in the pathogenesis of AD is the deposition of beta amyloid peptide, which is derived from the amyloid precursor protein by beta and gamma secretases. Cathepsin D (CTSD) is an acid protease with) and . secretases-like features in vitro. An exonic C→T polymorphism at position 224 of the CTSD gene has shown to be associated with the enzyme function of CTSD and to Alzheimer's disease. Two studies in German population reported a strong association of this polymorphism with an increased risk of developing AD, while other studies did not confirm this observation. We tested for this association in a case control study in 100 Iranian LOAD patients based on diagnostic criteria DSM-IV-TR and NINCDS-ADRDA and 100 normal controls without any personal and family history of Alzheimer's disease of other related dementias. PCR-RFLP was set up to detect this polymorphism. Our study demonstrated that T carrying genotype frequency in AD patients is significantly higher than in controls and there was 2.5 fold increased risk for developing AD in T carrying genotype compared to C/C genotype(OR=2.5, 95%CI: 1.2-5.1, P=0.01). Our results indicate that CTSD genotype is associated with the disease and significantly alter the risk for developing AD.

558T

Intersecting copy number variants and whole exome sequencing for diagnosis of *FA2H* mutations. *D.R. Simeonov, M. Sincan, T.C. Markello, D.A. Adams, C. Toro, G. Golas, C.F. Boerkoel, W.A. Gahl, T. Pierson.* Undiagnosed Diseases Program, National Institutes of Health, Bethesda, MD.

The spastic paraplegias are a genetically heterogenous group of disorders that overlap clinically. This clinical overlap confounds directed molecular testing. To circumvent this, the NIH Undiagnosed Diseases Program has been evaluating a combination of genomic tools for diagnosis of such genetically heterogeneous diseases. Exemplifying this, we present 10-year-old boy born to healthy unrelated parents who presented with spastic paraplegia, cerebellar ataxia, and cognitive deficits. At age 3, he developed progressive leg spasticity, ataxia, postural instability, impaired eye movements, and dysarthria. Extensive genetic and molecular testing did not reveal a definitive diagnosis. Muscle, skin, and nerve biopsies were unrevealing. MRI imaging of the brain showed non-specific T2 deep-white-matter hyper-intensity consistent with demyelination and evidence of mineralization of the globus pallidi. A sural axonal neuropathy was also present. Exome sequencing and SNP analysis identified two novel *FA2H* mutations: a paternally inherited c.707T>C (p.F236S) and maternally inherited hemizygosity for exons 3-7. FA2H encodes the enzyme fatty acid 2-hydroxylase and is important for CNS myelination. Fatty acid 2-hydroxylase deficiency causes fatty acid hydroxyl associated neurodegeneration (FHAN). In summary, we expand the clinical phenotype of FHAN, report the first instance of FHAN arising in a non-consanguineous family and show the CNV deletions can uncover recessive mutations in SPG-associated genes.

559T

Exome re-sequencing in familial hemiplegic migraine with linkage to 14q32. *M. VILA-PUEYO[1], E. CUENCA-LEON[1], I. GARCIA-MARTÍNEZ[1], F. CASTRO[2], I. GUT[2], M. BAYES[2], B. CORMAND[3], A. MACAYA[1].* 1) PEDIATRIC NEUROLOGY, INSTITUT DE RECERCA HOSPITAL VALL D'HEBRON, BARCELONA, BARCELONA, Spain; 2) CENTRO NACIONAL DE ANÁgLISIS GENÓMICO, BARCELONA, SPAIN; 3) DEPARTMENT OF GENETICS, FACULTY OF BIOLOGY, UNIVERSITY OF BARCELONA, SPAIN.

Familial hemiplegic migraine (FHM) is a rare and severe monogenic variant of migraine with aura, with an estimated prevalence of 1/10.000. Mutations in three genes, CACNA1A, SCN1A and ATP1A2, have been linked to both familial and sporadic hemiplegic migraine. However, genetic heterogeneity appears to be high and a substantial number of cases do not harbour mutations in any of these three genes, including families with known linkage to other chromosomal loci. We have previously reported a multigenerational family with FHM and MA following a dominant inheritance pattern, where a genome-wide linkage analysis mapped the disease-causing gene to a 4.15Mb locus on chromosome14q32 with a maximum two-point LOD score of 3.11 (Cuenca-León et al., Neurogenetics 2009). Direct sequencing of three candidate genes in the region (SLC24A4, ATXN3 and ITPK1) did not uncover pathogenic mutations. To further analyze the linkage region, in the present study we performed exome sequencing of the FHM index case. The Nimblegen SeqCap EZ Exome Library SR was used for exome enrichment. The capture library was sequenced on a Illumina Genome Analyzer IIx instrument using paired-end 75 bp reads. We attained a mean coverage depth of x15 for ~82% of the primary target (26.7 Mb). Under a dominant inheritance model, preliminary whole exome analysis identified 22,131 variants. After filtering through the dbSNP, 1000G and CNAG in-house databases, 156 predicted damaging variants were left, including those in several genes with known brain expression. However, none of them was found within the linkage critical interval on chromosome 14. We then focused on the 4.15 Mb linkage locus and identified 62 variants, although only one of them was not present in dbSNP or 1000G and it was not predicted to be damaging. We are currently inspecting the variants identified in the linkage region and in the rest of the exome to list a selection of plausible candidates for Sanger sequencing validation and for testing cosegregation with the disorder in the whole pedigree.

560T

Association of CNTNAP2 with specific language impairment related phenotypes. *S. Cheong[1], L. Hou[1], J. Flax[2,3], S. Petrill[1,4], P. Tallal[2], L. Brzustowicz[3], C. Bartlett[1].* 1) The Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Children's Hospital and Department of Pediatrics, The Ohio State University, Columbus, OH; 2) Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ; 3) Department of Genetics, Rutgers University, Rutgers University, New Brunswick, NJ; 4) Department of Human Development and Family Science, The Ohio State University, Columbus, OH.

Background. Specific Language Impairment (SLI) is a developmental failure to develop normal language in the absence of intellectual disability or other cognitive/medical explanations. With a prevalence of 7%, it has been associated with later academic difficulty/failure. In previous sibling studies a gene replicated as being involved in autism, CNTNAP2, was associated with SLI specifically with regard to phonological short-term memory (PSTM). In order to replicate and extend this finding, a series of extended pedigrees with SLI were assayed and assessed for association between CNTNAP2 and SLI phenotypes. **Methods.** The sample consisted of 17 US and Canadian extended families with multiple cases of SLI that were genotyped with Illumina SNP arrays (one of: Omni Express, 660W, or Omni 1M). A total of 133 SNPs in CNTNAP2 were used for linkage and association analysis to 8 reading and language phenotypes (categorical: language impairment, reading impairment; quantitative: PSTM, rapid auditory processing, reading comprehension, decoding, expressive language, and receptive language). For each phenotype we analyzed linkage and linkage disequilibrium using the program Kelvin's posterior probability of linkage/linkage disequilibrium. Phenotypic effects of CNTNAP2 were further characterized in a regression framework. **Results.** Three phenotypes (decoding, expressive language, and receptive language) show strong LD with CNTNAP2 (PPLDs > 60% for SNPs rs17586018, rs347180, and rs347223). Additionally, rs1608629 yielded a PPL of 10% with PSTM in line with previous findings. Phenotypes were regressed onto associated SNPs in a model selection framework including possible covariates for age, age2, sex, and performance IQ (PIQ). Variance accounted for by these models ranged in R2 from 0-14% when performing backward selection of hierarchical models assuming additive genotypic effects and accounting for multiple testing. The 3 SNPs associated with language accounted for as much variance in rapid auditory processing and decoding as the language measures (R2=4-9%). Additionally, genotypes at rs1608629 account for 14% of the variance in PSTM. **Conclusions.** CNTNAP2 shows equally strong genetic associations with expressive and receptive language abilities as well as decoding though regression models assuming additive effects also indicate an important role for rapid auditory processing. Additionally, additive effects of CNTNAP2 on PSTM are quite large in this sample.

561T

Association of 18q22.1 with Specific Language Impairment Susceptibility. *N. Li[1], L. Hou[1], J. Flax[2,3], S. Petrill[1,4], P. Tallal[2], L. Brzustowicz[3], C. Bartlett[1].* 1) 1The Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Children's Hospital and Department of Pediatrics, The Ohio State University, Columbus, OH; 2) Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ; 3) Department of Genetics, Rutgers University, Rutgers University, New Brunswick, NJ; 4) Department of Human Development and Family Science, The Ohio State University, Columbus, OH.

Background Specific Language Impairment (SLI) is a neurodevelopmental failure to develop normal language without explanatory factors such as low IQ or inadequate environment. Affecting ~7% of children entering school, it is associated with poor academic outcomes. To date, 4 SLI-associated genes have replicated. In order to discover additional genetic risk factors, genome-wide linkage and follow-up association analysis of a single extended pedigree was conducted. Methods A single large Caucasian pedigree consisting of 61persons from the US (phenotypic N=41, genotypic N=36) was genotyped at > 600,000 single nucleotide polymorphisms (SNPs) using the Illumina SNP arrays. Primary linkage analysis of 6 phenotypes (categorical: language impairment, reading impairment, quantitative: reading comprehension, expressive language, receptive language, non-word reading) were included in the primary analysis. Linkage was conducted using a modified version of McLink. Peak regions were included in an association analysis using all genotyped SNPs from the array including an additional 7 SLI extended pedigrees for follow-up linkage disequilibrium analysis in the program Kelvin. Additional genotype-phenotype characterizations were conducted by regression analysis. Results Four linkage peaks were detected including a PPL of 14.3% in 18q22.1 with the reading comprehension measure. Several SNPs showed linkage disequilibrium in the pedigree (highest PPLD=26%) Those SNPs were entered as independent variables in a regression analysis of reading comprehension. SNP rs957789 accounted for 7% of the variance in reading comprehension in discovery extended pedigree and 2.6% of the variance in reading comprehension for 7 independent SLI pedigrees from a replication analysis. In both cases, the A allele is associated with lower mean reading comprehension scores. This SNP is located in a miscellaneous RNA (miscRNA) transcriptional unit, which we found is expressed in an adult human brain cDNA library. Discussion This is the first report of a risk factor for language impairment in this region of chromosome 18. Further elucidation of the transcriptional system may provide possible mechanistic insights for further cognitive neuroscience studies. Key Words: genome-wide linkage study; language; reading abilities; specific language impairment (SLI).

562T

Linkage and candidate gene analysis identifies a rare variant in NRG1 associated with cannabis dependence in African-Americans. *S. Han[1], J. Gelernter[1,2], H. Kranzler[3,4], B. Yang[1].* 1) Department of Psychiatry, University School of Medicine, New Haven, CT 06511 and VA CT Healthcare Center 116A2; 950 Campbell Avenue; West Haven, CT 06516, USA; 2) Departments of Genetics and of Neurobiology, Yale University School of Medicine, New Haven, CT 06511, USA; 3) Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; 4) VISN 4 MIRECC, Philadelphia VAMC, Philadelphia, Pennsylvania.

Cannabis is the most commonly used illicit drug in the world. A genetic contribution to cannabis dependence (CaD) has been established from family and twin studies. The objective of the current study was to use linkage and candidate gene analyses to identify genetic variants associated with CaD. We first performed nonparametric linkage analysis for CaD in 384 African-American (AA) and 385 European-American (EA) families ascertained on the basis of sibling pairs affected with cocaine and/or opioid dependence. We then used the SAGE GWAS dataset to investigate the association between single nucleotide polymorphism (SNPs) in the region of the linkage peak and CaD, adjusting for sex, age, and population stratification principal components in AAs and EAs separately. In AAs, we identified genomewide suggestive evidence for linkage on chromosome 8p21.1 at 54.9 cM (lod=2.9, pointwise P = 0.00013, empirical genomewide P = 0.097). Weak linkage evidence was detected at the same location in EAs (lod= 0.62, pointwise P = 0.05). In the association analysis in the region of the linkage peak (2601 SNPs) in the SAGE sample, we identified three SNPs nominally significantly associated with CaD in both AAs and EAs. However, only one SNP (rs17664708), located at NRG1, which encodes neuregulin 1, variation in which has been implicated in risk of schizophrenia, showed consistent direction of effect in both AAs (minor allele frequency (MAF) = 0.02, OR=2.7, 95% CI=1.33-5.49, P=0.006) and EAs (MAF=0.098, OR=1.4, 95% CI=1.06-1.86, P=0.017). We replicated the association of rs17664708 with CaD in an independent AA sample (MAF=0.012, OR=2.76, 95% CI= 1.20-6.35, P=0.0085), but not in EAs (MAF=0.12, OR=0.99, 95% CI=0.73-1.35, P=0.96). The joint analysis of the two AA sample sets (SAGE and ours) demonstrated strong association between rs17664708 and CaD (OR= 2.86, 95% CI=1.73-4.75, P=1.3E-5). In conclusion, our study shows, for the first time, that NRG1 is probably a susceptibility gene for cannabis dependence, based on convergent evidence of linkage and replicated association in two independent samples of AAs. Further replication studies using a high density SNP panel or deep sequencing are necessary to confirm the role of NRG1 in CaD.

563T

Cytochrome P450 downregulation in Niemann-Pick disease type C. *C.V.M. Cluzeau[1], N. Al Eisa[2], D.E. Watkins-Chow[3], R. Fu[1], B. Borate[4], M.K. Dail[1], C.A. Wassif[1], F.M. Platt[2], W.J. Pavan[3], F.D. Porter[1].* 1) Program in Developmental Endocrinology and Genetics, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services, Bethesda, MD; 2) Department of Pharmacology, University of Oxford, UK; 3) Genetic Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD; 4) Molecular Genetics Section, Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD.

Niemann-Pick disease type C (NPC) is a lethal autosomal recessive neurodegenerative disorder, resulting from mutations in *NPC1* and *NPC2* genes, with no FDA approved therapy. Cholesterol and sphingolipids are trapped in late endosomes/lysosomes, resulting in liver disease and progressive neurodegeneration. Presenting signs and symptoms are nonspecific, and the diagnosis is frequently difficult and delayed. Despite intensive work, the pathological mechanisms leading to both brain and liver dysfunctions have not been fully defined. In order to identify functional pathways responsible for the NPC pathology, we carried out gene expression profiling in a murine *Npc1*$^{-/-}$ model, which closely recapitulates the human disorder. We isolated liver RNA for microarray analysis to compare gene expression in mutant and control females (N=4), at multiple pre-symptomatic (1-, 3- and 5-week old) and post-symptomatic ages (7-, 9- and 11-week old). Using this approach, we identified 222 significantly altered pathways, including many corresponding to metabolic processes, immune response and developmental signaling. We focused initially on 155 genes that were differentially expressed at all time points. Among these, 10 genes in the cytochrome P450 family were surprisingly downregulated in the mutant mice. This microarray result was validated by qPCR. Cytochrome P450 enzymes are involved in the metabolism of drugs and endogenous compounds, such as arachidonic acid (AA). AA metabolites include the pro-inflammatory molecules prostaglandins (PG) and leukotrienes (LT). The downregulation of cytochrome P450 family members, together with the upregulation of two major enzymes in PG synthesis, led us to hypothesize that dysregulation in AA metabolism may contribute to the inflammation observed in NPC. Cytochrome P450 enzyme downregulation is also a significant pharmacogenetic finding, since impaired P450 activity may result in altered drug metabolism by NPC patients and thus require alteration in medication dosing. Our preliminary data in the murine and feline NPC models showed a marked reduction in cytochromes P450 activity. Microarray analysis also identified 103 differentially expressed genes encoding secreted proteins, including 12 with a modified expression at all time points. These genes represent important candidate biomarkers to facilitate diagnosis or development of therapeutic trials. Analysis of these potential biomarkers in a 56-patient natural history trial is in progress.

564T

Age-dependent Parkinson disease risk assessment for GBA mutation carriers. *H.Q. Rana[1], M. Balwani[1], L. Bier[1], R.N. Alcalay[2].* 1) Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) Movement Disorders Division, Department of Neurology, Columbia University Medical Center, New York, NY.

Background: The association between GBA mutations and Parkinson disease (PD) is well established; however, the age-dependent risk for PD among mutation carriers is unknown. In the general population, PD risk by age 65 is estimated to be 1-1.5%. Objective: To obtain age-related history of PD in parents and children of Gaucher disease (GD) patients, who are obligate carriers of GBA mutations and who were not ascertained by PD history. Methods: We approached all previously genotyped GD patients evaluated at MSSM from 2009-2011. A validated family history of PD questionnaire was administered to GD patients; their parents' and children's age, history of PD, age-at-onset of PD, and ethnic background were obtained. Results: Of 106 GD patients with whom we established contact, 104 (98%) agreed to participate in the study. Mean age was 47.4 years (range 2-93 years old), 53 participants (51%) were male, 66 (63.5%) were N370S homozygotes, and 71 (69%) patients were on enzyme replacement therapy (ERT). One study participant, genotype N370S/L444P, who was diagnosed with GD at age 55 and started ERT at the same time, reported a personal history of PD diagnosed at age 63. Of the 206 parents of GD patients, (mean age 68.7, SD 15.9.), four were reported to have a definitive diagnosis of PD and one had Lewy body dementia (2.4%). The mean age of disease onset was at 64 years old. None of the children of GD patients (N=162, mean age 24.2 years, SD 16.6), were reported to have PD. Conclusion: While GBA mutations may increase the age-dependent risk for PD, the vast majority of GD patients and heterozygous mutation carriers may not develop the disease. Further studies are needed to assess PD risk among GBA mutation carriers in order to provide accurate genetic counseling.

565T

Endophenotype-based discovery of a novel risk locus for alcoholism: *CYP7B1*. *L. Almasy[1], M.A. Carless[1], R. Olvera[2], J. Kent[1], T.D. Dyer[1], M.P. Johnson[1], J.E. Curran[1], E.K. Moses[1], H.H.H. Göring[1], R. Duggirala[1], J. Blangero[1], D. Glahn[3].* 1) Texas Biomedical Research Institute, San Antonio, TX; 2) University of Texas Health Science Center at San Antonio, San Antonio, TX; 3) Yale University, New Haven, CT.

The maximum number of drinks an individual has ever consumed in a 24-hour period (maxdrinks) is highly correlated with risk for alcohol dependence and has been utilized in genetic studies of alcoholism, including successful gene localizations via linkage. However, this important endophenotype has yet to be studied using modern methods of genome scanning that take advantage of high density SNP data.

Maxdrinks was assessed in 1195 Mexican American participants in extended pedigrees from the San Antonio Family Study, along with lifetime history of abuse or dependence derived from the MINI-Plus psychiatric screening questionnaire. Participants were genotyped for approximately one million SNPs on Illumina microarrays. After removal of monoallelic SNPs and SNPs with low call rates, 931,219 markers remained. Genome-wide association analyses were conducted using an additive measured genotype model, correcting for the non-independence among family members.

The heritability of maxdrinks was 0.33 and its genetic correlation with lifetime history of alcohol abuse or dependence was 8_g=0.98 ($p=1.4\times10^{-10}$). The environmental correlation was 8_e=0.48 ($p=3.1\times10^{-9}$), implying that both shared genetic and shared environmental factors influence lifetime risk for alcoholism and maxdrinks. Genome-wide association identified one significant signal for max-drinks on chromosome 8 at rs17293712 ($\chi2$=29.5, $p=5.0\times10^{-8}$, MAF=0.096) which also showed nominal evidence of association with lifetime history of alcohol abuse (p=0.018). This SNP is located in the intron of the cytochrome P450, family 7, subfamily B, polypeptide 1 (CYP7B1) gene, which encodes a member of the cytochrome P450 superfamily. The cytochrome P450 proteins are monooxygenases that catalyze reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. Although *CYP7B1* has not previously been associated with alcoholism, it is highly expressed in human hippocampus, a brain region altered in individuals at risk for alcoholism. In addition, a closely related gene, *CYP2E1*, has been implicated in alcohol dependence in candidate gene studies. We are currently conducting sequencing studies in a subset of these families to identify putative functional variants in and around *CYP7B1*.

566T

Association between the *COL25A1* gene and anti-social personality disorder (ASPD) co-morbid with drug dependence. *D. Li[1], H. Zhao[2,3], HR. Kranzler[4,5], D. Oslin[4,5], RF. Anton[6], LA. Farrer[7], J. Gelernter[1,3,8,9].* 1) Department of Psychiatry, School of Medicine, Yale University, New Haven, CT; 2) Department of Epidemiology and Public Health, School of Medicine, Yale University, New Haven, CT; 3) Department of Genetics, School of Medicine, Yale University, New Haven, CT; 4) Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA; 5) VISN 4 MIRECC, Philadelphia VAMC, Philadelphia, PA; 6) Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina, Charleston, SC; 7) Departments of Medicine (Biomedical Genetics), Neurology, Ophthalmology, Genetics & Genomics, Biostatistics, and Epidemiology, Boston University Schools of Medicine and Public Health, Boston, MA; 8) VA CT Healthcare Center, West Haven, CT; 9) Department of Neurobiology, Yale University School of Medicine, New Haven, CT.

Antisocial Personality Disorder (ASPD) is a psychiatric disorder characterized by a long-term pattern of manipulating, exploiting or violating the rights of others. Subjects ascertained for genetic studies of drug addiction and diagnosed with ASPD and co-morbid drug dependence were included in a two-stage genetic association study. Selected single nucleotide polymorphisms (SNPs) mapped to 258 candidate gene loci were genotyped in a case-control cohort and family-based cohort at the discovery stage. After stringent quality control, one SNP, rs13134663, mapped to the collagen XXV alpha 1 gene (*COL25A1*), was significantly associated with ASPD in both African-Americans (AAs) and European Americans (EAs) (smallest *P* values were 0.0002 and 0.0004, respectively). The findings were further observed by including family samples using a unified association approach. Evidence of association with the same SNP was also revealed in independent case-control replications in both AA and EA subjects with co-morbid alcohol dependence (AD), cocaine dependence (CD), and marijuana dependence (MjD) (*P* = 0.035 and 0.033, respectively). Analysis of the combined set of case-control subjects including both AAs and EAs (in both the discovery and replication stages) yielded an allelic *P* value of 9×10^{-6} [odds ratio (95% confidence interval) = 1.3 (1.16, 1.47)]. The association was more significant in the AA subjects with co-morbid drug dependence. The *COL25A1* gene, located at chromosome 4q25, encodes the collagen-like Alzheimer amyloid plaque component precursor, a type II transmembrane protein specifically expressed in neurons; it co-localizes with A) in senile plaques in Alzheimer disease brains. This SNP maps to the transcription factor binding site and is conserved in 17 vertebrates, including mice and rats. Our findings suggest that the *COL25A1* gene may also be associated with ASPD, especially when it co-occurs with AD, CD and/or MjD. Further investigation of the gene using other methods is warranted.

567T

Genomic Characterization of Schizophrenia Candidate Gene Regions. *A. Nato[1], X. Kong[1,2], B. Byrne[3], J. Naus[4], D. Gordon[1], S. Buyske[1,4], L. Brzustowicz[1], T. Matise[1].* 1) Dept Genetics, Rutgers Univ, Piscataway, NJ; 2) GlaxoSmithKline, King of Prussia, PA; 3) Informatics Inst, UMDNJ, Piscataway, NJ; 4) Dept Statistics and Biostatistics, Rutgers Univ, Piscataway, NJ.

Schizophrenia (SZ) is a debilitating mental disorder with a lifetime prevalence of ~0.55-1%. Family, twin, and adoption studies have revealed that SZ has a genetic component. In this study, we extracted and analyzed data from the 47 published independent genomewide linkage scans for SZ between 1994 and 2010. We defined 22 schizophrenia candidate gene regions (SCRs) by employing three methods: single significant hit approach, disjoint approach, and smoothing method. For the single significant hit approach, an SCR was defined by the presence of a significant hit by extending 10 cM upstream and downstream of that hit. For the disjoint approach, we identified criteria-events based on clustering of hits within small regions by using a sliding window approach. For the smoothing method, we imputed a randomized range of p-values ($0.001697\leq p<1$) for the genome scans without a suggestive or significant result at each 0.1 cM interval. We combined these p-values with the genome scan results using three methods: geometric mean method, Fisher's method, and Stouffer's method. We characterized each SCR by identifying genes and genomic elements such as structural variations, regulatory elements, and functional noncoding RNAs. Total SCR coverage is 880 cM with SCR sizes ranging from 18 cM to 87 cM. There are 5,482 known genes, 1,954 SD pairs, 16,176 CNVs, and 24 rearrangement hotspots within these SCRs. SCRs with multiple peaks were divided into smaller subregions. We developed a ranking system to identify and prioritize SZ candidate genes by assigning weights to each of the following criteria: (a) located within SCRs that are weighted based on maximum -log10(p-values), (b) annotated in SZ association studies, microarray analyses, and meta-analyses, (c) associated with phenotypes or diseases, and (d) located within or near other genetic elements. We identified pathways that were most significantly associated with the top 10% of candidate genes after prioritization by utilizing the Ingenuity® Pathways Analysis (IPA). We generated an interactome for SZ based on the SZ candidate genes. Our procedure, which provides a novel approach to identify and prioritize candidate gene regions and genomic elements, is applicable to other complex diseases.

568T

Next generation sequencing follow-up study on the Late-Onset Alzheimer Disease susceptibility gene *MTHFD1L*. *J.R. Gilbert[1], M.A. Kohli[1], A.C. Naj[1], A.J. Griswold[1], J.M. van Baaren[1], T.E. Plitnik[1], P.L. Whitehead[1], G.W. Beecham[1], E.R. Martin[1], M.L. Cuccaro[1], C.B. Wright[1], E. Crocco[1], J.L. Haines[2], M.A. Pericak-Vance[1].* 1) John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL, USA; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN, USA.

Genome-wide association studies (GWAS) have identified many common variants with reproducible associations to a multitude of human complex diseases, however the underlying functional variant or molecular mechanism often remains undiscovered. Next generation sequencing (NGS) promises comprehensive variant discovery in a genomic region of established genetic association to human disease. We used long-range PCR to capture a 50 kb region on chromosome 6q25.1 for NGS on a Genome Analyzer II from Illumina. The selected region comprises a linkage disequilibrium (LD) block harboring rs11754661, a single nucleotide polymorphism (SNP) for which we previously reported genome-wide statistical significance in a GWAS on Late-onset Alzheimer's disease (LOAD) and subsequently replicated in a much larger GWAS. The SNP rs11754661 is an intronic SNP with a low minor allele frequency (MAF) of 0.07 in Caucasians, and is located in the 5' region of the 236-kilobasepair gene *MTHFD1L*, which is involved in the folate pathway and represents an interesting LOAD candidate since it may influence homocysteine levels, a significant risk factor for LOAD. We re-sequenced 50 LOAD cases and 34 age-matched cognitively normal controls all heterozygous for rs11754661 to increase the likelihood of also detecting rare LOAD relevant variants. We generated ultra-deep sequencing data and used MAQ for sequence read alignment and variant calling. We detected 264 putative variants which were all intronic or mapped 5' of *MTHFD1L*. Genotypes of 234 variants were clustered according to expected allelic read ratios and examined: 29% were novel (not in dbSNP132 annotation) and 55% were rare (MAF < 0.05). Excluded variants were shown to be false positives by Sanger sequencing mainly due to falling in repetitive regions. Examining single marker case-control data, several associations were observed, including rs4869953 (P=0.033), 151234519_A>G (P=0.048), and rs7765521 (P=0.049). Pooled rare variant case-control associations are currently being examined. In summary, NGS data provide a comprehensive catalogue of SNPs throughout the target region with reliable individual genotypes after filtering. Several challenges remain, including insertion-deletion genotype calling and finding functional candidate variants to explain the GWAS association, the latter of which may require analysis of resequencing data of the whole *MTHFD1L* gene region in more individuals, which is ongoing.

569T

Identification of autism risk variants through targeted next generation sequencing in a 1000 case and 1000 control cohort. *A.J. Griswold[1], D. Hedges[1], R. Chung[1], J. Rantus[1], P. Whitehead[1], I. Konidari[1], W. Hulme[1], S. Slifer[1], J. Jaworski[1], S.M. Williams[2], R. Menon[3], M.L. Cuccaro[1], E.R. Martin[1], J.R. Gilbert[1], J.L. Haines[2], J.P. Hussman[4], M.A. Pericak-Vance[1].* 1) University of Miami, Miami, FL; 2) Vanderbilt University, Nashville, TN; 3) Emory University, Atlanta, GA; 4) Hussman Foundation, Ellicott City, MD.

Genome-wide association studies (GWAS) and copy number variation screens have suggested roles for hundreds of genes in autism susceptibility. Since disease causing variants likely have not been genotyped, sequencing of associated regions to identify rare or low frequency variants contributing to autism is essential. To identify functional variants associated with autism, we are sequencing candidate regions in 1000 unrelated cases with autism and 1000 unrelated controls. Candidate regions were chosen from our recent analysis of two autism datasets which employed the GWAS noise reduction method in conjunction with prioritization of haplotype blocks based on the Truncated Product Method (TPM) (Hussman et. al., 2011). We designed an Agilent SureSelect probe set covering 17Mb corresponding to: 1) exons of 689 genes overlapping blocks with TPM p-values < 0.05 2) evolutionarily conserved regions in those genes plus 5kb from their transcriptional starts and ends 3) evolutionarily conserved regions within non-genic significant blocks 4) the entire blocks with TPM p-values < 0.01. Cases meet DSM-IV and ADI-R criteria for autism; controls were obtained from a cord blood bank or saliva samples from pediatric non-autistic individuals. The cases and controls are matched across three self-reported ethnicities, confirmed by Eigenstrat analysis, into 700 non-Hispanic Caucasians, 200 Hispanics, and 100 African Americans in each set. Sample preparation and capture hybridization of 96 samples is concurrently performed on the Caliper Sciclone G3 and sequenced on the Illumina HiSeq2000. Data are analyzed with a pipeline consisting of BWA alignment, variant calling with the GATK Unified Genotyper, and annotation using publicly available tables. Preliminary analysis of 214 sequenced samples (112 cases and 102 controls) reveal complex libraries with 4.8% ± 2.3 library duplication and 64.1% ± 5.1 of bases on target. 87.5% ± 4.3 of our targeted bases are covered at least 8X allowing for robust variant identification. We identified an average of 32685 ± 3226 single nucleotide variants per individual including 30084 ± 2785 variants in dbSNP version 132 or 1000-Genomes and 2601 ± 878 novel variants, including 54 ± 19 novel non-synonymous nonsense and missense or splice site mutations. This sequencing will provide a vast compendium of variation in autism associated genomic regions and allow for discovery of new variants in genes and genetic networks contributing to autism risk.

570T

Deep sequencing of the LRRK2 gene in 14,002 individuals to guide the clinical development of LRRK2 inhibitors. *J.P. Rubio[1], S. Topp[1], L. Warren[2], P.L. St Jean[2], J. Shen[2], D. Kessner[3], J. Novembre[3], D. Fraser[2], J. Aponte[2], K. Nangle[2], L.R. Cardon[4], M.G. `Ehm[2], S.L. Chissoe[2], J.C. Whittaker[1], M.R. Nelson[2], V.E. Mooser[4].* 1) Genetics, R&D, GlaxoSmithKline, Stevenage, Hertfordshire, United Kingdom; 2) Genetics, R&D, GlaxoSmithKline, Research Triangle Park, NC, USA; 3) Ecology and Evolutionary Biology, UCLA, Los Angeles, CA, USA; 4) Genetics, R&D, GlaxoSmithKline, King of Prussia, PA , USA.

Both rare and common genetic variation in the LRRK2 gene can predispose to Parkinson Disease (PD). The former observation set in motion discovery efforts for inhibitors of LRRK2 for the treatment of PD. LRRK2 has pleiotropic functions and is genetically associated with autoimmune diseases, so LRRK2 inhibitors may be indicated or contraindicated for non-PD conditions. In an attempt to answer these questions and guide the development of LRRK2 inhibitors, we applied the principle that natural variations within the drug target gene may be used as surrogates for pharmacological modulation of LRRK2 in humans. To that end, we deep-sequenced the 51 protein-coding exons of LRRK2 in 12 case collections (n = 9,582) including Alzheimer's disease, bipolar disorder, coronary artery disease, chronic obstructive pulmonary disease , dyslipidemia, epilepsy, irritable bowel syndrome, multiple sclerosis, osteoarthritis, rheumatoid arthritis, schizophrenia and unipolar depression, and in 4,420 population-based controls. We identified 739 single nucleotide variants (SNVs), one every 20 bp on average, including 260 non-synonymous (NS) variants and five nonsense mutations. In Europeans, 62% of SNVs were private to a single individual, whereas 6% had a minor allele frequency (MAF) ⁄ 1%. For the enzymatic domains of LRRK2 we found greater conservation across mammalian species and evidence of purifying selection against potentially damaging NS variants in humans. Case-control association analysis for 12 diseases identified 17 suggestive associations (P≤0.05) of small-modest effect that will require independent replication. We also identified 98 carriers (0.7%) of previously reported PD-associated variants amongst 14,002 individuals, including 31 carriers (0.22%) of pathogenic mutations. In conclusion, this study provides the most comprehensive evaluation of LRRK2 variation to date and provides important insights into evolutionary and population genetic aspects of LRRK2. Genetic association analysis in 12 non-PD common human diseases did not strongly point towards new opportunities for re-purposing of LRRK2 inhibitors, or any particular safety concerns.

571T

Whole-Exome Sequencing of 40 Obsessive-Compulsive Disorder Patients. *D. Trujillano[1], S. Ossowski[1], C. Tornador[1], P. Alonso[2], M. Gratacòs[1], X. Estivill[1].* 1) Genes and Disease Program, Center for Genomic Regulation (CRG-UPF), Barcelona, Catalonia, Spain; 2) Psychiatry Department, Bellvitge University Hospital, Barcelona, Catalonia, Spain.

Introduction: Fast progress in sequencing capacity focused on the coding regions (the exome) that occupy 2% of the genome has provided enormous possibilities to identify new disease genes. Obsessive-compulsive disorder (OCD) is a severe mental disorder characterized by unwanted and intrusive thoughts, images, or impulses (obsessions) and/or repetitive behaviors or mental acts (compulsions). OCD is recognized by the World Health Organization as a major cause of disability. However, little is known about its neurobiology and research still needs to study the genetic factors underlying this complex and heterogeneous disorder. **Methodology:** To reach this goal we carried out whole-exome sequencing of 40 OCD patients. Our heterogeneous cohort included patients with family history of OCD, early age at onset, severe cases, and also patients with good prognosis. The high depth of sequencing achieved by using the SureSelect Human All Exon Kit (Agilent) and sequencing using paired-end sequencing on a single lane of the Illumina GAIIX sequencer allowed us to confidently mine the data for single nucleotide variants (SNV) of interest and for novel SNV and short InDel discovery. **Results:** We identified potentially pathogenic variants in our cohort of patients, where further functional testing is necessary. Correlations are being sought between the different mutated genes and the clinical phenotypes of the patients. **Conclusions:** Whole-exome sequencing of OCD patients provides a high level of resolution of SNV and InDel discovery to dissect the molecular basis of OCD. Replication of SNV and InDel in additional patients should further substantiate these results. We expect that results from this project will contribute to design new strategies for treatment of OCD.

572T

Difficulties in prenatal Diagnosis of Metachromatic Leukudystrophy. *E. Khalili[1&2], M. Hooshmand[1&2], O. Aryani[1].* 1) National Institute for Genetic Engineering and , Tehran, Iran; 2) Genetic Dep. Special Medical Center, Tehran, Iran.

Two siblings of consanguinous parents were noted to have a neurologic syndrome marked by regression of psychomotor performance from the first year, marked spasticity and progressive central nervous system degeneration (clinical manifestation was like late infantile Metachromatic Leukodystrophy.) Markedly delayed nerve conduction times were evident. Brain MRI showed diffuse white matter lesions without involvement of subcortical U-fibers., activity of arylsulfatase A white blood cells and cultured skin fibroblasts were below 5%. Molecular investigation of ARSA gene showed a homozygote DNA alteration in A905G position in exon 5 which changes amino acide Lys302Arg. The parents were heterozygote for this alteration. Because of some problem with prenatal diagnosis with enzyme assay such as: 1) The presence of aryl sulphatase C in chorionic villi, an isoenzyme which may interfere in assays of aryl sulphatase A. 2) The presence of maternal enzyme in chorionic villus material illustrated by the detection of the A isoenzyme of B-hexosaminidase in chorionic villus from a pregnancy affected with Sandhoff's disease. 3) The finding of falsely normal levels of -iduronidase in chorionic villus samples from a pregnancy affected with Hurler's disease, probably due to contamination with maternal tissue which has relatively high levels of this enzyme compared with fetal chorionic material. 4) The inadequacy of indirect assays of incorporation of radiolabel into macromolecules using chorionic villi, for example propionate incorporation for prenatal diagnosis of methylmalonic aciduria. 5) Multiple sulfatase deficiency and their combination with other diseases. Molecular diagnosis according to sequencing finding was done for this family. Prenatal Diagnosis was done for this family and fetus was heterozygot for this mutation. This child was affected according to enzyme assay after birth. Molecular investigation for multiple sulfatase deficiency and Saposin B deficiency were negative in this family. Thus, we believe these patients may represent a new form of ARSA deficiency.

573T

Impaired FLNA expression disrupts radial glia functions leading to periventricular heterotopia in rats and humans. *A. Carabalona[1,2], S. Beguin[1,2], E. Pallesi-Pocachard[1,2], E. Buhler[1,2,3], C. Pellegrino[1,2], K. Arnaud[1,2], P. Hubert[4], M. Oualha[5], S. Khantane[1,2], I. Coupry[6], C. Goizet[6], A. Bernabe Gelot[7], A. Represa[1,2], C. Cardoso[1,2].* 1) INMED, INSERM U901, Parc Scientifique de Luminy, Marseille, France; 2) Université de la Méditerranee, Marseille, France; 3) Plateforme postgénomique INMED, INSERM, Parc Scientifique de Luminy, Marseille, France; 4) Université Paris Descartes, 12 rue de l'Ecole de Medecine, Paris, France; 5) Service de réanimation pédiatrique et de néonatologie, Hôpital Necker Enfants Malades, 149 rue de Sèvres Paris, France; 6) Laboratoire de Génétique Humaine, Université Victor Segalen Bordeaux 2, Bordeaux, France; 7) Département de Neuropathologie, Service d'Anatomie et Cytologie Pathologiques, Hôpital A. Trousseau, Paris, France.

Periventricular heterotopia (PH) is a brain malformation caused by defective neuronal migration that results in ectopic neuronal nodules lining the lateral ventricles beneath a normal appearing cortex. Most affected patients have epilepsy, with normal to borderline cognitive function. Mutations in the Filamin-A (FLNA) gene are the main cause of PH. However, the lack of FlnA animal models that mimic PH has delayed our understanding of the underlying pathological mechanisms. Here, we show that in utero RNA interference-mediated knockdown of FlnA expression leads to the genesis of PH in the developing rat brain. FlnA-knockdown disrupts the organisation and polarity of radial glia as revealed by the altered distribution of vimentin and) 1-integrin at the ventricular zone. The resultant defects alter cell cycle progression and delay migration of cortical neurons. We found similar alterations of radial glia in the post-mortem brain of a PH patient harboring a novel FLNA frameshift mutation (R2103QfsX56). Altogether, these findings highlight the critical role of FLNA in radial glia organization and function for neurogenesis and migration. We propose that alterations of these functions contribute to PH formation. Finally, FlnA-knockdown rats showed increased susceptibility to pentylenetetrazol-induced seizures thus providing a unique opportunity to investigate PH pathogenesis.

574T

Deep sequencing in mental retardation. *M. Gratacos[1], J. González[1], I. Madrigal[2], L. Rodríguez[2], M. Milà[2], X. Estivill[1], R. Rabionet[1].* 1) Genes and Disease, Center for Genomic Regulation, Barcelona, Spain; 2) Genetics Service, Hospital Clínic i Provincial de Barcelona, Barcelona, Spain.

Intellectual disability (ID) is a genetically heterogeneous disorder affecting 1-3% of the population. There are both syndromic and non-syndromic forms of ID, and although there is an elevated proportion of sporadic cases, it can also be inherited in both autosomal dominant and autosomal recessive forms. About 30% of cases of ID can be explained by large structural variants, visible either cytogenetically or by microarray, and over 100 genes have been implicated in its pathophysiology. Nevertheless, about 60% of the cases remain unexplained. A recent study has shown that *de novo* point mutations are a frequent cause of ID, which would make next generation sequencing technologies extremely powerful tools to identify rare *de novo* genetic causes of mental retardation. We recruited 37 cases of sporadic idiopathic mental retardation without dysmorphic features, all of them negative for the fragile X expansion and without cytogenetically visible abnormalities. We performed array comparative genomic hybridization (aCGH) in order to detect smaller structural variants potentially causing the ID, and we identified large sturctural variations (SV) in approximately 20% of the cases. We further performed long-insert mate pair sequencing in 15 cases in order to compare the results with aCGH. Samples negative for SV by aCGH or next generation sequencing were selected for exome sequencing. The exome was captured with Agilent sure select technology, multiplexed, and sequenced in an Illumina HISeq2000 lane. Potential damaging variants were detected by an in-house developed pipeline. Finally, the identified variants were tested in parental DNA to confirm that they were true *de novo* events and thus considered as potentially involved in the susceptibility to develop ID.

575T

Parent-child trio exome sequencing in non-syndromic intellectual disability. *F.F. Hamdan[1], H. Daoud[2], A. Dionne-Laport[2], D. Spiegelman[2], S. Dobrzeniecka[2], E. Henrion[2], O. Diallo[2], J.M. Capo-Chichi[1], S.L. Girard[2], G.A. Rouleau[2], J.L. Michaud[1].* 1) Centre of Excellence in Neuroscience of Université de Montréal (CENUM), Centre de Recherche du CHU Sainte-Justine, Montréal, Quebec, H3T1C5, Canada; 2) Centre of Excellence in Neuroscience of Université de Montréal (CENUM) and Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), and Department of Medicine, Université de Montréal, Montreal, Quebec, Canada, H2L 4MI.

Non-syndromic intellectual disability (NSID) is defined by the absence of associated morphologic, metabolic, or radiologic abnormalities. Recent studies suggest that de novo mutations are responsible for a large fraction of sporadic NSID cases. We have launched a project that aims to identify these mutations by performing exome sequencing in sporadic NSID cases and in their parents. We capture exomes with the Agilent SureSelect all exon capture kit (50Mb) and sequence them on SOLiD or Illumina HiSeq platforms. Stringent criteria are used for calling variants. After applying a series of variant filters to exclude synonymous, inherited and common variants, the remaining candidate amino-acid altering or splicing de novo variants are validated by Sanger sequencing. This analytic pipeline typically leads to the identification of less than 10 candidate variants per trio. As a proof of principle, we have identified a de novo truncating mutation (p.R157X) in the transcription factor gene *TCF4* in a female with NSID using this approach. Heterozygous truncating point mutations in *TCF4* have been previously reported in patients with Pitt-Hopkins syndrome (PHS), which is characterized by specific facial dysmorphism, severe intellectual disability, epilepsy, and intermittent hyperventilation. In contrast, our patient shows mild-to-moderate NSID without epilepsy or breathing abnormalities. Our finding shows for the first time that *TCF4* truncating point mutations can cause NSID, expanding the phenotypical spectrum associated with this gene. At this point in time, we have completed the sequencing in 11 other trios and are currently processing the datasets. We will discuss these results and provide an analysis of the yield of our approach.

576T

Mutations in SPTAN1 in intellectual disability and pontocerebellar atrophy. *J.L. Michaud[1], H. Saitsu[2], K. Nishiyama[2], J. Gauthier[3], S. Dobrzeniecka[3], D. Spiegelman[3], J.C. Lacaille[4], J.C. Décarie[5], N. Matsumoto[2], G.A Rouleau[3], F.F. Hamdan[1].* 1) Centre of Excellence in Neuroscience of Université de Montréal (CENUM), Centre de Recherche du CHU Sainte-Justine, Montréal, Quebec, H3T1C5, Canada; 2) Department of Human Genetics, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan; 3) Centre of Excellence in Neuroscience of Université de Montréal (CENUM) and Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), and Department of Medicine, Université de Montréal, Montreal, Quebec, Canada, H2L 4MI; 4) Le Groupe de Recherche sur le Système Nerveux Central, Department of Physiology, Université de Montréal, Montréal, H3C 3J7, Canada; 5) Department of Medical Imaging, CHU Sainte-Justine, Montréal, Quebec, H3T1C5, Canada.

Heterozygous in-frame mutations in the (-II subunit of spectrin (SPTAN1) were recently identified in 2 patients with intellectual disability (ID), infantile spasms, hypomyelination and widespread brain atrophy (Saitsu et al., 2010 *AJHG*). By screening *SPTAN1* in 95 patients with idiopathic ID, we found 2 novel de novo mutations. The first is an in-frame mutation, p.Q2202del, found in a patient with a milder phenotype than that found in the previously described patients, including less severe cognitive impairment, well-controlled generalized epilepsy and pontocerebellar atrophy. Expression of this mutant form of (-II spectrin in primary neurons resulted in the formation of aggregates containing) -II and) -III spectrin subunits in 20 percent of cells, whereas the previously described in-frame mutations induced aggregation in most cells. The milder phenotype of our patient thus correlates with the decreased ability of the mutation p.Q2202del to induce spectrin aggregations. The second de novo mutation is a missense, p.R566P, found in a patient with mild non-syndromic ID. The sister of this second patient was also diagnosed with non-syndromic ID but lacked p.R566P in her blood DNA, suggesting that she is a phenocopy or that p.R566P is not responsible for her brother's condition. The p.R566P mutant induced aggregates in a small number of neuronal cells (<5 percent). Double immunostaining revealed that these aggregates were composed of the) -II but not of the) -III spectrin subunit. This work indicates that in-frame mutations in the C-terminus of SPTAN1 cause a core set of manifestations that include severe ID, generalized epilepsy and pontocerebellar atrophy. Although it is unclear whether p.R566P is pathogenic, we found that this mutation induces a distinct pattern of spectrin aggregation. The study of spectrin aggregation in cultured cells provides a paradigm to validate candidate variants and to establish correlations between genotypes and phenotypes.

577T

Mutations in the *SHANK3* gene in Japanese autistic patients with severe delayed speech development and mental retardation. *C. Waga[1,2], N. Okamoto[3], H. Asano[1], Y. Goto[2], S. Uchino[1], S. Kohsaka[1].* 1) Department of Neurochemistry, National Institute of Neuroscience, Kodaira, Tokyo, Japan; 2) Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, Kodaira, Japan; 3) Department of Medical Genetics, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan.

The 22q13.3 deletion syndrome (Phelan-McDermid syndrome, PMS) is characterized by a significant delay in language development, mental retardation, hypotonia, and autistic features. Cumulative evidence has shown that haploinsufficiency of the *SHANK3* gene is a major cause of the neurological symptoms of the 22q13.3 deletion syndrome. Shank3, a multidomain protein containing the SH3 and PDZ domains, is located in the postsynaptic density and interacts with various synaptic molecules including PSD-95 and glutamate receptors. It is therefore thought that the Shank3 plays an important role in the formation and function of synapses. The *SHANK3* gene consists of 22 exons and has five CpG islands (CpG island-P, -2, -3, -4, -5) whose methylation is thought to be involved in regional- and temporal-specific expression of *SHANK3* gene. In this study, we initially analyzed the *SHANK3* gene in 128 autistic patients with manifestations similar to those seen in the 22q13.3 deletion syndrome and identified two novel heterozygous mutations; an 18 bp (6-amino acid) deletion upstream of the SH3 domain (1 patient) and the insertion (3 patients) or deletion (1 patient) of a repeated 10-bp GC sequence located 9-bp downstream from the 3' end of exon 11. None of these mutations was found in 288 control subjects. Since these mutations were located within an intragenic CpG island-2 (vicinity of exon11 encoding SH3 domain), we next examined the methylation of CpG island-2 in the developing mouse neocortex. The results showed that the methylation rate of CpG island-2 increased from birth until postnatal day 14, thereafter decreased gradually. Furthermore, we identified intragenic promoters whose activity was regulated by DNA methylation, and found the expression of several *SHANK3* transcripts. To clarify the effect of mutations of *SHANK3* gene in autistic patients on DNA methylation and promoter activity and elucidate the role of novel Shank3 transcripts in the developing brain, further study is now on going. This study was approved by the ethics committees in National Center of Neurology and Psychiatry and in Osaka Medical Center and Research Institute for Maternal and Child Health.

578T

Genomic methylation profile in peripheral blood DNA of Autism Spectrum Disorder (ASD): A case-control study. *A. Homs Raubert[1], I. Cusco[1,2], L.A. Perez-Jurado[1,2].* 1) Unitat de Genetica, DCEXS, Universitat Pompeu Fabra, Barcelona, Spain; 2) Centro de Inevstigacion Biomedica en Red de Enfermedades Raras, CIBERER, ISCIII, Spain.

Autistic Spectrum Disorders (ASD) are a group of neurodevelopmental conditions characterized by a triad associating impairments in social interactions, communication problems and restricted range of interests. ASD prevalence is apparently increasing, affecting nowadays 1/150 newborns, being a relevant Public Health burden. Despite strong evidence for a genetic basis and recent findings of single gene mutations, copy number changes and double hit combinations, only a small proportion of cases have a defined etiology. MZ twin concordance is less than 90%; with variable expressivity suggesting environmental factors are also important. To explore a possible role of epigenetic abnormalities in ASD, we have quantified global 5mC content by HPLC-MS and SssI/dam methylase assays and established genomic methylation patterns with the Illumina Human Methylation 450K array platform in blood DNA from ASD patients (n=34, males, 2-15 year old) and age and gender-matched controls (n=10). All patients had a diagnosis of idiopathic ASD after extensive clinical and genomic studies. We found that the genomic content of 5mC was significantly reduced in ASD with respect to age-matched controls (2.62±0.4 vs 4.06±0.16). After extensive filtering and statistical analysis of array data, we observed 293 genes containing differentially methylated CpGs (p-value<0.01; SD in controls<0.025, present at least in 2 samples and with no SNPs in the CpG), mostly showing relative hypomethylation (82%), in agreement with 5mC quantification. Functional annotation by enriched gene ontology-based analysis performed with the top 20 differential methylated genes revealed a primary role in regulation of brain development, organization and synaptic assembly (*CTNNA2*, *WNT7B* and *NCOR2* genes). Moreover, among the 293 loci, 21 lie in genes previously associated with ASD through different genetic or genomic rearrangements (mostly de novo events), further reinforcing their putative role in the disease. Replication in a larger cohort by scalable techniques is in process, along with expression studies in several samples and additional tissues, including brain. Our data suggest that epigenetic abnormalities may be pathogenically related with ASD in a significant proportion of cases.

579T

DNA methylation landscapes of the human brain in Alzheimer's disease. *S. Iraola-Guzman[1], R. Rabionet[1], M. Montfort[2], F. Mancuso[3], G. Roma[3], E. Martí[1], I. Ferrer[4], X. Estivill[1].* 1) Genes and Disease Program, Center for Genomic Regulation (CRG), and Pompeu Fabra University (UPF), Barcelona, Spain; 2) Genotyping Unit, CeGen-ISCIII, Center for Genomic Regulation (CRG), and Pompeu Fabra University (UPF), Barcelona, Spain; 3) Bioinformatics Unit, Center for Genomic Regulation (CRG), and Pompeu Fabra University (UPF), Barcelona, Spain; 4) Anatomo-pathological Clinical Service, Hospital of Bellvitge and Biomedical Research institute of Bellvitge (IDIBELL), Barcelona, Spain.

Alzheimer's disease (AD) is a neurodegenerative disorder with an estimated worldwide prevalence of 30 million people. Most patients with AD develop the disease after age 60, and are considered “late-onset AD” (LOAD). Several genes have been described as risk factors for LOAD (*APOE, CLU, CR1, PICALM and MTHFD1L*), but the variants in these genes explain a small proportion of the genetic component of the disorder. Alterations in the DNA methylation (DNAm) profile have been related to several diseases, and it has been proposed that they may play an important role in age-related diseases, such as Alzheimer's, Parkinson's, and Huntington's disease. We have attempted the evaluation of the DNAm profile of human brain samples in patients affected by LOAD. For this purpose, we selected four brain areas that are target regions for LOAD (cortex, hippocampus, amygdala and cerebellum), belonging to 40 individuals affected and non-affected with LOAD. To assess the DNAm status of more than 450,000 CpG sites (CpGs) we used the Humanmethylation450K Beadchip Illumina array, containing CpGs in coding and non-coding CpG islands (CGIs), CGIs shores, non-CGIs regions and miRNAs. We performed hierarchical clustering based on methylation values by Principal Component Analysis (PCA), which confirmed the previously reported clustering of all the cerebellum samples, in addition to a clustering by gender within each branch, probably due to X-chromosome inactivation. A second PCA, after removing the cerebellum samples and the CpG sites located in the X-chromosome, showed a trend to cluster by brain area (cortex, hippocampus and amygdala). Next, we assessed the feasibility to correlate DNAm values with either brain area or disease status, using Linear Discriminant Analysis (LDA), finding: 1) a combination of 38 CpGs that group all samples according to brain area; and 2) a different combination of 60 CpGs that differentiate unaffected and affected samples, even discriminating between the various stages of the disease. Finally, we performed differential analysis, finding 123 CpGs with DNAm values significantly altered in the cerebellum samples of LOAD-affected vs. unaffected individuals (p<0.05). Our findings strongly support the hypothesis of an epigenetic involvement in LOAD, and encourage further studies in this and other neurodegenerative disorders.

580T

Association analysis of polymorphisms in DNA MethylTransferase(DNMT) genes with Schizophrenia in a South Indian population. *K.R. SARADA LEKSHMI[1], N.V NEETHA[1], BALAN. SHABEESH[1], NAIR. CHANDRASEKHARAN[2], M.A PRIYA[3], K.R INDU[3], M. BANERJEE[1].* 1) HUMAN MOLECULAR GENETICS, RAJIV GANDHI CENTRE FOR BIOTECHNOLOGY, THIRUVANANTHAPURAM, KERALA, INDIA; 2) NAIRS HOSPITAL, COCHIN, KERALA,INDIA; 3) MENTAL HEALTH CENTRE, THIRUVANANTHAPURAM, KERALA, INDIA.

Schizophrenia is a complex biopsychosocial disorder affecting about one percent of world population. It encompasses severe mental disorders that result in loss of contact with reality along with major personality derangements. The etiology is complex involving a major genetic contribution as well as environmental factors interacting with the genetic susceptibility. Epigenetic processes may contribute to environmentally induced phenotypic variation by modifying gene expression. The capacity to respond to environmental cues is often heritable, indicating a genetic basis for epigenetic modifications. DNA methylation is the most common epigenetic modification and aberration that have been implicated in the pathophysiology of various complex disorders including schizophrenia. These variations might be due to extrinsic factors like diet, age etc or due to intrinsic variations in the genes involved in maintainance of DNA methylation.DNA methytransferases are a group of enzymes which catalyses the transfer of methyl group from S-Adenosyl methionine to the cytosine residue of DNA. The objective of the study was to investigate the role of polymorphisms in DNMT genes (DNMT1, DNMT2, DNMT3A, 3B and 3L) and their association with schizophrenia in a south Indian population and its effects in global methylation levels. Methodology: DNA was isolated from 250 patients and 250 healthy controls after obtaining informed consent. Only patients suffering from schizophrenia diagnosed by DSM-IV/ICD 10 have been enrolled for this study. Symptom severity was rated using BPRS-E and PANNS. Age, Sex and ethnicity matched controls were recruited for the study. We selected 19 polymorphisms from DNMT genes based on their functional relevance and tagging status. Samples were genotyped using allele specific amplification followed by fluorescence detection (KASPar) and PCR-Sequencing. Global DNA methylation levels were analysed using Methylamp™ Global DNA Methylation Quantification Kit from Epigentek. Statistical analysis was done using COCAPHASE and SPSS. Results: DNMT1 was found to be significantly associated with schizophrenia in our population. The rs 2114724 and rs 2228611 were associated with allelic, genotypic and haplotypic combinations. Global DNA methylation level was found to be higher in patients compared to controls.

581T

Gene and miRNA transcriptional profiling of cognitive endophenotypes in schizophrenia pedigrees. *M.A. Carless[1], J. Neary[1], M. Zlojutro[1], R.C. Gur[2], M.F. Pogue-Geile[3], K. Prasad[4], J. Blangero[1], V.L. Nimgaonkar[4], R.E. Gur[2], L. Almasy[1].* 1) Department of Genetics, Texas Biomedical Research Institute, 7620 NW Loop 410, San Antonio, TX, 78227; 2) Departments of Psychology, Neurology and Radiology, University of Pennsylvania Medical Center, 10th Floor Gates Building, Philadelphia, PA, 19104; 3) Department of Psychiatry, University of Pittsburgh, 210 S. Bouquet St, Pittsburgh, PA, 15260; 4) Department of Psychiatry, University of Pittsburgh Medical Center, 3811 O'Hara Street, Pittsburgh, PA, 15213.

Schizophrenia (SCZ) is a highly heritable complex disorder for which disease liability is poorly explained by known genetic variation. To better elucidate the biology of SCZ we are implementing a transcriptional approach to cognitive endophenotypes within large, multigenerational, multiplex SCZ pedigrees. We have a cohort of 677 samples from 42 families with 2 or more cases of SCZ or schizoaffective disorder, depressed type (SAD) and 273 healthy controls. We performed preliminary gene expression analysis on 158 individuals (23 SCZ/SAD, 122 unaffected family members, 13 healthy controls) using Illumina microarrays; 15,278 probes were tested against nine cognitive trait scores. Most notably, expression of *QSOX2* withstood Bonferroni correction for correlation with sensorimotor dexterity (SD, $p=1.72\times10^{-6}$). *QSOX2* plays a role in catalyzing oxidation of sulfhydryl groups in peptide and protein thiols, and is suggested to be a positive mediator of programmed cell death. We also saw suggestive significance for correlation between *CCNG1* expression and abstraction and mental flexibility (ABF) efficiency ($p=8.8\times10^{-4}$). *CCNG1* is located within a chromosome 5 linkage region that we had previously implicated in ABF in this cohort. It is involved in cell cycle regulation and expression differences have been demonstrated in hippocampus and inferior parietal lobule of individuals with mild cognitive impairment. Further, gene enrichment analyses indicated that related cyclin-dependent kinase inhibitors (CDKN, empirical $p=6.0\times10^{-3}$) were enriched for association with ABF. We also examined miRNA expression using the Illumina GA*IIx* next-generation sequencing platform; a total of 480 miRNAs were tested against cognitive traits. *miR-1248* withstood Bonferroni correction for correlation with ABF ($p=4.9\times10^{-5}$) and has many predicted target sites, including those within cell cycle regulatory genes (*CCND2*, *CDK2*). Other miRNAs showed suggestive significance with a number of traits, including *miR-505* with emotion ($p=5.9\times10^{-4}$), *miR-339-3p* with SD ($p=1.3\times10^{-4}$), *miR-4517* with facial (1.4×10^{-3}) and spatial ($p=6.1\times10^{-4}$) memory and *miR-182* with verbal memory ($p=8.8\times10^{-4}$). Our preliminary results suggest that gene and miRNA transcripts play an important role in cognitive deficits in schizophrenia, particularly with regard to cell cycle regulation in ABF. We are currently analyzing the full cohort to identify additional genes and miRNAs that may play a role in the etiology of schizophrenia.

582T

MicroRNA expression profile in mesial temporal sclerosis provides insight into underlying mechanisms. *D.B. Dogini[1], C.S. Rocha[1], C. Yassuda[2], H. Tedeschi[2], E. Oliveira[2], C.V. Maurer-Morelli[1], F. Cendes[2], I. Lopes-Cendes[1].* 1) Dept Medical Genetics, FCM - UNICAMP, Campinas, Sao Paulo, Brazil; 2) Dept Neurology, FCM - UNICAMP, Campinas, Sao Paulo, Brazil.

The main purpose of this study was to investigate the possible role of microRNA (miRNA) gene regulation in temporal lobe epilepsy (TLE) with mesial temporal sclerosis (MTS). MTS is mainly characterized by selective neuronal loss and gliosis of specific regions of hippocampus. Total RNA was isolated from hippocampal tissue of 4 patients who underwent selective resection of the mesial temporal structures for the treatment of clinically refractory seizures. In addition we used control samples from autopsy (n= 4) for comparison. RNA samples were used in real-time PCR reactions with the Human Panel Early Access™ kit (Applied Biosystems) to quantify 157 different miRNAs. Bioinformatic analyzes identified three miRNAs, which were differently expressed in patients as compared to controls: let-7d and miR-29b were over expressed in patients; whereas, miR-30d was down-regulated in patients. A possible target gene for let-7d is Nme6 which we also found to be down- regulated in patients. In addition, Mcl-1, the putative target gene of miR-29b was also down-regulated in patients. Mcl-1 is a potent multidomain anti-apoptotic protein of the Bcl-2 family and its tight regulation of protein levels is necessary, because insufficient Mcl-1 can result in inappropriate cell death. Nme6 belongs to NME (nm23 /nucleoside diphosphate kinase) gene family in humans and act as an inhibitors of p53-induced apoptosis. In conclusion, we have identified three miRNAs differently expressed in hippocampal tissue from patients with TLE and MTS. At least 2 of the genes regulated by these miRNAs play an important role in apoptosis.

583T

Transcriptional and posttranscriptional regulation of *SPG4*, encoding Spastin, the most frequently mutated gene in hereditary spastic paraplegia. *B.J. Henson[1], W. Zhu[2], K. Hardaway[1], J.L. Rupert[1], M. Stefan[1], K.M. Albers[3], R.D. Nicholls[1,2].* 1) Dept Pediatrics, Children's Hospital of Pittsburgh, Rangos Research Center, Pittsburgh, PA; 2) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 3) Department of Medicine, University of Pittsburgh, Pittsburgh, PA.

Hereditary spastic paraplegia (HSP) refers to a group of neurodegenerative disorders that display progressive spasticity of the lower extremities, due to axonal degeneration of the corticospinal motor tracts. HSP is both clinically and genetically heterogeneous. Clinically, HSP can be pure or present as complex forms that have other neurological or non-neurological features. HSP shows autosomal dominant inheritance in ~70-80% of cases, with additional cases being recessive or X-linked. To date, 48 chromosomal loci have been linked to pathogenesis in HSP, with the causal genes identified for half of these. The most common form of HSP arises from mutations in the *SPG4* (*SPAST*) gene, which occurs in ~40% of dominantly inherited cases and ~10% of sporadic cases. *SPG4* encodes Spastin, which possesses microtubule-severing ability and is a member of the AAA (ATPases Associated with diverse cellular Activities) protein family. Both loss of function and dominant-negative mutations in *SPG4* have been described, suggesting that precise or stoichiometric levels of Spastin are necessary for biological function. Therefore, we hypothesized that transcriptional and post-transcriptional regulatory mechanisms controlling expression of *SPG4* are important and presently unknown determinants of Spastin biology. We used phylogenetic methods to identify highly conserved transcription factor (TF) binding sites and miRNA targeting sequences in the *SPG4* promoter and 3'-UTR, respectively. Using chromatin immunoprecipitation (ChIP), luciferase reporter assays (with mutations in the TF binding sites), siRNA knockdown of TFs, and TF overexpression, we demonstrate that *SPG4* transcription is positively regulated by NRF1, SOX11, and Sp1. Indeed, our data shows that NRF1 regulates about one-third of known HSP loci. Furthermore, we show that miR-182 and miR-96 negatively regulate *SPG4* via effects on both mRNA stability and protein translation. Intriguingly, our data indicate that miR-182 and miR-96 also regulate *NRF1*, *SOX11*, and several other spastic paraplegia gene products, suggesting that these miRNAs play critical roles in the regulation of corticospinal neuronal functions. In conclusion, we have identified major transcriptional and miRNA regulatory mechanisms for Spastin synthesis. These regulatory elements provide new functional sequences for mutation screening in HSP. *Supported by the Spastic Paraplegia Foundation and NIH.*.

584T

miRNAs and Polyamine Gene Expression in the Brains of Suicide Completers. *J.P. Lopez[1,2], L.M. Fiori[2,3], V. Yerko[3], G. Turecki[1,2,3].* 1) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 2) McGill Group for Suicide Studies, Douglas Mental Health University Institute, McGill University, Montreal, Quebec, Canada; 3) Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada.

MicroRNAs (miRNAs) are small, non-coding RNA molecules that play an important role in the posttranscriptional regulation of mRNA. These molecules have been the subject of growing interest as they are believed to control the regulation of a large number of genes, including those expressed in the brain. Evidence suggests that miRNAs could be involved in the initiation and progression of neuropsychiatric disorders. Alterations in metabolic enzymes of the polyamine system have been reported to play a role in predisposition to suicidal behavior, and we have previously shown the expression of the polyamine genes SAT1 and SMOX to be down-regulated in the brains of suicide completers. We hypothesize that the dysregulation of these genes in suicide completers could be influenced by miRNA posttranscriptional regulation. Using five miRNA target prediction programs, we identified several miRNAs that target the 3' untranslated region (UTR) of SAT1 and SMOX. We profiled the expression of 10 miRNAs in the prefrontal cortex (BA44) of suicide completers (N=15) and healthy controls (N=15) using quantitative real-time polymerase chain reaction (qRT-PCR). We found that four miRNAs showed significant up-regulation in the prefrontal cortex of suicide completers compared to healthy controls: hsa-miR-139-5p (fold change: 1.84; $p < 0.01$), hsa-miR-195 (fold change: 1.68; $p < 0.05$), hsa-miR-34c-5p (fold change: 2.56; $p < 0.01$) and hsa-miR-320c (fold change: 1.95; $p < 0.01$). Furthermore, we demonstrated a significant correlation between these miRNAs, SAT1 and SMOX expression. Our results suggest a relationship between miRNAs and polyamine gene expression in suicide behaviors and postulate a mechanism for SAT1 and SMOX down-regulation by posttranscriptional activity of miRNAs. Ultimately, our results provide new evidence for the role of miRNAs in neuropsychiatric disorders.

585T

Identification of miRNAs associated with mood disorders using next-generation sequencing. *J.L. Neary[1], D.C. Glahn[2], M. Zlojutro[1], T.D. Dyer[1], J.E. Curran[1], L. Almasy[1], R. Duggirala[1], J. Blangero[1], M.A. Carless[1].* 1) Department of Genetics, Texas Biomedical Research Institute, San Antonio, TX; 2) Department of Psychiatry, Yale University, New Haven, CT.

MicroRNAs (miRNAs) are a family of short non-coding RNAs which post-transcriptionally modulate gene expression, and several have been proposed as viable targets for mood stabilizing medications. However, miRNA expression in affected individuals and the relationship between miRNA expression and neurophenotype have not been well-studied. Our GOBSF (Genetics of Brain Structure and Function, ~1,300 individuals within ~50 families) and GOBD (Genetics of Bipolar Disorder, ~100 individuals) studies collect blood samples and extensive neuroanatomical and neurocognitive data for all subjects, as well as measures of depression, anxiety, and substance abuse. Here we present preliminary results associating miRNA expression to neurocognitive and neuroanatomical measures of mood disorder.

For a subset (n=126) of individuals with complete phenotypic data, we employed multiplex-based NGS sequencing (Illumina TruSeq platform) to ascertain whether quantitative variations in lymphocyte-derived miRNA levels correlate to mood disorder endophenotypes. Several miRNAs were associated with major depressive disorder (MDD), recurrent MDD (rMDD) and the Beck Depression Inventory (BDI) at a nominal p-value of 0.05. For example, hsa-miR-1282 ($p=0.021$, $p=0.0015$, $p=0.045$), hsa-miR-486-3p ($p=0.023$, $p=0.0028$, $p=0.036$), and hsa-miR-1231 ($p=0.035$, $p=0.0023$, $p=0.040$) were associated with all three measures of depression respectively. Another miRNA, hsa-miR-4690-3p, was strongly associated with lifetime MDD ($p=0.0075$) and recurrent MDD ($p=0.00028$). In these cases, higher levels associated with increased risk. We also identified several miRNAs with evidence of decreased expression in bipolar disorder and some suggested targets of mood stabilizers. For example, hsa-miR-27b shows altered expression in bipolar disorder, MDD ($p=0.021$), and recurrent MDD ($p=0.045$). Although no associations met correction for multiple testing, we anticipate that expansion to a larger cohort will yield more powerful results, particularly for miRNAs that demonstrate consistency across multiple measures of depression or have prior evidence of affiliation with mood disorders.

586T

Identification of miRNAs and target mRNAs with deregulated expression in schizophrenia and bipolar brains. *B. Sadikovic, R. Pearson, L. Meng, A. Beaudet.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

While miRNA expression could play a role in the etiology of schizophrenia and bipolar disorder, current literature is limited and conflicting. The objective of this study was to identify deregulated miRNAs in the prefrontal cortex of schizophrenia and bipolar subjects, and analyze expression of their mRNA targets. Postmortem samples of BA8 region from 30 control, 22 schizophrenia, and 20 bipolar subjects were analyzed with the Agilent miRNA Microarray (V3) for >950 miRNAs. We identified 25 and 24 significantly deregulated miRNAs in schizophrenia and bipolar samples respectively, whose expression signature allowed robust clustering of disease vs. control brains. Validation of 20 candidate miRNAs with qRT-PCR across all samples confirmed the array findings in 17 miRNAs, with 13 showing significance ($p < 0.05$). These included miRNAs 223, 142-3p, 19b overexpressed, and 129-5p, 212, 181a, 132 26a, 24, and 487a underexpressed in schizophrenia, and 223, 7-1*, 454 overexpressed, and 26a, 29c, 22, and 1973 underexpressed in the bipolar samples. Bioinformatic analysis of binding sites identified significant enrichment for brain and neuron-specific genes as putative targets for schizophrenia miRNAs, and included AFF2, SEMA6A, CDK5R1, BPTF, and MAPT genes. Expression analysis using qRT-PCR showed significant increase in expression in all these genes in schizophrenia brains. In a concurrent set of experiments using qRT-PCR and Northern analysis, we show evidence of differential degradation of snoRNAs (that are often used to normalize miRNA data) relative to endogenous miRNAs in post-mortem brains, which create normalization artifacts, and may provide explanation for some of the discrepancies in current schizophrenia miRNA literature. In conclusion, these data provide the most thorough and comprehensive assessment of miRNA expression in schizophrenia to date and identify disease-specific miRNA signatures. In silico integration of miRNA and mRNA targets allowed us to predict and validate multiple brain-specific and neurodegeneration-related genes with increased expression in schizophrenia brains.

587T

Differential analysis of microRNAs expression profile between PBMCs from primary progressive and relapsing remitting multiple sclerosis patients for the identification of novel targets. *M. Sorosina[1], P. Brambilla[1], C. Fenoglio[2], G. Giacalone[1], D. Scalabrini[2], F. Esposito[1], M. Serpente[2], C. Cantoni[2], E. Venturelli[2], M. Rodegher[1], L. Moiola[1], B. Colombo[1], M. De Riz[2], V. Martinelli[1], E. Scarpini[2], G. Comi[1], D. Galimberti[2], F. Martinelli Boneschi[1].* 1) Institute of Experimental Neurology (INSPE), and Department of Neurology, Division of Neuroscience, San Raffaele Scientific institute, Milan, Italy; 2) Dept. of Neurological Sciences, "Dino Ferrari" Center, University of Milan, Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, Milan, Italy.

Multiple Sclerosis (MS) is a chronic autoimmune disease of the central nervous system, characterized by the loss of myelin and axonal degeneration. Identification of novel targets and biomarkers of MS could be extremely helpful to better understand the mechanisms underling the pathogenesis of the disease. About 85% of the MS patients experience the relapsing-remitting MS (RRMS) disease course, characterized by phases with neurological deficits followed by remission. The more severe form of MS is the primary progressive (PPMS), characterized by a progression of the neurological impairment from the onset. MicroRNAs (miRNAs) are single-stranded non-coding RNA molecules of 20-25 nucleotides involved in the fine regulation of the gene expression at the post-trascriptional level. They act binding specific 3'UTR sequences of target miRNAs leading to their down-regulation. By using an hypothesis-free approach, we tested the expression profile of 1146 miRNAs in peripheral blood mononuclear cells (PBMCs) of 7 PPMS patients and 6 RRMS patients using the Illumina® BeadArray Technology. We observed 64 differentially expressed miRNAs (FDR corrected $p \leq 0.05$ and Fold Change ≥ 2 or ≤ -2) between the two variants of MS: 30 and 34 miRNAs were respectively up- and down-regulated in PPMS. According to the microRNA.org database we downloaded a list of 37 predicted target genes (mirSVR score$\leq$-1) common to the top five upregulated miRNAs and a list of 159 target genes (mirSVR score$\leq$-1) common to the top five downregulated miRNAs. Subsequently we looked at the expression profile of these target genes obtained from a HumanHT-12 v.3 BeadChip microarray Illumina experiment performed on PBMCs from 13 PPMS and 12 RRMS patients. Among all the predicted common target genes, 10 genes satisfied either conditions: (i) they were significantly differentially expressed ($p \leq 0.05$) between PPMS and RRMS and (ii) they showed an opposite expression levels compared to those of the miRNAs they were targeted by. One gene (PRIC285) resulted downregulated in PPMS, and 9 genes (AP3M1, ATXN1, CGGBP1, DENND4C, EIF5, PRDM2, SPRED1, TMED7, UBE4A) were observed to be upregulated. Despite the limited sample size, we believe that this pilot study could suggest the presence of fine regulation mechanisms, carried out through miRNAs differentially expressed between PPMS and RRMS patients, toward common target mRNAs. Deeper analyses are needed to evaluate the role of these target genes in the two forms of MS.

588T

Mitochondrial genetics: Identification of variants in subjects with mood disorders and schizophrenia. *F. Mamdani[1], P.A. Sequeira[1], M.V. Martin[1], B. Rollins[1], E.A. Moon[1], W.E. Bunney[1], F. Macciardi[1], C. Magnan[2], M. van Oven[3], P. Baldi[2], M.P. Vawter[1].* 1) Psychiatry & Human Behavior, University of California, Irvine, Irvine, CA; 2) Computer Science, University of California, Irvine, Irvine, CA; 3) Forensic Molecular Biology, Erasmus MC, University Medical Center Rotterdam, The Netherlands.

The human mitochondrial DNA genome (mtDNA), independent from the nuclear genome, has been shown to accumulate mutations in the brain during the lifespan that can lead to biochemical dysfunctions and observable phenotypic changes. Mitochondrial diseases often are characterized by the presence of psychiatric symptoms suggesting a link between mutations in the mtDNA and the susceptibility to psychiatric disorders. Several lines of evidence support a role of mitochondrial dysfunction in mood disorders and schizophrenia, including postmortem brain, imaging and, genetic studies. Mitochondrial DNA (mtDNA) somatic and common polymorphisms were investigated in schizophrenia (SZ), bipolar disorder (BD), major depressive disorder (MDD) and controls using the Illumina GAII platform, as well as Affymetrix SNP 6 arrays. Previously, we compared a 4977 base pair mtDNA common deletion between SZ and controls, in eleven brain regions and in peripheral blood. This investigation was further extended to assess levels of the deletion in the dorsolateral prefrontal cortex (DLPFC) in a sample of 12 BD, 14 SZ, 15 MDD and 35 controls. We found the presence of the common deletion to be highly variable across the 11 brain regions; however, minimal differences were seen in peripheral blood. Instances of the common deletion in the DLPFC sample were increased in BD ($p=0.022$) compared to controls, but not in the other disorders. Full mtDNA genome resequencing identified 12 novel homoplasmic mutations in the haplogroups mtDNA of 23 subjects. Currently, we are conducting further experiments to confirm and reinforce our findings through sequencing of 40 additional subjects (10 BD, 10 SZ, 10 MDD, 10 controls) for DLPFC and investigating the common deletion across 11 brain regions from these same 40 subjects. This study reinforces that the brain shows an increased accumulation of mitochondrial structural variants in brain tissue within psychiatric populations compared to controls.

589T

Regulators of expression of heroin-dependence candidate genes and their association with gene networks involved in mouse opioid-induced behavioral changes. *R.F. Clark, E.O. Johnson.* RTI International, Research Triangle Park, NC.

Differences in gene expression in the brain influence behavior and disease susceptibility, yet the contribution of single genes to complex behavioral phenotypes is usually small. Describing such phenotypes in terms of networks of interacting genes is desirable but challenging in humans due to the low availability of appropriate tissues. Thus, a systems biology approach to linking human candidate genes with publicly-available large-scale gene expression data in mice may be a useful technique for identifying networks of co-expressed genes that may influence complex human phenotypes. In this study we used in silico systems genetics tools to identify genes that are co-regulated with mouse homologues of 20 human heroin-dependence candidate genes and to evaluate their overlap with genes associated with >100 opioid-induced behavioral changes in mice. We used expression-QTL mapping to identify the sources of variation in gene expression in the mouse hippocampus and nucleus accumbens (NA), and to enable global mapping of factors regulating such transcriptional responses. We investigated natural genetic variation in gene expression levels of >39,000 transcripts in related mouse strains in two RI panels of C57BL/6J and DBA/2J to gain insights into molecular networks that might alter the transcription of the candidate genes. We found that the expression of >18 genes proximal to Oprm1 (an important gene involved in opioid-dependence) on chromosome 10 in mice are coordinately expressed and cis-regulated (LRS > 20). We also found that several other candidate genes (e.g., Comt, Myocd, etc.) have statistically significant trans- QTL loci (LRS > 20). Some of these loci are found proximal to loci of genes within gene networks associated with several opioid-induced behaviors, such as differential morphine response to locomotion in an open field. These analyses suggest that we have found several good candidate genes for regulating hippocampal/NA expression of opioid-induced behavioral genes in mice (and potentially heroin-dependence in humans). All genes are highly expressed in the hippocampus/NA, as well as in several other brain regions. Furthermore, these genes are novel candidates for involvement in human heroin addiction pathways. These findings emphasize the utility of a systems biology approach to analyze complex data to model interacting biological processes.

590T

Access to Genetically Engineered Mouse Models. *S. Rockwood, C. Lutz, M. Sasner, L. Donahue.* Gen Res Sci, The Jackson Laboratory, Bar Harbor, ME.

Genetically modified mice have greatly contributed to our understanding of basic biology and disease processes. In order to facilitate access to these mice, the Mouse Repository at The Jackson Laboratory was established to serve as a centralized resource for the archiving and distribution of genetically engineered mice at a high health status. As the number and complexity of alleles increase, so does the importance of providing adequate information to facilitate their selection and use by researchers. New mouse strains with applications in a wide range of disciplines (immunology, neurology, metabolism, etc) continue to be made available to the scientific community through an easy to use, online database. Newly available strain types generally reflect the most compelling human disease concerns. New to the immunological class of mutants are *Batf3* knockout mice which feature an impaired development of splenic CD8($^+$ conventional dendritic cells. Conditional mutants continue to represent a significant portion of the new alleles reposited; a floxed Rptor strain makes possible the examination of a central node of the mammalian pathway that coordinates cell growth and availability of nutrients, energy and growth factors. Serving as companion strains to floxed models are a wide range of cre recombinase expressing strains; newly added is a transgenic line expressing cre under the direction of an *Adipoq* promoter, allowing researchers to control cre-mediated recombination effectively in white and brown adipose tissues. Another knock-in model at the *Sst* locus expresses cre in dendritic inhibitory interneurons, such as Martinotti cells and Oriens-Lacunosum-Moleculare (O-LM) cells. Numerous tool strains are available as well, such as a growing number of strains that incorporate channelrhodopsin technology, and the "R26R-Confetti" mice which serve as a stochastic multicolor Cre recombinase reporter of multiple fluorescent proteins expressed from a single genomic locus. Donating a strain to the Repository fulfills the NIH's requirements for sharing of mice. Researchers wishing to have strains considered for inclusion in the Repository are encouraged to submit their strains at: www.jax.org/donate-a-mouse.

591T

Evaluating Genetic Markers and Neurobiochemical Analytes for Antidepressant Response Using a Panel of Inbred Mouse Strains. *T. Wiltshire[1], C. Santos[1], B.H Miller[3], S. Skewerer[2], J.S. Marron[2], O. Suzuki[1], M. Pletcher[4].* 1) Sch Pharmacy, Univ North Carolina, Chapel Hill, NC; 2) Department of Statistics and Operations Research, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; 3) Department of Neuroscience, The Scripps Research Institute, Florida, Jupiter, FL, USA; 4) Compound Safety Prediction, Pfizer Global Research and Development, Groton, CT, USA.

Identification of biomarkers that can establish diagnosis or treatment response is critical to the advancement of research and management of patients with major depressive disorder. Biomarkers can be used objectively to evaluate clinical progression and response to antidepressant therapy. To identify genetic and neurobiochemical biomarkers of antidepressant response, we compared behavior, gene expression, and levels of thirty-six neurobiochemical analytes proposed to affect anxiety and mood disorders between water and fluoxetine-treated mice in a panel of genetically diverse mouse inbred lines. While responses in the open field (OF) and tail suspension test (TST) both contribute to baseline inter-strain differences, chronic fluoxetine treatment predominantly affected behavior in the TST, indicating that the TST is sensitive to the antidepressive effects of fluoxetine. We found that levels of glyoxylase1 (GLO1) and guanine nucleotide binding protein (GNB1) account for most of the covariance in baseline anxiety-like and depressive-like behavior. Overall difference in neurobiochemical levels were observed for positive and negative responders. Biochemical alterations following chronic fluoxetine treatment discriminated positive responders, while baseline neurobiochemical differences differentiated negative responders. Results from partial least squares (PLS) and distance weighted discrimination (DWD) analyses show that levels of S100) , GFAP, GSK3) , and HDAC5 contributed most to variable antidepressant response. These proteins are linked within a cellular growth/proliferation pathway, suggesting that positive responses to antidepressants are likely due to increased cellular genesis. In addition, a candidate genetic locus that associates with baseline depressive-like behavior contains a gene that encodes for cellular proliferation/adhesion molecule (Cadm1), supporting a genetic basis for the role of neuro/gliogenesis in depression.

592T

Pre-diagnostic parent experiences in Duchenne and Becker muscular dystrophies, congenital muscular dystrophies, and spinal muscular atrophies: A survey of the National Task Force for the Early Identification of Childhood Neuromuscular Disorders. *H. Peay[1], K. Mathews[2], National Task Force for the Early Identification of Childhood Neuromuscular Disorders.* 1) Parent Project Muscular Dystrophy, Richmond, VA; 2) University of Iowa Children's Hospital, Iowa City, IA.

Objective: Describe the pre-diagnostic experiences of parents of children with Duchenne, Becker, congenital muscular dystrophies and spinal muscular atrophy, including parents' descriptions of their earliest concerns. Background: We know little about how parents describe first concerns of neuromuscular disorders. Studies utilizing chart reviews indicate that childhood NMDs are often diagnosed well after symptoms emerge, and after parents express concerns to a clinician. Design/Methods: Cross-sectional, retrospective survey distributed electronically to parents of children diagnosed between ages 6m-9y. Analyses include chi-squared, T tests, ANOVA, correlation, and thematic coding. Results: Data were analyzed from 1087 parents: 651 DMD (60%), 244 SMA (22%), 105 CMD (10%), 87 BMD (8%). Quantitative data largely support published data and clinical experience. There were no significant differences between those with a positive family history and those without regarding whether parent had concerns before diagnosis (Chi-Square = 0.528, p-value = 0.467) or who had first concerns (Chi-Square = 2.035, p-value = 0.362). Parents with a family history reported significantly less time between first telling doctor about their worry and age at diagnosis (t = -2.171, p-value = 0.030). Analysis of open-ended questions about words parents use to describe concerns identified themes of milestone-associated concerns, comparing to siblings/peers, and non-specific intuition statements. Concerns clustered in infant/young child (SMA and CMD) and preschool/school age (B/DMD). We report specific words parents use to describe weakness, hypotonia, coordination, muscle hypertrophy, difficulty rising from floor, and others. Conclusions: These data support the previously reported diagnostic delay. The fact that there is a significantly shorter time between sharing concerns with providers and diagnosis in those with a family history suggests that providers are (rightly) taking family history into account when evaluating development. The effect of birth order may reflect a greater focus on motor milestones among first-born children and disinclination by parents, and/or dissuasion by providers, to compare the development of siblings. Unlike chart reviews, we report parents' words to describe their concerns as one step in facilitating earlier diagnosis. Funding Source: This survey was funded by the Centers for Disease Control and Prevention.

593T

Molecular analysis of *FA2H* gene mutations in patients with spastic paraplegia and thin corpus callosum. *D. Di Bella[1], E. Sarto[1], M. Plumari[1], S. Caldarazzo[1], B. Castellotti[1], M. Panzeri[1], A. Erbetta[2], L. Nanetti[1], C. Mariotti[1], F. Taroni[1].* 1) Unit of Genetics of Neurodegenerative & Metabolic Diseases, IRCCS Istituto Neurologico C Besta, Milan, Italy; 2) Unit of Neuroradiology, IRCCS Istituto Neurologico C Besta, Milan, Italy.

Hereditary spastic paraplegias (HSP) are a clinically and genetically heterogeneous group of neurodegenerative disorders. Pure and complicated forms of the disease have been described. Autosomal recessive HSP (AR-HSP) with thin corpus callosum (TCC) is a distinct form of familiar spastic paraplegia linked to the SPG11, SPG15, SPG21 and SPG5 loci. SPG35 is an AR-HSP caused by mutations in the gene encoding fatty-acid 2-hydroxylase (*FA2H*), an enzyme that produces free 2-hydroxy fatty acids. *FA2H* mutations have recently been associated not only with complicated forms of spastic paraparesis characterized by TCC and white matter abnormalities on MRI, but also with leukodystrophy and neurodegeneration with brain iron accumulation (NBIA). Eleven Italian HSP index patients, including 7 sporadic patients and 4 AR patients, were investigated for SPG35 mutations by direct sequencing and quantitative Real-Time PCR of the *FA2H* gene. All the patients have thin corpus callosum and are negative for mutations in SPG11, SPG15, SPG21 and SPG5 genes. Results: Two novel missense FA2H mutations (Y34C, T207M) were detected in one family, with 2 affected sibs carrying both variations and 2 healthy brothers heterozygous for the Y34C only or homozygous for the normal allele, respectively. These sequence variants were not found in 300 unrelated control chromosomes. The 2 affected brothers presented with slowly progressive adult-onset HSP (at 32 and 40 yrs) and manifested, a few years later, speech impairment and cognitive decline. Neurological examination at age 48 and 51 demonstrated severe lower-limb spasticity, Babinski sign, pes cavus, distal muscle weakness, dysarthria, ophthalmoplegia, and dementia. Electroneurography showed normal conduction velocities. MRI demonstrated prominent global atrophy (including cerebellum, brainstem, cerebral hemispheres, and corpus callosum) with enlarged ventricles. No micro-rearrangements in *FA2H* gene were identified. Conclusions: We have identified compound heterozygosis for two missense FA2H mutations in one AR-HSP family. This is the first report of FA2H mutations in adult-onset patients with a complicated form of HSP characterised by TCC in early phases, cognitive decline and profound pancerebral atrophy after 10 years of disease. Although SPG35 represents a rather rare form, our findings suggest that it should be considered in the diagnosis of complicated AR-HSP phenotypes with TCC, negative for other AR-HSP gene mutations.

594T

Analyses of compound heterozygous rearrangements of *parkin*. *M. Funayama[1, 2], H. Kusaka[2], H. Yoshino[1], Y. Li[1], K. Ogaki[2], H. Tomiyama[2, 3], N. Hattori[1, 2, 3].* 1) Research Institute for Disease, Graduate School of Medicine, Juntendo University, Tokyo, Japan; 2) Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan; 3) Department of Neuroscience for Neurodegenerative Disorders, Juntendo University School of Medicine, Tokyo, Japan.

Background and Purpose: Parkinson's disease (PD) is one of the most common neurodegenerative disorders affecting approximately 1.39 million people in Japan. Mutations in *parkin* are the most frequent cause of early onset autosomal recessive parkinsonism. Exonic rearrangements in *parkin* are frequently reported in Asian populations. In general, gene dosage analysis by quantitative PCR (qPCR) is used to detect exonic rearrangements. However, there is a problem with the method of qPCR, because compound heterozygous mutations for deletion and duplication in the same allelic exon could be determined to be normal. To verify the problem, we investigated parkin mutations of a Japanese family with early onset parkinsonism by detailed genetic analyses. **Methods:** A patient with early onset parkinsonism and the parents were investigated by qPCR using TaqMan probe, semi-qPCR and haplotype analysis using microsatellite, and RT-PCR. **Results:** None of point mutations or homozygous exonic deletions were found in any of the three family members. Heterozygous duplication of exon 6 and 7 (EX6-7 dup) of *parkin* was detected in the patient with parkinsonism by qPCR. However, the parents did not have the same mutation. Comparison of the peak height of each allele and genotyping results showed that the patient's mutation pattern seemed to be compound heterozygous with EX3-7 dup and EX3-5 del. RT-PCR and direct-sequencing were revealed that the patient had two of abnormal alleles (EX 3-7dup and EX3-5 del) which were inherited from the parents. We detected a sequence of the duplicated break point in the patient and father, and exon skipping was observed at the transcript level in the patient and mother. **Discussion:** In this study, we demonstrated the pseudo-heterozygous rearrangement mutation of *parkin* using qPCR assay due to the combination of duplication and deletion in the same allelic exons. It is difficult to avoid this erroneous decision by examining gene dosage in only patients with parkinsonism. **Conclusion:** In conclusion, to execute the correct decision for rearrangement mutation, we should examine the mutation analysis not only in the patient but also in the patient's family members and/or breakpoint analysis in genomic DNA and transcript level.

595T

***LRRK2* haplotype-sharing analysis in patients with familial Parkinson's disease reveals a novel mutation located in the COR domain.** *O. Lorenzo-Betancor[1,2], L. Samaranch[1], M. Ezquerra[3], E. Tolosa[3,4], E. Lorenzo[1], J. Irigoyen[1,2,4], C. Gaig[3,4], M.A. Pastor[1,2,4], A.I. Soto-Ortolaza[5], O.A. Ross[5], M.C. Rodriguez-Oroz[1,2,4], F. Vallderiola[3], M.J. Martí[3], M.R. Luquin[1,2], J. Perez-Tur[6], J.A. Obeso[1,2,4], P. Pastor[1,2,4].* 1) Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain; 2) Department of Neurology, Clínica Universidad de Navarra, University of Navarra School of Medicine, Pamplona, Spain; 3) Parkinson's Disease and Movement Disorders Unit, Neurology Service, Institut Clínic de Neurociències, Hospital Clínic de Barcelona, Spain; 4) CIBERNED, Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas, Instituto de Salud Carlos III, Spain; 5) Division of Neurogenetics, Department of Neuroscience, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL, USA; 6) Unitat de Genètica Molecular, Institut de Biomedicina de València-CSIC, Valencia, Spain.

Background and objective. Mutations in the *LRRK2* (Leucine-rich repeat kinase 2) gene at chromosome 12q12 are the most common genetic cause of sporadic and familial Parkinson's disease (PD). Our aim was to identify novel *LRRK2* mutations in late-onset PD (LOPD) families. **Design.** We analyzed chromosome 12p11.2-q13.1 haplotypes in 14 families with LOPD, none of whom carried the most common *LRRK2* mutations (p.G2019S, p.R1441G or p.R1441C). **Results.** Haplotype analysis identified 6 LOPD families in which the affected subjects shared chromosome 12p11.2-q13.1 haplotypes, and 6 families in which *LRRK2* haplotype-sharing among affected individuals was uncertain. Sequencing of the entire *LRRK2* encoding gene region in the probands of these 12 LOPD families led to the discovery of a novel co-segregating mutation in the *LRRK2* COR domain in Family 1256. No mutations in the *LRRK2* coding region were detected in the other 11 LOPD families. The novel *LRRK2* mutation is located within a highly conserved region of the COR domain, and could potentially deregulate LRRK2 kinase activity. The novel mutation was also detected in 2 out of 2501 unrelated PD subjects, but not in 2491 healthy controls analyzed. Mutation carriers developed levodopa-responsive asymmetrical parkinsonism, with variable age at onset (range: 37-72 years) suggesting age-dependent penetrance. **Conclusions.** These findings support that haplotype-sharing analysis is an efficient tool to identify infrequent *LRRK2* mutations causing familial LOPD.

596T

Identification of schizophrenia-associated *NPAS3* and *COMT* exonic variants in a pre-onset aged population. *L. Luoma[1], G. Macintyre[1], D. LaFreniere[2], A. Beierbach[2], F. Berry[1,3], S. Purdon[2], P. Tibbo[2,4], D.W. Cox[5].* 1) Dept Med Gen, Univ Alberta, Edmonton, AB, Canada; 2) Department of Psychiatry, University of Alberta, Edmonton, AB, Canada; 3) Department of Surgery, University of Alberta, Edmonton, AB, Canada; 4) Department of Psychiatry, Dalhousie University, Halifax, NS, Canada; 5) Department of Medicine, University of Alberta, Edmonton, AB, Canada.

Schizophrenia (SZ) is a psychotic disorder with an incidence of approximately 1% worldwide. SZ is contributed to by endogenous (biochemical, developmental, neurological, etc.) and exogenous (environmental) factors, both of which are influenced by genetic factors. Treatment response is similarly heterogenous and is more effective when implemented early in the course of psychosis. Identification of specific sub-clinical quantifiable phenotypes (endophenotypes) of genetic variants is critical to understanding SZ pathology and developing methods to identify at-risk individuals to maximize outcomes.

Variants in catechol-O-methyltransferase (COMT), an enzyme involved in the metabolism of catecholamines are associated with SZ, and have been shown to affect synaptic dopamine, cortical thickness and working memory. NPAS3 (neural PAS-domain containing 3) is a SZ-associated transcription factor implicated in neurodevelopment and adult neurogenesis in mice, and cross-disorder plieotropic effects in humans. Endophenotypes of the SZ-associated SNPs in *NPAS3* have yet to be determined. We hypothesize that we will identify an increased frequency of SZ-associated SNPs in *COMT* and *NPAS3* in our pre-onset aged population with a range of psychosis risk relative to a selected control population of neurologically normal adults.

Eighty-seven healthy teenagers (16-19 years) were recruited for volumetric MRI, MRS, neuropsychological and genetic analyses to identify endophenotypes of one *COMT* and three *NPAS3* SNPs. *COMT* rs4680 allele and genotype frequencies are in agreement with the HapMap CEU population (minor allele frequency [MAF] 0.48, $\chi 2$ P=0.80; genotype $\chi 2$ P=0.68). The allele frequency of *NPAS3* SNP rs12434716 differs from the reported frequency (normal control MAF=0.096; current cohort MAF=0.17; $\chi 2$ P=0.0016) likely due to the cohort being of pre-onset age. The genotype frequencies of SNPs rs12434716, rs10141940, rs10142034 are in agreement with those previously reported (Fisher's Exact P=0.10, 0.38 and 0.38). There is no association between rs4680 and any *NPAS3* SNP (Fisher's Exact P=0.31, 0.82 and 0.82). The variability observed in *NPAS3* and *COMT* allows for additional research to correlate putative biological and psychological endophenotype markers for psychosis. This information may be useful for identifying at-risk individuals and lead to earlier diagnosis and treatment.

597T

Clinicogenetic study of patients with FTDP-17 (*MAPT*) in Japan. *K. Ogaki[1], Y. Li[2], M. Takanashi[1], K. Ishikawa[1], T. Kobayashi[3], A. Nakanishi[4], T. Nonaka[5], M. Hasegawa[5], M. Kishi[6], H. Yoshino[2], M. Funayama[1,2], K. Shioya[7], M. Yokochi[8], R. Sasaki[9], Y. Kokubo[9], S. Kuzuhara[10], Y. Motoi[1], H. Tomiyama[1,11], N. Hattori[1,2,11].* 1) Dept of Neurology, Juntendo University School of Medicine, Tokyo, Japan; 2) Research Institute for Diseases of Old Age, Juntendo University School of Medicine, Tokyo, Japan; 3) Dept of Neurology, Fukuoka University School of Medicine, Fukuoka, Japan; 4) Dept of Radiology, Juntendo University School of Medicine, Tokyo, Japan; 5) Dept of Neuropathology and Cell Biology, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan; 6) Dept of Internal Medicine, Division of Neurology, Sakura Medical Center, Toho University, Sakura, Japan; 7) Dept of Neurology, National Hospital Organization Miyazaki Higashi Hospital, Miyazaki, Japan; 8) Dept of Neurology, Tokyo Metropolitan Health and Medical Treatment Corp., Ebara Hospital, Tokyo, Japan; 9) Dept of Neurology, Mie University Graduate School of Medicine, Tsu, Mie, Japan; 10) Dept of Medical Welfare, Faculty of Health Science, Suzuka University of Medical Science, Suzuka, Mie, Japan; 11) Dept of Neuroscience for Neurodegenerative Disorders, Juntendo University School of Medicine, Tokyo, Japan.

Background and Purpose: Mutations in the microtubule-associated protein tau (*MAPT*) cause frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). We investigated the genetic and clinical features in familial and sporadic patients with *MAPT* mutation. Methods: All exons were sequenced and multiplex ligation-dependent probe amplification (MLPA) was performed in *MAPT* in 75 Japanese patients suspected of FTDP-17; frontotemporal dementia (FTD), FTD with motor neuron disease, FTD with parkinsonism, primary progressive aphasia, progressive supranuclear palsy (PSP), corticobasal syndrome, dementia with Lewy bodies and Parkinson's disease, including consideration for family history. Patients with mutations underwent single photon emission computed tomography. Results: We identified 4 *MAPT* mutations in 6 families. The gene dosage of *MAPT* by MLPA study was normal. The frequency of *MAPT* mutation was 9.0%. All 6 patients with PSP phenotype and characteristically abnormal eye movements (such as visual grasping, oscillopsia with congenital nystagmus, and tonic upward fixation after eye closure) had *MAPT* mutations. Comparison of 3D-stereotactic surface projection images of *MAPT*-positive PSP phenotype patients with these distinct eye movements and duration-matched PSP patients lacking these distinct eye movements showed hypoperfusion in bilateral Brodmann area 8. Conclusions: *MAPT* should be analyzed when clinicians see patients with PSP suspected of having FTDP-17 (*MAPT*), after carefully examining the clinical presentation, especially characteristically abnormal eye movements. The distinct eye movements and hypoperfusion of Broadmann area 8 might be new hallmarks of FTDP-17 (*MAPT*).

598T

To identify, validate and characterize the gene on 18p which is responsible for causing Myoclonus-Dystonia. *M. Vanstone[1,2], T. Read[1,2], F. Han[1,2], D.A. Grimes[1], D. Bulman[1,2].* 1) Regenerative Medicine, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada; 2) Biochemistry Dept, University of Ottawa, Ottawa, Ontario, Canada.

Myoclonus-Dystonia (MD) is an inherited, rare, autosomal dominant movement disorder characterized by quick, involuntary muscle jerking or twitching (myoclonus) and involuntary muscle contractions that cause twisting and pulling movements, resulting in abnormal postures (dystonia). The onset of symptoms usually occurs within the first two decades of life. People with MD also have an increased risk of developing psychological conditions such as depression and anxiety. The first MD locus was mapped to 7q21-q31 and called DYT11; this locus corresponds with the *SGCE* gene. We have identified a second MD locus (DYT15) in a large kindred spanning 5 generations, which maps to a 3.18 Mb region on 18p11. The coding regions and intron-exon boundaries of all known REFSEQ genes in the critical region were sequenced and did not yield the causative mutation. In total, 110 kb of the critical region was sequenced and we identified 358 novel SNPs and insertion/deletions, all of which have been detected in control samples. Large deletions, duplications and inversions have been excluded as a cause of MD in our family. Two meiotically distant patients from our family were chosen to undergo next-generation sequencing. First, NimbleGen Sequence Capture technology was used to enrich for DNA from the chr18:4,619,674-7,800,480 critical region plus 50 kb flanking, then Roche 454 sequencing technology was used to sequence the targeted region on 18p11. We obtained >97% coverage of our critical region with an average depth of coverage >50 per patient. Using NextGENe™ software from SOFTGENETICS, we have identified 2,413 shared novel variants within the critical region. I have assigned a priority to this list of variants and am currently attempting to exclude candidates by confirming each one by Sanger sequencing and validating their disease-causing potential by testing for their occurrence in control patients. To date, 2,325 candidate variants have been excluded and 88 remain, one of which will be the causative mutation of MD in our family.

599T

Genetic analyses of myotonic dystrophy in Malaysia. *M.K. Thong[1], I. Taufik[1], K. Ambrose[2], L.H. Lian[2], A.A. Azlina[2], K.J. Goh[3], K.T. Wong[4].* 1) Dept Pediatrics, Univ Malaya Med Ctr, Kuala Lumpur, Malaysia; 2) Dept Molecular Medicine, Univ Malaya Med Ctr, Kuala Lumpur, Malaysia; 3) Dept Medicine, Univ Malaya Med Ctr, Kuala Lumpur, Malaysia; 4) Dept Pathology, Univ Malaya Med Ctr, Kuala Lumpur, Malaysia.

Introduction: Myotonic dystrophy (DM1) is an autosomal dominant condition characterized by progressive muscle weakness and myotonia. The congenital form leads to neonatal death. DM1 results from an unstable trinucleotide repeat (CTG) expansion in the DMPK gene. The age of onset and phenotypic severity correlates with the CTG expansion size. In Malaysia, molecular diagnosis of DM1 is not available. Objective of this study is to develop a polymerase chain reaction (PCR) analysis and Southern transfer approach to diagnose DM1 Method: Analysis of the CTG repeat length was carried out via polymerase chain reaction (PCR) and later confirmed with sequencing and Southern-blot. When an individual demonstrates two different normal size alleles on PCR, DM1 is excluded. The presence of a single band may indicate CTG expansion or an unaffected individual homozygous for a given normal allele. Therefore, Southern blotting test is performed. We determined the heterozygosity of DM1 alleles and the frequency distribution of various CTG repeats in normal Malays and optimized Southern blotting to determine the CTG repeats in affected families. Results: In 120 normal Malays, 58% were heterozygous while 42% were homozygous for the DM1 alleles. Amongst 240 normal chromosomes, a total of 14 alleles with different CTG lengths from 5 to 28 were found. The frequency of CTG > 18 alleles was 3.3%. We successfully determined the number of CTG repeats in two families who had several affected individuals. Conclusion: We developed an effective strategy to screen patients for DM1 using PCR analysis. To the best of our knowledge, this is the first study on the molecular genetics of DM1 in the Malay population. Individuals with DM1 homozygous alleles were subjected to Southern transfer for ascertainment of CTG repeats. Genetic counseling was provided for affected families.

600T

Prader-Willi Syndrome due familial paternal 224 Kb interstitial deletion upstream to SNORD108A. *M. Albalwi[1,2,4], I. Al Abdulkareem[2,4], B. Almuzaini[2], M. Almoalami[2], N. Alatawi[1,4], W. Eyaid[3], M. Aljumah[2,4].* 1) Div Molec Path & Lab Med, National Guard Hosp, Riyadh, Saudi Arabia; 2) King Abdullah International Medical Research Center, Molecular Biology, King Abdulaziz Medical City, Riyadh, Saudi Arabia; 3) Pediatric department, King Abdulaziz Medical City, Riyadh, Saudi Arabia; 4) King Saud bin Abdulaziz University for Health Sciences, College of Medicine, Riyadh, Saudi Arabia.

Prader-Willi syndrome is a neuro developmental disorder due to a number of different genetic mechanisms. All involve lacking of paternal genes expression within 4 Mb of chromosome 15q11q13. Recent studies narrowing PWS to the critical region involving cluster of imprinting SNORD16 gene. Although rare cases of PWS are reported due to inherited familial interstitial deletion compared to the majority of PWS sporadic cases. Here we report a baby girl clinically diagnosed with PWS. DNA Methylation studies and high resolution karyotyping analysis were normal. FISH analysis revealed presence of a dim florescent signal in both daughter and her father. High-resolution oligonucleotide array-CGH revealed approximately 224 Kb interstitial deletion upstream to SNORD108A paternally inherited. This deletion was further validated by Multiplex Ligation-dependent Probe Amplification (MLPA) assay. Using such advance molecular technology will provide better understanding of familial genetic alteration as well as providing better counseling for family with high recurrence risk of having PWS offspring.

601T

Custom capture in a large Primary Lateral Sclerosis family. *V. Belzil[1], S. Girard[1], H. Daoud[1], M. Sabbagh[1], C. André-Guimont[1], D. Spiegelman[1], P. Valdmanis[1], J. St-Onge[1], I. Bachand[1], P. Provencher[2], N. Dupre[2], P. Dion[1], G. Rouleau[1].* 1) Medicine Department, CHUM Research Centre, Univ Montreal, Montreal, Canada; 2) Neurosciences Department, CHAUQ Enfant-Jesus, Univ Laval, Quebec, Canada.

Background: Primary Lateral Sclerosis (PLS) is a neurodegenerative disease of upper motor neurons characterized by progressive spinal and bulbar spasticity. The lower limbs are generally first affected, followed by the upper extremities and the bulbar muscles. The average disease duration is 20 years, and its prevalence is 1 per 10 million. The average age of onset is 50 years old. PLS cases are mostly sporadic. We first identified in 2007 one French-Canadian family with twelve individuals displaying a PLS phenotype which segregates in an autosomal dominant fashion. A 550 marker genome scan was performed and a locus on chromosome 4 between the telomere and marker D4S2928 was identified (PLS1 locus). Twelve members of the family share the same haplotype. This region spans 10.2 Mb and encompasses 130 genes. We then identified a second small French-Canadian family with three members having the same haplotype found to be shared between the affected members of the first family. The two families actually shared the same ancestor three generations before; thus, the same mutation must cause the disease in the two pedigrees. Objectives: Identify the mutation responsible for the PLS phenotype in this large French-Canadian family, in order to better understand the pathological pathway involved in the degeneration of upper motor neurons. Methods: The UTRs and coding regions of the 14 most interesting candidate genes were sequenced and no causative mutation was identified. Also, no major deletion or duplication was identified after performing a copy number variant test. DNA extracted from blood from two patients of the first pedigree and one patient from the second pedigree was used to capture the 10.2Mb region of interest. The Agilent custom capture SureSelect XT kit (13.6 Mb) was selected to sequence the entire PLS1 locus; extra bates were also used to increase the coverage. Library preparation, capture and sequencing were performed at the Genome Quebec Innovation Centre (Montréal, Québec, Canada) using Illumina Hiseq Paired-end sequencing. Results: A list of variants was generated for each three affected members of the PLS family. The variants shared between the three patients were validated by Sanger sequencing and segregation in the two pedigrees was verified. Discussion/Conclusions: The analysis of variants found is undergoing.

602T

Analysis of Lysosomal Storage Disorder Genes in Lewy Body Disorders. *L. Clark[1], N. Park[2], M. Verbistky[1], A. Brown[1], JP. Vonsattel[3], K. Marder[3], L. Honig[3], J. Lee[2].* 1) Dept Pathology & Taub Inst, Columbia Univ, New York, NY; 2) Gertrude H. Sergievsky Center, Columbia Univ, New York, NY; 3) Dept Neurology, Columbia Univ, New York, NY.

Objective To determine the relationship of lysosomal storage disease (LSD) gene mutations to Lewy Body (LB) and Alzheimer disease (AD) pathology. Background: Mutations in the LSD gene, glucocerebrosidase (GBA), are associated with LB disorders. We hypothesized that other LSD genes in the same biochemical pathway may also represent susceptibility genes for LB. Methods: Brain tissue from 243 autopsies from the NYBB at Columbia University included: 128 brains with primary neuropathological diagnoses of LB disorders with or without AD changes, 79 brains with AD without significant LB pathology, and 36 control brains with neither LB or AD pathologies. To capture rare variants (MAF<1%), four LSD genes were sequenced, including GBA and 3 non-GBA genes: hexosaminidase A (HEXA), sphingomyelin phosphodiesterase 1 (SMPD1), and mucolipin 1(MCOLN1). Rare variant analysis was performed using the CCRaVAT. The CRaVAT program used a collapsing method that investigates a combined multiple variant alleles in our case-control study. Results: We found non-GBA variants in 103 (42.4%) brains (HEXA n=42 (17.3%), SMPD n=37 (15.2%), MCOLN1 n=67 (27.6%), of which 33 (13.6%) and 5 (2.1%) brains had variants in 2and 3 of the genes, respectively. Several of the variants that we identified including, c.1278insTATC (HEXA), c.672+30T>G (HEXA), S3S (HEXA), P330R (SMPD1) and E515V (SMPD1) have been reported previously as mutations in patients with lysosomal storage disorders. GBA, as previously reported was in 34 (16.9%) brains (23 without and 11 with non-GBA mutations). We showed an association of GBA mutations with presence of LB particularly cortical LB: OR=6.48, 95%CI, 2.45-17.6, p<0.001 adjusting for sex, death age, and ApoE4 status. At least one mutation in SMPD and MCOLN1, non-GBA gene, were significantly associated with LB.or LB with AD pathology. Multiple variants in SMPD and MCOLN1 were significantly associated with LB and LB with AD (p range: 0.01 - 0.0078. Conclusion: We showed that GBA mutations and at least one mutation in SMPD and MCOLN1 were significantly associated with LB or LB with AD pathology. This work shows evidence suggesting that the mutations in the lysosomal pathway may be associated with LB pathology. Funding: This study was funded by National Institutes of Health grants R21NS050487 (LNC), R01NS060113 (LNC), R01NS36630 (KM), UL1RR024156 (KM), R37AG15473 (RM) and P50AG08702 (M. Shelanski), the Parkinson's Disease Foundation (LNC, KM).

603T

Clinical features of four familial cortico-basal degeneration (CBD) and progressive supranuclear palsy (PSP) in south Fukuoka prefecture, Kyushu island in Japan. *H. Furuya[1], A. Watanabe[1], H. Arahata[1], Y. Kawano[1], E. Araki[1], H. Kumazoe[2], N. Fujii[1].* 1) Department of Neurology, National Omuta Hospital, Omuta, Fukuoka, Japan; 2) Department of Radiology, National Omuta Hospital, Omuta, Fukuoka, Japan.

Objective: We evaluate clinical features and genetic analysis for four unrelated familial CBD and PSP pedigrees in south Fukuoka prefecture. **Background:** Although, it is considered that 10-15% of Parkinson disease (PD) is familial, familial PSP and CBD is very rare. Recently, a decrease in myocardial uptake of meta-iodobenzylguanidine (MIBG), an analogue of norepinephrine, has been reported in PD and dementia with Lewy body disease (DLB) but not in PSP and CBD. This imaging approach is thought to be of significance in the diagnosis and characterization of akinetic-rigid syndromes with high sensitivity and specificity especially for PD and DLB, which infer the involvement of postganglionic sympathetic nerves failure in the heart caused by (-synucleopathy. **Methods:** We describe four unrelated families of CBD and PSP in this region with clinical feature and result of gene analysis (PARK 1, 2, 6, 7, 8 and MAP) and MIBG myocardial scintigraphy. **Results:** In this region, 18.5% (15/81) of PD and DLB, 16.7% of PSP and CBD (4/24) are familial. L-dopa was not effective in these four families and genetic analysis exclude the possibility of PARK 1, 2, 6, 7, 8 and MAP related PD. The myocardial uptake of MIBG is decreased only in one case. Heterogenous clinical phenotypes in these pedigrees are also observed. **Conclusions:** The incidence of familial CBD and PSP are higher in this region. Cardiac sympathetic denervation due to (-synucleopathy occurs rare in familial CBD and PSP.

604T

Novel microRNA and novel predicted gene targets in sporadic form of Creutzfeldt-Jakob disease (CJD). *D. Glavač, E. Bostjančič, M. Ravnik-Glavač, M. Popovič.* Department of Molecular Genetics, Faculty of Medicine, Vrazov trg 2, 1000, Ljubljana, Slovenia.

Prion diseases are a related group of rare, fatal brain diseases in humans and animals, also known as transmissible spongiform encephalopathies (TSE).The most common human prion disease is the sporadic form of Creutzfeldt-Jakob disease (CJD). MiRNAs are effectors of brain function with roles in ischemia and injury, neuroprotection and neurodegeneration. Compelling evidence also links miRNAs to the control of neuronal development and differentiation. We used a co-ordinated approach to integrate miRNA and mRNA profiling and bioinformatic predictions in order to profile miRNA expression changes in human brains during sporadic CJD.Total RNA was isolated from the brain tissue of 8 patients with diagnosed CJD and the brain tissue of 7 healthy individuals. Expression analysis was performed using hybridization to μParaflo microfluidics micorarrays in the Sanger miRBase database version Release 10.1. Our analysis revealed over one hundred differentially expressed (up- or down-regulated) miRNAs. Sixteen of them showed similar dysregulation in experimental animals, in brains of mice infected with mouse-adapted scarpie and in the brains of BSE-infected cynomolgus macaques as a model for CJD. In addition, we selected 15 miRNAs for microarray validation using real-time PCR. MiRNA miR-342-3p, which showed up-regulation in our microarray experiment, was also up-regulated in the brains of BSE-infected macaques. Neuronal-specific miRNA, miR-124a, was also up-regulated in our experiment; this miRNA is believed to have constant expression in adult neurons. Neuronal death would therefore have some impact on miR-124a expression. Other up-regulated miRNAs, miR-16 and miR-98, are involved in apoptosis as pro-apoptotic miRNAs, with miR-16 believed to be involved in the apotosis of developing neurons. Neuronal autophagy and apoptosis are mechanisms of programmed cell death. Since there is some information that autophagy and apoptosis are processes naturally occurring in experimentally induced scrapie and CJD, we believe that miRNAs regulating apoptosis may also play a role in transmissible spongiform encephalopathies. Computational analysis predicted numerous potential gene targets of these miRNAs, including some genes previously identified as being also de-regulated in mouse scrapie. In conclusion, we detected several novel miRNAs and novel predicted gene targets and confirmed their down-regulation in human CJD brain tissue.

605T

Mitochondrial morpho-functional dysfunction in SPG31 patients. *C. Goizet[1,2], G. Benard[2], C. Depienne[3,4,5,6], A. Boukhris[7], G. Solé[1], E. Mundwiller[3,4,5,6], I. Coupry[1], J. Pilliod[1], M-L. Martin-Négrier[8], E. Fedirko[6], S. Forlani[3,4,5], C. Cazeneuve[6], D. Hannequin[9], P. Charles[6], E. Leguern[3,4,5,6], A. Durr[3,4,5,6], A. Brice[3,4,5,6], D. Lacombe[1,2], R. Rossignol[1], G. Stevanin[3,4,5,6].* 1) Medical Genetics, CHU Pellegrin, Bordeaux, France; 2) Université Bordeaux Segalen, Laboratoire Maladies Rares: Génétique et Métabolisme (MRGM), EA4576, 33076 Bordeaux, France; 3) INSERM, U975, 75013 Paris, France; 4) Université Pierre et Marie Curie - Paris 6, UMR_S975, Centre de Recherche de l'Institut du Cerveau et de la Moelle épinière, GHU Pitié-Salpêtrière, 75013 Paris, France; 5) CNRS, UMR7225, 75013 Paris, France; 6) APHP, GHU Pitié-Salpêtrière, Département de Génétique et Cytogénétique, Fédération de Génétique, 75651 Paris cedex 13, France; 7) Service de Neurologie, Hôpital Habib Bourguiba, Sfax, Tunisia; 8) CHU Bordeaux, Département de Pathologie, 33076 Bordeaux, France; 9) INSERM U614, Service de Neurologie, CHU Rouen, France.

Hereditary spastic paraplegias (HSP) constitute a heterogeneous group of neurodegenerative disorders characterized at least by slowly progressive spasticity of the lower limbs. Mutations in REEP1 were recently associated with SPG31, a pure dominant HSP. We recently identified 12 different heterozygous mutations in REEP1 by screening 175 unrelated HSP index patients from kindreds with dominant inheritance (AD-HSP), associated with either a pure or a complex phenotype. The overall mutation rate in our clinically heterogeneous sample was 4.5% in French families with AD-HSP. The phenotype was restricted to pyramidal signs in the lower limbs in most patients but 9 had a complex phenotype. Interestingly, we evidenced abnormal mitochondrial network organization in fibroblasts of patients from two families in addition to defective mitochondrial energy production in both fibroblasts and muscle, but whether these anomalies are directly or indirectly related to the mutations remains to determine.

606T

Exome sequencing reveals mutations in VPS35, encoding a subunit of the retromer complex, as cause for Parkinson's disease. *E. Graf[1], A. Zimprich[2], A. Benet-Pagès[1], W. Struhal[3], S.H. Eck[1], M.N. Offman[4], D. Haubenberger[2], S. Spielberger[5], E.C. Schulte[1,6], P. Lichtner[1], S.C. Rossle[4], N. Klopp[7], E. Wolf[5], W. Pirker[2], S. Presslauer[8], R. Katzenschlager[10], T. Foki[2], E. Reinthaler[2], R. Kralovics[11], A. Peters[7], F. Zimprich[2], T. Brücke[8], W. Poewe[5], E. Auff[2], C. Trenkwalder[9,12], B. Rost[4], G. Ransmayr[3], J. Winkelmann[2,6,13], T. Meitinger[1,13], T.M. Strom[1,13].* 1) Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany; 2) Department of Neurology, Medizinische Universität Wien, Vienna, Austria; 3) Department of Neurology and Psychiatry, Allgemeines Krankenhaus, Linz, Austria; 4) Institute of Bioinformatics, Technische Universität München, Munich, Germany; 5) Department of Neurology, Medizinische Universität Innsbruck, Innsbruck, Austria; 6) Department of Neurology, Technische Universität München, Munich, Germany; 7) Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany; 8) Department of Neurology, Wilhelminenspital, Vienna, Austria-Department of Neurology, Wilhelminenspital, Vienna, Austria; 9) Paracelsus-Elena Klinik, Kassel, Germany; 10) Department of Neurology, Sozialmedizinisches Zentrum Ost-Donauspital, Vienna, Austria; 11) Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria; 12) Department of Clinical Neurophysiology, Georg-August-Universität Göttingen, Göttingen, Germany; 13) Institute of Human Genetics, Technische Universität München, Munich, Germany.

Parkinson's disease is the second most common neurodegenerative disorder affecting 1%-2% of the population above the age of 60. It is characterized by degeneration of dopaminergic neurons in the nigrostriatal pathway and other monoaminergic cell groups in the brainstem leading to bradykinesia, resting tremor, muscular rigidity, and postural instability as well as non-motor symptoms. By exome sequencing, we identified a single mutation, D620N, in VPS35, encoding a subunit of the retromer complex, in an Austrian family with 16 affected individuals. The mutation co-segregated with the disease in all seven affected family members that are alive. This is, after *LRRK2* and *SCNA*, the third gene in which mutations provide a high risk for late-onset PD. By screening additional PD cases, we saw the same variant co-segregating with the disease in an autosomal dominant mode with high penetrance in two further families with five and ten affected members, respectively. Genotyping showed that the shared haplotype extends across 65 kilobases around *VPS35*. Screening the entire *VPS35* coding sequence in 860 cases and 1014 controls by dye-binding/high-resolution DNA melting curve analysis revealed six further non-synonymous missense variants. Three were only present in cases, two only present in controls, and one present in cases and controls. D620N and a further variant detected in a PD case, R524W, were predicted to be damaging by sequence-based and molecular dynamics analysis. VPS35 is a component of the retromer complex which mediates retrograde transport between endosomes and the trans-Golgi network and which has recently been described to be involved in Alzheimer's disease.

607T

Development of high throughput targeted resequencing array for the diagnosis of hereditary neuropathie. *O. Jarinova[1], J. Warman[1,2], C. Goldsmith[1,2], N. Carson[1], E. McCready[1], G. Yoon[3], S. Baker[4], A.M. Innes[5], K. Boycott[1].* 1) Department of Genetics, Children's Hospital of Eastern Ontario, Ottawa, K1H 8L1; 2) Division of Neurology, Department of Medicine, The Ottawa Hospital, K1H 8L6; 3) Division of Clinical & Metabolic Genetics, The Hospital for Sick Children, Toronto, M5G 1X8; 4) Department of Medicine, Divisions of Physical Medicine and Rehabilitation, McMaster University, Hamilton, L8N 3Z5; 5) Department of Medical Genetics, Faculty of Medicine, University of Calgary, Alberta, T2N 4N1.

Hereditary neuropathies comprise a distinct group of inherited disorders in which peripheral nerves undergo progressive degeneration leading to significant physical disability of the hands and lower legs. Causative mutations can reside in any of a number of genes responsible for various aspects of maintenance of peripheral nerves and result in an overlapping clinical spectrum for these disorders. Currently available molecular technologies only allow the sequential genotyping of these genes and determining genetic cause for most cases is often time consuming and expensive.

We have developed a high-throughput cost-effective method of molecular detection to facilitate the diagnosis of inherited neuropathies (`Neuropathy Chip') based on the Affymetrix resequencing platform. The Neuropathy Chip v1.0 is primarily designed to discern genetic changes in the coding sequences of 42 genes. In addition, we targeted 18 regions of potential clinical importance residing in close proximity to coding sequences of genes that are most commonly altered in hereditary neuropathies, i.e. *PMP22*, *MPZ*, *GJB1* and *MFN2*. These regions exhibit sequence conservation in human, mouse and opossum and may contain yet uncharacterized exonic sequences and/or regulatory elements. Combined, Neuropathy Chip v1.0 interrogates over 167 kb of unique sequence with call rates above >98% for successfully hybridized amplicons and effectively detects point mutations and provides clues towards identifying insertion and deletions.

Resequencing data were obtained for 13 affected individuals with known mutations and 19 affected individuals with unknown molecular etiology. Generated resequencing data will be examined and detected variants of potential/established clinical significance will be confirmed by Sanger sequencing. Efficiency, performance and clinical validity of the assay will be assessed and results from this analysis will be presented.

We estimate that the cost for testing of all the genes associated with hereditary neuropathies in any given patient would be close to the fee currently charged for analysis of only one gene. Furthermore, this approach has a potential to discover novel causative mutations and establish missing heritability in neuropathy patients with unknown molecular etiology.

608T

Rare genetic variants in APP, PSEN1, PSEN2, GRN, APOE and MAPT are associated with familial and sporadic Early and Late-Onset Alzheimer's disease. *S. Jin[1], S. Cervantes[2], B. Benitez[1], P. Pastor[2], A. Goate[1], C. Cruchaga[1].* 1) Psychiatry, Washington University in St. Louis, St. Louis, MO; 2) Center for Applied Medical Research, University of Navarra, Spain.

It has been suggested that the combined effects of rare variants could explain a large proportion of the genetic susceptibility to Alzheimer's disease (AD). Early studies showed that mutations in APP, PSEN1, and PSEN2 contribute to early-onset familial AD. Mutations in GRN and MAPT have been found in clinically diagnosed AD cases. We have used next-generation sequencing to identify rare variants implicated in both early and late onset AD. Pooled-sample sequencing was performed in 141 individuals with sporadic early-onset AD, 48 with familial early-onset AD, and 47 with late-onset AD. The pooled PCR products were sequenced on Illumina HiSeq 2000. Sequencing reads were mapped back to the sample and the reference sequence by gapped alignment. The SPLINTER program was used not only to predict and quantify short insertions, deletions, and substitutions but to predict rare allele frequencies. SIFT2 was then used to predict the effect of variants on protein structure. Variants identified by next-generation sequencing were confirmed by direct genotyping using Sequenom technology. Novel variants were tested for segregation in the familial cases and were also genotyped in 1000 controls. A total of 40 coding variants were identified out of 357 non-intronic variants in the genes encoding APP, PSEN1, PSEN2, GRN, APOE, and MAPT. Among these 40 variants, 19 are non-synonymous variants, 19 are synonymous variants. Out of 19 non-synonymous, 8 are predicted to be tolerated and 11 damaging. Out of 357, there are 43 (12%) novel variants. Comparing to 1000 Genomes data and the AD & FTD Mutation Database, 4 non-synonymous variants are predicted to be novel and damaging and 3 novel and tolerated. In addition, we find 6 known non-causative variants in PSEN1 and MAPT, and 2 known where pathogenicity is unknown in pooled-sample. These results highlight the necessity of additional sequencing in these genes, because new pathogenic variants are still to be discovered.

609T

A Japanese hereditary spastic paraplegia patient with BSCL2 gene mutation. *M. KOHDA[1], K. ITOKAWA[2], T. HIRATA[1], N. ARAKI[2], Y. OKAZAKI[1].* 1) Division of Translational Research, Saitama Medical University, Japan; 2) Department of Neurology, Saitama Medical University, Japan.

OBJECTIVE AND BACKGROUND: Hereditary spastic paraplegia (HSP) is a neurodegenerative disorder defined clinically by progressive lower limb spasticity and weakness. HSP is a genetically highly heterogeneous condition with at least 46 gene loci identified so far, involving X-linked, autosomal recessive and autosomal dominant inheritance. For correct diagnosis molecular testing is essential since clinical parameters by themselves are not reliable enough to differentiate HSP types. Here we report a two-generation family with six patients in which the inheritance pattern is consistent with an autosomal dominant model. We performed a homozygosity haplotype method (HH) in all affected/unaffected family members followed by exome sequencing to find the causative mutation in this family. METHODS: We used the HH approach to identify shared chromosomal segments derived from a common ancestor, who had the causative mutation. The HH represents haplotypes of both copies of homologous autosomes, allowing for direct comparisons of the autosomes among multiple patients and enabling the identification of the shared segments. Then we performed exome sequencing which is capable of resequencing nearly a complete set of coding exons in an individual. RESULTS: HH analysis identified a certain amount of shared regions that segregated with spastic paraplegia in this family. We performed exome sequencing on single affected individuals to investigate causal nonsense variants. Using a combination of sanger sequencing of candidate genes and next-generation exome sequencing, we identified one mutation in BCSL2 (N88S), which lied in one of the shared region. This variant had been reported previously as pathogenic in SPG17 with incomplete penetrance. Sanger sequencing confirmed the co-segregation between disease-phenotype and this mutation. CONCLUSION: The HH successfully detected the shared segments from members of a family with spastic paraplegia, which is an autosomal dominant disease. Exome sequencing has greatly impacted the speed at which new disease genes are identified. Our strategy to identify causative disease genes using the HH followed by exome sequencing provides an efficient and effective approach in identifying potential disease linked region and causative mutation.

610T

Evaluation of Parkinson Disease Risk Variants as Expression-QTLs. *J.C. Latourelle[1], A. Dumitriu[1], T.C. Hadzi[1], T.G. Beach[2], R.H. Myers[1].* 1) Department of Neurology, Boston University School of Medicine, Boston, MA; 2) Civin Laboratory for Neuropathology, Banner Sun Health Research Institute, Sun City, AZ.

The recent Parkinson Disease GWAS Consortium (PDGC) meta-analysis and replication study reports association at several previously confirmed risk loci at *SNCA*, *MAPT*, *GAK/DGKQ*, and *HLA* and identified a novel risk locus at *RIT2*. None of the findings represents coding variation and most of the regions encompass many genes. We sought to examine modification of gene expression by the associated SNPs to identify the genes responsible for association. In this study, we examine the association between Parkinson Disease (PD) risk SNPs identified by the PDGC and gene expression levels in prefrontal cortex brain samples. 33 pathologically confirmed PD cases and 29 controls were run on an Agilent 60-mer Microarray. The samples were genotyped in the PDGC replication sample using a custom Illumina array including all top association results from the discovery meta-analysis. Twelve samples were removed after evaluation of microarray and genotyping QC, leaving 26 cases and 24 controls for the eSNP analysis. We selected for evaluation as potential eSNPs 92 SNPs, from 19 different loci, that showed nominal association to PD ($p<0.05$) in the PDGC replication sample. Association between the eSNPs and expression levels was evaluated using a 2df test of association between genotype and expression and difference in association between cases and controls, adjusted for relevant covariates. SNPs at each of the 19 loci were tested for cis-acting association to all probes within 250 kb of the most proximal and distal SNPs for each locus. As a secondary analysis, trans-effects of the SNPs on the 39,122 probes passing all QC on the microarray were also examined. From the cis analysis, several SNPs in the *MAPT* region show significant association to multiple nearby probes, including two strongly correlated probes, targeting the gene *LOC64424* and the duplicated genes *LRRC37A* and *LRRC37A2*, and a third uncorrelated probe targeting the gene *DCAKD*. Significant cis-associations were also observed between SNPs and two probes targeting genes in the *HLA* region on chromosome 6. Expanding the association to examine trans effects revealed an additional 20 SNP-probe associations reaching statistical significance ($p<1.9\times10^{-8}$) including SNPs from the *SNCA* and *RIT2* regions. These findings suggest that genes other than *MAPT* may be responsible for the SNP association to PD observed at 17q21 and give additional insight into which genes are responsible for association in ambiguous GWAS implicated regions.

611T

Comparison of pathway analysis approaches using lung cancer GWAS data sets. *G. Fehringer[1], G. Liu[2], L. Briollais[1], P. Brennan[3], C.I. Amos[4], M.R. Spitz[4], H. Bickeböller[5], H.-E. Wichmann[6], A. Risch[7], R.J. Hung[1].* 1) Prosserman Centre for Health Research, Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada; 2) Department of Medicine and Medical Biophysics, Ontario Cancer Institute/Princess Margaret Hospital, Toronto, Ontario, Canada; 3) International Agency for Research on Cancer (IARC), Lyon, France; 4) Department of Epidemiology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas, U.S.A; 5) Department of Genetic Epidemiology, University Medical Center, University of Goettingen, Goettingen, Germany; 6) Institute of Epidemiology, GSF-National Research Center for Environment and Health, Neuherberg, Germany; 7) Division of Epigenomics and Cancer Risk Factors, German Cancer Research Center, Heidelberg, Germany.

Pathway analysis has been proposed as a complement to single SNP analyses in GWAS. This study compared pathway analysis methods using two lung cancer GWAS data sets based on four studies: one a combined data set from Central Europe and Toronto (CETO); the other a combined data set from Germany and MD Anderson (GRMD). We searched the literature for pathway analysis methods that were widely used, representative of other methods, and had available software for performing analysis. We selected the programs EASE, which uses a modified Fishers Exact calculation to test for pathway associations, GenGen (a version of Gene Set Enrichment Analysis (GSEA)), which uses a Kolmogorov-Smirnov-like running sum statistic as the test statistic, and SLAT, which uses a p-value combination approach. We also included a modified version of the SUMSTAT method (mSUMSTAT), which tests for association by averaging χ^2 statistics from genotype association tests. There were nearly 18,000 genes available for analysis, following mapping of more than 300,000 SNPs from each data set. These were mapped to 421 GO level 4 gene sets for the pathway analysis. Among the methods designed to be robust to biases related to gene size and correlation among pathway SNPs (GenGen, mSUMSTAT and SLAT), mSUMSTAT identified the most significant pathways (9 in CETO and 1 in GRMD). This included a highly plausible association for the acetylcholine receptor activity pathway in both CETO (FDR ≤0.001) and GRMD (FDR=0.005). Few other replicated associations were found using any of these methods. Difficulty in replicating associations hindered our comparison, but results suggest mSUMSTAT has advantages over the other approaches. This method may be a useful pathway analysis tool that could be used alongside other methods such as the commonly used GSEA (GenGen) approach. Acknowledgements: D. Zaridze, Cancer Research Centre, Moscow, Russia; N. Szeszenia-Dabrowska, Institute of Occupational Medicine, Lodz, Poland; J. Lissowska, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland; P. Rudnai , National Institute of Environmental Health, Budapest, Hungary; E. Fabianova, Specialized Institute of Hygiene and Epidemiology, B. Bystrica, Slovakia; D. Mates, Institute of Public Health, Bucharest, Romania; V. Bencko, Charles University in Prague, Czech Republic; L. Foretova, Masaryk Memorial Cancer Institute, Brno, Czech Republic; V. Janout, Palacky University, Olomouc, Czech Republic.

612T

Identification of genetic factors that mediate inter-individual differences in the gut microbiome. *J.K. Goodrich[1], J. Bell[2], T. Spector[2], A.G. Clark[1], R.E. Ley[1].* 1) Cornell University, Ithaca, NY; 2) King's College, London, U.K.

Using fecal samples from the TwinsUK study cohort, we quantify the magnitude of heritable, inter-individual differences in microbiome composition. Next generation sequencing was applied to generate 16S rRNA gene sequences of microbes in the fecal samples of 128 dizygotic and 110 monozygotic twin pairs, with each sample yielding an average of ~6,000 quality-filtered sequence reads. These rRNA sequence data were used to catalog and quantify inter-individual variability in microbiota and produce a phylogenetic representation of the microbiome species composition. Multiple metrics to characterize the microbiome were examined, including representation of all universally-shared taxa, and proportional representation of some of the major phylogenetic groups (e.g. Firmicutes). Monozygotic twin samples and repeated measures of some individuals were used to quantify environmental stability of each microbiome metric. Using whole-genome SNP genotypes, we applied allele-sharing methods to identify DZ twin pairs that shared 0, 1, or 2 alleles Identical-by-Descent (IBD) for each segment of the genome. This information was used to test the null hypothesis that each genome segment had zero effect on each of the microbiome metrics. Significant rejections of this hypothesis establish a statistical association between regions of the human genome and the composition of the gut microbiome, indicating that genetic factors may in part be responsible for gut microbiome dysfunction.

613T

The Kaiser Permanente/UCSF Genetic Epidemiology Research Study on Adult Health and Aging: High Imputation Coverage of Low Frequency Variants using Dense Genotyping Arrays. *T.J. Hoffmann[1], M.N. Kvale[1], Y. Zhan[2], S. Hesselson[1], P. Kwok[1], C. Schaefer[3], N. Risch[1,3].* 1) University of California, San Francisco, San Francisco, CA; 2) Affymetrix Incorporated, Santa Clara, CA; 3) Kaiser Permanente Northern California Division of Research, Oakland, CA.

The Kaiser Permanente/UCSF Genetic Epidemiology Research Resource on Adult Health and Aging has assayed 675,000+ SNPs on each of 100,000 participants. With this unprecedented number of subjects, it is possible to have adequate power to detect disease/trait associations even for SNPs with low minor allele frequency (MAF) not assayed on the array via imputation of missing genetic variants. It has been previously shown that larger reference sample sizes increase the accuracy of imputing genetic variants, especially rare variants. However, these analyses have not directly addressed the question of how well imputation can predict variants as a function of their population frequency. The ability to impute a low frequency variant is likely to be a function of both its general population frequency as well as its representation in the reference sample, and possibly by other sample characteristics. Here we report results of analyses intended to explore the determinants of imputation accuracy for low MAF variants, stratifying these analyses by various different metrics. For these analyses, we use the SNP content of a European-ancestry array designed specifically for the Kaiser Permanente/UCSF project. For reference data, we use various subsets of recently released sequence data from the 1000 Genomes Project. For determination of MAF, we use a sample independent of the reference sample on which the imputation is based, to disentangle the confounding between minor allele count and MAF. We find that the primary determinant of imputation ability for low frequency variants is the minor allele count in the reference sample. For a given minor allele count, imputation accuracy is only modestly influenced by minor allele frequency and overall reference sample size (or major allele count). For MAF down to 2%, we project high imputation accuracy provided a sufficiently large reference sample is available. However, for detecting associations of variants with frequencies below this level, because of possible ethnic variation and mutational heterogeneity, direct sequencing will likely be preferable. Finally, we use these results to determine the average power of our project to detect associations for rare alleles given a large reference data set for imputation. For a SNP with MAF of 2%; or greater that contributes 0.1%; of the variance of a quantitative trait, for our sample of 100,000 individuals the power is greater than 90%; at a P-value of 10^-8.

614T

A meta-analysis of 16 genome-wide association studies identifies genetic variation in the *LEP* gene to be associated with blood leptin levels independent of body mass index. *T.O. Kilpeläinen[1], Q. Sun[2,3], Z. Kutalik[4], K. Kristiansson[5], M. Mangino[6], M. Su[7], F. Del Greco M.[8], D. Pasko[9], T. Tanaka[10,11], Y.J. Sung[12], O.T. Raitakari[13], L. Perusse[14], C. Ohlsson[15], M. Walker[16], S.M. Willems[17], R.H. Myers[18], J. Eriksson[19], V. Salomaa[20], H. Grallert[21], R.J.F. Loos[1] for the Leptin GWAS Consortium.* 1) MRC Epidemiology Unit, Institute of Metabolic Science, Cambridge, United Kingdom; 2) Department of Nutrition, Harvard School of Public Health, Boston, MA; 3) Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 4) University of Lausanne, Lausanne, Switzerland; 5) Public Health Genomics Unit, Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland; 6) Department of Twin Research & Genetic Epidemiology, King's College London, London, United Kingdom; 7) Department of Biostatistics, University of Washington, Seattle, WA; 8) Institute of Genetic Medicine, European Academy Bozen/Bolzano (EURAC), Bolzano, Italy; Affiliated Institute of the University of Lübeck, Lübeck, Germany; 9) Peninsula College of Medicine and Dentistry, University of Exeter, Exeter, United Kingdom; 10) Medstar Research Institute, Baltimore, MD; 11) Longitudinal Study Section, National Institute on Aging, Baltimore, MD; 12) Division of Biostatistics, Washington University School of Medicine, St. Louis, MO; 13) Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku and Department of Clinical Physiology, Turku University Hospital, Turku, Finland; 14) Division of Kinesiology, Department of Social and Preventive Medicine, Laval University, Ste-Foy, Quebec, Canada; 15) Center for Bone and Arthritis Research, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; 16) Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom; 17) Department of Epidemiology, Erasmus MC, Rotterdam, The Netherlands; 18) Genome Science Institute, Boston University, Boston, MA; 19) Diabetes Prevention Unit, Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland; 20) Chronic Disease Epidemiology and Prevention Unit, Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland; 21) Institute of Epidemiology, Helmholtz-Zentrum München, Neuherberg, Germany.

Background: Leptin is in adipocyte-derived hormone of which the circulating levels correlate closely with body fat. Leptin acts as a chronic signal to inform the brain about the stored body fat and as such it is involved in the regulation of long-term energy homeostasis. Complete leptin deficiency due to mutations in the leptin (*LEP*) gene causes severe obesity. We aimed to identify genetic variants regulating leptin levels in the general population. **Methods:** We performed a meta-analysis of associations between ~2.5 million SNPs and blood leptin levels from 24,778 individuals of white European descent using additive genetic model while adjusting for age, in men (n=9,835) and women (n=14,943) separately. To identify loci associated with leptin levels independent of body fatness, we performed an additional meta-analysis with adjustment for BMI. Both meta-analyses were performed in all individuals and in men and women separately. **Results:** Five independent loci reached $P<10^{-6}$ for their association with leptin. In the BMI-unadjusted analysis, variants in the *COBLL1* ($P=2x10^{-7}$) and *FTO* ($P=5x10^{-7}$) genes and near the *SLC19A3* gene ($P=6x10^{-7}$) showed association with leptin in all individuals, and variants in the *SLC2A2* gene showed association with leptin in men ($P=4x10^{-7}$). In the BMI-adjusted analysis, variants in the *LEP* gene showed genome-wide significant association with leptin in all individuals ($P=6x10^{-9}$). The associations of all 32 previously established BMI loci with leptin in the BMI-unadjusted analysis were directionally consistent with their known associations with BMI. **Conclusions:** Genetic variation in the *LEP* gene is associated with blood leptin levels irrespective of the overall body fatness. The association of variants in *FTO*, *COBLL1*, *SLC2A2*, and near *SLC19A3* with leptin may be dependent on BMI. While *FTO* is a well-known adiposity gene and is likely associated with leptin due to its effect on body fatness, the *COBLL1*, *SLC2A2*, and near-*SLC19A3* loci have not been linked with adiposity and may affect leptin levels through other mechanisms. The *COBLL1* gene is involved in neuronal development, and the *SLC2A2* and *SLC19A3* genes encode transmembrane transporters for glucose and thiamine, respectively. The replication of all loci reaching $P<10^{-6}$ for the association with leptin is underway. The identification of loci regulating leptin levels may provide new insights into the genetic regulation of appetite and obesity.

615T

Replication of GWAS signals and association of novel functional variants for serum uric acid and total serum bilirubin levels in an Irish population. *Y. Kim[1], C.D. Cropp[1], A.M. Molloy[2], J.L. Mills[3], P.N. Kirke[4], J.M. Scott[2], L.C. Brody[5], J.E. Bailey-Wilson[1], A.F. Wilson[1].* 1) Inherited Disease Research Branch, NHGRI, NIH, Baltimore, MD, USA; 2) School of Immunology and Biochemistry, Trinity College, Dublin, Ireland; 3) Division of Epidemiology, Statistics, and Prevention Research, NICHD, NIH, Bethesda, MD, USA; 4) Child Health Epidemiology Unit, Health Research Board of Ireland, Dublin, Ireland; 5) Genome Technology Branch, NHGRI, NIH, Bethesda, MD, USA.

Background: The measurement of blood and serum metabolites can provide insight into the metabolic processes underlying the variation of traits related to many diseases. The catabolic products of purine and hemoglobin metabolism can directly cause disease and serve as pathologic markers of several disease states. In order to better understand the genetic determinants of the product of these pathways, we measured serum uric acid (URIC, mmol/L) and total serum bilirubin (TBIL, umol/L) in a healthy, young population. Blood and dietary information were collected and metabolites were measured in 2490 healthy students at Trinity College Dublin in Ireland. **Methods:** URIC was adjusted for age, sex, and BMI, and log-transformed TBIL was adjusted for age and sex. High density genotyping was performed with the Illumina 1M HumanOmni1-Quad chip. After quality control assessment 2232 unrelated individuals and 757,533 SNPs were retained for association testing. Tests of association were performed with simple univariate linear regression, regressing each marker on each trait, assuming an additive genetic model as implemented in PLINK v1.0.7. Locus-specific heritability (h2) for the effect size of each SNP on phenotypic variation was calculated using R v2.12.1. **Results:** For URIC, we replicated previously reported SNPs in *SLC2A9* (rs6449213, h2 = 10%), *WDR1* (rs717615, h2 = 3%), and *ABCG2* (rs2199936, h2 = 2%) at genome-wide significance levels (p-value ≤ 5e-08). Additional SNPs were significant in these 3 reported candidate genes and *ZNF518B*. The most significant results were from rs13111638 (p-value = 4e-24, h2 = 9.5%) in an intronic region and in two coding SNPs in *SLC2A9* (rs10939650 and rs13113918 (p-value = 2e-22, 1e-21; h2= 4.4%, 5.6% respectively). For TBIL, strong *UGT1A* gene family signals were found including replication of rs887829 (p-value = 4e-156, h2=28%). Additionally, two non-synonymous SNPs (rs6431631 and rs1500480 (p-value = 3e-09, 2e-08 respectively) were found to be associated in *LOC339766*. However, there were no genome-wide significant signals in *SLCO1B1* a known candidate gene for TBIL (minimum p-value = 7e-07). **Conclusion:** Previously published common variants associated with URIC and TBIL levels with high h2 were replicated and additional new coding genetic variants were found in this healthy, young Irish population. These findings provide additional evidence for future analyses to identify the mechanism of genetic contributions to these phenotypes.

616T

Exploration of pleiotropic effects of inflammation-related disease GWAS SNPs with C-reactive protein levels in the PAGE study. *J.D. Kocarnik[1], S.A. Pendergrass[2], C. Carty[1], J. Pankow[3], F. Schumacher[4], I. Cheng[5], P. Durda[6], N. Cook[7], S. Liu[8], J. Wactawski-Wende[9], A. LaCroix[1], B. Jackson[10], T. Manolio[11], D. Crawford[2], M. Gross[12], U. Peters[1] on behalf of the PAGE Consortium.* 1) Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 3) Department of Epidemiology, University of Minnesota, Minneapolis, MN; 4) Keck School of Medicine, University of Southern California, Los Angeles, CA; 5) University of Hawaii Cancer Center, Honolulu, HI; 6) Department of Pathology, University of Vermont, Burlington, VT; 7) Department of Epidemiology, Brigham & Women's Hospital, Boston, MA; 8) Department of Epidemiology, University of California Los Angeles, Los Angeles, CA; 9) Department of Social and Preventive Medicine, University at Buffalo, Buffalo, NY; 10) Department of Internal Medicine, Ohio State Medical Center, Columbus, OH; 11) Office of Population Genomics, NHGRI, NIH, Bethesda, MD; 12) Masonic Cancer Center, University of Minnesota, Minneapolis, MN.

Introduction: Inflammation is an important health measure related to many common complex diseases. C-reactive protein (CRP) is a circulating biomarker indicative of systemic inflammation. Genome-wide association studies (GWAS) have successfully identified several loci important to inflammation-related diseases including cardiovascular disease (CVD), diabetes and obesity. However, the associations between these variants and CRP have not been fully explored. We evaluated whether CRP levels are associated with previously identified variants from GWAS of inflammation-related diseases, and whether such associations differ by race. Methods: We included 9088 participants of the Women's Health Initiative who had baseline high-sensitivity CRP measurements and were genotyped as part of the Population Architecture using Genomics and Epidemiology (PAGE) Consortium: 6309 White, 1564 Black, 714 Hispanic, 418 Asian/Pacific Islander, and 83 American Indian women. We evaluated 95 single nucleotide polymorphisms (SNPs) previously shown to be associated with inflammation-related diseases. We used linear regression stratified by race to evaluate the association between serum CRP level and each SNP, using an additive genetic model adjusted for age and global ancestry, using the first two principal components from ancestry informative markers. We corrected for multiple comparisons using Bonferroni adjustment ($p<5.3x10-4$). Results: Three SNPs had statistically significant positive associations with CRP: rs429358 T allele (APOE) in Whites ($p=1.2x10-20$), Blacks ($p=2.6x10-7$), and Hispanics ($p=3.3$ x10-7); rs4420638 A allele (APOC1) in Whites ($p=7.7x10-14$) and Hispanics ($p=8.7$ x10-5); and rs1260326 T allele (GCKR) in Whites ($p=6.2$ x10-6). No SNPs were significantly associated with CRP in Asian/Pacific Islanders or American Indians, though sample sizes were small. Conclusions: This preliminary analysis indicates that several SNPs previously associated with inflammation-related disease are also associated with circulating CRP levels, which appear to differ by race/ethnicity. Future efforts will include incorporating data from the other three studies of PAGE, to further detect and replicate and SNP-inflammation associations in each ethnic group. PAGE includes the Multiethnic Cohort (MEC), Epidemiologic Architecture of Genes Linked to Environment (EAGLE), and the CALiCo Consortium (see pagestudy.org).

617T

Two-stage multi-locus association analysis - collapsing variants with the adjustment for effect directions. *C. Kuo, D. Zaykin.* Biostatistics Branch, National Institute of Environmental Health Sciences, Durham, NC.

Complex diseases are likely to be influenced by multiple genetic loci among which the majority are undetectable using single-locus approaches, due to small marginal effects. Multi-locus approaches jointly modeling several loci at a time may capture gene-gene interactions but most of them have low power to detect joint effects of rare variants. A number of approaches have been proposed, based on the idea of adding up the number of rare alleles for each individual and using the resulting “allelic score” in an association test. However, such approaches implicitly assume that susceptibility of an allele is inversely related to its frequency. To avoid this assumption while maintaining statistical power, we introduce a two-stage analysis. In the first stage, we use a portion of the data to compute an overall P-value of association (P1) while recording effect directions for each variant. In the second stage, we compute a one-sided P-value (P2) from the “allelic score”, with possibly corrected effect directions, based on what have been observed in the first stage. Finally, we combine P1 and P2 in a weighted manner. We conduct simulations by considering a variety of genetic models with different patterns of linkage disequilibrium (LD). When the number of variants increases and a substantial portion of them are either causal variants or indirectly associated with the disease due to the LD, the two-stage analysis using ~30% of cases and controls for the first-stage has similar or higher power than a test based on the total sample.

618T

Optimal test for rare variant effects in sequencing association studies. *S. Lee[1], M. Wu[2], X. Lin[1].* 1) Biostatistics, Harvard School of Public Health, Boston, MA; 2) Biostatistics, University of North Carolina, Chapel Hill, NC.

With development of massively parallel sequencing technologies, rare variants association tests are increasingly conducted. Burden tests, which assume all rare variants in the target region have effects on the phenotype in the same direction and of similar magnitude, are not powerful if there are many non-causal rare variants, or if there exist variants with different association directions. Recently proposed Sequence Kernel association Test (SKAT) (Wu, Lee, et al, AJHG, 2011) provides a robust test and is particularly powerful in the presence of protective and deleterious variants and null variants. However, SKAT is less powerful than burden tests when a large number of variants in a region are causal and in the same direction. It is hence of substantial practical interest to develop a test that is optimal for both scenarios. In this paper, we generalize SKAT by incorporating a correlation structure of variants effects through a particular family of kernels. The generalized SKAT includes burden tests as a special case when the effects of variants are perfectly correlated. Based on this new family of kernels, we have developed the optimal testing procedure (SKAT-O) by estimating the correlation parameter to maximize the power. We show that SKAT-O outperforms burden tests and SKAT in a wide range of scenarios. The results are illustrated using simulation studies and triglyceride data from the Dallas Heart Study.

619T

HLA-DRB1 gene and type 1 diabetes: meta-analysis. *Y. Lee[1,2,4], C. Huang[2], W. Ting[2], F. Lo[3], T. Chang[1].* 1) Dept Med Res, Mackay Memorial Hosp, Tamshui District, New Taipei City, Taiwan; 2) Dept Pediatrics, Mackay Memorial Hospital, Taipei, Taiwan; 3) Dept Pediatrics, Chang Gung Memorial Hospital, Taoyuan, Taiwan; 4) Dept Pediatrics, Taipei Medical University, Taipei, Taiwan.

The concordance rate of type 1 diabetes (T1D) is ~50%; in monozygotic twins but only ~5%; in dizygotic twins. These observations suggest that genetic factors are important in the pathogenesis of T1D. The DRB1 gene in the HLA complex has been found to be associated with T1D. However, there are wide discrepancies among different ethnic groups. Therefore a meta-analysis on the gene may help know the reasons. **Materials and Methods** We searched the PubMed database for all genetic association studies of DRB1 and T1D using search terms “Diabetes Mellitus, Type 1”[Mesh] AND “DRB1”. The references of all identified publications were also searched for additional studies. According to the MOOSE (Meta-analysis Of Observational Studies in Epidemiology) guidelines, authors' names, year of publication, ethnicity of the study subjects, region/country where the study subjects resided, numbers of patients and controls, age at diagnosis of T1D for patients and at sampling of DNA for controls, manner in which the controls were selected, and number of subjects with each genotype or allele in both cases and controls were extracted. A meta-analysis was by Comprehensive Meta-Analysis Version 2. All P values are two-tailed and significant if <0.05. **Results** The literature search yielded 101 eligible articles. The meta-analysis revealed that risk factors are DRB1*04 and *03 in Africans and African Americans; *03 and *04 in Arabs; *03, *04 and *09 in Asians; and *03 and *04 in Caucasians. Protective factors are DRB1*11 in Africans; *15 in African Americans; *14, *13, *15 and *11 in Arabs; *12, *10 and *15 in Asians; and *15, *07, *11 and *14 in Caucasians. **Conclusions** The alleles in the DRB1 gene conferring susceptibility to or protection against T1D vary in different ethnic populations. The results of a case-control study in a population might not be completely replicated in another populations with different ethnicities.

620T

The role of cis-acting genetic modifiers in phenotypic variability of familial amyloid polyneuropathy FAP ATTRV30M. *C. Lemos[1,2], T. Coelho[3], J.L. Neto[1], J. Pinto-Basto[1,2], J. Sequeiros[1,2], I. Alonso[1,2], A. Sousa[1,2].* 1) UnIGENe, IBMC, Porto, Portugal; 2) Unidade Clínica de Paramiloidose, Centro Hospitalar do Porto (CHP), Porto, Portugal; 3) ICBAS, Instituto Ciências Biomédicas Abel Salazar, Univ. Porto Portugal.

Familial amyloid polyneuropathy (FAP) ATTRV30M is an autosomal dominant systemic amyloidosis, due to a point mutation in the transthyretin (TTR) gene. More than 100 mutations have been found in the TTR gene but V30M is by far the most common. The initial description of Portuguese cases (Andrade, 1952) characterized FAP as a disease of the young adult (onset 25-35yrs). A much wider variability in age-at-onset (AO) of FAP has been uncovered, including among Portuguese patients [17-80yrs]. So far, very few consistent results have been produced to explain that variability and most of the approaches have compared early (≤30) and late-onset (⁄ 50) as two separate groups. However, early and late-onset cases are not separate entities, since they often coexist in the same family, with offspring showing a much earlier AO than their affected parent - anticipation. Late-onset parents remain asymptomatic for several years and often have offspring with early onset, while the reverse has never been observed. Focusing on variation between generations of the same family and identifying genetic modifiers associated with variability in AO may have important clinical implications and is a very promising avenue. Our aim is to identify genetic modifiers within or closely linked to the TTR locus. Haplotype analysis families may indicate the presence of modifiers in TTR neighbourhood. Haplotype analysis is on-going in 100 families, using intragenic SNPs and flanking STRs for extended haplotypes. Sixteen tagging SNPs were selected based on a data dump from the HapMap Project (www.hapmap.org) and using Haploview v4.1, with a minor allele frequency (MAF) of 0.1% and covering 60 Kb around the TTR locus. SNP genotyping is currently underway by SNaPshot, using a multiplex approach. Eight microsatellite markers were also selected, encompassing 11.4 Mb. STRs genotyping is being performed by PCR, using fluorescent-labelled primer pairs and genotypes will be determined using GeneMapper version 4.0 software. Haplotypes will be constructed in informative families and inferred, using PHASE v2.1, when the phase cannot be directly determined. Some variants or regions that may confer protection to a number of generations (in families with late-onset patients or aged asymptomatic carriers) are expected to be found which when lost (by recombination or mutation) result in early onset in the offspring and therefore, may explain part of the variability of age-at-onset observed in FAP ATTRV30M.

621T

Differential call rates in genotype imputation and their effect on genetic association studies. *N. Liu, B. Zhang.* Dept Biostatistics, Univ Alabama at Birmingham, Birmingham, AL.

Genotype imputation is the process of inferring genotypes that are not directly genotyped in a cohort of individuals. Imputation offers a promising way to infer untyped genotypes in genetic studies and has been widely adopted to boost power, fine-map associations, and synchronize the genotype data from studies using different platforms. However, the quality of the imputed data is vital to the downstream analyses and is largely ignored. One of these issues is the differential call rates among the imputed genotypes: AA, Aa, and aa (assuming a is the minor allele at a locus). In this work, we compare the call rates among the genotypes from imputation and evaluate the effect of differential call rates on genetic associate studies. We further provide guideline for genetic association studies when imputation is used. Our analysis based on the WTCCC and HapMap data indicates that the call rates for Aa and aa can be as low as 33% and 35% of that for AA when the minor allele frequency (MAF) is low. MAF is the major cause of such difference, although other factors such as untype rate, homogeneity of study samples, and threshold for accepting the “calling” from imputation can also affect the call rates. Simulation studies show that the differential call rates can considerably lower the power of the association test by 66% when background prevalence, MAF, and sample size are small. Relative risk of the disease, untyped rate and threshold for accepting an imputed genotype can also affect the power. We suggest that a study should balance between the sample size, untyped rate, and threshold used for imputation. For rare variants less than 5%, a study may consider a combined genetic score instead of using single marker for association test.

622T

A Nonparametric Approach to Population Based Association Tests. *S.M. Lutz[1], W. Yip[3], J. Hokanson[2], N. Laird[3], C. Lange[3].* 1) Biostatistics Dept, University of Colorado at Denver, Aurora, CO; 2) Epidemiology Dept, University of Colorado at Denver, Aurora, CO; 3) Biostatistics Dept, Harvard School of Public Health, Boston, MA.

In population-based genetic association studies, the standard approach is to model the phenotype of interest as a function of the offspring genotype. We propose an alternative approach based on conditional score-tests that treats the genetic information as the random variable and conditions upon the phenotypic information. The flexible structure of the approach enables the straight-forward application to standard and complex phenotypes. By treating the phenotype data as deterministic, the validity of the approach does not depend on the correctness of any assumptions about the phenotype. This makes the approach especially suitable for complex phenotypic models and the analysis of secondary phenotypes in studies that applied ascertainment conditions to the primary phenotype. If both phenotypes are correlated, the ascertainment conditions can cause a perturbation of the distribution of the secondary phenotypes. Based on theoretical considerations and on simulation studies, we show that our approach is robust against mis-specification of phenotype assumptions and, at the same time, achieves the same or higher power level as standard genetic association tests for population-based designs.

623T

Comparing the statistical power of joint and meta-analysis association testing with rare variants. *C. Ma, M. Boehnke, L.J. Scott.* Biostatistics, University of Michigan, Ann Arbor, MI.

Meta-analysis has become the standard approach to combine GWAS information across multiple studies, allowing simple sharing of results and study-specific covariate adjustment. For association testing of common genetic variants, Lin and Zeng (2010) showed that using meta-analysis to combine results from multiple studies has comparable power to joint analysis of the complete individual-level data. However, it is unclear whether meta-analysis remains statistically efficient compared to joint analysis for variants with small per-study minor allele counts (MAC). To address this question, we compared the power of joint and meta-analysis for disease-marker association testing of less common variants (minor allele frequency [MAF] < .05). We simulated datasets with 10000 cases and 10000 controls, assuming a single multiplicative causal genetic variant with varying expected MAC and a binary covariate. We analyzed the data using (1) logistic regression (LR), (2) Fisher's exact test (FET) with covariate stratification, and (3) exact logistic regression (ELR). We performed (a) joint analysis of the complete dataset and (b) meta-analysis of results from the same dataset partitioned and analyzed as 10 equal-sized sub-studies.

For common variants (per sub-study MAC ⁄ 400; MAF ⁄ .1), both joint and meta-analysis had near nominal type I error and equivalent power. However, for less common variants (MAC ≤ 40; MAF ≤ .01) and for all analysis methods, type I error for joint and meta-analysis was conservative but more conservative for meta-analysis. Furthermore, power was greater for joint analysis than meta-analysis. For example, for variants with MAC = 4 (MAF = .001) and genotype odds ratio = 5, power estimates were: 83% joint vs. 38% meta (ELR), 82% joint vs. 19% meta (FET), and 55% joint vs. 2% meta (LR). Overall, exact-inference tests (ELR and FET) had greater power than asymptotic tests (LR) for both joint and meta-analysis. Without covariate adjustment, ELR and FET had similar power for both joint and meta-analysis, but with covariate adjustment, ELR had greater power. For each test, meta-analysis has lower power and type I error due to the smaller number of possible statistically-significant configurations for the small number of alleles per sub-study. Since ELR can directly adjust for covariates and handle imputed allelic dosages, we recommend ELR for testing low frequency variants, preferably using joint analysis over meta-analysis.

624T

Genome-wide association analysis of rare variants with Crohn's disease. *R. Magi, A.P. Morris.* Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK.

Genome-wide association studies have been successful in identifying novel Crohn's disease (CD) susceptibility loci. However, the joint effects of common variants account for no more than 23% of the heritability of the disease. The aim of this study was to assess the evidence for association of CD with rare genetic variation, defined here to have minor allele frequency (MAF) less than 1%, through imputation up from a scaffold of existing GWAS genotyping data.

We performed imputation in 1,748 CD cases and 2,940 controls of European descent, genotyped using the Affymetrix GeneChip 500K Mapping Array Set by the Wellcome Trust Case Control Consortium. Imputation was undertaken using IMPUTEv2 and the European reference panel from the 1000 Genomes Project (August 2010 release). We tested for association of CD with accumulations of minor alleles at rare variants within genes using GRANVIL software, which models disease status as a function of the proportion of rare variants at which an individual carries at least one minor allele in a logistic regression framework.

The strongest signal of association of rare variants with CD was observed for *PTGER4* ($p=1.3x10^{-6}$, genome-wide significant correcting for 30,000 genes). This gene contains common variants that have been previously associated with CD and plays an important role in immune response. The gene contained 28 rare variants (mean MAF = $4.9x10^{-3}$), with odds ratio of 0.73 (0.64-0.83) per minor allele. Strong evidence of association of rare variation with CD ($p<10^{-5}$) was also observed for *CD247* ($p=2.5x10^{-6}$). Defects in the gene are a primary cause of primary T-cell immunodeficiency and common variants have been previously associated with systemic sclerosis. The gene contained 19 rare variants (mean MAF = $5.7x10^{-3}$), with odds ratio of 1.23 (1.13-1.34) per minor allele.

Our analysis has highlighted strong evidence of association of Crohn's disease with rare variation in 2 genes which warrant follow-up in independent cohorts. Our results highlight the potential for the identification of rare variant associations using existing GWAS genotyping data, supplemented with imputation from publicly available high-density reference panels, without the need for costly re-sequencing experiments.

625T

Transferability of European Fasting Glucose Quantitative Trait Loci to African American Populations: the Candidate Gene Association Resource (CARe). *J.B. Meigs[1], C.T. Liu[2], J. Dupuis[2], A. Adeyemo[3], S. Bielinski[4], I. Borecki[5], B. Cade[6], Y.I. Chen[7], M. Fornage[8], M.O. Goodarzi[7], S.F.A. Grant[9], X. Guo[7], T. Harris[10], L. Kao[11], E. Kabagambe[12], J.R. Kizer[13], Y. Liu[14], J. Nettleton[8], M. Ng[14], J. Pankow[15], S. Patel[16], E. Ramos[3], L. Rasmussen-Torvik[17], S.S. Rich[18], C. Rotimi[3], D. Siscovick[19], J. Zmuda[20], J.C. Florez[1], J.G. Wilson[21], D.W. Bowden[14].* 1) Massachusetts Gen Hosp, Boston, MA; 2) Boston University School of Public Health, Boston, MA; 3) National Institute of Health, Bethesda, MD; 4) Mayo Clinic, Minneapolis, MN; 5) Washington University, St. Louis, MO; 6) Harvard Medical School, Boston, MA; 7) Cedars Sinai Medical Center, Los Angeles, CA; 8) University of Texas Health Science Center at Houston, Houston, TX; 9) Children's Hospital of Philadelphia, Philadelphia, PA; 10) National Institute on Aging, Bethesda, MD; 11) Johns Hopkins University, Baltimore, MD; 12) University of Alabama, Bimingham, AL; 13) Weill Cornell Medical College, New York, NY; 14) Wake Forest University, Winston-Salem, North Carolina; 15) University of Minnesota, Minneapolis, MN; 16) Brigham and Women's Hospital, Boston, MA; 17) Northwestern University, Chicago, IL; 18) University of Virginia, Charlottesville, VA; 19) University of Washington, Seattle, WA; 20) University of Pittsburgh, Pittsburgh, PA; 21) University of Mississippi Medical Center, Jackson, MS.

Background: Hyperglycemia disproportionately affects African Americans (AfA). Genetic variants associated with glycemic traits in European Ancestry (EuA) populations may be the same in AfA. We investigated population differentiation, evidence for selection, transferability and fine-mapping in the AfA CARe cohorts for loci previously associated with fasting glucose (FG) in EuA genome-wide association studies. Methods: We meta-analyzed (inverse-variance weighting approach) genome-wide single nucleotide polymorphism (SNP) associations with FG in 5,984 non-diabetic AfA from 5 CARe cohorts: ARIC, CARDIA, CFS, JHS, MESA. We tested transferability of 16 Index EuA FG-associated SNPs (from MAGIC, Dupuis et al 2010) in AfA using the meta-analysis results by 1) calculating allele frequency differences (|EuA-AfA/EuA|), fixation index (Fst) and integrated haplotype scores (iHS) in EuA and AfA, 2) testing directly 16 EuA index SNPs and 3) interrogating SNP-FG associations within ±250kb flanking regions around each index SNP to account for differences in LD structure. Significant evidence of transferability for index SNPs was defined as the same direction of effect and a P value adjusted for the effective number of independent tests. Results: Index SNP frequencies in each group differed by 2% (0.28 AfA, N=5,984 vs. 0.29 EuA, N=46,186; TCF7L2) to 77% (0.87 AfA vs. 0.49 EuA, CRY2). Fst was >0.15 at 1/16 loci in CARe but 6/16 loci in HapMap 2, indicating modest population differentiation, and all iHS were <2, suggesting no recent major selection at any locus. Only 3/16 EuA index SNPs (at G6PC2, GCK and MTNR1B) were nominally associated with FG in AfA (13 SNP associations were P>0.05). However, after accounting for LD differences, 15 SNPs flanking EuA loci were directionally consistent and nominally significantly associated with FG (P<0.05), with three which were multiple-test-adjusted significantly associated (at GCK, P=5.8x10-8, MTNR1B, P=8.5x10-9, FADS1, P=2.2x10-4). At each locus the SNP in AfA was only weakly correlated (r2 <0.3) with the EuA index SNP. Conclusions: Despite substantial allele frequency variation and modest index SNP transferability, after accounting for LD differences most FG-associated loci showed at least nominal evidence of similarity in AfA and EuA, with 4 loci (G6PC2, GCK MTNR1B, FADS1) associated with FG in both AfA and EuA. Accounting for varying LD across ancestral groups identified signals that may aid functional variant fine-mapping.

626T

The lipid-associated rs4846914 GALNT2 gene variant confers a highly elevated risk for type 2 diabetes. *B. Melegh[1], P. Kisfali[1], A. Maasz[1], E. Baricza[1], B. Duga[1], M. Mohas[2], I. Wittmann[2], N. Polgar[1].* 1) Department od Medical Genetics, University of Pecs, Pecs, Hungary; 2) 2nd Department of Internal Medicine and Nephrology Center, University of Pecs, Pecs, Hungary.

The UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2 (GALNT2) is indirectly contributing to plasma triglyceride level modifications as it has been recently implicated in the modulation of antgiopoietin-like protein - 3 (ANGPTL3) activity via O-glycosylation and ANGPTL3 is an inhibitor of lipoprotein lipase, the enzyme responsible for triglyceride hydrolysis. In addition, recent genome-wide association studies found GALNT2 variant rs4846914 to be associated with blood lipid level alterations. As the GWAS studies do not provide functional information, we studied the possible association of this variant in type 2 diabetes mellitus (T2DM) and metabolic syndrome (MS) patients. A total of 308 type 2 diabetic patients, 394 metabolic syndrome patients and 246 healthy controls were genotyped by PCR-RFLP method. A significantly higher ratio of GG homozygotes was detected among both T2DM and MS patients compared to controls. Furthermore, in multiple regression analysis adjusted for body mass index, triglyceride as well as age and sex, the GG genotype was found to be associated with the development of type 2 diabetes, with an unusually high odds ratio of 5.218 (CI 95%: 2.811-9.683; p<0.001), while carriers of the G allele demonstrate a lower risk for T2DM (OR 1.659 [CI 95%: 1.027-2.678], p=0.038). The results revealed a differentiated risk nature of the lipid profile modifying GALNT2 natural gene variant rs4846914, as in our cohort the variant did not show an association with metabolic syndrome, but seems to confer an exceptionally high risk for type 2 diabetes, which strong association has not been reported in the literature available.

627T

Coexpression network analysis in abdominal and gluteal adipose tissue reveals regulatory genetic loci for metabolic syndrome and related phenotypes. *J.L. Min[1], G. Nicholson[2], I. Halgrimsdottir[2], K. Almstrup[3], A. Petri[3], P. Keller[3], A. Barrett[4], M. Travers[4], N.W. Rayner[1,4], R. Mägi[1], F.H. Pettersson[1], J. Broxholme[1], M.J. Neville[4,5], Q.F. Wills[2], J. Cheeseman[4], The. GIANT Consortium[6], The. MolPAGE Consortium[7], M. Allen[4], C.C. Holmes[1,2], T.D. Spector[8], J. Fleckner[3], M.I. McCarthy[1,4,5], F. Karpe[4,5], C.M. Lindgren[1], K.T. Zondervan[1].* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 2) Department of Statistics, University of Oxford, Oxford, United Kingdom; 3) Department of Molecular Genetics, Novo Nordisk A/S, Maaloev, Denmark; 4) Oxford Centre for Diabetes, Endocrinology & Metabolism, Churchill Hospital, Oxford, United Kingdom; 5) NIHR Oxford Biomedical Research Centre, ORH Trust, Churchill Hospital, Oxford, United Kingdom; 6) The GIANT Consortium; 7) The MolPAGE Consortium; 8) Twin Research Unit, King's College London, London, United Kingdom.

Metabolic Syndrome (MetS) is a highly prevalent disorder with considerable public health concern, but its underlying genetic factors remain elusive. To identify gene networks involved in its pathogenesis, we conducted whole-genome expression and genotype profiling on abdominal (ABD) and gluteal (GLU) adipose tissue, and whole blood (WB), from 29 MetS cases and 44 controls. Co-expression network analysis for each tissue independently identified nine, six and zero MetS-associated modules of coexpressed genes in ABD, GLU and WB, respectively. Of 8,992 probesets expressed in ABD or GLU, 685 (7.6%) were expressed in ABD and 51 (0.6%) in GLU only. Differential eigengene network analysis of 8,256 shared probesets detected 22 shared modules with high preservation across adipose depots, ($D_{ABD-GLU}$=0.89), seven of which were associated with MetS (FDR P<0.01). The strongest associated module, significantly enriched for immune response related processes, contained 109/620 (18%) genes with inter-depot differences. In an independent cohort of 145/141 twins with ABD and WB longitudinal expression data, median variability in ABD due to familiality was greater for MetS-associated vs un-associated modules (ABD: 0.48 vs 0.18, P=0.08; GLU: 0.54 vs 0.20, P=0.002); median variability due to shared hospital visits was greater in WB than in ABD (0.34 vs 0.15, $P=1.9*10^{-6}$). *Cis*-eQTL analysis of probesets associated with MetS (FDR P<0.01) and/or inter-depot differences (FDR P<0.01) provided evidence for 32 eQTLs. Corresponding eSNPs were tested for association with MetS-related phenotypes in two GWAS of >100,000 individuals; rs10282458, affecting expression of *RARRES2* (encoding chemerin), was associated with body mass index (BMI) ($P=6.0*10^{-4}$); rs2395185, affecting inter-depot differences of *HLA-DRB1* expression, was associated with high-density lipoprotein ($P=8.7*10^{-4}$) and BMI-adjusted waist-to-hip ratio ($P=2.4*10^{-4}$). Since many genes and their interactions influence complex traits such as MetS, integrated analysis of genotypes and coexpression networks across multiple tissues relevant to clinical traits is an efficient strategy to elucidate their pathogenesis.

628T

Pleiotropic Relationships among Measures of Body Composition in Afro-Caribbeans from Tobago. *R.L. Minster[1], A.L. Kuipers[2], C.M. Kammerer[1], A.L. Patrick[3], V.W. Wheeler[3], J.M. Zmuda[1,2].* 1) Dept of Human Genetics, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; 2) Dept of Epidemiology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; 3) Tobago Health Studies Office, Scarborough, Tobago, Trinidad and Tobago.

Covariation between bone, muscle and fat tissue could be due to developmental, homeostatic, mechanical or geriatric processes, some of which may be due to pleiotropy. Little is known about such pleiotropic effects or the specific genes involved. In this study we explored the pleiotropic relationships among 22 measures of arm and leg bone mineral density and geometry, arm and leg lean mass and arm and leg fat mass using data from two populations of Afro-Caribbeans from the island of Tobago: a population sample of 1,937 unrelated men aged ⁄ 40 years and a set of 470 men and women aged > 18 years in seven extended pedigrees (mean family size = 67; 4,206 relative pairs). We first characterized phenotypic relationships via hierarchical clustering (HC) and then derived composite traits using principal components (PC) analyses. Subsequently, we estimated residual heritabilities (h_r^2) for the individual and composite traits, estimated genetic correlations (δ_G) among the 22 individual traits, and conducted genomewide linkage analysis using data on 1,516 autosomal SNPs (median spacing: 1.92 cM). HC and PC analysis revealed that the traits fall into three groups: (1) a “geometry group” that comprises mostly bone geometry traits and lean mass (PC1); (2) a “density group” that comprises mostly bone mineral density traits (PC2); and (3) a “fat mass group” that comprises measures of fat mass (PC3). Estimates of h_r^2 ranged from 0.206 to 0.763 (all $p < 0.007$) for all individual and composite traits. Genetic correlations were high among measures of lean mass and fat mass (δ_G: 0.43-0.84) and among measures comprising PC1 (δ_G: 0.36-0.89). Linkage analysis revealed significant evidence for QTLs on two chromosomes: (1) a QTL for PC1 that overlapped with a QTL for tibial periosteal circumference on 10q (LOD = 3.45 and 3.12 respectively) and (2) a QTL for PC3 that overlapped with a QTL for arm fat mass on 21q (LOD = 2.82 and 3.66 respectively). Leg fat mass and arm and leg lean mass also showed peaks on 21q (LOD < 2.5). Bivariate linkage analysis of arm and leg fat mass demonstrated suggestive linkage at this locus (LOD = 2.65) as did arm lean mass and arm fat mass (LOD = 2.92). The current analyses present evidence for specific QTLs with pleiotropic effects on multiple body composition traits. Additional mapping of variation in these regions could reveal genes that jointly influence susceptibility to osteroporosis, sarcopenia and obesity.

629T

Association Tests for Rare and Common Variants: an approach based on Genotypic and Phenotypic Measures of Similarity between Individuals. *I. Mukhopadhyay[1], J. Zhao[2], A. Thalamuthu[2].* 1) Human Genetics Unit, Indian Statistical Institute, 203 B T Road, Kolkata 700108, India; 2) Human Genetics, 60 Biopolis Street 02-01, Genome Institute of Singapore, Singapore 138672.

Gene-based association tests are gaining importance for the analysis of Genome-wide Association Studies (GWAS) because it reduces multiple testing burdens and also provides directions for future functional studies. The common variants used in GWAS account only for a small fraction of phenotypic variance or heritability. Along with the common variants, many rare variants are also identified through next generation sequencing technology. These rare variants are expected to have significant effects in the disease pathogenesis and may contribute significantly to the fraction of heritability that is still unexplained. So, within a gene or any genomic region of interest, testing of the joint association of both rare and common variants would be of utmost importance to determine their synergistic effects. Based on the idea of our recently proposed kernel based association test (KBAT) for binary trait, here we propose powerful methods to test such joint association. The association test for quantitative trait is based on both genotypic and phenotypic measures of similarity between individuals. We also have developed a modified version of KBAT for rare variants association. In fact, this method also has the potential to combine both rare as well as common variants to see whether any gene is associated with the disease etiology. The basic idea is to construct a number of groups of individuals depending on the similarity (phenotypic and / or genotypic) score between two individuals and test whether the group specific effects are same for these groups. Thus the usual idea of analysis of variance (ANOVA) technique is used here with careful modification. However, in contrary to ANOVA, we have shown that the proposed test statistics do not follow any known probability distribution, even when the null hypothesis of absence of association is true. Hence we use extensive simulation and permutation based type I error rate and power to evaluate the performance of the tests, under several genetic models. Our newly proposed approach seems to be very robust and can be extended in various directions.

630T

The effect of plasma fibrinogen on the risk of venous thromboembolism among African American and White women using oral contraceptives. *C.S. Neal[1], F.D. Mili[2], W.C. Hooper[2], C. Lally[3], H. Austin[3].* 1) Pediatric Genetics Team, National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; 2) Research Laboratory Branch, Division of Blood Disorders, National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; 3) Department of Epidemiology, Emory University Rollins School of Public Health, Atlanta, Georgia, USA.

Venous thromboembolism (VTE) is a condition that includes deep vein thrombosis and pulmonary embolism and has both hereditary and acquired risk factors. A strong association between Factor V Leiden mutation and VTE has been shown to exist in individuals of European descent. The mutation is uncommon and therefore of lesser importance in individuals of African descent and, to date, few genetic risk factors for VTE have been defined in this population. Data from the Genetic Attributes and Thrombosis Epidemiology (GATE) study, a matched case-control study conducted in Atlanta, Georgia, provided evidence that oral contraceptive (OC) use increased the risk of VTE in African American women and that the increased risk was even greater among African American women with sickle cell trait. Levels of plasma fibrinogen have a major genetic component and previous studies have suggested that high plasma fibrinogen (equal to 3g/L or above) was associated with increased risk for VTE. We sought to investigate the effect of plasma fibrinogen on the association between OC use and unprovoked, first VTE among African American versus White American women between 18 and 49 years of age. We defined unprovoked VTE as an episode of VTE where certain factors known to promote VTE (such as surgery or prolonged immobility) were absent. We analyzed data from the GATE study, and calculated odds ratios (OR) and 95% confidence intervals (CI) using conditional logistic regression adjusted for income and age. Our analysis included 269 African American women, [63 case patients (with VTE) and 206 control patients (without VTE)] and 192 White American women [43 case patients and 149 control patients]. Our findings indicated that the association between OC use and unprovoked, first VTE was stronger in African American women with high plasma fibrinogen (OR=5.5, 95% CI 1.7—18.0; P= 0.005) compared to those with normal fibrinogen levels (OR= 0.89, 95% CI 0.13—6.0; P=0.90). However, the association between OC use and VTE was not significantly different between White American women with high and normal plasma fibrinogen (OR=1.2 vs. OR=0.56, respectively). The P for interaction between African American women with high fibrinogen levels versus White American women with high fibrinogen levels was statistically significant at 0.037. Our preliminary results indicated that high fibrinogen levels might put African American women at a significantly greater risk for VTE from OC use.

631T

Improving discrimination of true associations from tracking associations: a test using simulated disease data structured by real genomic data. *G. Nelson[1], P. An[2], C.A. Winkler[2].* 1) BSP/CCR Genetics Core, SAIC Frederick, NCI-Frederick, Frederick, MD; 2) Basic Research Laboratory, SAIC-Frederick, NCI-Frederick, Frederick, MD.

Successful genetic association searches, e.g. GWAS, commonly lead to identification of a region of association or a set of associated polymorphisms. If multiple polymorphisms are associated they will probably be in strong linkage disequilibrium (LD), making it difficult or impossible to distinguish factors functionally associated with disease from factors tracking the functional polymorphism by LD. Multivariate regression is a standard approach for finding the best association among correlated variables, but is strongly subject to noise when the explanatory variables are highly correlated, leading to instability in the results: small changes in the data can flip the results, so that a factor strongly associated in one analysis can lose all association in an analysis with slight changes in the data. This instability is heightened when automatic variable selection is used. We considered that bootstrapping might improve the stability and the accuracy of this analysis by partially suppressing the effect of noise. The chemokine receptor cluster on chromosome 3 contains a number of identified polymorphisms affecting AIDS progression, along with additional candidate polymorphisms for an influence on AIDS. As many of these are in strong LD, this region illustrates the association analysis problem. To test the ability of alternate methods in the context of a real pattern of LD, we combined real genomic data for polymorphisms in this receptor cluster with simulated AIDS progression data, generated assuming a Weibull distribution for genetic effects on AIDS progression. We show that bootstrapped forward automatic variable selection of the Cox model regression is the most successful strategy, among 6 bootstrapped and non-bootstrapped approaches, at correctly distinguishing the simulated associated factor or factors from the tracking factors. This contradicts a prevalent judgment that backward selection is preferable in epidemiological analysis, but is plausible for genetic factors, most of which will have no real association with the disease being studied.

632T

Japanese Population Structure Estimated from the Japanese Multi-institutional Collaborative Cohort (J-MICC) Data. *T. Nishiyama[1], N. Hamajima[2], S. Suzuki[3], H. Kishino[4], the Japan Multi-institutional Collaborative Cohort (J-MICC).* 1) Clinical Trial Management Center, Nagoya City University Hospital, Japan; 2) Departments of Preventive Medicine/Biostatistics and Medical Decision Making and Public Health/Health Information Dynamics, Nagoya University Graduate School of Medicine, Japan; 3) Department of Public Health, Nagoya City University Graduate School of Medicine, Japan; 4) Laboratory of Biometry and Bioinformatics, Graduate School of Agriculture and Life Sciences, University of Tokyo, Japan.

Background: Because population structure can cause spurious association in case-control studies, clarifying the population structure is important. The previous study of Japanese population with the use of genome-wide SNPs (Yamaguchi-Kabata et al, 2008) revealed that Japanese individuals fell into two main clusters, called as Hondo and Ryukyu. In this study, we examined Japanese population structure, using the Japanese Multi-institutional Collaborative Cohort (J-MICC) Data. Our main focus is to determine to which cluster Amami islands belong, because Amami islands is located just midway between regions covered by two clusters.

Methods: 234 SNPs in 4514 Japanese individuals from J-MICC were analyzed using principal-component analysis (PCA). We also used the multi-dimensional scaling (MDS) method to examine relatedness among individual using PLINK (Purcell et al, 2007).

Results: The PCA plot with the first and second components showed no separated cluster. The MDS method also indicated no separation in this sample.

Conclusions: These results have suggested that Amami islands midway between two sub-populational regions belong to the Hondo cluster, which includes most of the individuals from the main islands in Japan. Therefore, population stratification is unlikely to cause confounding in case-control analyses of J-MICC data.

633T

Cross-sectional Gender-stratified Blood Pressure Analyses in Children: Results from a Genome-wide Association Study. *P.G. Parmar[1], H.R. Taal[2], V. Aalto[3], E. Thiering[4], N.J. Timpson[5], M. Bustamante[6], N.G. Martin[7], G. Verwoert[2], T. Lehtimäki[8], A.G. Uitterlinden[2,9], L. Briollais[10], C. Stoltenberg[11], C. Power[12], J.P. Newnham[1], D.I. Boomsma[13], J. Viikari[14], M.W. Gillman[15], H.N. Lyon[16], J.J. Hottenga[13], A. Hofman[2], M. Kähönen[8], M.J Tobin[17], O. Raitakari[18], V.W.V. Jaddoe[2], M-R. Jarvelin[19], L.J. Beilin[20], J. Heinrich[4], C.M. van Duijn[2], C.E. Pennell[1], L.J. Palmer[10,21], EArly Genetics and Lifecourse Epidemiology(EAGLE) and CHARGE Consortiums.* 1) The Western Australian Pregnancy Cohort (Raine) Genetic Epidemiology Team, School of Women's and Infants' Health The University of Western Australia, Perth WA, Australia; 2) The Generation R Study Group and Departments of Epidemiology and Pediatrics Erasmus Medical Center, Rotterdam, The Netherlands; 3) The Cardiovascular Risk in Young Finns Study, Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland; 4) Lisa Plus Helmholtz Zentrum Muenchen, German Research Centre for Environmental Health, Institute of Epidemiology, Neuherberg, Germany; 5) The Avon Longitudinal Study of Parents and Children (ALSPAC), The Medical Research Council (MRC) Centre for Causal Analyses in Translational Epidemiology, Department of Social Medicine, University of Bristol, Oakfield House, Bristol, UK; 6) Spanish INMA—INfancia y Medio Ambiente—(Environment and Childhood) Project Centre for Research in Environmental Epidemiology, 08003 Barcelona, Catalonia, Spain, Municipal Institute of Medical Research (IMIM), 08003 Barcelona, Catalonia, Spain; 7) Australian Twin Study, Queensland Institute of Medical Research, 300 Herston Road, Q 4029, Australia; 8) The Cardiovascular Risk in Young Finns Study, Department of Clinical Physiology, University of Tampere and Tampere University Hospital, Tampere, Finland; 9) Internal Medicine Erasmus Medical Center, Rotterdam, The Netherlands; 10) Samuel Lunenfeld Research Institute University of Toronto, Ontario, Canada; 11) The Norwegian Mother and Child Cohort Study (MoBa), Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway; 12) NCDS (National Child Development Study), Centre for Paediatric Epidemiology and Biostatistics, MRC Centre of Epidemiology for Child Health, University College of London Institute of Child Health, London, UK; 13) Netherlands Twin Register, Department of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands; 14) Department of Medicine, University of Turku and Turku University Hospital; 15) Project Viva, Obesity Prevention Program, Department of Population Medicine, Harvard Medical School and Harvard Pilgrim Health Care Institute, Boston, Massachusetts, USA; 16) Project Viva, Obesity Prevention Program, Division of Genetics, Program in Genomics, Children's Hospital, Boston, Massachusetts, USA; 17) Departments of Health Sciences & Genetics, University of Leicester, United Kingdom; 18) The Cardiovascular Risk in Young Finns Study, Department of Clinical Physiology, University of Turku, Turku, Finland; 19) The Northern Finland Birth Cohort (NFBC), Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, UK., Institute of Health Sciences, University of Oulu, Oulu, Finland, Biocenter Oulu, University of Oulu, Oul; 20) The Western Australian Pregnancy Cohort (Raine) Genetic Epidemiology Team, School of Medicine and Pharmacology Royal Perth Hospital Unit The University of Western Australia, Perth WA, Australia; 21) Ontario Institute for Cancer Research University of Toronto, Canada.

Purpose: The aim of this project was to define genes associated with childhood blood pressure (BP) so as to better understand key biological pathways potentially related to hypertension in adulthood. **Methods:** Sex-stratified cross-sectional GWAS were conducted on children of European descent from the EAGLE Consortium across three pre-defined time frames; pre-pubertal [4-7 years], pubertal [8-12 years] and post-pubertal [13-19 years]. Each cohort created a Z-score for systolic and diastolic blood pressure (SBP and DBP) by removing the first BP measure and averaging the remaining at each time point, three models (M1-M3) were analysed. All models adjusted for age and assumed an additive model for SNP based main effects (M1). M2 additionally adjusted for height and weight. M3 additionally adjusted for birth weight and weight. Preliminary meta-analyses (in METAL(www.sph.umich.edu/csg/abecasis/metal)) using GWA data from studies; Raine, GenerationR, Young Finns Study and Lisa Plus are presented here. Avon Longitudinal Study of Parents And Children, Australian Twin Study and Spanish INMA will be contributing their GWAS analyses. The Netherlands Twin Register and Northern Finnish Birth Cohort will also supply genetic data for the purpose of replication. **Results:** A number of SNPs reached had a p-value of at least p≤1x10-5 across all models analysed (M1-M3). ***Males*** Pre-pubertal analyses revealed three SNPs in ARHGAP42 and one in SEL1L3 that were associated with SBP and DBP respectively. Pubertal analyses revealed two SNPs in HIVEP3 that were associated with SBP. Three SNPs in PAPPA2 were associated with DBP. Post-pubertal analyses revealed two SNPs in LINGO2 that were associated with DBP. ***Females*** Pre-pubertal analyses revealed a SNP in CDC45 was associated with SBP. Two SNPs in AFF3 and five SNPs in CSMD1 were associated with DBP. Pubertal analyses revealed a SNP in AKAP13 was associated with DBP. Post-pubertal analyses revealed a single SNP in both LPAR1 and SNX31 was associated with DBP. **Conclusion:** The findings were consistent within each time frame but not across time frames, supporting the concept that complex gene-environment interactions underlie the developmental origins of hypertension. Future studies with larger sample sizes will be required to replicate these findings (and in adult consortium: Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE)) to identify further biological pathways related to hypertension.

634T

Meta-analysis of genome-wide association studies on atopic dermatitis identifies three novel risk loci. *L. Paternoster[1], M. Standl[2], C-M. Chen[3], A. Ramasamy[4,5,6], K. Bønnelykke[7], L. Duijts[8,9,10], M-R. Jarvelin[5,11,12], M.A. Ferreira[13], A. A. G. C.[30], H-E. Wichmann[2,14], D. Strachan[15], J.P. Thyssen[16], E.A. Nohr[17], D.L. Jarvis[4], B. Feenstra[18], P. Sleiman[19], D. Glass[20], L.J. Palmer[21], N.M. Probst-Hensch[22], B. Jacobsson[23,24], J.A. Curtin[25], D.I. Boomsma[26], G.H. Koppelman[27], A. Sääf[28], H. Bisgaard[7], J. Heinrich[2], D.M. Evans[1], S. Weidinger[29] on behalf of the EAGLE Eczema Consortium.* 1) MRC CAiTE, University of Bristol, Bristol, United Kingdom; 2) Institute of Epidemiology I, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany; 3) Hannover Medical School, Department for Paediatric Pneumology, Allergy and Neonatology, Hannover, Germany; 4) Respiratory Epidemiology and Public Health, Imperial College London, UK; 5) Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, UK; 6) Department of Medical and Molecular Genetics, King's College London, Guy's Hospital, London, UK; 7) COPSAC, Copenhagen Prospective Studies on Asthma in Childhood; Health Sciences, University of Copenhagen and Copenhagen University Hospital, Gentofte, Denmark; 8) The Generation R study group, Erasmus Medical Center, Rotterdam, The Netherlands; 9) Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands; 10) Department of Paediatrics, Division of Respiratory Medicine, Erasmus Medical Center, Rotterdam, The Netherlands; 11) Institute of Health Sciences, Biocenter, University of Oulu, Finland; 12) National Institute of Health and Welfare, Finland; 13) Queensland Institute of Medical Research, Brisbane, Australia; 14) Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians Universitat, Munich; Klinikum Grosshadern, Munich, Germany; 15) Division of Population Health Sciences and Education, St George's University of London, London, UK; 16) National Allergy Research Centre, Department of Dermato-Allergology, Gentofte Hospital, University of Copenhagen, Denmark; 17) Aarhus University, Institute of Public Health, Denmark; 18) Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark; 19) The centre for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA, USA; 20) Department of Twin Research and Genetic Epidemiology, King's College London; 21) Ontario Institute for Cancer Research,Toronto; University of Toronto, Canada; 22) Swiss Tropical and Public Health Institute (Swiss TPH), Basel; University of Basel, Switzerland; 23) Department of Obstetrics and Gynecology, Sahlgrenska University Hospital, Sahlgrenska Academy, Göteborg, University, Sweden; 24) Norwegian Institute of Public Health, Department of Genes and Environment, Division of Epidemiology, Oslo, Norway; 25) The University of Manchester, Manchester Academic Health Science Centre, NIHR Translational Research Facility in Respiratory Medicine, University Hospital of South Manchester, NHS Foundation Trust, Manchester, UK; 26) Department of Biological Psychology, VU University, Amsterdam, The Netherlands; 27) Department of Epidemiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; 28) Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; 29) Department of Dermatology, Allergology and Venerology, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany; 30) The Australian Asthma Genetics Consortium.

Atopic dermatitis (AD), or eczema, is one of the most common chronic skin diseases with a high heritability, complex mode of inheritance and an unclear genetic relationship with other atopic conditions such as asthma. Apart from filaggrin (*FLG*), the genes that underlie susceptibility to AD are largely unknown. In a large international consortium, the EAGLE Eczema Consortium, we conducted a genome-wide association meta-analysis on 5,606 Caucasian cases and 20,565 Caucasian controls from 16 population-based cohorts from across Europe, North America and Australia. Each contributing study conducted a case control genome-wide analysis of ~2.5 million imputed SNPs and we then used inverse-variance fixed effects meta-analysis (in METAL) to test for the associations in the overall study population. The ten most strongly associated novel markers were tested in a further 5,419 cases and 19,833 controls from 14 replication studies. Two variants were associated with AD susceptibility with genome-wide significance in the discovery sample (rs7000782 on chromosome 8q21·13, near the zinc finger and BTB domain containing ten gene *ZBTB10*, $p=1{\cdot}6x10^{-8}$ and rs9050 on chromosome 1q21·3, near the trichohyalin gene *TCHH*, $p=1{\cdot}9x10^{-8}$). Analysis adjusting for FLG null mutations suggested that the association of rs9050 was driven by these *FLG* variants and so rs9050 was excluded from further analyses. The locus reported to be associated in the only other GWAS on AD to date (11q13·5) showed nominal association in our meta-analysis (rs7927894, p=0·008), but of the 15 loci reported to be associated with asthma or total serum IgE levels in a recent GWAS, only two showed nominal association (5q13·1 *IL13*: rs1295685, p=0.0008; 12q13·3 *STAT6*: rs167769, p=0·0379). Of the ten SNPs taken through to replication, three showed association in the replication stage and reached genome-wide significance in the combined analysis: rs479844 on chromosome 11q13·1, near *OVOL1* (OR=0·88, $p=1{\cdot}1x10^{-13}$); rs2164983 on chromosome 19p13·2, near *ACTL9* (OR=1·17, $p=2{\cdot}4x10^{-10}$); rs2897442 on chromosome 5q31·1, in *KIF3A* (OR=1·10, $p=3{\cdot}0x10^{-8}$). We have identified three novel genetic loci associated with AD. The implicated genes for these associations underline the importance of epidermal barrier function in AD pathogenesis.

635T

Segregation Analysis of Isolated Cleft Lip in a high-prevalence cluster of South America: uncommon high-risk allele and implications for genetic association studies. *F.A. Poletta[1,2,3], E.E. Castilla[1,2,3,4], I.M. Orioli[3,5], J.C. Mereb[6], F.M. Carvalho[3,5], C.A. Brandon[7,9], J.M. Resick[7,9], A.R. Vieira[7,8,9], M.L. Marazita[7,9], J.S. Lopez-Camelo[1,2,3,10].* 1) ECLAMC (Estudio Colaborativo Latino Americano de Malformaciones Congénitas) at CEMIC (Centro de Educación Médica e Investigaciones Clínicas-CONICET), Buenos Aires, Argentina; 2) CONICET (National Research Council of Argentina); 3) INAGEMP (Instituto Nacional de Genética Médica Populacional); 4) ECLAMC at Laboratório de Epidemiologia de Malformações Congênitas, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil; 5) ECLAMC at Laboratório de Malformações Congênitas, Departamento de Genética, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; 6) ECLAMC at Hospital Zonal El Bolson, El Bolson, Rio Negro, Argentina; 7) Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, USA; 8) Department of Pediatric Dentistry, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, USA; 9) Center for Craniofacial and Dental Genetics, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, USA; 10) IMBICE: Instituto Multidisciplinario de Biologia Celular (CIC-CONICET), La Plata, Argentina.

The genetic contribution to the etiology of orofacial clefts especially cleft lip and cleft palate (CL/P) is complex and heterogeneous. An area with high prevalence of CL/P was previously identified in Argentine Patagonia, probably associated with Amerindian ancestry and low socioeconomic status. The aim of this work was to estimate the mode of inheritance and the number of loci involved in CL/P families from Patagonia prior to planned for linkage/association studies. The sample included 117 extended pedigrees (2,835 total people) ascertained from CL/P probands registered by ECLAMC (The Latin-American Collaborative Study of Congenital Malformations) hospitals in Patagonia. Family Risk Ratios (FRR) were estimated for first-, second-, and third-degree relatives of CL/P probands, and Complex Segregation Analyses (CSA) were conducted using Pointer and SAGE software. CSA excluded the Sporadic, Environmental and Multifactorial threshold models, and provided evidence that CL/P is most likely determined by a dominant major gene with incomplete penetrance and with residual familial effects on affection status. Furthermore, FRR for relatives equate well with a major gene (or multiple additive or independent loci). One or two loci interacting epistatically with an poligenic background was also shown to be a plausible alternative. The high-risk allele frequency estimates of 1 to 9% have important implications with regards to the feasibility of identifying causative loci in this population through the typical commercial platforms and methods used in genome-wide association studies. Newer methodological approaches including customized genotyping platforms and analysis methods for association involving rare variants should be taken into account for future association studies to be conducted in this population. The identification of susceptibility genes associated with the occurrence of oral clefts in this high-prevalence cluster will eventually provide practical advice for genetic counseling of these families that will be more accurate than the current empiric recurrence risk estimates used. Support: DE016148.

636T

Classification of anthropometric validated associations from GIANT using effects on childhood growth. *R.M. Salem[1,2,3], J.N. Hirschhorn[1,2,3], Early Growth Genetics (EGG) Consortium.* 1) Broad Institute, Cambridge, MA; 2) Children's Hospital Boston, Boston, MA; 3) Harvard Medical School, Boston, MA.

Background: The recent GIANT consortium meta-analysis recently revealed many validated loci associated with height (180 loci) and BMI (32 loci). Some of these variants have been shown to have effects on childhood traits (birth weight, birth length and puberty timing), but it is not clear whether the associated loci have similar effects on childhood growth, or show distinct patterns of association. We hypothesized that the pattern of associations across multiple traits can be used to classify the height- and BMI-associated loci into different groups representing different biological processes. **Methods:** Association data for the validated height and BMI associations from GIANT was provided by the EGG consortium for birth length and birth weight, and by REPROGEN for age at menarche. Cluster analysis was performed using a multivariate vector consisting of beta values or beta z-scores (beta / SE) for each childhood trait, in addition to GIANT height and BMI values. Heatmaps and dendrogram plots were generated to review clustering pattern and determine the number of clusters. Pathway analysis was performed using GRAIL. **Results:** The BMI SNPs clustered into two groups and the height SNPs into 11 groups (1-51 SNPs) based on review of heatmap and dendrogram plots. The two BMI clusters were differentiated most strongly by SNP effect on height (increasing and decreasing betas). The height clusters were more complicated, with varying patterns of effects across the traits. The subgroups identified by clustering fell into distinct pathways as noted with GRAIL pathway analysis, such as growth-hormone related genes, hedgehog- related genes, etc. Suggesting that cluster analysis across phenotypes can be used to identify SNPs that mark nearby genes that fall into distinct biological pathways, though GRAIL p values were generally not improved by division of the loci into subgroups. **Conclusion:** Association data for childhood traits could be used to classify the height- and BMI-associated SNPs into groups. Classifying variants by their effects on multiple traits might be a useful method to disentangle the genetic architecture of complex traits, and may inform pathway analysis to identify loci that play important roles in different biological processes.

637T

Imprinting and maternal genotype effects of 4q35 genetic variants on combined asthma-plus-rhinitis phenotype. *C. Sarnowski[1,2,3], G. Malerba[4], C. Laprise[5], K. Rohde[6], M. Moffatt[7], P. Jeannin[1,2,3], M-H. Dizier[1,2,3], P.F Pignatti[4], W.O.C Cookson[7], M. Lathrop[8], F. Demenais[1,2,3], E. Bouzigon[1,2,3].* 1) INSERM, U946, PARIS, France; 2) Univ Paris Diderot, Sorbonne Paris Cité, Institut Universitaire d'Hématologie, Paris, France; 3) Fondation Jean Dausset-Centre d'Etude du Polymorphisme Humain (CEPH), Paris, France; 4) Section of Biology and Genetics, Department of Mother and Child, and Biology-Genetics, University of Verona, Verona, Italy; 5) Université du Québec à Chicoutimi, Chicoutimi, Canada; 6) Max-Delbrück-Center for Molecular Medicine (MDC), Berlin, Germany; 7) National Heart Lung Institute, Imperial College, London, UK; 8) CEA-CNG, Evry, France.

A previous genome-wide linkage scan conducted in 640 families from European ancestry (French, British and Italian) detected linkage of 4q35 to a combined phenotype asthma-plus-rhinitis, with increased evidence when taking into account imprinting (LOD=3.14, $P=2.5x10^{-5}$). We investigated further this region by genotyping a panel of 3,000 SNPs (spanning 20Mb) in 161 families (206 offspring) from the French EGEA study (Epidemiological study on the Genetics and Environment of Asthma). To test for association between these SNPs and asthma-plus-rhinitis phenotype, we used two different methods aiming to detect parent-of-origin and/or maternal genotype effects: 1) the Monte-Carlo Pedigree Parent-Asymmetry-Test (MCPPAT) and 2) the Parent-of-origin-Likelihood ratio Test (PO-LRT). Irrespective of the method used, we identified 50 markers associated with asthma-plus-rhinitis with P-value < 0.005. These associations were replicated in 245 French Canadian families (Saguenay-Lac-Saint-Jean) for four SNPs with P-values ranging from 0.06 to 0.005 under the same model as in the discovery set. The combination of P-values (P_{comb}) from the EGEA and SLSJ samples using Fisher's method enhanced the evidence for association of asthma-plus-rhinitis with SNPs in two genes. The most significant SNP in one gene had $P_{comb}=2x10^{-4}$ under a parent-of origin effect model while the best SNP in the other gene had $P_{comb}=5x10^{-4}$ under a maternal genotype effect model. This study highlights that taking into account complex mechanisms, such as imprinting and maternal genotype effect, facilitates the identification of new susceptibility genes. Funded by French Min Education & Research, AFSSET, ANR-CEBS, ANR-CEST, GABRIEL.

638T

The Kaiser Permanente Research Program on Genes, Environment and Health: A Resource for Genetic Epidemiology Research Linking Electronic Health and Environmental Risk Data to Genomics Data in a Large Cohort Based in a Health Plan. *C. Schaefer[1], S. Rowell[1], L. Walter[1], C. Somkin[1], S. Van DenEeden[1], C. Quesenberry[1], L. Croen[1], L. Kushi[1], R. Whitmer[1], C. Iribarren[1], M. Henderson[1], D. Smethurst[1], M. Sadler[1], D. Ranatunga[1], L. Shen[1], S. Sciortino[1], D. Ludwig[1], D. Olberg[1], K. Lapham[2], J. Lin[2], S. Miles[1], S. Hesselson[3], T. Hoffman[3], M. Kvale[3], E. Blackburn[2], P. Kwok[3], N. Risch[1,3].* 1) Division of Research, Kaiser Permanente Northern California, Oakland, CA; 2) Dept. of Biochemistry and Biophysics, UCSF, San Francisco, CA; 3) Institute for Human Genetics, UCSF, San Francisco, CA.

Over the past decade, we have developed the Research Program on Genes, Environment, and Health (RPGEH), a large, population-based resource for genetic epidemiologic research based within the Kaiser Permanente Northern California (KPNC) membership of 3.3 million. The objective of the RPGEH is to facilitate research on determinants of disease and treatment response by linking information from comprehensive electronic medical records, genomic data from biospecimens, and environmental exposure data from surveys and geographic information system (GIS) databases on 500,000 broadly consented KP members. Over 430,000 adults have completed a survey that ascertained health-related behaviors and other risk factors. To date, we have obtained approximately 170,000 biospecimens (primarily saliva) from RPGEH survey participants. In 2009, we received a Grand Opportunity award from the NIH to perform genome-wide genotyping and telomere length analysis of 100,000 RPGEH participants, which we have now successfully completed. The sample is approximately 25% minority and 75% white. The mean age of participants is 65 years, and 51% have been KPNC members for 20 years or more. In addition to the genotype and telomere data, there is extensive health, environmental exposure, demographic and behavioral data on the cohort of 100,000. A unique feature of this resource is the comprehensive and longitudinal clinical, laboratory, radiology, pharmacy and other health data on all participants, beginning in 1995 and continuously updated as the cohort ages. The health data include many repeated measures of standard risk factors such as body mass index, blood pressure, lipid panels and other tests, as well as large numbers of subjects with a variety of chronic as well as infectious diseases with concomitant pharmacy and treatment data. GIS databases are available to map participants to environmental data such as air pollution and pesticide exposures, as well as the built environment. The resource will provide countless unique opportunities to advance knowledge of genetic and environmental predictors of disease and treatment response. Because a sizeable proportion (14%) are age 80 years and older, this cohort also provides unique opportunities to understand the factors underlying age-related disease and the processes of aging. To ensure best use of this resource, the RPGEH will provide collaborative access through application procedures or through dbGaP.

639T

Rare variants in *ANKRD31* and *CXADR* are associated with blood pressure in African-Americans. *P.B. Shetty[1], H. Tang[2], T. Bamidele[3], A.C. Morrison[4], C.L. Hanis[4], D.C. Rao[5], R.S. Cooper[3], N. Risch[6], X. Zhu[1].* 1) Department of Epidemiology and Biostatistics, Case Western Reserve University School of Medicine, Cleveland, OH; 2) Department of Genetics, Stanford University School of Medicine, Stanford, CA; 3) Department of Preventive Medicine and Epidemiology, Loyola University of Chicago Stritch School of Medicine, Chicago, IL; 4) Division of Epidemiology, Human Genetics and Environmental Sciences, The University of Texas Health Science Center School of Public Health, Houston, TX; 5) Division of Biostatistics, Washington University in St. Louis School of Medicine, St. Louis, MO; 6) Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA.

Background: Common genetic variants for hypertension have been identified through genome-wide association studies, but they are associated with modest effect sizes. Some of the missing heritability in hypertension may be attributed to rare variants of larger effect sizes, as they are expected to be functionally important. To address this, we assessed rare variants for association with blood pressure and body-mass-index (BMI).

Methods: We genotyped functional SNPs identified from a public database in genes in regions on chromosomes 5, 6, 8 and 21 where association evidence for hypertension or BMI was identified in our previous admixture mapping studies. We conducted gene based association analysis for systolic blood pressure (SBP) and diastolic blood pressure (DBP) adjusted for treatment and BMI in 1735 unrelated African-Americans. We collapsed the minor alleles of each rare functional SNP in a gene and conducted regression analyses. We adjusted for age, age^2, sex, and BMI, as applicable. To address population stratification, we adjusted for an individual's African ancestry estimated from 2,507 ancestry-informative markers. We corrected for multiple comparisons by adjusting for the number of genes in each region using the Bonferroni correction.

Results: Rare variants in *ANKRD31* were associated with SBP and DBP (nominal p = 0.0011 and 0.0001, respectively). Rare variants in *CXADR* were associated with SBP and DBP (nominal p = 0.0002 and 0.0008, respectively).

Discussion: The result in *CXADR* seems consistent with published findings that this gene plays a role in electrical conduction of the heart, and it is also associated with viral myocarditis and subsequent dilated cardiomyopathy [1, 2]. In *ANKRD31*, 3 of the 4 SNPs are non-synonymous, so they may result in functional changes. These two genes may be important in predicting the risk of hypertension, so future replication and functional analyses are warranted.

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640T

Gene-based tests that account for covariates and pedigree: The GLOGS / ACR pipeline for GWAS of complex traits. *S. Stanhope, M. Abney.* Department of Human Genetics, The University of Chicago, Chicago, IL.

In most GWAS, associations between markers and a trait are evaluated individually. Corrections for multiple comparisons are based on the number of markers, and after a significant association is obtained the chromosomal region containing the associated marker is studied. Presumably it contains a coding gene, with other markers in the region also showing association with the trait due to LD. We suggest that the analysis of individual markers is wasteful in terms of power. An approach that analyzes genes directly is preferable and more easily interpreted.

We describe a computational pipeline for gene-wise GWAS of complex traits in populations with arbitrary kinships and non-genetic risk factors. Our approach uses GLOGS (Genomewide LOGistic regression-Score test) to score associations between individual markers and the trait, and a novel code, ACR (Aggregation-Covariance estimation-Randomization test) that 1) aggregates marker scores, which are chi-squared distributed under the null hypothesis of no association with the trait, into gene scores; 2) calculates intra-marker score covariances due to LD, kinships, and the sensitivity of marker scores to changes in genotypes; and 3) performs randomization tests of the null hypothesis of no association between any marker in a gene with the trait. These tests utilize the fact that under the null the gene score is a function of covarying chi-squared random variables, and so its distribution may be sampled.

Using the GLOGS / ACR pipeline, we investigate the type I error, power, and sensitivity to misspecification in estimated intra-marker score covariances of several gene scoring methods. Although all methods yield correct type I error, there are differences in power with respect to the proportion of non-null markers within a gene, and in the sensitivity of a scoring method to covariance misspecification. In timing tests, we show that GLOGS / ACR, which is implemented for use on cluster, multi-core and GPGPU systems, is computationally tractable - a GWAS can be completed in under two hours. We conclude with gene-wise GWAS of several phenotypes in the Hutterites. We show that gene-based analyses can reveal associations between genes and traits that would be missed in marker-based GWAS, and that these associations can replicate those observed previously.

641T

Clinical and epidemiological analyses of nonsyndromic craniosynostosis. *C.G. Stevens[1], G. Yagnik[1], L. Qi[2], E. Cherkez[1], P.A. Sanchez-Lara[3], V. Kimonis[4], J. Stoler[5], M. Cunningham[6], J.M. Graham[7], S.A. Boyd[1].* 1) Section of Genetics, Department of Pediatrics, University of California Davis, Sacramento, CA 95817; 2) Division of Biostatistics, Department of Public Health Sciences, University of California Davis, Davis, CA 95616; 3) Division of Genetics, Department of Pediatrics, Children's Hospital of Los Angeles, University of Southern California, Los Angeles, CA 90027; 4) Division of Genetics, Department of Pediatrics, University of California Irvine, Irvine, CA 92697; 5) Division of Genetics, Children Hospital Boston, Harvard University, Boston, MA 02115; 6) Division of Craniofacial Medicine, Department of Pediatrics, University of Washington, Seattle, WA 98195; 7) Division of Clinical Genetics and Dysmorphology, Medical Genetics Institute, Cedars Sinai Medical Center, Los Angeles, CA 90048.

Craniosynostosis is the premature fusion of one or more cranial sutures. It is a common malformation (3 to 5 per 10,000 live births) and may present as an isolated anomaly (nonsyndromic) or with associated defects (syndromic). Through the International Craniosynostosis Consortium (genetics.ucdmc.ucdavis.edu/icc.cfm), we enrolled 699 families with at least one individual affected with craniosynostosis and collected clinical and epidemiological data using a 10 page self-administered bilingual (English/Spanish) survey. Of the families that provided sufficient information, 429 were found to have a proband with nonsyndromic craniosynostosis. Our primary aim was to characterize the clinical features of these affected individuals and to screen for potential causative environmental factors. We analyzed parental data, teratogenic exposures, pregnancy complications, indicators of fetal constraint, and associated congenital anomalies and developmental delays. Results were compared to National Vital Statistics data to determine if any of the above factors showed significant deviation from the control population. All factors were assessed statistically for the entire study cohort, as well as within suture-specific subgroups. We observed an association with advanced parental age (maternal, p=4.8 x 10-5; paternal, p=8.3 x 10-4) as well as with plurality (p=6.1 x 10-6). The latter may indicate intrauterine constraint as a risk factor for the malformation.

642T

Descriptive analysis of skeletal dysplasias in the Utah population. *D. Stevenson[1], J.C. Carey[1], J.L.B. Byrne[1,2], S. Srisukhumbowornchai[3], M.L. Feldkamp[1,3].* 1) Dept Pediatrics, Univ Utah, Salt Lake City, UT; 2) Dept Obstetrics and Gynecology, Univ Utah, Salt Lake City, UT; 3) Utah Birth Defect Network, Utah Department of Health, Salt Lake City, UT.

The Utah Birth Defect Network (UBDN) collects population-based data for Utah on births from all resident women. Our aims were to investigate the prevalence of skeletal dysplasias and perform a descriptive analysis of affected pregnancies/infants within the Utah population. Cases categorized as a skeletal dysplasia from all live births, stillbirths, and pregnancy terminations (TAB) between 1999-2008 were reviewed by 3 clinical geneticists. After case review, 153 were included for analysis (88% live births, 3% stillborn, 9% TAB). Cases were categorized by individual diagnosis and by etiologic groups (2010 Nosology and Classification of Genetic Skeletal Disorders). Epidemiologic characteristics/outcomes (e.g. death, prenatal diagnosis, family history, genetic testing) were evaluated. The overall prevalence for skeletal dysplasia was 3.0/10,000 births. The prevalence for skeletal dysplasia for stillbirths was 20.0/10,000 stillbirths. The most common diagnoses were osteogenesis imperfecta (OI) (n=40; 0.79/10,000), thanatophoric dysplasia (n=22; 0.43/10,000), achondroplasia (n=18; 0.35/10,000), and cleidocranial dysplasia (n=6; 0.12/10,000). The most common etiologic groups were the FGFR3 chondrodysplasia group (n=41; 0.81/10,000), the OI/decreased bone density group (n=40; 0.79/10,000), and the type 2 collagen group (n=10; 0.2/10,000). Prenatal diagnosis was made in 53.6% (mean gestational week of diagnosis = 23 weeks). Of those deceased, 88% were prenatally diagnosed; of those alive 29% prenatally diagnosed. Paternal age was increased compared to the general population (mean 30.5 yrs; $p<0.05$). Cesareans were performed in 44%. 53% of mothers living in rural areas delivered at an urban hospital. Only 16% of cases were transferred to a different facility. Age of death for live born individuals: thanatophoric dysplasia (median 1.5 days; n=16 live births), OI type 2 (median 1 day; n= 6 live births). Of those deceased, 78% had a known lethal diagnosis. Median age of postnatal diagnosis was 30 days (range 1-2162). Postnatal genetic consult was obtained in 30% with and 55% without a prenatal diagnosis. Previously reported prevalence rates of skeletal dysplasia vary, but our data provide a population based approach not limited to the perinatal/neonatal period. OI was the most common skeletal dysplasia, differing from other epidemiologic studies. This investigation also provides data on outcomes and associated evaluation and referral patterns for skeletal dysplasias.

643T

Association testing for rare variants via pooled design. *I. Tachmazidou[1], M. De Iorio[2], M. Falchi[2].* 1) The Wellcome Trust Sanger Institute, Hinxton, United Kingdom; 2) Imperial College, London, United Kingdom.

It is thought that much of the genetic susceptibility to complex diseases is due to rare variants. Next generation sequencing in large populations facilitates the detection of rare variants association to disease risk. Although sequencing costs have dropped, they are still high for large-scale studies. DNA pooling could prove a cost effective approach for identifying associations between rare variants and disease.

In this work, we propose to use pooled DNA samples to estimate allele and haplotype frequencies for rare SNPs, and we investigate the performance of collapsing methods in the context of a binary trait. In particular, we consider haplotype-based association tests and collapsing methods based on estimated allele frequencies. We use the EM algorithm of Yang et al 2003 to estimate haplotypes from pooled DNA, and we use a logistic regression framework to model the case/control outcome as a function of i) the presence or absence of a minor allele at any causal variant (RVT1, Li and Leal 2008, Morris and Zeggini 2009), and ii) the proportion of causal variants at which a minor allele is present (RVT2, Morris and Zeggini 2009). This approach is compared to the collapsing method (as described in Li and Leal 2008) with the estimated allele frequencies in an extended simulation study of N individually genotyped subjects versus N*K pooled subjects, where K is the pool size and N is the number of pools. We also introduce error in the allele and haplotype frequency estimates due to unequal amounts of probe material.

We find the allele frequency test to be more powerful than the haplotype-based tests even in the presence of high LD. The differences in power become smaller as N and/or the genetic signal increases. Moreover, the two collapsing haplotype-based methods perform similarly with RVT2 having a slight advantage. The methods also seem robust to pooling error. In conclusion, next generation sequencing of DNA pools provides a cost-effective approach for studying association of disease with rare SNPs, offering an advantage in terms of ability of detecting rare variants and power for association testing over individual DNA sequencing.

644T

Additive polygenic effects on longevity and their functional meaning. *S.V. Ukraintseva[1,2], D. Wu[1], K.G. Arbeev[1], A.I. Yashin[1,2].* 1) Center for Population Health and Aging, Duke University, Durham, NC; 2) Duke Cancer Institute, Duke University, Durham, NC.

Recently we have shown that a substantial portion of genetic influence on lifespan can be explained by joint genetic effects involving large numbers of common SNP alleles, each with small and/or low significant effect (Yashin et al. 2010). Using various statistical approaches to selecting individual pro-longevity alleles from data on genome-wide genotyping of 550K SNPs in Framingham Study participants we identified a core set of 27 SNP alleles that manifest both substantial and significant additive influence on life span, regardless of statistical method used for their selection. This suggests potentially important functional roles of respective SNPs for achieving longevity. We found that majority of these SNPs (74%) are located within genes, compared to only 40% SNPs in the original 550K set, and most of them are intronic, which may also indicate functional significance of the selected SNPs such as in regulating protein concentrations, rather than in changes in protein structure. We performed trough search of literature (200+ relevant research papers) and online information data bases, to investigate functional properties of genes closest to 27 longevity SNPs. We found that these genes are largely involved in cell proliferation, apoptosis, cell adhesion, and brain information processing, as well as in cancer and brain disorders. Cell proliferation, apoptosis, and cell adhesion are also major outcomes of both aging and cancer pathways. The results indicate that the aggregate effect of aging and cancer related cell responses in body is essential for determining lifespan. We have also shown that “longevity” or “aging” genes identified in other studies often represent the same cell responses/pathways as genes in our study. E.g., a SNP linked to longevity in Newman et al. (2010) is near MINPP1 gene, which is involved in regulating cell proliferation, same as five genes from our study; one of longevity SNPs from our study is in CDH4 cell adhesion gene, and SNP in the same gene was linked to brain aging in Seshadri et al. (2007). This indicates that it may be reasonable to address replication of gene-longevity associations on the level of pathways and their outcomes rather than on the level of individual SNPs.

645T

Association of a TOMM40 Variable-length Polymorphism with Risk and Age at Onset of Alzheimer Disease is not Independent of APOE. *B.N. Vardarajan[1], G. Jun[1,2,3], J. Buros[1], L. Cantwell[10], K.L. Lunetta[2], T. Foroud[6], R. Mayuex[7], J. Haines[8], M.A. Pericak-Vance[9], G. Schellenberg[10], L.A Farrer[1,2,3,4,5], Alzheimer Disease Genetics Consortium.* 1) Department of Medicine, Boston University, Boston, MA; 2) Department of Biostatistics, Boston University, Boston, MA; 3) Department of Ophthalmology, Boston University, Boston, MA; 4) Department of Epidemiology, Boston University, Boston, MA; 5) Department of Neurology, Boston University, Boston, MA; 6) Department of Medical and Molecular Genetics, Indiana University, Indianapolis, IN; 7) Taub Institute on Alzheimer's Disease and the Aging Brain, Department of Neurology, Columbia University, New York, NY; 8) Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN; 9) The John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL; 10) Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA.

The APOE , 4 allele is the strongest and most consistently replicated genetic risk factor for Late-Onset Alzheimer Disease (LOAD). Recently, a repeat-length polymorphism (poly-T), rs10524523, in the translocase of outer mitochondrial membrane 40 homolog (TOMM40) gene located in the APOE region was associated with age-at-onset (AAO) of LOAD, and this association was independent of the effect of APOE. We examined the association of the poly-T variant with risk and AAO of in 3 Caucasian cohorts containing 1,258 AD cases and 1,715 age-matched cognitively normal controls. One dataset is almost exclusively comprised of younger autopsy-confirmed cases and age-matched controls (mean age=74.3±8). The other two datasets contain relatively older clinically-confirmed AD patients (mean age=81.7±5.9). The poly-T repeat lengths were grouped into three classes based on the number of T residues: Small (S) =14-16, Long (L) = 19-30 and Very Long (VL) =30-36. Models including covariates for age, sex and population substructure were evaluated in each cohort and the results were combined using an inverse-variance meta-analysis approach. The dosages of the S, L and VL variants were very significantly associated with AD (p= 5.9e-08, 3.87e-33 and 1.86e-08 respectively). However, when adjusted for the number of APOE , 4 alleles, only the L variant revealed marginal association with AD (p=0.87 for S, p=0.02 for L and p=0.35 for VL). Similarly, none of the poly-T variants was associated with AAO after adjusting for , 4 status (p=0.87 for S, p=0.21 for L and p=0.63 for VL). Within the subgroup of APOE , 3/3 subjects, there was no evidence of association with AD or AAO with any of the TOMM40 variants. Among subjects with at least one , 4 allele, AD was associated with the S (p=0.002) and L (p=2.47e-06) variants. We tested the association of the APOE , 4 allele in subjects stratified by presence and absence of the L form of TOMM40 to determine whether the effect of TOMM40 is independent of APOE. , 4 was strongly associated (p= 3.7e-06) with AD in subjects with the TOMM40 L variant. The correlation of the dosages of , 4 and the L allele is more than 90%in the three datasets. Our results indicate that the association of the TOMM40 poly-T polymorphism with AD risk and AAO is indistinguishable from the effect of APOE probably because of strong linkage disequilibrium between these loci. Functional studies are needed to demonstrate a role of TOMM40 in AD pathogenesis.

646T

Review and re-analysis of all schizophrenia multiplex families in the NIMH repository substantially alters overall linkage findings. *K.A. Walters[1], K. Tobin[2], M. Azaro[2], T. Lehner[3], L.M. Brzustowicz[2], V.J. Vieland[1].* 1) Battelle Center for Mathematical Medicine, Research Institute Nationwide Children's Hospital, Columbus, OH; 2) Department of Genetics, Rutgers University, Piscataway, NJ; 3) Genomics Research Branch, NIH/NIMH, Bethesda, MD.

We have undertaken an exhaustive review of all available genome-wide schizophrenia data in the NIMH Human Genetics Initiative (HGI) (1,417 multiplex families from 6 studies) and analyzed the newly cleaned data using the PPL statistical framework, which is uniquely designed to accumulate genetic information across multiple sets of potentially heterogeneous data. Our results differ from previously published results for these data sets as well as from meta-analysis across the studies. Differences include substantial reductions in previously reported linkage peaks; large newly observed peaks; and substantial shifts in localization for previously detected loci. Here we examine the causes of differences in results: whether cleaning genotypes (GT-clean), cleaning phenotypes (PT-clean), and/or changing statistical methods (METHOD) had the greatest effects on results. Data cleaning included: review and correction of family structures based on genotypes; application of various filters including markers < 10%; missingness and individuals < 20%; missingness; and construction of by-study marker maps and allele frequency estimation. Phenotypes were carefully reviewed and in a large number of cases revised. We required at least one person affected with schizophrenia (SZ), one additional person affected with SZ or schizoaffective disorder (SA), classified families clinically based on presence/absence of SA, and used sequential updating to accumulate evidence across studies and clinical groups. In all we filtered out 27%; of families (N= 1,029 families in “clean” analyses). Here we compare 4 versions of the data: RAW (original data, N=1,417 families), GT-clean (PT-raw), PT-clean (GT-raw), CLEAN (N=1,029 families with PT- and GT-clean); and we compare the PPL with previously reported methods (primarily NPL) and meta-analysis (GSMA). The results illustrate specific ways in which any one of the cleaning steps or METHOD can substantially affect results, and show that in aggregate the overall picture of the SZ genome that emerges is substantially different from what would be gleaned from a review of the previous literature on these studies. This work underscores that revisiting retrospective data with the benefit of hindsight for purposes of cleaning data and applying new statistical techniques can extract important new genetic information from existing data resources.

647T

Study design and power considerations for exome sequencing of near-Mendelian traits. *M.E. Weale, M.A. Simpson, R.C. Trembath.* Department of Medical & Molecular Genetics, King's College London, London, United Kingdom.

Exome sequence scans have successfully identified the causal variants of many simple dominant Mendelian disorders. However, a causal variant will not be unique to all cases if its variant-specific `homogeneity' (defined as one minus genocopy/phenocopy rate) is <1, and will not be excluded from screened controls if its penetrance is <1. Even for `near-Mendelian' traits (where homogeneity and penetrance are close but not equal to one), simple filtering and sharing approaches to variant discovery are likely to fail. This problem is exacerbated when considering unscreened controls, recessive inheritance, and protocol-specific sequencing errors between cases and controls.

Here we investigate the use of simple exact tests for gene-level rare variant burden in cases and controls, thus avoiding a heavy dependence on variant filtering. We perform empirically-based power calculations to define the success space of exome scans for near-Mendelian traits. We compare the use of only 1000 genomes data for controls against a combined 1000 genomes + additional exome data (sequenced at King's College London using an Agilent capture + Illumina sequencing protocol) to correct for protocol-specific sequencing errors. We show that with a case exome pool of 10, power to detect a genome-wide significant signal is greater than 80% provided penetrance >0.5 and gene-level homogeneity >0.75. This provides reassurance that some near-Mendelian traits will be mapped by a modest increase in exome sequencing effort.

648T

Manifold learning and causal inference in genome-wide integrated genetic and epigenetic analysis. *M. Xiong, Y. Zhu.* Dept Biostatistics, Univ Texas Hlth Sci, Houston, TX.

Understanding cause-effect relationships between genotype and phenotypes is of primary interest in unraveling mechanism of diseases. Usually intervention is used to discover cause-effect relationships. Unfortunately, in human genetics experimental intervention is infeasible. The goal of this report is to infer cause-effect from high dimensional observed genetic and phenotype data. We present a novel semiparametric method to estimate causal effects of genetic variants. Specifically, a variant to be examined for causal effects is taken as a primary variable and modeled as a linear relationship between the variants and a phenotype in a semiparametric model. All remaining genetic variants in high dimensional space which include confounding factors will be projected into a low dimensional space while preserving neighborhood structure among genetic variants by manifold learning which will be modeled as a nonlinear function. These confounding variables can be viewed as nuisance variables. A novel inference procedure and new test statistic will be developed to estimate the direct causal effect of the primary variable while removing the correlation between the primary variable and confounding variables from the primary variable. To evaluate its performance, we first compare the proposed method with the sparse graphical model for causal inference by extensive simulations. The simulation results show that the genetic causal effects on phenotypes can be consistently estimated by two approaches. The proposed method is then applied to two TCGA datasets: Glioblastoma Multiform dataset with expressions of 12,042 genes and 470 miRNA and typed 561,278 SNPs in 198 tumor samples, and ovarian cancer dataset with expressions of 12,042 genes and 799 miRNA, and typed 906,600 SNPs in 512 tumor samples. Multi-level networks with gene co-expression networks, miRNA co-expression networks, miRNA target networks, quantitative trait networks as its components and their consensus network in two datasets were identified. The causal effects were estimated. The results demonstrate that the network structure is stable in bootstrap analysis and their causal effects are quite different from regression analysis. Finally, our experience in simulations and real data analysis demonstrates that the proposed manifold learning combined with inference on the primary variables in the semiparametric model is more computationally fast than sparse graphical approach.

649T

Simultaneous association testing of genome-wide genes using sequence data. *H. Xu.* Dept Biostatistics, Georgia Health Sciences University, Augusta, GA.

Sequence data have been increasingly available for population-based association studies to investigate the genetic basis of complex diseases. Association methods have been developed to handle the vast majority of rare genetic variants in sequence data, for example, by combining information in a genomic region. However, for multi-factorial complex diseases, it is preferable to test the association for the genes across the genome simultaneously. We developed such an approach based on supervised principal component analysis. We compared its performance with the LASSO based approach through simulations. Results show that it has smaller error rate and better power than the LASSO based approach. It is also robust with respect to missing data and sequencing errors. Our method has great potential in genome-wide association studies using (re)-sequencing data.

650T

Do endothelial NOS haplotypes influence neonatal respiratory distress in premature infants? *K. Yanamandra[1], S.A. Ursin[1], H. Chen[1], J.A. Bocchini Jr.[1], R. Dhanireddy[2].* 1) Pediatrics, LSU Health Sciences Center, Shreveport, LA; 2) Pediatrics, UT Health Science Center, Memphis, TN.

Apnea and ineffective respiration are common clinical findings in preterm infants and infants who have experienced a peripartum hypoxic insult. Failure to clear fluid from the lungs delays normal cardiopulmonary transition and may result in Transient Tachypnea of the Newborn (TTN). Another risk for neonatal respiratory distress is the Persistent Pulmonary Hypertension of the Newborn (PPHN), a common pathophysiologic pathway in many diseases that compromise the pulmonary and cardiac systems of newly born infants. Asphyxia occurs when there is impairement of function within the lungs. In the fetus, asphyxia results from decreased placental blood flow or maternal hypoxia. Asphyxia occurs either when there is alveolar hypoventilation or impaired pulmonary blood flow. The initial response to hypoxemia includes a vigorous effort to breathe with preservation of heart rate and blood pressure. It is important to recognize that the sequence of these events begin in utero and continue after delivery. Several genetic factors including gene polymorphisms have been implicated in neonatal respiratory distress. Our laboratory has been studying endothelial nitric oxide synthase (eNOS) gene polymorphisms in premature neonates in the etiology of sepsis, retinopathy of prematurity (ROP), and necrotizing enterocolitis (NEC) for the past several years. In the present investigation we studied 169 premature infants with respiratory distress and 114 without the condition for the association of haplotypes in the eNOS gene (T-786C in the promoter region, 27-bp VNTR b/a in intron 4, and G894T in exon 7) with respiratory disease using microplate PCR-RFLP genotyping methods and Allele specific PCR methods. Mutant haplotype C-a-T frequency was represented significantly higher in infants with respiratory distress as compared to controls in Caucasian infants (odds ratio 2.2, CI 1.6-3.1, pValue <0.00001). The findings were similar in African American infants. Earlier data have shown that the mutant eNOS genotypes result in decreased levels of nitric oxide. Based on our data we conclude that the endothelial NOS haplotypes, dictating endogenous nitric oxide production, have a significant role in the development of the neonatal respiratory system. Stratified data and statistics with various genotypes, ethnicities, and disease condition will be presented.

651T

A Genome-wide association (GWA) study of Cloninger's Temperament and Character Inventory (TCI): Do genetic variants influence personality? *S. Yang[1], M. Lee[1], D.H. Lee[1], J.H. Kim[2], H.L. Kim[5], H.N. Kim[5], Y.M. Song[3], K. Lee[4], J. Sung[1].* 1) Genetic Epidemiology Branch, Department of Epidemiology and Institute of Environment and Health, School of Public Health Seoul National University,Korea; 2) Department of Psychiatry, Samsung Medical Center, Sungkyunkwan University, School of Medicine , Seoul, Korea; 3) Department of Family Medicine, Samsung Medical Center, and Center for Clinical Research, Samsung Biomedical Research Institute, SungKyunKwan University School of Medicine, Seoul, Korea; 4) Department of Family Medicine, Busan Paik Hospital, Inje University College of Medicine, Korea; 5) Department of Biochemistry, School of Medicine, Ewha Womans University, Seoul, Korea.

Whether or not, and what extent of, human personality is influenced by genes, have been a question. Previous research has demonstrated that genes accounted for 30%~60% of variation in personality measurements. A report from a consortium identified genes related to domains of human character measured by Big 5 (RASA1, KATNAL2). A previous GWA study on TCI has identified potential associated SNPs, but none established genome-wide significance with four temperament domains. Specific aims of this study are to estimate overall genetic contribution to human character measured by TCI's seven dimensions: Novelty seeking (NS), Harm Avoidance (HA), Reward Dependence (RD), Persistence (PE), Self-Directedness (SD), Cooperativeness (CO), Self-Transcendence (ST), and to identify genetic variants explaining TCI. A total of 3079 individuals (1217 men) including 661 families, in the Healthy Twin Study in Korea: a twin-family cohort, were involved in this study. Heritability of each dimensions of TCI was estimated using the variance component method. The Affymetrix (1 million SNPs) platform was used for generating genetic marker information. The GWA study was conducted by combining transmission test (FBAT) and population mean effects of founders (PLINK). Heritability of the each seven dimension of TCI ranged 0.25~0.40 (ACE Model). Several genes were replicated with previous GWA study on TCI; BTBD3 on 20p12.2 (“PE”, p=4.80E-4), NCALD on 8q22.2 (“HA”, p=2.20E-3), DENND1A on 9q33.3 (“PE”, p=6.3E-3), SUCLG2 on 3p14.1 (“PE”, 7.2E-3). A GWA result measured by Big5 was also compared. A correlation between Openness to Experience in Big5 measured in subsample (n=349) and “PE” in TCI was significant (r^2=0.2 p= 0.0001). RASA1 gene on 5q14.3, which is associated with Openness to Experience of Big5, was replicated with “PE” domain (p=2.8E-3, 5.0E-3 etc.) Some novel genetic variants were also identified; ATP2B4 on 1q32.1 (“RD”, p=8.9E-6), CACNB2 on 10.q12.33 (“SD”, p=1.2E-6). Also seven SNPs within MAP4K3 on 2p22.1 (rs10865149, p=1.7E-6, rs3770670, p=1.7E-6, etc.) were found to have high association to “CO”. Our findings suggest that Temperament and Character dimensions are genetically influenced. Also novel GWA findings suggest several candidate genes that can be associated to personality. This suggests that genetic component may contribute to personality trait variations, and determine one's personality.

652T

GWAS for childhood tooth decay implicates novel genes for pit and fissure and smooth surfaces. *Z. Zeng[1], J.R. Shaffer[1], X. Wang[2], M. Lee[2], K.T. Cuenco[2], M.M. Barmada[1], D.E. Polk[3], R.J. Weyant[3], R. Crout[4], D.W. McNeil[5], D.E. Weeks[1], E. Feingold[1], M.L. Marazita[1,2].* 1) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 2) Center for Craniofacial & Dental Genetics, Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA; 3) Department of Dental Public Health and Information Management, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA; 4) Department of Periodontics, School of Dentistry, West Virginia University, Morgantown, WV; 5) Dental Practice and Rural Health, West Virginia University, Morgantown, WV.

Dental caries (i.e. tooth decay) is one of the most common chronic diseases in children. Caries contributes to several associated health and social problems including chronic pain, tooth loss, difficulty hearing, eating, and sleeping, failure to thrive, poor school performance, poor social relationships, and decreased success later in life. As a multi-factorial disease, it is the result of a complex interplay of genetic and environmental factors. Despite the high heritability (30-60%) of caries, few specific risk genes have been identified. A genome-wide association study (GWAS) was conducted to explore the role of genes on dental caries in the primary dentition (i.e. “baby” teeth). Carious lesions on pit and fissure (PF) and smooth (SM) tooth surfaces were investigated separately. 596 self-reported whites were included for analysis of dental caries on SM surfaces (ages 3 to 12 years); 572 were included for PF surfaces (ages 4 to 14 years). 589,735 SNPs were genotyped on the Illumina Human610-Quadv1_B BeadChip and used to impute additional markers based on the HapMap Phase III reference panel. A total of 1.4 million SNPs were tested for association with separate counts of the number of carious SM and PF surfaces. All analyses were adjusted for age and age^2 effects. Genomic inflation factors (λ) calculated from all the 1.4 million SNPs were 1.10 for SM carious surface counts and 1.08 for PF. For SM caries, two SNPs passed the genome-wide significance threshold: rs7523195 on chromosome 1 (p = 2.16E-9) located in a “gene desert” and rs16867579 (imputed) on chromosome 8 (p = 1.11E-9) near *ZHF706*, *GRHL2*, and pseudogene *NACAP1*. These genes have no previously known role in cariogenesis. Several plausible caries genes were identified near “suggestive” association signals (i.e. p < 10E-6), including *PLUNK*, *BPIL1*, *BPIL3*, and *BASE* genes (involved in defense against pathogens in oral and nasal cavities) on chromosome 20q11, *PERP* (a regulator of tooth enamel formation) on chromosome 6q23, and *TRAF4* (involved in odontogenesis in mouse) on chromosome 17q11. For PF caries, no genome-wide significant associations or suggestive associations near plausible caries genes were observed. In conclusion, our GWAS discovered significant associations of a number of novel genes and loci with SM caries in the primary dentition. Replication studies in other samples are underway. Support: DE018903, DE014899, DE021425.

653T

Candidate pathway based analysis for cleft lip with or without cleft palate. *T. Zhang, I. Rucinski, T.H. Beaty, International Cleft Consortium.* Johns Hopkins University, Baltimore, MD.

Objectives: The objective of this research was to identify potential biological pathways associated with non-syndromic cleft lip with or without cleft palate (NSCL/P), and to explore the potential biological mechanisms underlying these associated pathways on risk of NSCL/P. Methods: This project was based on the dataset of a previously published genome-wide association (GWA) study on NSCL/P (Beaty et al. 2010). Case-parent trios used here originated from an international consortium (The Gene, Environment Association Studies consortium, GENEVA) formed in 2007. A total of 5,742 individuals from 1,908 CL/P case-parents trios (1,591 complete trios and 317 incomplete trios where one parent was missing) were collected and genotyped using the Illumina Human610-Quad. The main procedure of pathway-based analysis is based on the algorithm gene set enrichment assessment (GSEA) and candidate pathways were selected from gene list of 356 genes that may be related to oral clefts. We conducted a permutation-based test to assess the statistical significance of the nominal p-values of 42 candidate pathways. Results: In total, 42 candidate pathways, which included 1,707 genes and 42,919 SNPs were tested. Pathway-based analysis revealed several pathways yielding nominally significant p-values. However, after estimating the family wide error rate (FWER), none of these pathways could retain statistical significance. Nominal p-values of these pathways were concentrated at the lower tail of the distribution, with more than expected low p-values. A permutation based test for examining this type of distribution pattern was yielded an overall p-value of 0.029. Conclusion: We successfully examined a set of 42 pathways to assess their possible roles in the etiology of NSCL/P yielded intriguing evidence of association. Careful analysis of the top six most significant pathways indicated some genes in these top pathways, including PCDH18, ARHGAP8, EPHA3, and TP63 may be causally related to NSCL/P. Further development of pathway based approaches may help to incorporate and weigh tests of association to reflect prior biological knowledge and lead to the identification of novel susceptibility genes and mechanisms to NSCL/P.

654T

A combined principal components analysis and propensity score approach for population stratification adjustment in genome-wide association. *H. Zhao[1, 2], N. Mitra[1], T. Rebbeck[1].* 1) Biostatistics & Epidemiology, University of Pennsylvania, Philadelphia, PA; 2) Westat, Rockville, MD.

In population based genetic association studies, confounding due to population stratification (PS) arises when differences in both allele and disease frequencies exist in a population of mixed racial/ethnic subpopulations. Propensity scores are often used to address confounding in observational studies. However, they have not been adapted to correct bias due to PS in genetic association studies. Currently, genomic control, structured association, principal components analysis, and multidimensional scaling approaches have been proposed to address this bias using genetic markers. We propose a genomic propensity score approach to correct for bias due to PS that considers both genetic and non-genetic factors such as patient characteristics. We further propose an extended genomic propensity score approach that allows one to estimate a genotype effect under various genetic models in candidate gene studies. Finally, we propose a new approach that combines principal components analysis and the propensity score (PCAPS) to correct for bias due to PS in genome-wide association studies (GWAS). Simulations show that our approach can adequately adjust for bias due to confounding and preserve coverage probability, type I error and power. We illustrate these approaches in a case-control GWAS of testicular germ cell tumors. We provide a novel and broadly applicable strategy for obtaining less biased estimates of genetic associations.

655T

Genetic architecture of biochemical markers for disease. *B. Benyamin, RP. Middelberg, GW. Montgomery, PM. Visscher, NG. Martin, JB. Whitfield.* Queensland Institute of Medical Research, Brisbane, Australia.

Biochemical markers measured in blood are widely used indicators of a current disease or predictors of disease risk. Genetic factors are significant sources of individual difference in biochemical markers with heritability ranging from 25 to 75%. Genome-wide association studies (GWAS) have identified a number of single nucleotide polymorphisms (SNPs) associated with biochemical markers. However, the genetic architecture of the biochemical phenotypes remains poorly understood. The aims of this study are to: 1) assess the spectrum of allelic effect sizes; 2) assess the contribution from SNPs whose associations do not reach genome-wide (GW) significance; 3) compare the sum of SNP-associated effects against heritability; 4) contrast the results across phenotypes. We analysed 16 biochemical markers measured in serum from ~11,000 individuals from QIMR twin and family studies, in which genome-wide (~2.4 million) SNPs data were available. These include markers for cardiovascular disease, such as triglycerides, LDL- and HDL- cholesterol. For most biochemical markers, GWAS found several GW-significant SNPs that in total account for <5% of the phenotypic variance. For some phenotypes, including bilirubin, cholinesterase, transferrin and uric acid, GW-significant SNPs explain between 10 to 20% of the phenotypic variance. On the other hand, no GW-significant SNPs were identified for alanine aminotransferase, total protein or albumin. By considering all SNPs simultaneously (Yang et al Nat Genet 42:565-9, 2010), we found that common SNPs account for 20 - 80% of the heritability (except for albumin where common SNPs do not explain any of the heritability). For most phenotypes there is still some `heritability gap' - presumably due to incomplete LD between causal variants and tagging SNPs.

656T

Genetic Associations with the Variation in Aging from the SardiNIA/Progenia Project. *J. Bragg-Gresham[1], S. Sanna[2], S. Kardia[3], G. Abecasis[1], M. Uda[2,4], C. Sidore[1,2,4], F. Cucca[2,4], A. Cao[2], A. Mulas[2], F. Busonero[1,2], F. Reinier[5], R. Berutti[2,5], A. Maschio[1,2], M. Urru[5], M. Marcelli[5], R. Cusano[2], M. Oppo[2], M. Pitzalis[5], M. Zoledziewska[5], A. Angus[5], C. Jones[5], D. Schlessinger[6].* 1) Center for Statistical Genetics, Ann Arbor, University of Michigan, MI, USA; 2) Istituto di Ricerca Genetica e Biomedica (IRGB), CNR, Monserrato, 09042, Italy; 3) Michigan Center for Genomics & Public Health, Ann Arbor, University of Michigan, MI, USA; 4) Universita' degli Studi di Sassari, Dipartimento Scienze Biomediche, Sassari, 07100, Italy; 5) CRS4, Laboratorio di Genomica, Parco tecnologico della Sardegna, Pula; 6) Laboratory of Genetics, National Institute on Aging, Baltimore, MD, USA.

Age is associated with decline in many physiological functions eventually leading to increased susceptibility to diseases. The rate of aging varies among individuals. This study aims to quantify variation associated with aging by comparing an individual's chronological age to a predicted age, based on health indicators from the SardiNIA/Progenia longitudinal cohort study, which was designed to explore both gene-finding and longitudinal analyses of age-associated conditions. The cohort contains data on approximately 6,400 individuals, including 711 family units. Longitudinal data has been collected in excess of 100 quantitative traits, of which 46 were chosen for this analysis. Employing a mixed linear regression, accounting for pedigree structure of the data, a parsimonious predictive model for age was determined. The model accounted for ~64% of the variation in age within our sample. We then considered the difference between the predicted age (based on physiological indicators) and chronological age as a quantitative trait. This difference between predicted and chronological age was significantly heritable, with an estimated heritability of 29%. We are currently performing a genome-wide analysis study (GWAS) for this trait using ~13M genotyped or imputed SNPs.

657T

Meta-analysis of 15 genome-wide association studies with ~8,000 cases and ~50,000 controls identified multiple novel loci associated with age-related macular degeneration. *W. Chen[1], L.G. Fritsche[2], Y. Yu[3], M. Schu[4], B.L. Yaspan[5], AMDGene Consortium.* 1) Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI; 2) Institute of Human Genetics, University of Regensburg, Regensburg, Germany; 3) Ophthalmic Epidemiology and Genetics Service, Department of Ophthalmology, Tufts Medical Center, Boston, MA; 4) Department of Medicine Biomedical Genetics, Boston University School of Medicine, Boston, MA; 5) Center for Human Genetics Research, Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN.

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss in the elderly population in Western countries. Several genes and pathways have been discovered to be associated with the disease through linkage and genome-wide association studies (GWAS). However, individual GWAS does not have sufficient power to detect genetic variants with small effect size or low minor allele frequency. We conducted a meta-analysis of ~ 2.5 million imputed HapMap SNPs in 15 GWAS worldwide with a total of 7,650 cases and 51,844 controls from European and Asian ancestries. For our primary study outcome of advanced AMD, we confirmed eight previously identified loci including *ARMS2/HTRA1* ($p<10^{-308}$), *CFH* ($p<10^{-289}$), *C2/CFB* ($p<10^{-54}$), *C3* ($p<10^{-26}$), *CFI* ($p<10^{-9}$), *TIMP3* ($p<10^{-12}$), *CETP* ($p<10^{-11}$), and *LIPC* ($p<10^{-8}$). Five additional loci on chromosomes 3 ($p=6\times10^{-10}$), 6 ($p=9\times10^{-12}$), 8 ($p=7\times10^{-10}$), 19 ($p=4\times10^{-15}$) and 22 ($p=3\times10^{-8}$) reached genome-wide significance. Some of the top loci fit with ease into known pathways mediating AMD pathogenesis, whereas the biological role of others remains to be determined. For example, the top hit on chromosome 6 is near *VEGFA*, a gene that influences vascular permeability and vasculogenesis, confirms the importance of new vessel formation in advanced AMD. For secondary outcomes of specific AMD lesions, including large drusen (LD), geographic atrophy (GA) only, and choroidal neovascularization (CNV) only, we identified a number of gene loci which were close to genome-wide significance level, indicating possibly different roles they play in the etiology of AMD. Replications of these new findings are currently underway with ~15,000 cases and ~8,200 controls mainly from European ancestry. Our data strongly support a multigenic hypothesis for AMD pathogenesis and may provide new insights into the biology of the retina.

658T

A novel analysis method based on gene-gene interactions in pathways defined by protein-protein interaction networks. *R.H. Chung[1,2], W.K. Scott[1,2], J.M. Vance[1,2], E.R. Martin[1,2].* 1) John P. Hussman Inst for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami, FL.

Pathway analysis in GWAS has become increasingly popular under the premise that collectively testing a set of genes with individual modest effects might increase the power for identifying disease genes. Current pathway analysis methods mostly focus on testing for main effects in a set of genes on disease. However, for complex diseases, gene-gene interactions might play an important role in disease etiology. Identifying gene-gene interactions in GWAS is difficult because of the multiple testing burden. Because protein products of genes within a pathway often interact, testing for gene-gene interactions in gene sets created using protein-protein interaction (PPI) information offers an informed way to reduce the number of gene pairs tested and may reveal associations missed by approaches focused only on main effects of genes. We developed the Pathway analysis method Using Protein-Protein Interaction network for case-control data (PUPPI). PUPPI computes the PLINK fast-epistasis statistics for a pair of genes if (a) the two genes are in the same pathway, (b) public PPI databases indicate the protein products of the two genes interact and (c) the two genes are on different chromosomes or are more than a pre-defined distance apart on the same chromosome. The maximum test statistic from all pairs of SNPs between the two genes is used as the score for the gene pair and the score is adjusted for gene size by correcting for the effective number of SNPs tested. The PUPPI statistic for a pathway is the sum of the scores of gene pairs in the pathway producing p-values less than a pre-specified threshold. A permutation procedure is used to approximate the distribution of the PUPPI statistic. We used simulations to demonstrate that PUPPI has correct type I error rates and high power under different disease models. We applied PUPPI to data from a Parkinson disease (PD) GWAS (Edwards et al 2010), consisting of 1,752 cases with PD and 1,745 controls and 443,234 autosomal SNPs. We tested 208 KEGG pathways. Interestingly, the top pathway (hsa05012) is the PD pathway defined by KEGG (PUPPI p-value=0.0005). The significance is mainly derived from interactions between nuclear-encoded NADH dehydrogenase subunits that localize to the mitochondrial membrane. Our analyses demonstrate that pathway analysis using only gene-gene interaction information can be powerful for identifying disease pathways and reveal gene-gene interactions that may not be detected at a genome-wide level.

659T

Use of posterior probability of linkage in weighted false discovery rate analysis of cleft lip with or without cleft palate (CL/P) families. *M. Govil[1], N. Mukhopadhyay[1], T.H. McHenry[1], T.H. Beaty[2], J.C. Murray[3], M.L. Marazita[1].* 1) University of Pittsburgh, Department of Oral Biology, Pittsburgh, PA; 2) Johns Hopkins University, School of Public Health, Baltimore, Maryland, USA; 3) The University of Iowa, Department of Pediatrics, Iowa City, IA.

Non-syndromic CL/P is a common birth defect with a complex etiology. In the past several years, our group and others have done much to shed light on a variety of aspects of CL/P including heterogeneity of the phenotype and its refinement to include sub-clinical phenotypes, identification of environmental factors influencing risk, and accumulating evidence for the role of genes in CL/P etiology, including IRF6, FOXE1, MSX1, and TGFA. Concurrently, a variety of sophisticated statistical methods for both linkage and association have been developed to improve our ability to map such complex traits. The weighted false discovery rate (w-FDR) utilizes evidence for linkage as a prior probability to weight association p-values. DATA and METHODS: The posterior probability of linkage (PPL) is a class of likelihood-based, model-free statistics to measure of the strength of evidence for/against linkage. PPLs were pulled from a 10 cM, 401 marker genome scan (Govil et al, 2005) carried out on 487 multiplex CL/P families drawn from 7 countries. Association p-values were generated for 1,028 trios from 7 ethnicities, using 589,945 GWAS SNPs described earlier (Beaty et al, 2010). For each of these analyses, the families were divided into two groups: Asian (A) and European (E). The PPL was sequentially updated by population within each group. The w-FDR p-values were then generated for the entire marker panel using the PPLs as user-defined weights. RESULTS: The original sequentially updated PPLs had identified multiple regions of linkage, with peak scores for Asians on chr. 6 and Europeans on chr. 9. These results corresponded to previous multipoint HLOD results for the same data (Marazita et al, 2009). The original GWAS, with a different set of families, identified regions primarily on chr. 1, 11 (A) and 8 (E), with a peak on chr 6 (A) ~63 Mb from the linkage peak, but found no significant results on chr. 9. Using the PPL as a user-defined weight in w-FDR, additional SNPs on both chr. 6 (A) and 9 (E) approached the w-FDR corrected genome-wide significance levels (2.71e-6, A; 3.42e-6, E) with p-values<3.6e-5 for SNPs under the linkage peak. While we continue to refine this method, our results show the potential power of this approach to identify genes associated with complex traits. Support: DE016148, DE008559, DE018085, DE018085-01A2S1, DE018993.

660T

Detecting Genetic Association with Rare Variants in Admixed Populations. *X. Mao[1], Y. Li[2], M. Li[1].* 1) Departments of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA; 2) Department of Genetics, Department of Biostatistics, University of North Carolina, Chapel Hill, North Carolina 27599-7264, USA.

Recent studies suggest that rare variants play an important role in the etiology of many traits. Analysis of rare variants holds the promise of explaining some of the missing heritability left from GWAS. Although a number of methods have been developed for genetic association analysis of rare variants, all assume a relatively homogeneous population under study. Such an assumption may not be valid for samples collected from admixed populations as various studies have shown that there is a great extent of local variation in ancestry in admixed populations. While such variation may be useful for admixture mapping, it imposes a challenge for the analysis of rare variants because rare variants are more likely to be influenced by distinct local genetic background. To ensure valid and more powerful rare variant association tests performed in admixed populations, we have developed a local-ancestry-based weighted dosage test for the analysis of rare variants. Our test shares similarity with WHaIT, a recently developed method for the analysis of rare variants, in that both take into account of the direction of association by allowing for risk and protective alleles. The key difference between our method and WHaIT is that we are able to adjust the weighted dosage score by local ancestry. In our earlier publication, we have shown that regardless of the mechanism of population stratification, whether it is due to local or global ancestry difference, adjustment of local ancestry is sufficient to remove the effect of population stratification. Through extensive simulations, we confirm that our proposed test has controlled type I error rates when population stratification is induced by local ancestry difference among study subjects, whereas naïve application of existing rare variants tests leads to inflated type I error rates. When there is moderate population stratification such as 80%-20% admixture in case samples vs 75%-25% admixture in control samples, the existing rare variants tests without adjusting for ancestry has type I errors of 22% while our proposed test has a type I error under 5% (both at 5% level). Our results suggest that it is important to appropriately control for potential population stratification induced by local ancestry difference in the analysis of rare variants in admixed populations.

661T

Mapping Genes for Longitudinal Data Phenotypes: A Study of Type I Error and Power. *A. Musolf[1], D. Londono[1], K. Chen[2], R. Wang[3], T. Shen[3], J. Brandon[4], J.A. Herring[5], C.A. Wise[4,6,7], H. Zong[8], M. Jin[8,9], L. Yu[1,10], S.J. Finch[3], T. Matise[1], D. Gordon[1].* 1) Department of Genetics, Rutgers, The State University of New Jersey, 145 Bevier Road, Piscataway, NJ 08854, USA; 2) Department of Statistics, Rutgers, The State University of New Jersey, 110 Frelinghuysen Road, Piscataway, NJ 08854-8019, USA; 3) Department of Applied Mathematics and Statistics, Stony Brook University, Stony Brook, NY 11794-3600, USA; 4) Seay Center for Musculoskeletal Research, Texas Scottish Rite Hospital for Children, 2222 Welborn Street, Dallas, TX 75219, USA; 5) Department of Orthopedic Surgery, Texas Scottish Rite Hospital for Children, 2222 Welborn Street, Dallas, TX 75219, USA; 6) Department of Orthopaedic Surgery, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390, USA; 7) McDermott Center, University of Texas Southwestern Medical Center, 6000 Harry Hines Blvd, Dallas, TX 75390, USA; 8) Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China; 9) Shanghai Bio Corp, Shanghai, China; 10) Center of Alcohol Studies, Rutgers, The State University of New Jersey, 607 Allison Road, Piscataway, NJ 08854, USA.

To date, there have been relatively few GWAS for longitudinal data phenotypes. Yet, knowledge of genes that influence longitudinal patterns may inform about disease course and offer the ability to predict disease progression. We have developed a systematic GWAS procedure for testing association between SNP genotypes and longitudinal phenotype data. We used growth mixture models (GMMs) to fit longitudinal data to a small number of estimated trajectory curves. We determined Bayesian posterior probabilities of belonging to specific trajectory curves and used these probabilities as the values for quantitative trait loci. We then tested for genome-wide association with SNPs. We evaluated the type I error and power of our method using data that was simulated as a function of multiple parameters under null and alternative scenarios. Our alternative scenarios mimicked different modes of inheritance including single disease marker at full penetrance, a single disease marker at reduced penetrance under both a dominant and multiplicative model, and multiple disease loci. We also performed association tests under alternative scenarios for different minor allele frequencies and variances. Our principal findings are: (i) our method maintains the correct empirical type I error rate for all simulations and (ii) most of our alternative simulations produce ~ 95 - 100% power. For example, we observe a type I error rates of 0.0101, 0.0496, 0.0931, and 0.1398 for (= 0.01, 0.05, 0.1, and 0.15, respectively. We also observed 100% power for the single marker full penetrance and the multi-locus model at all (. In addition, 94-98% power was obtained for the dominant reduced model and 97-99% power for the multiplicative reduced model. Thus, we conclude that our method is effective for identifying allelic association with disease for multiple genetic modes of inheritance using longitudinal data. We believe that this method is clinically significant as it may allow doctors to predict disease progression based on genotype and alter treatment accordingly.

662T

Genome-wide linkage and association analyses reveal quantitative trait loci that influence thyroid-related hormones. *J. Singh[1], L. Yerges-Armstrong[2], J.R. Shaffer[1], J. Curran[3], M. Carless[3], S. Cole[3], J. Kent[3], T. Dyer[3], L. Almasy[3], M. Mahaney[3], J. Blangero[3], C.M. Kammerer[1], P.B. Samollow[4].* 1) University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; 2) University of Maryland School of Medicine, Baltimore, MD; 3) Texas Biomedical Research Institute, San Antonio, TX; 4) Texas A&M University, College Station, TX.

Thyroid hormones play major roles in the regulation of protein synthesis, basal metabolism, lipid metabolism, and higher order characteristics such as neural and skeletal development, cardiac output, thermogenesis, and other traits that are critical to health; but the genetic basis of normal thyroid hormone regulation is not well known. In this study, we performed genome-wide linkage (GWL) and association (GWA) analyses to detect quantitative trait loci (QTLs) influencing six thyroid hormone related traits: free and total thyroxine (FT4 & TT4), total triiodothyronine (TT3), thryoxine-binding globulin (TBG), thyrotropin (TSH), and thyroglobulin (TG). Phenotypic and genotypic data were available on 1002 euthryroid individuals (420 males and 582 females) from 35 Mexican-American families collected as part of the San Antonio Family Heart Study. Prior to performing GWL and GWA analyses, effects of significant environmental components (e.g., sex, age, diabetes status, alcohol consumption, physical activity and smoking habits) were identified and removed. GWL revealed a QTL influencing TG on chr 2 at location 160cM pter (LOD=5.28, p=0.0007). Analyses (correcting for familial correlations) of 46032 SNPs under the linkage peak for TG, revealed multiple SNPs with suggestive associations ($P < 10^{-5}$) within a 600kb region. This genomic region includes the genes *TFCP2L1, RNU4ATAC, CLASP1, MKI67IP*, and *TSN*. We next performed WGA and detected associations for (1) TBG and SNPs within the intronic region of *ANKS1B* gene on 12q23 ($P<10^{-7}$) and (2) TSH and SNPs in the 5'-UTR of the *DDX18* gene on 2q14 ($P<10^{-7}$). None of these genes are known to be associated with thryroid function. These findings illustrate that linkage and association, two complementary approaches for detecting QTLs, can enhance our understanding of genetic control of thyroid hormone variation by revealing genomic locations that harbor previously unrecognized genetic features that influence thyroid-related hormone metabolism and function, thereby facilitating their identification.

663T

Population Stratification in Burden Tests for Rare Variant Associations. *M. Zawistowski[1,2], D. Wegmann[3], M.G. Ehm[4], M.R. Nelson[4], J. Novembre[3], S. Zöllner[1,2,5].* 1) Department of Biostatistics, The University of Michigan, Ann Arbor, MI; 2) Center for Statistical Genetics, The University of Michigan, Ann Arbor, MI; 3) Department of Ecology and Evolutionary Biology / Interdepartmental Program in Bioinformatics, University of California, Los Angeles, CA, USA; 4) GlaxoSmithKline, Department of Genetics, RTP, NC; 5) Department of Psychiatry, The University of Michigan, Ann Arbor, MI.

Next generation sequencing datasets will dramatically improve our ability to identify rare variants contributing to disease risk. Such datasets are typically assumed to be drawn from a homogeneous population but, in practice, are often unintentionally heterogeneous with respect to population of origin. Correlation between population of origin and both phenotypes and genotypes can result in population stratification, a known cause of spurious genetic associations. Genomic control and principal components work well to correct for population stratification in single marker tests. However, tests for excess accumulation of rare variants within a genomic region in cases or controls, so-called burden tests, are more powerful than single marker tests of rare variants. In this analysis, we use real sequence data and population genetic models to determine the effects of population stratification on burden tests and identify factors likely to cause stratification. We used real sequence data from the exons of 202 drug target genes in >14,000 subjects to simulate case-control datasets containing no direct genotype-phenotype association. However, we induce an indirect association via population stratification by differentially sampling cases and controls with respect to population of origin. The diverse European and non-European populations included in this study make it ideal for considering realistic levels of rare variation across a wide range of stratification scenarios. We show that analyses of these datasets using a variety of existing burden tests have severely inflated false positive rates even when closely related populations are considered. Moreover, existing correction methods, such as those used in the genotype-phenotype analyses of the drug target genes, often fail to control for the stratification. We also perform a simulation analysis using population genetic models to determine the cumulative effects of forces such as growth, migration and selection that can each affect population-specific levels of rare variation and drive stratification in burden tests. Our results are used to predict genes and study designs likely to suffer from stratification, highlighting conditions under which existing correction methods fail. We suggest novel methods to control for rare variant stratification and, ultimately, interpret the genotype-phenotype analyses of the 202 drug target genes in this and future rare variant studies.

664T

A genome scan for vesicoureteric reflux reveals a new recessive locus on chromosome 10 with an HLOD score of >6. *J.M. Darlow[1, 2], M.G. Dobson[1, 2], M. Hunziker[2, 3], C.M. Molony[4], P. Puri[2, 3], D.E. Barton[1].* 1) Natl Ctre Med Gen, Our Lady's Children's Hosp, Dublin, Ireland; 2) National Children's Research Centre, Our Lady's Children's Hosp, Dublin, Ireland; 3) National Children's Hospital, Tallaght, Dublin 24, Ireland; 4) Merck & Co., Inc., 1 Merck Drive, Whitehouse Station, New Jersey, USA 08889.

Vesicoureteric reflux (VUR), the retrograde flow of urine from the bladder towards the kidneys, results from a developmental anomaly of the vesicoureteric valve mechanism, and is often associated with other urinary tract anomalies. It is the most common urological problem in children, with an estimated incidence at birth of 1-2%. Though it may be symptomless and often resolves with age, about 8% of affected individuals develop renal failure, in childhood or adult life, accounting for ~25% of all renal failure. Several genome scans have been performed, with conflicting results, including one by us with 4,700 SNP markers on 129 VUR families. We have now performed a new scan on 246 families (530 cases (514 children 15 parents and 1 grandparent) and 435 other family members, 119 with a history or family history of urinary tract problems) with 900,000 markers on the Affymetrix SNP Array 6.0. Numerous analyses have been carried out - singlepoint and multipoint, parametric and non-parametric, counting and not counting probable carriers as affected, and at the time of abstract deadline we are not yet ready to give exact details, but we are confident that our previous non-parametric linkage peaks on 2q, 6q and 10q are confirmed (though with slightly altered position) and are all dominant, the highest being on 10q with an HLOD of ~5, and our previous peak on 7q is confirmed with HLOD >3 on a common recessive model but also some linkage on a very rare dominant model. However, the most exciting finding is a very narrow recessive peak in a different position on 10q with an HLOD >6 that was previously missed because the markers were too far apart. There is also a dominant peak on 22q in one analysis. Numerous other smaller peaks remain to be examined. Our new results did not replicate the peaks on chromosomes 5, 13, and 18 found by Briggs et al.(2009)(even though we had previously found some linkage on 13q) nor the recessive peak of Weng et al. (2009) on 12p11-q13, nor linkage to ROBO2 or anywhere in the region identified on Chromosome 3 by Conte et al. (2007). The novel linkage peak of HLOD >6 on chromosome 10 is of particular interest and will focus attention on novel positional candidate genes for VUR. Analysis of the data for association and copy-number variation is ongoing.

665T

The Kaiser Permanente/UCSF Genetic Epidemiology Research Study on Adult Health and Aging: Efficient and Accurate Genome-wide Genotyping of 100,000 Subjects using the Affymetrix Axiom Genotyping System. *S. Hesselson[1], M. Kvale[1], Y. Cao[1], J. Eshragh[1], R. Lao[1], G. Mathauda[1], T. Shenoy[1], E. Wan[1], S. Wong[1], M. Patil[2], D. Chan[2], M. Mittmann[2], M. Purdy[2], J. Gollub[2], T. Webster[2], S. Miles[3], S. Rowell[3], L. Walter[3], W. McGuire[3], S. Connell[3], C. Zau[3], D. Ranatunga[3], L. Shen[3], D. Smethurst[3], A. Finn[2], C. Schaefer[3], N. Risch[1], P.-Y. Kwok[1].* 1) Institute for Human Genetics, UCSF, San Francisco, CA; 2) Affymetrix Inc., Santa Clara, CA; 3) Kaiser Permanente Northern California Division of Research, Oakland, CA.

As part of the Kaiser Permanente/UCSF Genetic Epidemiology Research Resource on Adult Health and Aging, we needed to genotype 100,000 saliva-based DNA samples successfully (i.e. passing strict QC and call-rate thresholds) in a 14 month period using the Affymetrix Axiom genotyping system. This required a processing rate of nearly 24 plates (2,304 samples) per week. This high, consistent throughput was achieved with 6 laboratory staff, 4 Affymetrix Gene Titans, and 3 Beckman FX robots. Critical to the continuous throughput was rapid return of genotyping results in order to detect and diagnose problems as they arose. Array plates were hybridized continuously. Three Gene Titans were used full time; the availability of a fourth was critical to the throughput due to periodic machine breakdown that would otherwise compromise the ability to wash hybridized plates in a timely manner. Two plates were washed on each of 3 Gene Titans per day. The data were then transferred to a computer cluster and genotyped automatically. Plate-by-plate reports were evaluated in real time, to allow for a rapid respond to any apparent problems. In the short term, problems were detected by identifying plates with higher than average failure rates. One of the most informative indicators of a problem on a plate is the distribution of dQC and call rate values. Problems were also detected by examining individual images on every plate. The most common causes of increased failure rates (in order of temporal occurrence) were pellets that wouldn't resuspend at the end of DNA preparation, array lot performance variation, leaking of fluidics reservoirs, and scanner malfunctions. We deviated from Affymetrix's standard protocol at times, the most notable being an increase in the hybridization time from 24 hours to 48 hours per plate. This modification was implemented due to global decrease of the number of passing samples on all Gene Titans over many plates. Increased hybridization time raised the mean dQC from .896 to .910, and led to a nearly 50% decrease in the number of failing samples (dQC<.82) per plate. To allow for the inclusion of more minority subjects, we also tested assay robustness to decrease in DNA concentration. We found that reducing concentrations from 30 to 15 ng/μl had little impact on genotyping success rates. We achieved an overall success rate of 94% on this saliva-based cohort.

666T

Efficient capture of allele frequency spectra in sequencing studies by selection of independent chromosomes. *T. Edwards[1], C. Li[2].* 1) Center for Human Genetics Research, Division of Epidemiology, Department of Medicine, Vanderbilt University, Nashville, TN; 2) Center for Human Genetics Research, Department of Biostatistics, Vanderbilt University, Nashville, TN.

Sequencing studies using whole-genome or exome scans are still more expensive than GWAS on a per-subject basis for accurately calling genotypes. As a result, a subset of subjects from a larger study is often selected for sequencing studies. The choice of study subjects should capture as many ancestral lineages as possible to avoid redundant information from regions that were inherited identical by descent (IBD) from a common ancestor, estimable through genotype data or pedigree structure. We present SampleSeq2, which can select a subset of optimally unrelated subjects with minimal IBD sharing and estimate the number of independently inherited chromosomes in a sample, GT, or alternately select the minimum number of subjects for a target GT. We evaluated SampleSeq2 compared to a random draw by simulation and using the Amish Study of Successful Aging. We simulated a random mating island population growing 10.5% per generation from 500 founders for 30 generations to N=10,000. The simulated genome was 3,000cM, with 30 recombinations expected per meiosis. Comparing the known value of GT to the value estimated for GT, the estimate was close to the true value of GT, and SampleSeq2 provided an increase in GT relative to a random draw across a range of sample sizes. In the Amish study, there were 4,995 subjects in the pedigree, with 827 in the Aging study. The average IBD among the 827 subjects was 2.4%. Among the 341551 subject pairs, 83.1% pairs had >1% genome expected to be shared IBD, 5.3% pairs had >5% genome shared IBD, 1.1% pairs had >10% genome shared IBD, and 810 pairs of subjects shared⁄ 50% of the genome. We compared our approach with the alternative of random selection for K subjects (K=50, 100). For K=50, the average GT was 41.5 using SampleSeq2 and 29.7 for random selection. On average, our algorithm resulted in 11.8 additional independent genomes, a 39% increase. While random selection resulted in subsets with 2.4% average IBD sharing, our method resulted in 0.8% average pairwise IBD, a 67% reduction. For K=100, the average GT was 60.6 using SampleSeq2 and 39.9 for random selection. On average, our algorithm resulted in 20.7 extra independent genomes, a 52% increase. The average pairwise IBD sharing was 2.40% for random selection and 1.11% for our method, a 54% reduction. This increase in chromosomes will improve power of association tests, mitigate effects of relatedness on parameter estimates, and increase the yield of alleles from sequencing.

667T

Whole-genome sequencing of six canine disease models. *F.H.G. Farias[1], G.S. Johnson[1], T. Mhlanga-Mutangadura[1], R. Zeng[1], J.F. Taylor[2], D.P. O'Brien[3], R.D. Schnabel[2].* 1) Veterinary Pathobiology, University of Missouri, Columbia, MO., USA; 2) Division of Animal Sciences, University of Missouri, Columbia, USA; 3) Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, USA.

Whole-genome sequencing is a comprehensive approach to the detection of genetic variants. Improvement of next generation sequencing technology coupled with drastic reductions in cost has opened an opportunity for a fast and effective way of identifying causal mutations for Mendelian diseases. We have begun the search for these disease-causing mutations by sequencing the whole genomes of six dogs from different breeds that are individually affected with different inherited diseases including: Polymicrogyria, Multisystem degeneration, Cerebellar ataxias (two distinct types), Fanconi syndrome and Paroxismal dyskinesia. Four of the six disease loci have previously been mapped by genome-wide association studies either using microsatellites or SNP assays. Each of the six dogs has been sequenced to 12-23X genomic coverage on either an Illumina GAIIx or Hi-Seq 2000. We have used NextGENe® software to align the sequences to build 2.1 of the NCBI canine reference genome sequence and identify sequence variants (SV). Aggregated across dogs, approximately 6-8 million unique SV were produced from these data. Since each disease is believed to be the result of a founder event or de novo mutation within a breed, we expect the causal variant to be present in the case animal while the remaining animals serve as controls and must lack the causal variant. As such, we have developed algorithms to identify the SV present in the case dog that fit the disease mode of inheritance and which are not present in the remaining five control dogs. For the disease loci which have previously been mapped to a genomic region we have reduced the candidate SV from millions genome-wide to approximately 2000 total in the mapped region. Further filtering SV to include only those present in coding regions reduces the causal candidate set to less than 20 variants. For the two diseases that have not previously been mapped we are able to reduce the candidate causal mutations to approximately 1500 variants within the coding regions genome-wide. We are currently evaluating mutations for each disease by testing for associations between genotype and disease phenotype in pertinent canine populations.

668T

Detecting sample contamination using array-based genotype data. *M. Flickinger[1], G. Jun[1], K.F. Doheny[2], J. Romm[2], K.N. Hetrick[2], G.R. Abecasis[1], M. Boehnke[1], H.M. Kang[1].* 1) Department of Biostatistics, University of Michigan, Ann Arbor, MI 48109-2029; 2) Center for Inherited Disease Research (CIDR), Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

While advances in array-based genotyping and next generation sequencing have resulted in lower costs per genotype and reduced error rates, DNA sample contamination remains a threat to data quality. Here we describe methods to detect whether a sample contains DNA from two or more individuals. Early detection of sample contamination is both a useful quality control step for genotype data, and an effective way to pre-screen samples prior to re-sequencing so resources are not wasted on lower-quality samples. High levels of contamination are often easily discovered by low SNP call rates or unexpectedly high heterozygosity rates in GWAS, but low-levels of contamination (say <10%) may have been previously undetected. Our goal is to detect low-levels of contamination using data available from existing array-based genotyping methods. We propose two methods to detect contamination. The first uses a regression-based analysis to detect systematic shifts in the Illumina reported B-allele frequencies (BAF) associated with changes in the minor allele frequency (MAF) in the population across all assayed SNPs. In contaminated samples, as the population MAF increases, it becomes increasingly likely that the contaminated samples will have different alleles at a particular SNP, shifting the BAF of homozygous SNPs from the expected value of 0 or 1 towards the expected heterozygote value of 0.5. Our second method models the Illumina reported normalized intensity data using a Gaussian mixture model and compares the likelihoods across different mixture proportions to estimate the true contamination fraction. To validate these methods, we analyzed mixtures of DNA samples from HapMap individuals created to have specific, known levels of contamination ranging from 0.5%-10%. We genotyped these samples using the Metabochip genotype array (a custom Illumina iSelect SNP array with ~200,000 markers in regions previously shown to be associated with metabolic traits). Our results show that the regression-based method is more sensitive to lower levels of contamination while the mixture model is better at quantifying contamination fraction at higher levels of contamination. We find that these methods are valuable for pre-screening samples prior to re-sequencing and as a quality control check for genotype data.

669T

Design of DNA pooling to allow incorporation of covariates in rare variants analysis. *W. Guan[1], C. Li[2].* 1) Division of Biostatistics, Univ of Minnesota, Minneapolis, MN; 2) Department of Biostatistics, Vanderbilit University.

Rapid advances in next-generation sequencing technologies facilitate genetic association studies of an increasingly wide array of rare variants. To capture the rare or less common variants, a large number of individuals will be needed. However, the cost of a large scale study using whole genome or exome sequencing is still high. DNA pooling can serve as a cost-effective approach, but with a potential limitation that the identity of individual genomes would be lost and therefore individual characteristics and environmental factors could not be adjusted in association tests, which may result in power loss and a biased estimate of genetic effect, especially at presence of gene-environment interactions. For case-control studies, we propose a design strategy for pool creation and an analysis strategy that allows covariate adjustment. In our approach, we first calculate a propensity score for each individual, which is the estimated odds of disease given a set of potential confounders [Rosenbaum and Rubin, 1983]. We then group cases and controls based on their propensity scores, and divide them into case/control pools with desired size. After sequencing is done for pooled DNA, we impute genotypes and values of covariates for each individual, and perform association tests between phenotype and genotype while adjusting for the covariates, using multiple imputation technique. Simulations show that our approach can obtain consistent estimate for genotypic effect with only slight loss of power compared to the more expensive approach of sequencing individual genomes. For example, given a causal variant with minor allele frequency 0.01 and odds ratio (OR) of 3.0 and an environmental factor with OR of 1.5, at a significance level of 5%, a study of 600 cases and 600 controls has 76% power to detect the causal variant using individual sequencing, and 73% power using our approach at a fraction of the cost (with pool size 12 and an average depth of 200). Our design and analysis strategies enable more powerful and cost-effective sequencing studies of complex diseases, while allowing incorporation of covariate adjustment.

670T

Genotype calling from next-generation sequencing data in pedigrees. *S.C. Heath, E. Raineri.* CNAG, Barcelona, Spain.

We present a Hidden Markov Model (HMM) based approach for simultaneously calling genetic variants from genome wide sequence data in related individuals. The method combines information across individuals and between nearby genomic regions to increase the precision of genotype calling compared with individual genotype calling algorithms. Alternatively, it allows the accuracy of genotype prediction to be maintained while reducing the amount of sequence data required. The cost of genome wide sequencing in families can therefore be reduced, expanding the range of projects for which sequencing is feasible. Our approach follows that of linkage analysis programs using HMMs to model inheritance patterns along the chromosome. At a given locus let $\boldsymbol{S}$ be the inheritance pattern at a locus, $\boldsymbol{Y}$ the sequence data and $\boldsymbol{G}$ the (true) genotypes. The HMM has one node per observed locus along the chromosome, with the possible states at each node corresponding to the possible states of $\boldsymbol{S}$. The transmission probabilities for the HMM are the same as for regular linkage calculations, but the emission probabilities are calculated conditional on the sequence rather than on the genotype data. The emission probabilities can be written as $p(\boldsymbol{Y}|\boldsymbol{S})$. To calculate this we note that conditional on the true genotypes, $p(\boldsymbol{G}|\boldsymbol{S})$ is simple to calculate (and forms the basis of the use of HMMs for linkage). Also, $p(\boldsymbol{Y}|\boldsymbol{G})$ is the probability of the observed sequence data given the genotypes, and any parametric model used for calling genotypes from sequence data can be used here. $p(\boldsymbol{Y}|\boldsymbol{S})$ can therefore be calculated by the summation $\Sigma_G p(\boldsymbol{Y}|\boldsymbol{G})p(\boldsymbol{G}|\boldsymbol{S})p(\boldsymbol{G})$ over all possible values of $\boldsymbol{G}$ (where $p(\boldsymbol{G})$ is the prior of $\boldsymbol{G}$). The structure of the pedigree can be utilized to perform this summation in an efficient manner, even for general pedigrees with possible complex structures. Direct inference is made of the joint distribution of the inheritance relationships between pedigree members along the chromosome, and genotypes for pedigree members at each location can be estimated conditional on the most likely inheritance pattern and the sequence data. The method has been implemented and tested on a human nuclear family with 3 siblings, where exome sequencing had been carried out on all family members. The estimation procedure was performed both with and without the sequence data on the parents showing the the effect of the missing data on the precision of the estimates of genotypes and inheritance patterns.

671T

Single nucleotide polymorphisms in the promoter of tumor necrosis factor—(gene in korean retired workers exposed to inorganic dusts. *J. Hwang, K. Lee, J. Shin, J. Lee, B. Choi.* Research Dept, Occupational Lung Diseases Institute, Ansan-si, Gyeonggi-do, Korea.

Pneumoconiosis is a restrictive lung disease caused by the inhalation of inorganic dusts, often in mines that leads to inflammation and fibrosis. Tumor necrosis factor-((TNF-( ) plays an important pro-inflammatory cytokine in pneumoconiosis. Single nucleotide polymorphisms (SNPs) in the promoter region of TNF-(gene have been associated with regulating its production and effect. The aim of this study was to investigate the SNPs at position -308 and -238 in the promoter of TNF-(gene in 163 korean retired workers exposed to inorganic dusts. 96 of participants (58.9%) were pneumoconiosis and 67 (41.1%) non-pneumoconiosis patients by the pneumoconiosis review committee of Korea Worker's Compensation & Welfare Service. These alleles were in linkage disequilibrium ($p<0.001$), and so were not independent. But, there were no significant differences between pneumoconiosis and non-pneumoconiosis group at position -308 (16.7% vs 13.4%, Fisher's exact p value=0.662)and -238 (9.3% vs 13.4%, Fisher's exact p value= 0.453) in the promoter of TNF-(gene. The association between SNPs at position -308 and -238 in the promoter of TNF-(gene and pneumoconiosis need to be clarified by the involvement of a large number of subject as well as follow-up study.

672T

Finding genes in Mendelian disorders using sequence data: methods and applications. *I. Ionita-Laza[1], V. Makarov[2], J.D. Buxbaum[2], D.L. Nicolae[3], X. Lin[4].* 1) Columbia University, New York, NY; 2) Mount Sinai School of Medicine, New York, NY; 3) University of Chicago, Chicago, IL; 4) Harvard University, Boston, MA.

Many sequencing studies are now underway to identify the genetic causes for both Mendelian and complex traits. Using exome-sequencing, genes for several Mendelian traits including Miller Syndrome, Freeman-Sheldon Syndrome and Kabuki Syndrome have already been identified. The underlying methodology in these studies is a multi-step algorithm based on filtering variants identified in a small number of affected individuals depending on whether they are novel (not yet seen in public resources such as dbSNP, and 1000 Genomes Project), shared among affected (possibly related) individuals, and other external functional information available on the variants. While intuitive, these filtering-based methods are non-optimal and do not allow for uncertainty to be quantified in the form of P-values. We describe here a formal statistical framework that has several distinct advantages: (1) provides analytical, fast computation of approximate P-values, (2) accommodates designs based on both affected relative pairs and unrelated affected individuals, (3) adjusts for the background variation in each gene so that large genes do not rise to the top based on their sheer size alone, and (4) allows for natural incorporation of functional or linkage-based information. We show via simulations that the proposed approach achieves a substantially better ranking of a disease gene when compared with currently used filter-based approaches, especially in the presence of disease locus heterogeneity. We revisit recent studies on three Mendelian diseases that used filter-based approaches to identify the corresponding disease genes and show how the proposed approach can be applied in such cases, resulting in the disease gene being ranked first in all three studies, and approximate P-values of E-12 for the gene for the Miller Syndrome, 3E-4 for the gene for the Freeman-Sheldon Syndrome, and 1.4E-4 for the gene for the Kabuki Syndrome.

673T

A Powerful and Efficient Two-Stage Design for Next Generation Sequencing Data Analysis Using Extreme Phenotype Sequencing. *G. Kang, D. Lin, M. Li, J. Chen.* Department of Epidemiology and Biostatistics, University of Pennsylvania, Philadelphia, PA 19104.

Rare variants have been conjectured to contribute to a large proportion of genetic variation in complex traits that is not yet explained by associated genes identified from genome-wide association studies. The next-generation sequencing technology provides an unprecedented opportunity to discover rare variants. Currently, it is not financially feasible to perform whole-genome sequencing on a large number of subjects, and a two-stage design has been advocated to be a practical option. In stage I, variants are discovered by whole-genome sequencing on a small set of carefully selected individuals. In stage II, the discovered variants are genotyped on a large set of individuals for screening associations. Extreme phenotype sequencing is one of the primary designs for selecting stage I individuals. Using simulated data for unrelated individuals, we explore two important aspects of this design: the efficiency of discovering both common and rare SNPs in stage I, and the impact of incomplete SNP discovery in stage I on the power for testing associations in stage II. We apply a sum test and a sum of squared score test to perform gene-based association tests for evaluating the power of the two-stage design. Simulations and application to GAW17 data set show that, given the sample size, using individuals at both a cutoff ranging from 0.3% to 1.9% tails of the quantitative trait, 1) less than half of the total SNPs were discovered, including all common SNPs, nearly all of the less common SNPs, and less than half of the rare SNPs, which included nearly half of causal SNPs; 2) more than half of the total cost could be saved; 3) the two-stage method could achieve the same as or even higher power than the one-stage method if the rare variants contribute to the trait with large effect sizes.

674T

The Empirical Power of Rare Variant Association Methods: Results from Sanger Sequencing in 1,998 Individuals. *M. Ladouceur[1,2], Z. Dastani[2,3], Y.S. Aulchenko[4,5], M.T. Greenwood[2,3,7], J.B. Richards[1,2,6,8].* 1) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Quebec, Canada; 3) Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, Quebec, Canada; 4) Department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands; 5) Institute of Cytology and Genetics SD RAS, Novosibirsk, Russia; 6) Department of Medicine, Jewish General Hospital, McGill University, Montreal, Quebec, Canada,; 7) Department of Oncology, McGill University, Montreal, Quebec, Canada; 8) Twin Research and Genetic Epidemiology, King's College London, London, United Kingdom.

The role of rare genetic variation in the etiology of complex disease remains unclear. However, the development of next-generation sequencing technologies offers the experimental opportunity to address this question. Several novel statistical methodologies have been recently proposed to assess the contribution of rare variation to complex disease etiology. Nevertheless, no empirical estimates comparing their relative power are available. We therefore assessed the parameters that influence their statistical power in 1,998 individuals Sanger-sequenced at seven genes by modeling different distribution of effect, proportions of causal variants, and direction of the associations (deleterious, protective or both) in simulated phenotypes. Our results demonstrate that the power of recently proposed statistical methods depend strongly on underlying assumptions about each of these three factors. No method demonstrates consistently acceptable power despite this large sample size, and the performance of each method depends upon the underlying assumption of the relationship between rare variants and complex traits. Sensitivity analyses are therefore recommended to compare the stability of the results arising from different methods, and promising results should be replicated using the same method in an independent sample. These findings provide guidance in the analysis and interpretation of the role of rare base-pair variation in the etiology of complex disease.

675T

Quality assurance of variant calling (SNV and Indels) using next-generation sequencing (NGS) data. *H. Ling, H. Kurt, E. Pugh, J. Romm, B. Craig, B. Marosy, K. Doheny.* Center for Inherited Disease Research (CIDR), Johns Hopkins University, Baltimore, MD.

CIDR provides high quality NGS, genotyping and statistical genetics consultation to investigators working to discover genes that contribute to disease. The constantly evolving NGS technology and bioinformatics tools have greatly improved the accuracy of SNP and genotype calling using deep sequencing data (>=30x). However, the nature of sequencing technology and data processing pipelines have made variant calling platform specific and involve lab-to-lab, batch-to-batch and even sample-to-sample variations. This brings new challenges for variant calling in terms of quality assurance and filtering as even low level genotype errors can lead to a strong decrease in power for association mapping of complex traits for which the strategies focus on collapsing rare/low frequency variants into a compound variant based on gene, region or pathway. Therefore, high quality variant calling is essential for meaningful association mapping involving NGS data, and even more critical for family studies that look for variants shared among affected but not unaffected family members to narrow down candidate variants. After raw variants are called by the CIDRSeqSuite pipeline, a set of filters are applied to remove uncertain calls with quality metrics below a pre-determined threshold. These variants are then filtered by different BED files to define NearBait, OnBait and OnTarget calls for either WES or custom-targeted resequencing(CTS). Less than 2% raw variants from a WES are removed due to biases in strand, BaseQ, MapQ or tail distance. In the remaining 91K variants, 39%(35K) and 21%(19K) fall on Bait and Exon respectively. To investigate whether the filtered out set contains the majority of false positive calls, we compare a number of QC metrics between the filtered and the remaining sets at varying level of the filtering thresholds which include TiTv ratio, %dbSNP, SNP and genotype calling reproducibility for blind and HapMap duplicates, along with metrics obtained by comparing NGS against GWAS such as concordance and heterozygote sensitivity. In most cases, we found a fixed cutoff is effective in eliminating false positives. But under some circumstances, such as CTS with a much higher depth, the optimal cutoff for many filters can vary by sequencing depth. By choosing the optimal threshold for filtering parameters and examining data from blind duplicates and HapMap controls, we are able to release high quality variant lists and perform rapid troubleshooting for NGS data.

676T

Smoothed Functional Principal Component Analysis for Next-Generation Association Studies. *L. Luo[1], Y. Zhu[2], H. Kang[1], C. Stidley[1], E. Boerwinkle[2], M. Xiong[2].* 1) Division of Epidemiology and Biostatistics, University of New Mexico, Albuquerque, NM; 2) Human Genetics Center, University of Texas School of Public Health, Houston TX.

There is a consensus that common diseases are caused by an entire allele frequency spectrum of genetic variations including common and rare variants, such as SNPs, CNVs and other structural variations. To systematically investigate the genetic architecture of common diseases requires complete knowledge of the human DNA sequence variation across the entire spectrum of allele frequencies and types of DNA alterations. The current single marker association analysis platform is being shifted to joint multiple marker association analysis where a combination of common and rare variants from multiple genomic regions is taken as the unit for association analysis. Functional principal component analysis (FPCA) was recently developed as a tool to test the association of either common or rare SNPs or CNVs within a genomic region with disease. However, we often observe substantial variability of the principal component curves. These will be sensitive to sampling and dramatically reduce the power of FPCA. To overcome this limitation, we develop a statistical method which incorporates smoothing into FPCA. With the majority of smoothed FPCA methods focusing on the estimation procedures of FPCs, the statistics using smoothed FPC scores for inference has yet to receive much attention and have not been well developed for next-generation association studies. The goal of this research is to develop a novel smoothed FPCA-based statistic for inference and to apply it to testing the association of genetic variants generated by next-generation sequencing. This new statistic can jointly test for the association of risk increasing variants and protective variants within a genomic region without compromising power. We also develop a modified smoothed FPCA which takes population stratification into consideration. Extensive simulations using software based on coalescent theory are conducted to evaluate type 1 error rates and power. The simulation results show that the newly developed statistic has type I error rates not appreciably different from the expected nominal level, and has much higher power than the FPCA-based statistic and other existing methods for various disease models under a wide range of disease risk, allele frequencies and linkage disequilibrium patterns. The newly developed statistic is also applied to the Dallas Heart Study and the Framingham Heart Study. Our method identifies more significant genomic regions and yields much smaller p-values than existing methods.

677T

Genome-wide DNA Methylation Analysis for Osteoporosis Risk. *C. Qiu[1,2], H. Shen[1,2], J. Li[1,2], H.W. Deng[1,2].* 1) Center for Bioinformatics and Genomics, Department of Biostatistics, School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA 70112, USA; 2) School of Medicine, University of Missouri-Kansas City, Kansas City, MO 64108, USA.

Osteoporosis is a common disease mainly characterized by low bone mineral density (BMD) and increased risk of fractures. Peripheral blood monocytes (PBM) may act as precursors of osteoclasts, the bone resorption cells, and also produce cytokines important for osteoclast activity, and thus represent major systemic target cells for bone metabolism. Alterations in DNA methylation has been implicated as a key regulatory mechanism in the etiology of human complex diseases. However, the DNA methylation profiles in humans underlying osteoporosis risk are largely unknown. In this study, we performed a comparative genome-wide DNA methylation analysis of PBMs from 18 unrelated Caucasian postmenopausal females with extremely high (n =9) or low (n =9) BMD. By coupling methylated DNA immunoprecipitation with next-generation sequencing (MeDIP-seq), we generated over 282 million uniquely mapped reads for all the 18 samples. Genome-wide DNA methylation signals of each sample were normalized and quantified using MEDIPS analysis package. Differentially methylated regions (DMRs) were identified by comparing the genome-wide methylation profiles between the high and the low BMD subjects. The PBM methylome profile shows similar patterns as that in other somatic cells. For instance, most of the promoters are unmethylated or partially methylated, especially for promoters with high CpG density. In addition, while only 9% of promoter CGIs are methylated, over 30% of intragenic and intergenic CGIs are methylated. A total of 122 regions showed significant difference ($p \leq$ 1E-5, fold change$\geq$ 2) in methylation levels between the high and the low BMD groups, such as the promoter of SNTB1 gene (p<1E-6, fold change=3.6) and the promoter of BLVRA gene (p<1E-6, fold change=4.5). Interestingly, a number of known bone-related genes also showed differential methylation between the high and the low BMD subjects, such as the BMP7 gene (p=7.4E-5, fold change=10.4) and the RUNX2 gene (p=3.0E-5, fold change=2.3). Hierarchical clustering analysis using the top 100 genome-wide DMRs clearly separates the 18 samples into two distinct groups that are consistent with their BMD status. In summary, we conducted the first genome-wide DNA methylation analysis for PBMs and identified a number of novel epigenetic-regulated candidate genes for osteoporosis risk.

678T

Study design considerations to improve power in association tests for rare variants. *I. Ruczinski, R. Mathias.* Johns Hopkins Univ, Baltimore, MD.

The assumption that common complex diseases are attributable in part to allelic variants that are reasonably common in a population is often termed the “common disease, common variant” hypothesis, and is the underlying rationale for genome-wide association studies (GWAs). While GWAs have been successful identifying hundreds of such genetic variants associated with many complex diseases, the individual variants typically only represent a small increment in risk for any particular disease, and together, can usually explain only a small proportion of the familial clustering (heritability) observed. Thus, the paradigm has shifted somewhat towards whole exome and whole genome sequencing approaches to assess the effects of rare variants (with possibly larger effect sizes), which are poorly tagged by standard genotyping arrays. In this presentation, we focus on family and population based study design considerations, and show how family records can be leveraged to improve power even in population based studies.

679T

Detection of identity by descent using next-generation sequencing data. *S. Su, E. Jorgenson.* Ernest Gallo Clinic and Research Center, UCSF, Emeryville, CA.

Identity by descent (IBD) has played a fundamental role in the discovery of human disease genes. Both pedigree-based and population-based linkage analyses rely on estimating recent IBD, and evidence of ancient IBD can be used to detect population structure in genetic association studies. Various methods for detecting IBD, such as those implemented in the software programs fastIBD and GERMLINE, have been proposed for use with population genotype data from microarray platforms. Now, next-generation DNA sequencing data is becoming increasingly available, enabling the comprehensive analysis of genomes, including identifying rare variants. These sequencing data may provide an opportunity to detect IBD with higher resolution than previously possible, potentially enabling the detection of disease causing loci that were previously undetectable with sparser genetic data. Here, we conduct a comprehensive evaluation and comparison of these methods for detecting IBD using sequencing data. We investigate the resolution of detectable IBD for data with different levels of variant coverage. This includes micorarray genotype data from the WTCCC study, denser genotype data from the HapMap Project, low coverage sequencing data from the 1000 Genomes Project, and deep coverage complete genome data from our own projects. With high power, we can detect segments of length 0.4 cM or larger using fastIBD and GERMLINE in sequencing data. This compares to similar power to detect segments of length 2.0 cM or higher with microarray genotype data. We find that GERMLINE has slightly higher power than fastIBD for detecting IBD segments using sequencing data, but also has a much higher false positive rate. We further quantify the effect of variant density, conditional on genetic map length, on the power to resolve IBD segments. The results of our analysis may help guide the design of future next generation sequencing studies that utilize IBD.

680T

Genome-wide association study of African Americans implicates multiple lung and inflammatory disease-associated loci in sarcoidosis susceptibility. *I. Adrianto[1], C.P. Lin[1], J.J. Hale[1], A.M. Levin[2], I. Datta[2], R. Parker[1], A. Adler[1], J.A. Kelly[1], K.M. Kaufman[1,3,4], C.J. Lessard[1], K.L. Moser[1,3], M.C. Iannuzzi[5], B.A. Rybicki[2], C.G. Montgomery[1].* 1) Arthritis and Clinical Immunology Research 1Arthritis and Clinical Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104, USA; 2) 2Department of Biostatistics and Research Epidemiology, Henry Ford Health System, Detroit, MI 48202, USA; 3) Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA; 4) Oklahoma City VA Medical Center, Oklahoma City, OK 73104, USA; 5) Department of Medicine, SUNY Upstate Medical University, Syracuse, NY 13210, USA.

Sarcoidosis is a systemic granulomatous disease characterized by the formation of granulomas mainly in the lungs, but also in multiple organ systems. While the etiology of this disease remains elusive, the causal chain likely involves a dysregulated immune response to an environmental agent in a genetically susceptible host. This study sought to identify loci associated with sarcoidosis in African Americans using a large cohort of both families and independent cases and controls. We genotyped 1487 cases and 1504 controls using the Illumina HumanOmni1-Quad platform for 1.1 million SNPs across the genome. After applying sample and SNP quality control, the final set comprised 2918 samples (1273 cases and 1645 controls) including 180 HapMap YRI and ASW controls from the Illumina iControlDB and 887,296 SNPs. We assessed single SNP association to sarcoidosis using the Efficient Mixed-Model Association eXpedited (EMMAX) software that simultaneously controls for both pairwise genetic relatedness between individuals and population stratification under an additive model. Regions associated at a suggestive level of $p < 1 \times 10^{-4}$ were imputed using IMPUTE2 program and the 1000 Genomes Project reference panels. We identified 5 regions associated at the genome-wide significance level of $p < 5 \times 10^{-8}$ including the previously identified HLA-DRA and HLA-DRB1, and novel effects at NOTCH4 ($p = 4.30 \times 10^{-9}$), HLA-DQA1 ($p = 1.04 \times 10^{-11}$), and HLA-DQB1 ($p = 3.12 \times 10^{-10}$). Ninety-eight regions were associated at a suggestive level and 12 were associated with other inflammatory or lung diseases including FRMD3 ($p = 1.39 \times 10^{-6}$), PRDM1 ($p = 1.07 \times 10^{-5}$), and DMBT1 ($p = 8.70 \times 10^{-5}$). These genes have been associated with lung cancer (FRMD3) and inflammatory bowel disease (PRDM1 and DMBT1). We also found association within sarcoidosis susceptibility loci: BTNL2 ($p = 1.24 \times 10^{-5}$) and near RAB23 ($p = 9.47 \times 10^{-5}$). Pathway analysis of these data highlighted the calcium-dependent phospholipid binding pathway, which contained two genes (DOC2A and MCTP2) with $p < 1 \times 10^{-4}$ and seven of the Annexin (ANX) genes, one of which, ANXA11, has been associated with sarcoidosis. In this study, we expanded the number of putative candidate sarcoidosis susceptibility loci and replicated sarcoidosis associations in multiple gene regions. Future replication and sequencing studies are required to further elucidate the functional variants that may underlie these novel associations.

681T

Fine-scale association mapping of the xMHC-region in celiac disease cases and controls. *R.S. Ahn[1], A. Adamson[2], X. Deng[2], H. Gao[2], C. Garner[1], S. Neuhausen[2].* 1) Epidemiology Department, Sprague Hall, Room 318, University of California, Irvine, Irvine, CA 92697-3905; 2) Beckman Research Institute of City of Hope, 1500 E. Duarte, Duarte, CA 91010.

Celiac disease (gluten-sensitive enteropathy) is a chronic inflammatory disease of the small intestine caused by an autoimmune response to ingestion of dietary gluten peptides. It is a common disease with a prevalence of approximately 1% in populations of European descent. Classic symptoms include diarrhea, abdominal distension, as well as developmental delays in affected children. The majority of celiac cases present with minimal classic symptoms. Complications include anemia and osteoporosis, as well as other autoimmune disorders. The only known treatment is to remove all gluten from the diet. Multiple studies have shown a strong association with human leukocyte antigen (HLA) as well as associations with 39 non-HLA risk loci. More than 90% of celiac disease cases express the HLA-DQ2 heterodimer and 5% express HLA-DQ8. The alleles that encode for HLA-DQ2 are HLA-DQA1*05 and HLA-DQB1*02 and HLA-DQ8 is encoded for by HLA-DQA1*03 and HLA-DQB1*0302. We have carried out a fine-scale association analysis of the 7.6 Mb extended major histocompatibility region (xMHC) on chromosome 6p using a set of 1,898 single nucleotide polymorphisms (SNPs) genotyped in 1,695 celiac disease cases and 520 controls from the U.S. The purpose of the analysis was to search for evidence of novel genetic determinants of celiac disease within the xMHC that are independent of the known high-risk HLA-DQ genotypes. Association analysis of all 1,898 SNPs was followed by SNP selection based on linkage disequilibrium and the rank-order of p-values in order to generate a minimal set of SNPs that appear to have independent effects on celiac disease risk. We present the genetic and statistical evidence for novel celiac disease susceptibility loci within the xMHC. Furthermore, the study highlights the analytical challenges and pitfalls of fine-scale association mapping within the xMHC, where known strong disease risk genotypes, complex linkage disequilibrium patterns and extensive genetic variation predominate.

682T

Common Variants Identified by Meta-Analysis of 110,238 Individuals Associate with Serum Urate Concentrations. *E. Albrecht[1], A. Köttgen[2,3], A. Teumer[4], G. Pistis[5], V. Vitart[6], C. Hundertmark[2], D. Ruggiero[7], T. Tanaka[8], Q. Yang[9], T. Haller[10], J.C. Chambers[11], A. Tin[3], W.H. Kao[3], C.S. Fox[12], C. Gieger[1], CARe Consortium, Global Urate Genetics Consortium.* 1) Institute of Genetic Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany; 2) Renal Division, Freiburg University Hospital, Freiburg, Germany; 3) Dept. of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, USA; 4) Interfaculty Institute for Genetics and Functional Genomics, University of Greifswald, Germany; 5) San Raffaele Scientific Institute, Division of Genetics and Cell Biology, Milan, Italy; 6) MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, Edinburgh, UK; 7) Istitute of Genetics and Biophisics “Adriano-Buzzati Traverso”, CNR, Napoli, Italy; 8) Clinical Research Branch, National Institute on Aging, Baltimore, USA; 9) Department of Biostatistics, Boston University, Boston MA, USA; 10) Estonian Genome Center, University of Tartu, Estonia; 11) Dept Epidemiology and Biostatistics, School of Public Health, Imperial College London, UK; 12) National Heart, Lung, and Blood Institute, Framingham, USA.

Elevated urate levels are associated with gout and are considered a risk factor for cardiovascular diseases, metabolic syndrome and type II diabetes. To investigate the polygenic basis of serum urate levels, we conducted a meta-analysis of genome-wide association studies of approximately 2.5 million genotyped or imputed SNPs, combining data from 48 studies, totalling 110,238 participants of European descent. The detected loci were subsequently investigated among study participants of different ancestries from independent studies. We identified 26 independent loci reaching genome-wide significance at a level of 5×10^{-8} in the discovery analysis, including all previously reported loci, as well as 16 novel loci. The effects of the discovered risk alleles on urate concentrations ranged between 0.04 ($p=7.3 \times 10^{-9}$, *ORC4L*) and 0.38 ($p=1.2 \times 10^{-786}$, *SLC2A9*) mg/dl. In addition to the known sex-specific effects of variants in *SLC2A9* and *ABCG2*, no additional gender associations were identified. The strongest signals, in or near *SLC2A9*, *ABCG2*, *SLC17A1*, *GCKR* and *SLC22A11* also showed robust and direction-consistent associations in 9,867 participants of Indian ancestry () =0.10-0.39, $p=5.0 \times 10^{-8}$-4.3×10^{-21}). In a lookup among 7,129 African American participants from the CARe consortium, only variants at *SLC2A9* () =0.30, $p=8.62 \times 10^{-29}$) and *SLC22A11* () =0.15, $p=2.16 \times 10^{-4}$) showed significant associations. However, the effects of the risk alleles among European ancestry participants were consistent in their direction for 21/24 loci among Indian ancestry and 21/25 loci in African American participants. Taken together, these newly identified associations highlight additional genetic regions that are associated with serum urate levels across groups of different ancestries.

683T

Performance of different balancing score methods in case-control genetic association studies. *A. Barhdadi[1], MP. Dubé[1,2].* 1) Montreal Heart Institute, Montreal, Quebec, Canada, PHD; 2) Faculté de Médecine, Université de Montréal.

Background Confounding can be a major source of bias in non-experimental research. The propensity score is a popular method to control for confounding in prospective observational studies. Its counterpart for the case-control design is the stratification score which is a retrospective balancing score. Our study examines the performance of different balancing score methods in case-control genetic association studies. Methods and Results Three different methods for the stratification score have been described in the literature: stratification on the balancing score (SB), matching on the balancing score (MB) and covariate adjustment using the balancing score (CAB). We compared the performance of SB, MB and CAB to simple covariate adjustment with 1000 simulated datasets of 4000 subjects. We randomly generated replicates of 3 continuous covariates and 3 binary covariates that were imbalanced between cases and controls. For each of the 4000 subjects, we simulated genotypes at 9 single nucleotide polymorphisms (SNPs) of varying the minor allele frequency (MAF) and odds ratios (OR). We calculated the stratification score using 5 strata. Each of the 3 methods were evaluated for their ability to identify the simulated genetic effect based on the mean relative bias (distance of the method OR estimator to the true value of OR divided by OR). We find that the power to detect the causal SNP was 100% for all methods with a MAF of 0.30. Type I error rate was (0.053, 0.049 and 0.053) for CAB, SB and MB respectively. The relative bias mean was (0.08±0.07, 0.08±0.06, 0.12 ±0.09). With MAF of 0.05, the power to detect the associated SNP was 49.9%, 52.8%, and 16.4% for CAB, SB and MB respectively. Type I error rate was 0.014, 0.024 and 0.010and the relative bias mean was 0.58 ±0.83, 0.58+0.80, 0.62 ±0.69 for CAB, SB and MB respectively. Conclusion CAB, SB and MB methods outperformed simple covariate adjustment to identify a simulated genetic factor at varying levels of MAF and OR. The SB method had more power, lower false positive error rate and lower relative bias when used to adjust for covariates in the context of a case-control genetic association study. Keywords Association, Propensity score, Balancing score, Case-control, SNP.

684T

Replication and novel suggestive associations in meta-analyses of lipid traits in Mexican and Mexican Americans cohorts from Mexico City and Starr County Texas, as well as tissue specific enrichment of expression quantitative trait loci among top signals. *J.E. Below[1], E.J. Parra[2], E.R. Gamazon[3], J. Escobedo[4], A. Valladares[5], J. Garcia-Mena[6], P.M. McKeigue[7], J. Kumate[8], C. Liu[9], D.M. Hallman[10], D.L. Nicolae[1,3,11,12], G.I. Bell[1,12], N.J. Cox[1,3,12], C.L. Hannis[10], M. Cruz[5].* 1) Dept Human Gen, Univ Chicago, Chicago, IL; 2) Department of Anthropology, University of Toronto Mississauga, Ontario, Canada; 3) Section of Genetic Medicine, Univ Chicago, Chicago, IL; 4) Unidad de Investigacion en Epidemiologia Clinica. Hospital General Regional 1, “Dr Carlos McGregor”, IMSS, Mexico; 5) Unidad de Investigacion Medica en Bioquimica, Hospital de Especialidades, Centro Medico “Siglo XXI”, IMSS, Mexico; 6) Departamento de Genetica y Biologia Molecular, Cinvestav-IPN, Mexico; 7) Public Health Sciences Section, Division of Community Health Sciences, University of Edinburgh Medical School, Edinburgh, UK; 8) Fundacion IMSS, Mexico; 9) Dept of Psychiatry and Behavioral Neuroscience, Univ Chicago, Chicago, IL; 10) Human Genetics Center, The University of Texas Health Science Center at Houston, Houston, Texas, USA; 11) Dept Statistics, Univ Chicago, Chicago, IL; 12) Dept Medicine, Univ Chicago, Chicago, IL.

At least 95 loci have been associated with plasma lipid traits in European populations. Importantly, the common variants identified using large-scale GWA studies explain a substantial amount of the total variance of these traits (10-12% of the total variance, which represents approximately 25-30% of the genetic variance). These efforts have led to the discovery on new regulatory pathways in lipid metabolism, opening new potential targets for therapeutic intervention, and demonstrated the relevance of the GWA approach from the clinical point of view. We report here the results of the first meta-analysis of GWA studies for lipid traits (LDL-C, HDL-C, Total-C and Triglycerides) in populations of Mexican ancestry. We identified several previously identified loci with genome-wide significant associations, as well as other regions showing suggestive associations (p-value< 10-5). Top associations include genome-wide significant signals predicting LDL-C within the 3-prime untranslated region of the CELSR2 gene (rs964184, p= 1.2x10-9). In the analysis of Total-C, we approach genome-wide significance in the same region (rs7528419, p=5.9x10-8). We identify genome-wide significant signals for triglycerides at SNPs in a known region: 116.0-116.3 Mb; the lowest p-value falls between ZNF259 and BUD13 (rs964184, p= 2.3x10-19. Suggestive associations replicate regions APOB, DOCK6, and TRIB1. We also identify suggestive evidence in several novel regions including TNS1, SPOCK3, EPHA7, MNT, KCNJ15, and RUNX1, as well as several intergenic regions. To explore the functional relevance of our top findings, we conducted simulations to test for an enrichment of eQTLs (p-value < 1×10-4) among the top signals (p-value < 1×10-3) associated with lipid levels by generating 1000 random SNP sets, each of the same size as the original list of LD-pruned top signals (r2>0.30), containing variants matched on MAF distribution, sampled without replacement. We observe tissue specific enrichment of eQTLs for each lipid phenotype.

685T

Genome wide association study identifies several regions associated with malignant mesothelioma susceptibility. *G. Cadby[1,2], S. Mukherjee[3], A. Reid[4], M. Garlepp[4,5], B.W.S. Robinson[4], N. de Klerk[4,5], A.W. Musk[3,4], L.J. Palmer[1,2].* 1) Ontario Institute for Cancer Research, Toronto, Ontario, Canada; 2) Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada; 3) Sir Charles Gairdner Hospital, Perth, Western Australia; 4) University of Western Australia, Perth, Western Australia; 5) Telethon Institute for Child Health Research, Perth, Western Australia.

Malignant mesothelioma (MM) is a malignant tumour which arises from the single layer of mesothelial cells which line the pleural and peritoneal cavities. The main environmental risk factor for MM is exposure to asbestos, however only 5-10% of individuals exposed to high levels of asbestos develop MM, indicating the role of genetic variation in MM predisposition. Despite the evidence of genes in MM predisposition, there has been little consistency in the published findings from genetic studies of MM. The aim of this study is to perform a genome wide association study (GWAS) of MM cases and population-based controls to identify genetic variants associated with MM susceptibility.

Clinical data and DNA samples were obtained from individuals diagnosed with MM who attended a hospital clinic in Perth, Western Australia (WA) between 1995 and 2010. Case status was confirmed via the WA Mesothelioma Registry and expert review of clinical notes. Control DNA samples were obtained from a general population cohort of residents from the town of Busselton, Western Australia, who were part of the 1994/1995 Busselton Health Study. Genotyped and imputed single nucleotide polymorphisms (n~2.5 million) were compared between MM cases and controls using mach-2dat. All models were adjusted for age and sex. Adjustment for population stratification was made by including significant eigenvectors as covariates in the regression.

The GWAS has been performed on 428 individuals with MM (89% male) and 1,269 general population controls (42.6% male). Mean age of cases was 67 years (±10 years SD) compared to 54 years (±17 years SD) for controls. All cases and controls were confirmed as Caucasian-European. Preliminary analyses have identified several genes which appear to be associated with MM susceptibility, including SDK1 and RASGRF2.

This is the first GWAS for MM. Despite the limited sample size, several regions have been identified which may increase the risk of developing MM. Further analyses are planned, including using asbestos exposed controls, analysing gene-environment interactions in cases, and replicating results in other MM samples. These results will improve our understanding of the complex relationships between genes and asbestos, and their role in MM susceptibility, progression and response to therapy.

686T

Integrated analysis of variants and pathways for genome-wide association studies. *P. Carbonetto, M. Stephens.* Depts of Statistics and Human Genetics, University of Chicago.

Motivated be the shortcomings of conventional, “hypothesis-free” approaches to the analysis of genome-wide association studies, researchers have developed “pathway analysis” methods, grounded on the theory that complex diseases arise from the accumulation of genetic effects acting in common biological pathways. The aim of pathway analysis is to identify pathways that are “enriched” for disease. This can give us greater ability to identify relevant genetic factors, and can enhance interpretation of an association study. But pathway analysis does not give us feedback about associated variants within pathways; findings at the level of variants (``what are the variants associated with disease risk?'') are often more useful than results at the level of pathways (``what are the enriched pathways?''). We address this limitation by jointly analyzing variants and pathways in a genome-wide association study. We take a Bayesian approach to joint analysis because it offers a coherent way to resolve the interdependency of associations and enrichments. We demonstrate joint analysis of variants and pathways for genome-wide association studies of two chronic autoimmune disorders, Crohn's disease and type 1 diabetes.

687T

Identification of Genetic Variants Related to Hepatitis B Virus Infection in Hepatocellular Carcinoma Families and Chronic Liver Diseases in Han Chinese. *S. Chang[1], C. Hsu[1], A. Hsieh[1], C. Fann[1], D. Tai[2].* 1) IBMS, Academia Sinica, Taipei, Taiwan; 2) Liver Research Unit, Chang Gung Memorial Hospital, Chung Gung University College of Medicine, Taipei, Taiwan.

Hepatitis B is a major health problem worldwide and a potentially life-endangering liver infection. Previous studies have shown that male gender was a significant host factor related to hepatitis severity. However, a large part about the biomedical mechanism of persistence and protection for HBV infection is still unknown. In this study, we conducted a multi-stage genome-wide association analysis on 625 male Taiwanese. The first-stage sample comprised 321 HBsAg positive cases and 304 HBsAg negative controls with genotype data from Illumina HumanHap550K and 610K beadchips. A total of 456,262 SNPs were used in the GWAS analysis after quality control filters with an average call rate of 99.95%. We performed single-locus association tests and identified 61 SNPs that were potentially associated with persistent HBV infection (P < 10-5). Among those, 38 out of the 61 markers were clustering in the HLA Class II region on chromosome 6. One SNP at HLA-DQB2 locus was replicated in the two independent cohorts (with 410 cases/282 controls and 709 cases/419 controls, respectively). The underlying function remains uncertain and requires further investigation.

688T

Population analysis of asthma genome-wide association data using GenAMap. *R.E. Curtis[1,2], S. Wenzel[4], D.A. Myers[5], E. Bleecker[5], E.P. Xing[3].* 1) Joint Carnegie Mellon - University of Pittsburgh PhD Program in Computational Biology, Carnegie Mellon University, Pittsburgh, PA; 2) Lane Center for Computational Biology, Carnegie Mellon University, Pittsburgh, PA; 3) Machine Learning Department, Carnegie Mellon University, Pittsburgh, PA; 4) University of Pittsburgh Medical Center Montefiore, Pittsburgh, PA; 5) Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC.

Structured association mapping has the potential to discover weaker associations by leveraging structures inherent in GWAS and eQTL data. Population stratification is one such structure that must be considered in any genome-wide association study. Although geneticists frequently analyze association strengths between different populations in GWAS data, this is often done one population at a time as geneticists are limited by the lack of tools and strategies available to compare results between populations. In order to facilitate population analyses, we added new analysis and visualization tools into GenAMap, a visual analytics software platform for structured association mapping. We integrated association mapping methods including the Multi-population Group Lasso (MPGL), Wald test (plink), likelihood ratio test, and t-test. New interactive charts, tables, and plots provide both an overview and details of the associations in a population analysis, which allow geneticists to analyze and compare association strengths between different populations and tests. We used GenAMap to perform a structured association analysis using data collected through the Severe Asthma Research Program (SARP) and Cooperative Study for the Genetics of Asthma (CGSA). Both datasets include individuals from African American and non-Hispanic white populations. As has been previously appreciated, population analysis using GenAMap also revealed that the two populations have different patterns of association. Among the top 50 SNPs associated with asthma in each population, none of the SNPs were associated with asthma in the other population. These results suggest that different SNPs are affecting the acquisition of asthma in the two populations. Despite a large number of SNPs associated with asthma at the 1e-4 significance level, we used GenAMap to look at each population separately. By comparing the results from four tests, we identified twelve SNPs associated with asthma. Finally, we used GenAMap to run MPGL to find associations while accounting for population structure. We found support for an association between the SNP rs7661051 and the clinical lung function trait “Forced Vital Capacity (FVC), % predicted” in the non-Hispanic white population. Cases have a lower level of FVC (p-value=4.1e-17), thus rs7661051 could potentially impact asthma in non-Hispanic whites with effects to alter FVC. rs7661051 is located on chromosome 4 in a non-coding region near the genes CXCL13 and CCNG2.

689T

A novel variant in the MCF2L gene is associated with osteoarthritis. *A.G. Day-Williams on behalf of the arcOGEN Consortium and the replication data sets.* Human Genetics, Wellcome Trust Sanger Institute, Hinxton, United Kingdom.

Osteoarthritis (OA) is the most common form of arthritis, with the sibling recurrence risk (λ_s) estimated to be approximately 5 in the UK. We recently completed stage 1 of a large UK-based genome-wide association (GWAS) scan of knee and/or hip OA (the arcOGEN study) and detected no novel replicating signals. Here, we used 1000 Genomes Project (1KGP) pilot 1 data of 60 CEU individuals as a reference set and imputed 1KGP-identified variants into the stage 1 arcOGEN GWAS of 3,177 cases and 4,894 UK controls. After removing rare variants (with minor allele frequency [MAF] < 0.01) and SNPs with low imputation quality, 7,258,070 variants were tested for association with OA. We selected eight SNPs with $p < 1x10^{-5}$ from six loci for validation in the original arcOGEN data, and for de novo replication in independent sample sets. We first genotyped an independent set of 5,165 arcOGEN-collected cases and 6,155 population-based controls from the UK. Seven out of the eight SNPs were successfully genotyped in the replication samples and one SNP, rs11842874 on chromosome 13q34, replicated with $p=2.60x10^{-3}$ (OR 1.17 [1.06-1.30]) and consistent effect direction as the original scan. We subsequently sought replication of this signal in 6 further sample sets: the UK-based GOAL study (1,686 total joint replacement cases, 743 non-OA controls); newly-collected UK-based arcOGEN data (2,409 cases, 2,319 population-based controls); two from the Netherlands (RSI: 1,950 cases and 3,243 controls; RSII: 485 cases and 1,460 controls); one from Estonia (EGCUT: 2,617 cases and 2,619 controls); and one from Iceland (deCODE: 1,552 cases and 3,071 controls). In all seven replication sets combined, rs1184874 associated with OA with $p=3.0x10^{-5}$ (OR 1.13 [1.07 - 1.20]). Combined with the discovery sample set, the overall fixed effects meta-analysis (19,041 cases and 24,504 controls) established association at this variant with $p=2.07x10^{-8}$ (OR 1.17 [1.11-1.23]). rs11842874 is one of several highly-correlated SNPs at 13q34 constituting the association signal and they reside in intron 4 of the guanine nucleotide exchange factor-encoding gene MCF2L. Mcf2l studies in rat models of OA have shown expression in articular chondrocytes. In human cells MCF2L regulates neurotrophin-3 induced cell migration in Schwann cells. Neurotrophin-3 is a member of the nerve growth factor (NGF) family, and inhibition of NGF has shown reduction of pain in knee OA.

690T

Genetic loci implicated in erythroid differentiation and cell cycle regulation are associated with red blood cell traits. *K. Ding[1], K. Shameer[1], H. Jouni[1], D.R. Masys[2], G.P. Jarvik[3], A.N. Kho[4], M.D. Ritchie[5], C.A. McCarty[6], I.J. Kullo[1].* 1) Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN; 2) Departments of Medicine and Biomedical Informatics, Vanderbilt University, Nashville, TN; 3) Departments of Medicine (Medical Genetics) and Genome Sciences, University of Washington, Seattle, WA; 4) Department of Medicine, Northwestern University, Chicago, IL; 5) Center for Human Genetics, Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN; 6) Center for Human Genetics, Marshfield Clinic Research Foundation, Marshfield, WI.

Disorders involving red blood cells (RBC), such as anemia, are common and associated with adverse health outcomes. We performed a genome-wide association study for six RBC traits, including hemoglobin (HGB), hematocrit (HCT), RBC count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), in 12,486 patients of European ancestry in the electronic Medical Records and Genomic (eMERGE) network (www.gwas.net). We developed an electronic medical record-based algorithm which included individuals who had RBC measurements obtained for clinical care, and excluded RBC trait values measured in the setting of hematopoietic disorders, comorbid conditions or medications known to affect red cell production, or a history of blood loss. We identified six novel genetic loci associated with at least one RBC trait at $P<5\times10^{-8}$ in individuals of European ancestry and replicated nine previously reported loci. Notably, four of the six newly identified loci (*KLF1*, *THRB*, *PTPLAD1*, *CDT1*) and four of the nine replicated loci (*CCND3*, *SPTA1*, *FBXO7*, *TFR2/EPO*) are implicated in erythroid differentiation and regulation of cell cycle in hematopoietic stem cells. These findings provide insights into the molecular basis underlying variation in RBC traits, in particular highlighting that genes in the erythroid differentiation and cell cycle regulation pathways influence such variation.

691T

Strategy for genotype imputation in Hispanic-Americans in the Women's Health Initiative SNP Health Association Resource (WHI-SHARe). *J. Divers[1], W.M. Brown[1], L. Yang[1], A.P. Reiner[2,3], T.A. Thornton[4], Y. Li[5], H. Tang[6], M.Z. Vitolins[7], B.M. Snively[1].* 1) Dept Biostatistical Sci, Wake Forest Univ, Winston-Salem, NC; 2) Department of Epidemiology,University of Washington, Seattle, WA; 3) Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 4) Department of Biostatistics, University of Washington, Seattle, WA; 5) Department of Genetics, University of North Carolina Chapel Hill, Chapel Hill, NC; 6) Department of Genetics, Stanford University, Stanford, CA; 7) Dept Epidemiology, Wake Forest Univ, Winston-Salem, NC.

Questions remain about the number and combination of reference panels to use for genotype imputation in admixed populations. Imputation in Hispanic-Americans is particularly challenging because of the ancestral diversity that typically underlies this group. We compared the performance of various imputation strategies using different combinations of the CEU, YRI, MEX and JPT+CHB panels in HapMap3. Comparisons were made using chromosome 22 markers, where 9,584 SNPs were genotyped as part of WHI-SHARe; we also randomly masked 25%; of the genotyped markers. SNPs were imputed using each panel separately and all 6 two-panel combinations. Finally, we considered the union of the best performing combinations, which led to a three-panel reference and evaluated its benefits over the two-panel combinations. We used two metrics for judging the performance of each combination: percentage of SNPs that had $R^2 \geq 0.5$, and concordance rate between directly genotyped and imputed SNPs among those with $R^2 \geq 0.5$. We ran MACH on 3,586 self-reported Hispanic participants, and on more homogenous subsets of individuals, which were created from estimated ancestry proportions. We defined 11 subsets whose composition ranged from having individuals with at least 85%; ancestry from one ancestral group, to subsets of individuals with substantial ancestry from all 4 groups. When a single HapMap panel was considered, the percentage of SNPs with $R^2 \geq 0.5$ and the concordance between genotyped and imputed SNPs with $R^2 \geq 0.5$ were (88%, 99.2%), (88.8, 98.1), (90.8, 99.3) and (81, 98.3) using respectively the CEU, YRI, MEX and JPT+CHB panel. All two-panel combinations led to at least 90%; of the SNPs with $R^2 \geq 0.5$ and concordance of at least 99.5% The (YRI, CEU) and (YRI, MEX) had the best performance with (99, 99.7) and (99.3, 99.6), respectively. The (YRI, MEX, CEU) panel led to 99.7%SNPs with $R^2 \geq 0.5$ and concordance of 99.8% for a small improvement in performance. As expected, these metrics decreased somewhat in the masked set, with (92.2, 96.7) for (YRI, MEX, CEU). The three-panel combinations required 3 times the RAM space and took 3 times longer to run than any of the two-panel combinations. Performing the imputation on the more homogenous subsets did not affect the concordance in most sets, which suggests that the (YRI, MEX) and the three-panel combination would lead to reliable genotype imputation in many Hispanic-American populations. These results need validation in other study populations.

692T

Improving the power of genomewide association scans through two-step approaches using external summary data. *F. Dudbridge[1], J.M. Wason[2].* 1) London School of Hygiene and Tropical Medicine, London, United Kingdom; 2) Medical Research Council Biostatistics Unit, Cambridge, United Kingdom.

Two step approaches have been proposed to increase the power of genomewide association scans in some specific settings. In the first step a liberal significance threshold is applied to a test statistic that may not be robust to model assumptions. SNPs that pass this threshold are then analysed using an unbiased test in the second step, which is independent of the first step and has a reduced multiple testing burden. This approach has been developed for specific applications, in particular for family-based association and gene-environment interaction. We give a general formulation of this strategy in terms of pairs of estimators of an underlying parameter, which allows the idea to be extended to a very wide range of analyses. We show how the power of case/control studies can be improved by using external estimates of allele frequencies in the first step. This approach is robust to population differences between the external data and the study at hand, though we find that fairly small differences lead to lower power relative to a standard analysis. An illustration using the WTCCC1 data shows several regions, subsequently validated, which reach genomewide significance in the two-step approach but do not in a standard analysis. With the advent of biobanks and repositories for sharing large scale summary data, two-step analysis will become an important option for all types of exploratory study.

693T

Bayesian Hierarchical Modelling of SNPs and Pathways for Identifying Associated Pathways. *M. Evangelou[1], F. Dudbridge[2], L. Wernisch[1].* 1) MRC-Biostatistics Unit, University of Cambridge, Cambridge, Cambridgeshire, United Kingdom; 2) Department of Non-communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine, University of London, London, United Kingdom.

Pathway analysis incorporates the available biological knowledge of SNPs and genes for revealing the underlying genetic structure of the studied phenotype. Several methods have been proposed for identifying pathways associated with a phenotype. Some of these methods combine the pathway SNP p-values into a single pathway p-value, for example Fisher's Method (FM) and Tail Strength Measure (TSM). Other pathway analysis methods look for enrichment of pathways within the list of top ranking SNPs, e.g. Fisher's Exact Test (FET).

We propose a Bayesian Hierarchical framework that includes the pathway membership of SNPs for modelling both pathway level effects and SNP effects within pathways. The phenotype of each individual is assumed to depend both on its genotype data and on the sum of its alleles within each pathway. The goal of our framework is to identify pathways associated with the phenotype; SNP parameters are considered as nuisance parameters and integrated out of the analysis. A sparse normal distribution and a standard normal distribution are considered and tested for the pathway parameters.

We carried out a simulation study for testing the performance of the proposed Bayesian Hierarchical framework and comparing it with other methods like FM, TSM and FET. The results of the study show that our proposed framework with a sparse normal distribution for the pathway parameters outperforms the standard normal distribution as well as the other tested methods.Our proposed framework does not rely on the results of single-SNP analysis, unlike the other methods, and uses the advantage of hierarchical modelling to decrease the signal-to-noise ratio.

694T

Genome-wide association scans for ocular axial length of East Asian populations in Singapore. *Q. Fan[1], X. Zhou[1], CY. Cheng[1,2,3], LK. Goh[1,4,5], VA. Barathi[2], CC. Khor[2,6,7,8], MK. Ikram[1,2,4,9], WT. Tay[2], XL. Sim[7], KS. Sim[7], RT. Ong[1,10], KS. Chia[1,7,10], JJ. Liu[6], E. Vithana[2,3], ES. Tai[1,11], T. Aung[2,3], TY. Wong[1,2,3,12], YY. Teo[1,6,10,13], SM. Saw[1,2,3,10].* 1) Department of Epidemiology and Public Health, National University of Singapore, Singapore; 2) Singapore Eye Research Institute, Singapore National Eye Centre, Singapore; 3) Department of Ophthalmology, National University of Singapore, Singapore; 4) Duke-National University of Singapore Graduate Medical School, Singapore; 5) Department of Medical Oncology, National Cancer Centre Singapore, Singapore; 6) Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore; 7) Centre for Molecular Epidemiology, National University of Singapore, Singapore; 8) Department of Pediatrics, National University of Singapore; 9) Department of Ophthalmology, Erasmus Medical Center, Rotterdam, the Netherlands; 10) NUS Graduate School for Integrative Science and Engineering, National University of Singapore, Singapore; 11) Department of Medicine, National University of Singapore, Singapore; 12) Centre for Eye Research Australia, University of Melbourne, Australia; 13) Department of Statistics and Applied Probability, National University of Singapore, Singapore.

Ocular axial length (AL) is an endophenotype and major contributor of myopia, which causes vision loss and poses a significant public health concern. AL is highly heritable but the genetic etiology remains unknown. So far, two linkage scans found AL is linked to chromosome 2p24 and 5q; whereas no genome-wide association study (GWAS) has been reported. We conducted a meta-analysis of three GWAS for AL quantitative trait on 3,540 Singaporean individuals including 2,155 Malay adults, 928 Chinese children and 457 Chinese family trios (n=1,163). 460,528 autosomes SNPs genotyped by Illumina 550-Duo or 600-Quad BeadArray were present for the three cohorts after stringent quality control. We identified a significant association of common variants on chromosome 2p13 ($P_{meta} = 3.27 \times 10^{-8}$). The association between the identified SNPs and refractive error remained significant through candidate gene approaches ($P_{meta} = 2.14 \times 10^{-4}$). Further functional analysis revealed that the associated genetic locus encompassing top SNPs significantly up-regulated the myopia progression in the induced mouse model ($P = 7.1 \times 10^{-12}$, fold change of gene expression = 2.16). Our data suggest common genetic variants on chromosome 2p13 might influence susceptibility for ocular axial length and myopia.

695T

An Empirical Approach for Inferring Polygenic Contributions in Genome-wide Case-Control studies. *S. Feng[1], A. Mulas[2], R. Nagaraja[3], C. Sidore[2], M. Uda[2], S. Sanna[2], F. Cucca[4], D. Schlessinger[3], G.R. Abecasis[1], H.M. Kang[1].* 1) Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, MI; 2) Istituto di Neurogenetica e Neurofarmacologia del CNR, Cagliari, Italy; 3) Laboratory of Genetics, National Institute on Aging, Baltimore, MD, USA; 4) Dipartimento di Scienze Biomediche, Università di Sassari, Sassari, Italy.

Variance component models have been successfully applied to family-based quantitative trait analysis such as linkage studies and heritability estimation for many decades. The availability of high-density genotype data allows us to more precisely estimate the subtle genetic relatedness between reportedly unrelated individuals, and these information has been successfully used in inferring polygenic contributions from genome-wide studies of complex quantitative traits such as human heights or lipid levels via variance component models. Estimation of polygenic contributions from dichotomous traits, however, has been more challenging, because of the heavy computational burden required to make inferences under generalized linear mixed model (GLMM) for large data sets. Lee et al (2011) suggested an approach to estimating polygenic contributions using linear mixed model (LMM) and analytically adjusting it to be unbiased under probit-normal GLMM. We propose a flexible simulation-based approach to estimate polygenic contributions to dichotomous traits under GLMM. Our method can handle a wide range of generative models including logit-nomal and probit-normal GLMMs with an arbitrary ascertainment procedure. Our method empirically adjusts estimates from LMM into GLMM via computationally efficient simulations. Although inference under LMM is still in general computationally demanding, our method leverages the fact that given the spectral decomposition of variance components, parameter estimation under LMM can be reduced into linear time complexity, expediting the inference in orders of magnitude than a naïve approach. We evaluated our approach using extended families of >6,000 Sardinians genotyped with the Metabochip at 196,725 SNPs. Simulations under various distributions of causative loci and their effect sizes, across both logit-normal and probit-normal models, demonstrate that our method is accurate and computationally efficient in estimating polygenic contributions of complex dichotomous traits via a variance component model.

696T

Assessing the causes of heterogeneity in GWAS by combining various eQTL analyzes. *T. Flutre[1,2], X. Wen[1,3], M. Stephens[1,3].* 1) Human Genetics, University of Chicago, Chicago, IL; 2) Plant Breeding, Institut National de la Recherche Agronomique, France; 3) Statistics, University of Chicago, Chicago, IL.

By using intermediate phenotypes, e.g. gene expression, current research in quantitative genetics aim at bridging the gap between genotypes and phenotypes at the organismal level, e.g. disease status. Thanks to high-throughput technologies, such analyzes can be performed genomewide, assessing the association of millions of genetic variants with thousands, or more, phenotypic features. Given the high dimension of such datasets and the low fraction of genetic variants expected to be truly associated, several statistical methods have been proposed, notably Bayesian, emphasizing their advantages over frequentist approaches. As a result, numerous quantitative trait loci have been detected, and insights have been gained on the regulation of gene expression.

However, these results were obtained from various populations and cell types, using data measured on different platforms. As it is desirable to combine different studies to improve power, a Bayesian method for single-SNP analysis has been previously developed in our lab to deal with meta-analysis and gene-by-environment interactions. In this work, we apply this method on several existing datasets, each involving several subgroups, e.g. different populations (Stranger et al, 2007) or different cell types (Dimas et al, 2009). Genotype data from each study are imputed with IMPUTE2, using the same, deep and dense, set of genetic variants combining HapMap3 and the pilot project of the 1000 Genomes. Bayes factors and effect size estimates for each subgroup will be made available, e.g. as a custom track for the UCSC genome browser.

From a biological point of view, the aim of this work is to study the amount and extent of heterogeneity among the effect sizes of genetic variants. Indeed, several questions still remain unanswered, such as which genetic variants are functional in which cell types, and how (dis)similar functional variants are among populations. From a statistical point of view, the aim is to assess the reliability of effect size estimates when combining heterogeneous datasets, and to improve prior specification, e.g. via empirical Bayes approaches capitalizing on available data from the ENCODE project.

697T

eHF-algorithm: a fast and scalable method for hidden factor analysis in eQTL studies. *C. Gao[1], J.G. Mezey[1,2].* 1) Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY; 2) Department of Genetic Medicine, Weill Cornell Medical College, NY, NY.

We present an expression Hidden Factor (eHF-)algorithm for estimating and accounting for unmeasured environmental factors in an eQTL analysis when simultaneously analyzing thousands of expression probes. Our approach is to include factors without a pre-defined loading matrix within a multivariate factor analysis model, where both the hidden factors and the genotypic effects are treated as random effects with the same underlying distribution. We use a likelihood treatment of this model and implement an Expectation-Maximization (EM) algorithm for the simultaneous analysis of genetic associations and factors, where we provide an exact likelihood ratio test for assessing the pleiotropic effects of eQTL. Using simulated data, we demonstrate that our method can correctly account for an unknown number of hidden confounding factors and, compared to a two-step procedure, a simultaneous analysis of genetic markers and hidden factors is essential for reducing the identification of false positives. With our simulation analysis, we also demonstrate that our method has better scaling and performance than a proposed variational Bayes and a mixed model method designed for hidden factor analysis. We also analyzed existing datasets used for eQTL analysis, including the immortalized cell lines harvested from the HapMap individuals and gene expression measurements in human tissues affected by disease, and demonstrate the ability of our method to identify hidden factors and boost performance in the detection of eQTL.

698T

Genotype imputation accuracy for Mexican Americans using the HapMap Phase 3 reference panels. *X. Gao[1], P. Marjoram[2], R. Mckean-Cowdin[2], M. Torres[1], W.J. Gauderman[2], R. Varma[1].* 1) Department of Ophthalmology and Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033; 2) Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033.

Genotype imputation is a vital tool in genome-wide association studies (GWAS) and meta-analyses of multiple GWAS results. Imputation enables researchers to increase genomic coverage and to pool data generated using different genotyping platforms. Based on a reference panel of genetically similar population samples genotyped at a dense set of single nucleotide polymorphisms (SNPs), imputation methods infer genotypes at markers that were not directly typed in a study sample that was genotyped at only a subset of these SNPs. HapMap samples are often employed as the reference panel. Multiple GWAS are targeting Mexican Americans (MA), the most populous and fastest growing minority group in the US. Some consortia are also planning to meta-analyze the MA GWAS results. However, genotype imputation resources for MA are rather limited compared to European Americans at present, largely because of the lack of good reference data. One choice of reference panels for MA is one derived from the population of MA in Los Angeles contained in the HapMap Phase 3 project (MXL). However, a detailed evaluation of the quality of the imputed genotypes derived from the MXL reference panels has not yet been reported. Using the Los Angles Latino Eye Study (LALES) pilot GWAS samples genotyped using the Illumina OmniExpress BeadChips (~733K markers), and simulation studies, we evaluated the accuracy of genotype imputation in MA samples based on the MXL reference panel. Our results will provide insight for genotype imputation in MA using the MXL reference panel.

699T

Genome-Wide Association Study Identifies Four Loci Associated with Eruption of Permanent Teeth. *F. Geller[1], B. Feenstra[1], H. Zhang[1], J.R. Shaffer[2], T. Hansen[3], H.A. Boyd[1], E.A. Nohr[4], N.J. Timpson[5,6], D.M. Evans[5,6], R.J. Weyant[7], S.M. Levy[8], M. Lathrop[9,10], G. Davey Smith[5,6], J.C. Murray[11], T. Werge[3], M.L. Marazita[2,12], T.I.A. Sorensen[13], M. Melbye[1].* 1) Dept. of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark; 2) Dept. of Human Genetics, Univ. of Pittsburgh, PA, USA; 3) Research Institute of Biological Psychiatry Sct Hans, Roskilde, Denmark; 4) Institute of Public Health, Aarhus Univ., Denmark; 5) MRC CAiTE centre, Univ. of Bristol, UK; 6) School of Social and Community Medicine, Univ. of Bristol, UK; 7) Dept. of Dental Public Health, Univ. of Pittsburgh, PA, USA; 8) Dept. of Preventive and Community Dentistry, Univ. of Iowa, IA, USA; 9) Centre National de Genotypage, Evry, France; 10) Foundation Jean Dausset, Paris, France; 11) Dept. of Pediatrics, Univ. of Iowa, IA, USA; 12) Center for Cranofacial & Dental Genetics, Univ. of Pittsburgh, PA, USA; 13) Institute of Preventive Medicine, Copenhagen, Denmark.

The sequence and timing of permanent tooth eruption is thought to be highly heritable, and can have important implications for the risk of malocclusion, crowding, and periodontal disease. We conducted a genome-wide association study of number of permanent teeth erupted between age 6 and 14 years, analyzed as age-adjusted standard deviation score averaged over multiple time points, based on childhood records for 5,104 women from the Danish National Birth Cohort. Four loci showed association at $P<5 \cdot 10^{-8}$, and were replicated in four independent study groups from the United States and Denmark with a total of 3,762 individuals, all combined p-values were below 10^{-11}. Two loci agreed with previous findings in primary tooth eruption and were also known to influence height and breast cancer, respectively. The two other loci point to genomic regions without any previous significant GWAS results. However, the intronic SNP in *ADK* could be linked to gene expression in monocytes. The combined effect of the four genetic variants was most pronounced between age 10 and 12 years, where children with 6 to 8 delayed tooth eruption alleles had on average 3.5 (95%CI: 2.9-4.1) fewer permanent teeth than children with 0 or 1 of these alleles. During the age period of permanent tooth eruption many important developmental processes take place. Thus, we suggest following up the four reported SNPs in other growth related traits to further elucidate the genetic background of maturation.

700T

Genome-IBDLD: Estimating Pairwise Relatedness and Individual Inbreeding Coefficients and Discovery of Identity by Descent. *L. Han, M. Abney.* Human genetics, University of Chicago, Chicago, IL.

SNP data collected from natural populations allows information on genetic relationships to be established without referencing an exact pedigree. Many methods have been developed to use marker data for this purpose. These methods however are appropriate only when the analyzed pair is very distantly related or when there is no linkage disequilibrium (LD) between markers. We present a method, Genome-IBDLD, of estimating pairwise relatedness and individual inbreeding coefficients for any kind of pair given dense SNP genotypes. We use a new method based on ridge regression of unphased genotypes to account for LD in the identity by descent (IBD) estimation, that does not require phased data. Monte Carlo simulations show that Genome-IBDLD obtains as good an estimate as the IBDLD method based on pedigree information for sibling, avuncular, 1th cousin, 2nd cousin and more distantly related pairs and obtains more accurate genetic relationship matrix than GCTA and PLINK. The method is appropriate also for finding IBD tracts for any kind of pair. Simulations show that it is not only similarly efficient for finding short tracts of IBD as fastIBD/Beagle for distantly related pairs, but also has high power, a low FDR, large sensitivity for finding IBD tracts for closely related pairs and is highly robust to genotype errors. We use Genome-IBDLD to generate highly accurate estimates of genome-wide IBD sharing and discover IBD tracts in the HapMap data. Genome-IBDLD can be applied to thousands of samples with genome-wide SNP data. This is useful for estimation and adjustment for relatedness in association studies. Genome-IBDLD will be incorporated in the freely available IBDLD software package.

701T

Use of Diverse Electronic Medical Record Systems for a Genome-wide Association Study of Type 2 Diabetes in European- and African-ancestry populations. *M.G. Hayes[1,2], A. Kho[3], L.L. Armstrong[1], M.D. Ritchie[4], J.A. Pacheco[2], L. Rasmussen-Torvik[5], E.M. Just[6], J. Denny[7,8], D.C. Crawford[4], P. Peissig[9], L.V. Rasmussen[9], W. Wei[10], M. de Andrade[10], I.J. Kullo[11], D.R. Crosslin[12], D. Mirel[13], A. Crenshaw[13], K.F. Doheny[14], E. Pugh[14], W.A. Wolf[2,15], W.L. Lowe[1], D.M. Roden[7,16,17], R.L. Chisholm[2].* 1) Division of Endocrinology, Metabolism, and Molecular Medicine, Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL; 2) Center for Genetic Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL; 3) Division of General Internal Medicine, Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL; 4) Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN; 5) Department of Preventive Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL; 6) Northwestern Medical Enterprise Data Warehouse, Northwestern University Clinical and Translational Sciences (NUCATS) Institute, Northwestern University, Chicago, IL; 7) Department of Medicine, Division of Clinical Pharmacology, Vanderbilt University, Nashville, TN; 8) Department of Biomedical Informatics, Vanderbilt University, Nashville, TN; 9) Biomedical Informatics Research Center, Marshfield Clinic, Marshfield, WI; 10) Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN; 11) Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN; 12) Division of Medical Genetics, University of Washington, Seattle, WA; 13) Broad Institute of Harvard & MIT, Cambridge, MA; 14) Center for Inherited Disease Research, Johns Hopkins University, Baltimore, MD; 15) Division of Genetics, Children's Hospital Boston, Boston, MA; 16) Office of Personalized Medicine, Vanderbilt University, Nashville, TN; 17) Department of Pharmacology, Vanderbilt University, Nashville, TN.

Accurate phenotyping is key to the success of genome-wide association studies (GWAS). As part of the electronic Medical Records and Genomics (eMERGE) network, we identified type 2 diabetes (T2D) cases and controls for GWAS, using data captured through routine clinical care across five institutions with different electronic Medical Record (EMR) systems. We developed an algorithm using a combination of ICD9 codes, oral T2D medications, and abnormal glucose or glycated hemoglobin (HbA1c) lab tests. We excluded patients diagnosed with type 1 diabetes and those who were currently being treated with insulin only, had never been on a T2D medication, and did not have abnormal glucose or HbA1c. Controls were required to have ⁄2 clinical visits, no diabetes-related billing codes, no oral T2D medications or insulin, ⁄1 normal glucose values, and no family history of T2D. Our stringent criteria for source and specificity of the data achieved a minimum of 98 and 100% positive predictive values for identification of T2D cases and controls, respectively, compared against clinician review when the T2D algorithm was validated across three eMERGE sites. By collaboratively developing, standardizing and implementing the algorithm across sites, we reliably identified a total of 3,266 cases and 3,286 controls of African-ancestry (AA) and European-ancestry (EA) (AA: 810 cases and 873 controls genotyped on Illumina 1M BeadChips; EA: 2413 cases and 2392 controls genotyped on Illumina 660W BeadChips). Genotype data were collaboratively quality-controlled across all five sites. Logistic regression analyses were performed for T2D case-control status under an additive model, adjusting for age, sex, BMI, study site, and the first two principal components of ancestry in the AA and EA cohorts separately. These results were then combined using standard meta-analytic methods. Our most significant association in the joint meta-analysis, as well as in both the separate AA and EA cohorts, was the previously reported T2D associated SNP rs7903146 in *TCF7L2* (EA $p = 3.0 \times 10^{-10}$, AA $p = 5.3 \times 10^{-7}$, combined meta-analysis $p = 2.1 \times 10^{-15}$). T2D odds ratios for the minor allele were nearly identical to recently published meta-analyses (1.41, 1.64, and 1.46 in the EA, AA, and combined cohorts respectively). This study demonstrates that data derived from EMRs can accurately and reliably identify T2D cases and controls for genetic studies across multiple institutions.

702T

FaST Linear Mixed Models for Genome-Wide Association Studies. *D. Heckerman[1], C. Lippert[1], Y. Liu[1], C. Kadie[2], R. Davidson[2], J. Listgarten[1].* 1) Microsoft Res, Los Angeles, CA; 2) Microsoft Res, Redmond, WA.

Linear mixed models (LMMs) are among the richest class of models used today for genome-wide association studies as they are capable of correcting for population structure, family structure, and cryptic relatedness. Although their popularity is rapidly increasing for this reason, their use on contemporary data sets is limited because the required computations are prohibitive when many individuals are analyzed, with runtime increasing as the cube of the number of individuals and memory footprint increasing as the square of the number of individuals. We introduce a mathematical reformulation of LMMs called FaST-LMM to overcome this barrier, wherein results remain exact, but both runtime and memory footprint become linear in the number of individuals. On data from the Wellcome Trust with 15,000 individuals, our approach is an order of magnitude faster than the state-of-the art LMM implementation. On a data set containing 120,000 individuals, the state-of-the-art implementation is unable to run, whereas FaST-LMM completes in just a few hours.

703T

Genetics of Obesity in Mexican-Americans in Starr County, Texas. *H.M. Highland[1], J.E. Below[2], E.R. Gamazon[2], G.I. Bell[2], N.J. Cox[2], C.L. Hanis[1].* 1) Human Genetics Center, University of Texas, Health Science Center at Houston, Houston, Texas 77030, USA; 2) Department of Medicine, Section of Genetic Medicine, University of Chicago, Chicago, Illinois, 60637, USA.

The prevalence of obesity is increasing at an alarming rate across the United States and most developed countries. Current estimates indicate that 66% of Americans are either overweight or obese. Obesity increases risk for many diseases including type 2 diabetes, cardiovascular disease, stroke, sleep apnea and some cancers. Currently, 32 loci have been reproducibly associated with obesity in genome wide association studies. Only one genome-wide association study has previously been conducted in Hispanic-Americans, a population at particularly high risk of being obese. This makes the present study more important for understanding obesity genetics across populations. The current study utilizes a sample of 813 Starr-County Mexican-Americans with previously undiagnosed diabetes or without diabetes to identify genetic variants associated with obesity related traits. Samples were genotyped on the Affymetrix Genome-Wide Human SNP Array 6.0 and imputed to the HapMap resource using MACH (Li et al. 2006) to a total of 1.97 million SNPs. MaCH2qtl (Li et al. 2006) was used to analyze BMI and identify 22 SNPs with p-values less than $1x10^{-5}$. Top signals are rs10793047 and rs7731488 ($p=8.61x10^{-7}$ and $p=1.24x10^{-6}$, respectively), which map to hypothetical proteins on chromosomes 11 and 5 respectively. Other top SNPs are located in SUMF1 (rs12629402, rs17036134, and rs17036162, $p=1.26x10^{-6}$, $6.90x10^{-6}$ and $9.76x10^{-6}$, respectively) and KCNH5 (rs17100176 and rs1119595, $p= 1.85x10^{-6}$ and $2.89x10^{-6}$, respectively). Further LCL eQTL enrichment was observed for the BMI SNPs with $p<0.001$ ($p=0.0375$). Similar analyses have been conducted for a composite measure of obesity that includes BMI, waist circumference, hip circumference and percent body fat calculated from bioimpedance measures. Through this analysis an additional 17 SNPs with p-values less than $1x10^{-5}$ were identified.

704T

The PhenX Toolkit: Facilitating Cross-study Analysis in Genomic Studies. *W. Huggins[1], H. Pan[1], K.A. Tryka[2], M.J. Phillips[1], N. Whitehead[1], V. Bakalov[1], J. Levy[1], Y. Qin[1], M. Zmuda[1], D. Jackman[1], D. Nettles[1], J. Pratt[1], J.A. Hammond[1], T. Hendershot[1], D. Maiese[1], W.R. Harlan[3], J. Haines[4], H. Junkins[5], E. Ramos[5], L.C. Strader[1], C.M. Hamilton[1].* 1) RTI International, Research Triangle Park, NC; 2) National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD; 3) National Library of Medicine, Chevy Chase, MD; 4) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 5) National Human Genome Research Institute, Bethesda, MD.

Cross-study analyses of genome-wide association studies (GWAS) are needed to increase statistical power and replicate results. However, these analyses have been hindered by the lack of standard measures for risk factors, covariates, and outcomes that are shared by common, complex diseases. To address this need, the PhenX (consensus measures for Phenotypes and eXposures) Toolkit (https://www.phenxtoolkit.org/) offers high-quality well-established measures of phenotypes and exposures for use by the scientific community. The Toolkit contains 295 measures drawn from 21 research domains (fields of research). The measures were selected by Working Groups of domain experts using a consensus process that included input from the scientific community. Because measures relevant to complex diseases (e.g., Cardiovascular and Diabetes) can be found in multiple domains, measures are also organized into Collections, which are groups of measures related to a specific topic or target population (e.g., Diet and Nutrition and Body Size and Composition). For each PhenX Measure, the Toolkit provides a description of the measure, the rationale for including the measure in the Toolkit, protocol(s) for collecting the measure, and supporting documentation. Users can browse by measures, domains, or collections or search the Toolkit using the Smart Query Tool (SQT). Once users have selected some measures, they can download a customized Data Collection Worksheet (DCW) that specifies what information needs to be collected and a Data Dictionary (DD) that describes each variable included in their DCW. To help researchers share data, PhenX measures and variables are being mapped to multiple data standards, including the Unified Medical Language System (UMLS) and Cancer Data Standards Registry and Repository (caDSR). To help researchers find studies with comparable data, PhenX measures and variables are being mapped to studies in the database of Genotypes and Phenotypes (dbGaP). In collaboration with dbGaP staff at the National Center for Biotechnology Information (NCBI), 13 studies from the Gene Environment Association Studies (GENEVA) consortium and 3 studies from the electronic Medical Records and Genomics (eMERGE) network have been mapped to PhenX measures and variables. These mappings are displayed in dbGaP and highlight opportunities for cross-study analysis for researchers who adopt PhenX measures. *Supported by: NHGRI, Award No. U01 HG004597-01.*.

705T

MethLAB - A GUI Analysis Package for Array-based DNA Methylation Data. *V. Kilaru[1], R.T. Barfield[2], J.W. Schroeder[3], A.K. Smith[1,3], K.N. Conneely[2,4].* 1) Psychiatry & Behavioral Sci, Emory University, Atlanta, GA; 2) Biostatistics and Bioinformatics, Emory University, Atlanta, GA; 3) Genetics & Molecular Biology Program, Emory University, Atlanta, GA; 4) Human Genetics, Emory University, Atlanta, GA.

DNA methylation is a type of epigenetic modification that has been associated with numerous complex traits and diseases. The advent of array-based methods to interrogate DNA methylation has led to an explosion of epigenetic data in the literature. These methods include the popular Illumina GoldenGate and Infinium platforms that interrogate DNA methylation of an individual sample across the genome and output beta values that represent the proportion of DNA methylated at a given CpG site. These arrays generate enormous amounts of data, but there are currently no standardized methods available to analyze the data. This obstacle may reduce reproducibility among studies. We have developed a software package called MethLAB using R, an open source statistical language, which can be edited to suit the needs of the user. MethLAB features a front end graphical user interface (GUI) designed to accept and manipulate input data in a flexible format. The back end analyzes the association between beta values and a designated phenotype by fitting a separate linear fixed or mixed effects model for each CpG site. This package can incorporate both continuous and categorical covariates, as well as fixed or random batch or chip effects. It accounts for multiple tests by controlling FDR at a user-specified level. MethLAB outputs a flat text file containing the test statistics (t-statistics and p-values) for each CpG site and indicators for FDR-significant sites. The package produces quantile-quantile plots with confidence intervals to allow users to visually assess whether there is an excess of associated CpG sites. For FDR-significant CpG sites, MethLAB automates plotting of the beta values against the phenotype, with regression lines based on the model specified by the user. Considering the growing interest in DNA methylation studies and the increasing complexity in analyzing methylation data, there is a great need for user-friendly open source software to perform these analyses. With MethLAB, we present a timely and important resource that will allow users with no experience using statistical software to implement flexible and powerful analyses of DNA methylation data.

706T

No evidence for common genetic basis for clustering of metabolic syndrome traits. *K. Kristiansson[1,2], M. Perola[1,2,3], E. Tikkanen[1,2], J. Kettunen[1,2], I. Surakka[1,2], A.S. Havulinna[4], A. Stančáková[5], C. Barnes[6,7], E. Kajantie[8,9], J.G. Eriksson[8,10,11], J. Viikari[12], T. Lehtimäki[13], O.T. Raitakari[14], A-L. Hartikainen[15], A. Pouta[16], A. Jula[17], A.J. Kangas[18], P. Soininen[18,19], M. Ala-Korpela[18,19,20], S. Männistö[4], P. Jousilahti[4,21], L.L. Bonnycastle[22], M-R. Järvelin[16,23,24,25], F.S. Collins[22], M. Laakso[26], M.E. Hurles[27], A. Palotie[2,27,28,29], L. Peltonen[1,2,27,29,30], S. Ripatti[1,2], V. Salomaa[4].* 1) Public Health Genomics Unit, Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland; 2) Institute for Molecular Medicine Finland FIMM, University of Helsinki, Finland; 3) The Estonian Genome Center, University of Tartu, Estonia; 4) Chronic Disease Epidemiology and Prevention Unit, Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland; 5) Department of Medicine, Institute of Clinical Medicine, University of Eastern Finland; 6) Centre for Bioinformatics, Division of Molecular Biosciences, Imperial College London, London, UK; 7) Institute of Mathematical Sciences, Imperial College London, London, UK; 8) Diabetes Prevention Unit, Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland; 9) Hospital for Children and Adolescents, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland; 10) Unit of General Practice, Helsinki University Central Hospital, Helsinki, Finland; 11) Folkhalsan Research Centre, Helsinki, Finland; 12) Department of Medicine, University of Turku and Turku University Hospital, Finland; 13) Department of Clinical Chemistry, University of Tampere and Tampere University Hospital, Tampere, Finland; 14) Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku and Department of Clinical Physiology, Turku University Hospital, Finland; 15) Institute of Clinical Medicine/Obstetrics and Gynaecology, University of Oulu, Finland; 16) Department of Children, Young People and Families, National Institute for Health and Welfare, Oulu, Finland; 17) Population Studies Unit, Department of Chronic Disease Prevention, National Institute for Health and Welfare, Turku, Finland; 18) Computational Medicine Research Group, Institute of Clinical Medicine, University of Oulu and Biocenter Oulu, Oulu, Finland; 19) NMR Metabonomics Laboratory, Laboratory of Chemistry, Department of Biosciences, University of Eastern Finland, Kuopio, Finland; 20) Department of Internal Medicine and Biocenter Oulu, Clinical Research Center, University of Oulu, Oulu, Finland; 21) International Affairs Unit, National Institute for Health and Welfare, Helsinki, Finland; 22) National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, 20892, USA; 23) Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, Faculty of Medicine, London, United Kingdom; 24) Institute of Health Sciences, University of Oulu, Oulu, Finland; 25) Biocenter Oulu, University of Oulu, Oulu, Finland; 26) Department of Medicine, Institute of Clinical Medicine, University of Eastern Finland and Kuopio University Hospital, Finland; 27) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambs, CB10 1SA, UK; 28) Program in Medical and Population Genetics and Genetic Analysis Platform, The Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA; 29) Department of Medical Genetics, University of Helsinki; 30) Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA.

Dyslipidemia, hypertension, insulin resistance, and measurements of obesity are key components in definition of the metabolic syndrome (MetS). To date, genome-wide association (GWA) studies have identified several susceptibility genes for these MetS component traits, but there is little evidence on genes contributing to the syndrome as an entity rather than on individual component traits. We conducted a genome-wide association (GWA) study on metabolic syndrome and its component traits in four Finnish cohorts consisting of 2,637 MetS cases free of diabetes and 7,927controls, and followed the top loci in an independent sample with transcriptome and NMR-based metabolome data. Furthermore, we tested for loci associated with multiple MetS component traits using factor analysis and built a genetic risk score for MetS risk. A previously known lipid locus, *APOA1/C3/A4/A5* gene cluster region (SNP *rs964184*), was associated with MetS in all four study samples (P=7.23x10^{-9} in meta-analysis). The primary source of the association was the SNPs effect on triglyceride (TG) (P=2.59x10^{-31}) and high-density lipoprotein (HDL) levels (P=5.83x10^{-8}), which was further supported by a serum metabolite analysis, where *rs964184* associated with various VLDL, TG, and HDL metabolites (P=0.024-1.88x10^{-5}). 22 previously identified susceptibility loci for individual MetS component traits were replicated in our GWA and factor analysis but none of these loci significantly associated with two or more uncorrelated MetS components. A genetic risk score (GRS), calculating the number of alleles in loci associated with individual MetS traits, was strongly associated with MetS status. Our findings suggest that common genetic variation does not explain the clustering of metabolic syndrome traits. The genetic predisposition is likely to result from relatively small effects of multiple genes predisposing to dyslipidemia and abdominal obesity, possibly complemented by low frequency and rare variation, and/or epigenetic modifications.

707T

Genome-wide association studies using Bayesian classification with singular value decomposition identify that nine of the known Crohn's disease susceptibility loci are associated with disease prognosis and the need for surgery. *S. Kwon, D. McGovern, M. Dubinsky, T. Haritunians, S. Targan, K. Taylor, P. Fleshner, A. Ippoliti, J. Rotter, X. Guo.* Med Gen Inst, Cedars-Sinai Med Ctr, Los Angeles, CA.

Genome-wide association studies (GWAS) involve large numbers (p) of single nucleotide polymorphisms (SNPs) and a relatively small number of subjects (n) (p>>n). Conventionally, statistical methods analyze a single SNP at a time, resulting in problems related to multiple testing. Penalized logistic regression methods have been proposed for analyzing multiple SNPs, but the number of SNPs per analysis is limited. To analyze all SNPs simultaneously, we developed the Bayesian classification with singular value decomposition (BCSVD) method. The method achieves a massive dimension reduction by applying singular value decomposition to the design matrix. It utilizes a binary probit model and fits the model using Markov chain Monte Carlo with Gibbs sampler. The significance of SNPs/genes can be determined with the test statistic generated by permutation. Here, we applied the BCSVD method to Crohn's disease (CD) patients to identify genes associated with the need for surgery, specifically intestinal resection. Disease phenotype, and specifically type and indication for surgery, were ascertained by chart review according to agreed criteria. Both single SNP association analysis and the BCSVD method were performed on 521 CD patients (311 with surgery and 210 without surgery) recruited through the Cedars-Sinai IBD center. 412,309 SNPs passed genotype quality controls (HWE <10-5 and MAF<0.05). Among the top 10 SNPs associated with the need for intestinal resection identified by the BCSVD method, 9 SNPs had p-value<10-5 from single SNP association analysis (even though they didn't reach genome-wide significance level). 57 out of 71 CD susceptibility loci that have been reported in recent GWAS studies were either present in our genotype data or had proxies available. With the BCSVD method, we were able to identify that 9 (7 genotyped, 2 proxies) disease predisposing loci (IL12B, SLC22A4, ATG16L1, JAK2, SMAD3, C13or31, PTPN22, and 2 unknown positional candidate genes (14q35 and 5q31)) were also associated with a disease complication, the need for surgery. For each of the 7 genotyped SNPs, the same risk alleles were found associated with the need for surgery and CD susceptibility. This finding implies that disease risk alleles also predict natural history. Since the individual SNPs did not reach nominal significance, but the aggregation did by the BCSVD, this suggests that the BCSVD method is a powerful tool for identifying risk genes in modest size clinical samples.

708T

Identification of gene networks for caries of pit and fissure vs. smooth tooth surfaces through GWAS and protein network integration. *M. Lee[1], K.T. Cuenco[1], Z. Zhen[2], J.R. Shaffer[2], X. Wang[1], M.M. Barmada[2], R.J. Weyant[3], R.J. Crout[4], D.W. McNeil[5], D.E. Weeks[2], E. Feingold[2], M.L. Marazita[1,2].* 1) Center for Craniofacial & Dental Genetics, Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA; 2) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 3) Department of Dental Public Health and Information Management, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA; 4) Department of Periodontics, School of Dentistry, West Virginia University, Morgantown, WV; 5) Dental Practice and Rural Health, West Virginia University, Morgantown, WV.

The Center for Oral Health Research in Appalachia (COHRA) began evaluating risk factors for dental caries in a population with limited health care access and elevated oral disease. COHRA study families were included in the NIH Gene, Environment Association Studies Consortium (GENEVA), for a genome-wide association study (GWAS) of dental caries. Dental caries is heritable even after adjustments for shared environment. Tooth surfaces subject to caries are divided into pit and fissure (PFS, anatomic landmarks where the enamel folds inward) and smooth surfaces (SMS, any surfaces other than PFS), with heritability estimated as 19-53% and 17-42%, respectively. Multiple genes may control PFS and SMS. GWAS has been conducted on PFS and SMS measures in COHRA-GENEVA subjects, but single SNP results did not evaluate interactions between different genes. We attempted to detect the combined effects of multiple markers/genes included from GWAS. We identified corresponding gene networks by integrating GWAS of PFS and SMS from our study population with existing protein interaction databases.

Demographics and caries status on PFS and SMS in primary dentition (PFS: n=572 and SMS: n=596) were obtained from COHRA-GENEVA subjects. Subjects self-reported as Caucasian and 3-14 years old were included. Illumina 610-Quad platform genotypes were generated by the Center for Inherited Disease Research. Genotype quality control was conducted by the GENEVA Coordinating Center and U. Pittsburgh using standardized protocols. Single-SNP GWAS results were combined with the Protein Interaction Network Analysis (PINA) database using dmGWAS software. dmGWAS identified gene networks through an agglomerative search favoring networks containing low p-value genes from the GWAS.

SNP GWAS results suggest several chromosome regions may be associated with PFS and SMS. By applying network search algorithms that combine GWAS and protein pathway data, we have identified additional genes influencing PFS and SMS caries. Identified modules provide insight into additional gene relationships and help prioritize genes for further inquiry. Support: DE018903, DE014899, DE020127.

709T

Estimating the magnitude and influence of genetic marker errors by comparing conventional WTCCC guideline and further quality control using family and twin relationship. *D.H. Lee[1], M.K. Lee[1], Y.M. Song[2], K. Lee[3], J. Sung[1].* 1) Complex Disease and Genetic Epidemiology Branch, Department of Epidemiology and Institute of Environment and Health, School of Public Health Seoul National University; 2) Department of Family Medicine, Samsung Medical Center, and Center for Clinical Research, Samsung Biomedical Research Institute, SungKyunKwan University School of Medicine, Seoul, Korea; 3) Department of Family Medicine, Busan Paik Hospital, Inje University College of Medicine, Korea.

An increasing number of genetic studies are incorporating dense genome-wide single nucleotide polymorphism (SNP) marker information to explore gene-disease associations. The Wellcome Trust Case Control Consortium (WTCCC) guideline is a widely used quality control method for genetic marker cleaning, but the accuracy and the influence of marker errors evading the WTCCC cleaning methods has not been adequately evaluated. WTCCC method mainly depends on the call rates, minor allele frequency and Hardy-Weinberg equilibrium (HWE) test. Any of the three criteria, however, are methods developed to detect genotype errors. In the Healthy Twin Study of Korea, with dense SNP markers (Affymetrix GeneChip v6) and family relationship, we were able to test the validity of each marker using Mendelian (single point) and Non-Mendelian (multipoint) compatibility checks. Additionally, the presence of both genotyped monozygotic twins (MZ) (15 twin pairs) provides index of accuracy among initial 905,017 SNPs (excluding 3,014 duplicated markers) applying WTCCC guideline excluded 124,520 markers (exclusion - HWE incompatibility and call rate <95%). When we further applied Mendelian and Non-Mendelian inconsistency check, 87,608 markers were deleted additionally. Genotyping accuracy was calculated by confidence score and by MZ concordance rate. Confidence score on the Affymetrix platform using birdseed ranged from 0 (most confident) to 1 (least confident). Confidence score (SD) and MZ concordance rate are 0.008 (0.004) and 99.63 for WTCCC guideline application whereas 0.060 (0.047) and 96.52 for no application. Likewise, Confidence score and MZ concordance rate are 0.010 (0.015) and 99.79 for Mendelian and Non-Mendelian inconsistency check application whereas 0.038 (0.040) and 96.87 for no application. Previous studies showed most of genotyping errors removed by WTCCC guideline, but it is not. Mendelian and Non-Mendelian errors still remains. Consequentially, Mendelian and Non-Mendelian compatibility checks of genotype data by using family and twin relationship in GWAS will continue to be an important aspect of human genetics research.

710T

An evaluation of the accuracy of 1000 Genomes-based imputation. *M. Lemire, J. Rangrej, V. Peltekova.* Ontario Institute for Cancer Research, Toronto, ON, Canada.

Genotype imputation is routinely applied in the analysis of genome-wide association studies (GWAS). Genotypes derived from low-coverage, whole-genome sequencing data is available for 629 individuals in release 20100804 of the 1000 Genomes project. Of these, 283 are of European descent (EUR) and includes the CEPH samples from Utah (CEU) that are also available in all releases of HapMap as well as samples from Tuscany, Great Britain, Finland, Puerto Rico and Mexico. Because this data is derived from low-coverage sequencing data, misclassification of the genotype calls may occur. Moreover it is unclear if imputation based on a large set of potentially heterogeneous samples outperform that based on a smaller set of homogeneous samples. We thus aimed to evaluate the accuracy of 1000 Genomes-based imputation. We used data derived from a large-scale sequencing study of colorectal cancer (CRC) cases and controls. Eleven susceptibility regions from published GWAS, totalling 2.2 Mbp, were sequenced in 120 samples. The large majority of the SNPs identified were then genotyped in ~1200 cases of CRC and ~1200 controls using a custom iSelect array from Illumina; 5511 polymorphic SNPs were available after quality control. To evaluate the accuracy of the imputation, we pretended we only had genotyped a typical commercial high-density genotyping array, and used these SNPs along with the 1000 Genomes reference set to impute our remaining SNPs. The accuracy was measured by comparing the actual genotypes to the most-likely imputed calls, using Cohen's Kappa measure of agreement (k). We compared the use of the CEU to the use of the EUR reference sets, and compared MACH and BEAGLE software. Using MACH and EUR, there was more than 90% agreement (k>0.9) between the real genotypes and the most likely imputed calls for 59% of the imputed SNPs (84% among the SNPs with “imputation quality” r2>0.5, following MACH's guidelines). Based on minor allele frequency (MAF), the proportions of SNPs with k>0.9 are 86%, 39% and 14% for common (MAF>5%), uncommon (1%<MAF<5%) and rare SNPs (MAF<1%), respectively. Using CEU as the reference set, these proportions are slightly smaller. We found that MACH generally outperformed BEAGLE. Our results show that imputation is generally accurate, but that the accuracy declines dramatically with allele frequency, and that the potential heterogeneity of the EUR samples does not have a negative impact on accuracy.

711T

Rapid optimization of genotype imputation using parallelization. *J.L. Levy[1], D.B. Hancock[1], G.P. Page[2], E.O. Johnson[1].* 1) RTI International, Durham, NC; 2) RTI International, Atlanta, GA.

Genotype imputation of untyped markers is a computationally-intensive operation that improves power of genome-wide association studies and allows results to be combined across studies. We sought to evaluate imputation using 15 different Hapmap phase III reference panel combinations with optimization criteria of minor allele frequency (MAF), MACH imputation quality (r2), and concordance rate of imputed vs. actual genotype for 10 sets of randomly-masked SNPs. Because of the large number of imputations (15 reference panel combinations x 10 repetitions = 150) we devised a 3-step parallelized imputation procedure using our 172-core computing cluster to both speed performance and generate the optimization metrics. In Step 1 we used MACH to estimate crossover and error rates, which is parallelizable by chromosome. In Step 2 we split the individuals and imputed each one separately on its own compute core, using a version of MACH that we modified for calculating MAF and r2, which were originally not available from MACH when parallelized. In Step 3 we merged output from step 2, and calculated MAF, r2, and concordance. Another modified MACH algorithm, MINIMAC, speeds computing time and produces MAF and r2 values but it does not give the output needed to calculate concordance. We compared total run time of our 3-step strategy on our cluster ("split") vs. a 2-step MACH strategy on a single core ("unsplit") using two different sets of input genotypes (from 595 and 1,071 individuals) and 6 different sets of reference haplotypes (from 116, 124, 134, 250, 366 and 638 individuals), giving a total of 12 split and 12 unsplit times. On chromosome 22, the split runs showed up to a 4-fold reduction in computing time over the unsplit runs, with time savings increasing with larger reference panels or number of input genotypes. The greatest reduction in computing time came from step 2 (up to almost 114-fold), where we split individuals onto separate compute cores. The difference in computing time was even greater for larger chromosomes. In summary we have devised a parallelization strategy for generating metrics for optimizing imputation reference panel selection that shows a substantial improvement over imputation on a single machine. This strategy will also reduce computing time for genome-wide genotype imputation by parallelizing across chromosomes and individuals.

712T

Meta-analysis of genome-wide association studies identifies additional susceptibility loci for psoriasis. *Y. Li[1], S.L. Spain[4], J. Knight[4], P.E. Stuart[2], E. Ellinghaus[3], H.M. Kang[1], W. Chen[1], T. Tejasvi[2], J. Barker[4], G.R. Abecasis[1], J.T. Elder[2,5], R.P. Nair[2], A. Franke[3], R. Trembath[4].* 1) Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, Michigan 48109, USA; 2) Department of Dermatology, University of Michigan, Ann Arbor, Michigan 48109, USA; 3) Institute of Clinical Molecular Biology, Christian-Albrechts-University, Kiel, 24105, Germany; 4) Department of Medical and Molecular Genetics, Division of Genetics and Molecular Medicine, King's College London School of Medicine, London SE1 9RT UK; 5) Ann Arbor Veterans Affairs Hospital, Ann Arbor, Michigan 48105, USA.

Psoriasis is a chronic and debilitating disorder of the skin, nails, and joints, affecting about 2% of people of European descent. Its clinical characteristics include epidermal hyperplasia and inflammation. To evaluate the contribution of common genetic variants to psoriasis disease susceptibility, we carried out a meta-analysis of 3 independent recent genome-wide association studies (meta-GWAS): the Collaborative Association Study of Psoriasis (**CASP**, Nair, R.P. *et al., 2009*), a German study conducted in Kiel, (**KIEL**, Ellinghaus E, *et al., 2010*) and a third from the Wellcome Trust Case Control Consortium 2 (**WTCCC2**, Strange *et al., 2010*). Together, these studies constitute a European-origin discovery sample of 4011 psoriasis cases and 7721 controls. Genotypes in each study were imputed using the most recently released (Nov 2010) 1000 Genomes Project CEU haplotypes as a reference. Association analysis was performed on the 8 million imputed SNPs separately within each cohort, followed by adjustment for population stratification (genomic control parameters **CASP** 1.06, **KIEL** 1.09, **WTCCC2** 1.04), and meta-analysis. The discovery results confirmed 15 previously reported psoriasis associated loci: *HLA-C* (p<10-300), *IL12B* (p<10-25), *TRAF3IP2* (p<10-20), *TNIP1* (p<10-16), *LCE3D* (p<10-14), *TNFAIP3* (p<10-13), *IL23R* (p<10-13), *IL23A* (p<10-11), *REL* (p<10-10), *TYK2* (p<10-9), *RPS26* (p<10-9), *NFKBIA* (p<10-8), *RNF114* (p<10-8), *IFIH1* (p<10-8), and *ERAP1* (p<5x10-8), and showed strong support for another 3 known loci: *NOS2* (p~6x10-8), *FBXL19* (p~8x10-7), and *IL13* (p~8x10-6). We also identified a novel locus passing the genome-wide significance threshold and several other novel loci approaching it. Current replication efforts are being prioritized based on p-values, involvement in IBD (Inflammatory Bowel Disease), and functional relationships with known psoriasis loci as determined by GRAIL (Gene Relationships Across Implicated Loci). Our study validates and extends the catalog of psoriasis susceptibility loci and will eventually provide a better understanding of the pathogenesis of psoriasis.

713T

Identification of 6 Novel Susceptibility Loci for Androgenetic Alopecia and Their Association with Common Disease: A Genome-Wide Association Study. *R. Li[1], F. Brockschmidt[2,3], A. Kiefer[4], H. Stefansson[5], D.R. Nyholt[6], K. Song[7], S.H. Vermeulen[8,9], S. Kanoni[10], D. Glass[11], D. Waterworth[7], F. Geller[12], S. Hanneken[13], S. Moebus[14], C. Herold[15], G.W. Montgomery[6], P. Deloukas[10], N. Eriksson[4], A.C. Heath[16], T. Becker[15,17], M. Mangino[11], P. Vollenweider[18], T.D. Spector[11], G. Dedoussis[19], N.G. Martin[6], L.A. Kiemeney[8,20,21], V. Mooser[7], K. Stefansson[5], D.A. Hinds[4], M.M. Nöthen[2,3], J.B. Richards[1,11].* 1) Departments of Medicine, Human Genetics, Epidemiology and Biostatistics, Lady Davis Institute, Jewish General Hospital, McGill University, Montreal, Quebec, Canada; 2) Institute of Human Genetics, University of Bonn, D-53127 Bonn, Germany; 3) Department of Genomics, Life & Brain Center, University of Bonn, D-53127 Bonn, Germany; 4) 23andMe, Mountain View, CA; 5) deCODE genetics, Sturlugata 8 IS-101, Reykjavík, Iceland; 6) Queensland Institute of Medical Research, Brisbane 4029, Australia; 7) Genetics Division, GlaxoSmithKline, King of Prussia, Pennsylvania 19406, USA; 8) Department of Epidemiology, Biostatistics and HTA, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB, Nijmegen, the Netherlands; 9) Department of Genetics, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB, Nijmegen, the Netherlands; 10) Genetics of complex traits in humans, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, CB10 1SA, UK; 11) Twin Research and Genetic Epidemiology, King's College London, London, United Kingdom; 12) Department of Epidemiology Research, Statens Serum Institute, Artillerivej 5, 2300 Copenhagen S, Denmark; 13) Department of Dermatology, University of Düsseldorf, D-40225 Düsseldorf, Germany; 14) Institute for Medical Informatics, Biometry and Epidemiology, University Duisburg-Essen, D- 45122 Essen, Germany; 15) German Center for Neurodegenerative Diseases (DZNE), D-53127 Bonn, Germany; 16) Washington University Medical School, St Louis , USA; 17) Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Sigmund-Freud-Str. 25, D-53127,Bonn,Germany; 18) Department of Internal Medicine, CHUV University Hospital, Lausanne 1011, Switzerland; 19) Department of Dietetics-Nutrition, Harokopio University, Athens, Greece; 20) Department of Urology, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB, Nijmegen, the Netherlands; 21) IKNL, Location Nijmegen, PO Box 1281, 6501 BG, Nijmegen the Netherlands.

Male pattern baldness, also known as androgenetic alopecia (AGA), is a highly heritable condition, and has been associated with coronary heart disease and prostate cancer. Previous susceptibility loci have been described on chromosomes 20 and the X chromosome, but these loci explain a minority of its heritable variance. We therefore conducted a large-scale meta-analysis of seven genome-wide association studies for male pattern baldness in 12,806 individuals of European ancestry. Genotype scores were created by combining the risk alleles of novel and established susceptibility loci. We identified six novel susceptibility loci in or near 17q21.31, *HDAC4*, *HDAC9*, *AUTS2*, *TARDBP* and *SETBP1* that reached genome-wide significance ($P = 2.62 \times 10^{-9}$ - 1.01×10^{-12}) and confirmed known loci. Unexpectedly, the 17q21.31 locus harbored genome-wide significant alleles in the *MAPT* gene, which have also recently been described to be associated with Parkinson's disease at a genome-wide significant level. Further other genome-wide significant alleles at 17q21.31 have been demonstrated to be under negative selection pressure and are associated with decreased fertility in women. *HDAC4* and *HDAC9* act as transcriptional co-repressors and play an important role in prostate cancer progression. Individuals in the highest risk quartile of the genotype risk score had a five-fold increased risk of male pattern baldness (OR = 5.61, $P = 2.7 \times 10^{-20}$) compared to the individuals in the lowest quartile. In conclusion, our results highlight unexpected associations between AGA, Parkinson's disease and fertility, as well as expand our understanding of the role of the androgen pathway in susceptibility to male pattern baldness.

714T

The impact of genomic coverage on the power to detect additional variants with genome-wide association studies. *K.J. Lindquist[1], E. Jorgenson[2], T.J. Hoffmann[1], J.S. Witte[1].* 1) Department of Epidemiology and Biostatistics, Institute for Human Genetics, University of California, San Francisco, CA; 2) Ernest Gallo Clinic and Research Center, Department of Neurology, University of California, San Francisco, CA.

Genome-wide association studies (GWAS) have identified many variants that are associated with complex human diseases and traits. Most of these associations have been discovered by genotyping common single nucleotide polymorphisms (SNPs) using one of the early generation of GWAS microarray SNP chips. The latest generation of GWAS chips promises to offer better coverage of the genome by genotyping more SNPs that are in linkage disequilibrium (LD) with a larger proportion of the variants across the entire genome. This improvement is made possible by the availability of the 1000 Genomes Project data, which has identified many novel, less common SNPs in diverse populations. The new GWAS chips should enable the discovery of some additional associations that may have been missed by previous studies, but it is not clear how many more. In this study, we estimate how many additional SNPs might be found to be associated with several complex diseases that have been studied and recorded in the Catalog of Published Genome-Wide Association Studies (www.genome.gov/gwastudies). The studies all used a popular GWAS chip measuring close to a million SNPs, and the genomic coverage of this chip was calculated using the 1000 Genomes pilot data as a reference (1000 Genomes Project Consortium, Nature 467(7319), 2010). The power to detect the effect size of each SNP associated at p<1x10^-5 with each disease was calculated using a method that takes the coverage of the chip into account (Jorgenson and Witte, Am J Hum Genet 78(5), 2006). The power to detect the same effect size for each SNP was calculated using one of the newer commercially available chips with approximately 2.5 times as many SNPs and better coverage of the genome. The number of false negatives was compared under the two scenarios to determine how many additional SNPs might be associated using the larger chip. For eight diseases (breast cancer, chronic lymphocytic leukemia, coronary heart disease, hypertension, major depression, schizophrenia, age-related macular degeneration, and type 2 diabetes) and 31 SNPs included in this study, we estimated that an additional 7 SNPs could be found to be associated using the larger chip for these diseases combined. Future work will explore the impact of genomic coverage on the power to detect associations using additional diseases and chips with different SNP densities.

715T

Association analyses of 198,846 individuals identifies 11 new loci associated with body mass index (BMI). *A.E. Locke[1], S. Vedantam[2,3], S. Berndt[4], F. Day[5], S. Gustafsson[6], D.C. Croteau-Chonka[7], D. Shungin[8,9], A. Scherag[11], E. Wheeler[13], C. Willer[12], I. Barroso[13,14], K.E. North[15], M.I McCarthy[16], C.M. Lindgren[16], E. Ingelsson[6], K.L. Mohlke[7], R.J.F. Loos[5], E.K. Speliotes[17], J. Hirschhorn[2,3,10], The Genetic Investigation of Anthropometric Traits (GIANT) Consortium.* 1) Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI; 2) Division of Genetics and Enodcrinology and Program in Genomics, Children's Hosptial Boston, Boston MA; 3) Broad Institute, Cambridge, MA; 4) Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD; 5) MRC Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK; 6) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; 7) Department of Genetics, University of North Carolina, Chapel Hill, NC; 8) Genetic and Molecular Epidemiology Group, Department of Public Health and Clinical Medicine, Section of Medicine and Department of Odontology, Umeå University, Umeå, Sweden; 9) Department of Clinical Sciences, Skåne, University Hospital, Lund University, Malmö, Sweden; 10) Department of Genetics, Harvard Medical School, Cambridge, MA; 11) Institute for Medical Informatics, Biometry and Epidemiology, University of Duisburg-Essen, Essen, Germany; 12) Division of Cardiovascular Medicine, Department of Internal Medicine, University of Michigan, Ann Arbor, MI; 13) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK; 14) University of Cambridge, Metabolic Research Labs, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK; 15) Department of Epidemiology and Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, NC; 16) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; 17) Division of Gastroenterology, Department of Internal Medicine and Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI.

Obesity and its resulting complications comprise a major health care burden. To find additional genetic contributors to obesity, the Genetic Investigation of ANthropometric Traits (GIANT) Consortium is conducting a meta-analysis of association data with BMI in 198,846 adults of European ancestry. This effort uses data from 45 existing genome-wide association studies (GWAS) and 29 studies genotyped for the Metabochip, a custom-designed SNP genotyping array of ~200,000 markers focused on metabolic and cardiovascular traits. Metabochip includes approximately 5,000 of the most strongly associated SNPs from the previous meta-analysis for BMI (Speliotes et al., 2010) that did not reach genome-wide significance. In a meta-analysis of only Metabochip data (n=79,285), we confirmed at genome-wide significance ($p<5*10^{-8}$) ten loci previously associated with BMI and three novel regions (*GPR61*, *AGBL4*, and *CADM1*). 73% of independent SNPs ($r^{2<0.1} < 0.1$) on the Metabochip selected as potentially associated with BMI showed consistent effect direction with the recent GIANT BMI GWAS meta-analysis (1,730 of 2360 compared to 1180 expected; $p<1*10^{-100}$). In a combined meta-analysis of all 198,846 individuals on the 100,077 SNPs available in both GWAS and Metabochip meta-analyses, we identified 39 apparently independent loci reaching genome-wide significance ($p<5*10^{-8}$). These include 28 of 32 (88%) previously established BMI-related loci and eleven regions not previously reported as associated with obesity-related or metabolic traits. New association signals include *AGBL4*, *HIP1*, *OLFM4*, and *NLRC3*, genes associated with inflammatory diseases, and near *FHIT*, *AMPD2*, and *AK5* associated with purine metabolism. We also identified new associations near genes for cell adhesion (*CADM1*), cell-cell interactions (*INADL*), and tyrosine metabolism (*C9orf4*). An association with BMI at the diabetes-associated *TCF7L2* variant indicates a link between obesity and glucose and insulin homeostasis. Collectively, these new loci suggest additional pathways and mechanisms underlying the biology of obesity, potentially opening new avenues for research and therapeutic intervention. In summary, our extensive follow-up effort using the Metabochip has been effective in identifying novel BMI-associated loci, and suggests that many of the loci not reaching conventional levels of genome-wide significance likely contain additional true associations.

716T

Identification of novel associations using genome-wide penalized regression for height, white blood cell count, and platelet count in African-Americans within the Women's Health Initiative. *B.A. Logsdon, C.L. Carty, A.P. Reiner, J.Y. Dai, C. Kooperberg.* Public Health Sciences Div., Fred Hutchinson Cancer Research Center, Seattle, WA.

Genome-wide association studies (GWAS) to date have identified over 1000 common variants associated with human traits. For many complex traits, including height and platelet count, the majority of these variants have a small effect, leaving a significant proportion of the heritable variation unexplained. To increase statistical evidence for additional associations beyond those that reach a stringent genome-wide significance threshold of marginal association, we use a penalized regression methodology. Our methodology incorporates all approximately 900,000 genotyped single nucleotide polymorphisms (SNPs) within the study in a single multiple regression model, where the identified solutions are constrained to be highly sparse based on our specific choice of penalty. Using data from the Women's Health Initiative SNP Health Association Resource (SHARe) GWAS of African-Americans, we demonstrate that our penalized regression method has power to detect novel associations beyond those that reach a stringent Bonferroni cutoff of marginal association. We run multiple empirical null simulations to choose a significance cutoff for the output of our algorithm to control the Type-1 error rate. Our methodology, which we call “variational Bayes model selector” (VBMS), is designed specifically to identify sparse, multiple locus models, as well as the degree of sparsity of the models, such that there are few false positives included within a model for a given ultra-high dimensional GWAS data-set. This is achieved through a stringent “spike-and-slab” prior and a fast and highly scalable variational Bayes approximate inference algorithm. Combined with empirical null simulations to control the Type-1 error rates, we demonstrate that the VBMS algorithm is a powerful tool in the analysis of GWAS for elucidating additional loci contributing to heritable variation for body height, platelet count, and white blood cell count within a minority cohort.

717T

Leveraging resources in biobanks from the eMERGE network: a genome-wide association study of thyroid stimulating hormone levels in European Americans and African Americans. *J.R. Malinowski[4], J.C. Denny[1,2], S.J. Bielinski[5], M.A. Basford[6], Y. Bradford[4], H.S. Chai[7], P.L. Peissig[8], D. Carrell[9], J. Pathak[7], R.A. Wilke[2], L. Rasmussen[8], X. Wang[6], J.A. Pacheco[13], A. Kho[10], N. Weston[9], M. Matsumoto[7], K.M. Newton[9], R. Li[11], I.J. Kullo[12], C.G. Chute[7], R.L. Chisholm[13], E.B. Larson[9], C.A. McCarty[14], D.R. Masys[1], D.M. Roden[2,15], M. de Andrade[7], M.D. Ritchie[1,3,4], D.C. Crawford[3,4] on behalf of the eMERGE Network.* 1) Dept. of Biomedical Informatics, Vanderbilt University, Nashville, TN; 2) Dept. of Medicine, Vanderbilt University, Nashville, TN; 3) Dept. of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN; 4) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 5) Division of Epidemiology, Dept. of Health Sciences Research, Mayo Clinic, Rochester, MN; 6) Office of Research, Vanderbilt University, Nashville, TN; 7) Division of Biomedical Statistics and Informatics, Department of Health Services Research, Mayo Clinic, Rochester, MN; 8) Biomedical Informatics Research Center, Marshfield Clinic Research Foundation, Marshfield, WI; 9) Group Health Research Institute, Seattle, WA; 10) Dept. of Medicine, Northwestern University, Chicago, IL; 11) Office of Population Genomics, National Human Genome Research Institute, Bethesda, MD; 12) Division of Cardiovascular Diseases, Dept. of Health Sciences Research, Mayo Clinic, Rochester, MN; 13) Center for Genetic Medicine, Northwestern University, Chicago, IL; 14) Center for Human Genetics, Marshfield Clinic Research Foundation, Marshfield, WI; 15) Dept. of Pharmacology, Vanderbilt University, Nashville, TN.

Thyroid stimulating hormone (TSH) hormone levels are normally tightly regulated within an individual; relatively small individual variations may indicate thyroid disease. Genome-wide studies (GWAS), primarily in European descent populations, have suggested that variants in *PDE8B* and *FOXE1* are associated with TSH levels. Variants in *FOXE1* have been previously associated with increased thyroid cancer risk. *PDE8B* may influence TSH levels through regulation of cAMP signaling. The Electronic Medical Records and Genomics (eMERGE) Network is a collaboration across institutions with biobanks linked to electronic medical records (EMRs); eMERGE members are Group Health Cooperative/University of Washington, Marshfield Clinic, Mayo Clinic, Northwestern University, Vanderbilt University, and the eMERGE Administrative Coordinating Center. The eMERGE Network uses EMR-derived phenotypes to perform genomic association studies in diverse populations. At each site, GWAS was conducted for complex diseases (e.g. dementia, cataracts, peripheral arterial disease (PAD), type 2 diabetes) and medically relevant quantitative traits (e.g. cardiac conduction); overall, approximately 17,000 subjects were genotyped. Because eMERGE DNA samples are linked to EMRs, the network can “reuse” the genotype data to conduct network-wide GWAS for additional diseases and traits. In this report, we performed a “no additional genotyping” GWAS for a quantitative trait—serum TSH level—in ~5000 European Americans (EAs) and ~500 African Americans (AAs). Tests of association were performed using linear regression and adjusted for age, sex, and principal components, assuming an additive genetic model. Our results replicate the known association of *PDE8B* in EAs (rs1382879 $p=1.52\times10^{-16}$,) =0.07823) and *PDE8B* SNPs were the only SNPs to reach genome-wide significance in EAs. *FOXE1* variants failed to reach genome-wide significance in the EAs (rs1443434: $p=2.04\times10^{-5}$,) =-0.0404). No SNPs in *PDE8B* or *FOXE1* were significant in AAs including rs1382879 (p=0.398,) = -0.03151). No SNPs reached genome-wide significance in AAs, with the most significant being *MX2* rs886451 ($p=2.61\times10^{-6}$,) =-0.3637). These results support the previously reported association of *PDE8B* with serum TSH levels in EAs while highlighting the variability of association study results in diverse populations with differing allelic spectrums and linkage disequilibrium patterns.

718T

Genome-wide Analysis Uncovers Putative Associations for Diabetic Retinopathy in Individuals Identified from Biobanks Linked to Electronic Medical Records. *C.A. McCarty[1], R. Goodloe[2], G. Pesicka[3], L. Rasmussen[4], J.C. Denny[5, 6], C. Blanquicett[6], C. Waudby[1], M.A. Brantley[7], M.D. Ritchie[2, 6, 8].* 1) Center for Human Genetics, Marshfield Clinic Research Foundation, Marshfield, WI; 2) Center for Human Genetics Research, Vanderbilt Univ, Nashville, TN; 3) Department of Opthamology, Marshfield Clinic Research Foundation, Marshfield, WI; 4) Biomedical Informatics Research Center, Marshfield Clinic Research Foundation, Marshfield, WI; 5) Department of Medicine, Vanderbilt Univ, Nashville, TN; 6) Department of Biomedical Informatics, Vanderbilt Univ, Nashville, TN; 7) Ophthalmology and Visual Sciences, Vanderbilt Univ, Nashville, TN; 8) Department of Molecular Physiology & Biophysics, Vanderbilt Univ, Nashville TN.

Diabetic retinopathy (DR) is the most common ocular complication of diabetes and is estimated to cause 5% of blindness and low vision in White adults in the US and up to 14.5% in African Americans. Methods for the prevention and treatment of diabetic retinopathy include control of blood glucose and retinal laser photocoagulation for proliferative retinopathy and macular edema. The prevalence rates for people with diabetes have been estimated to 40.3% for retinopathy and 8.2% for vision-threatening retinopathy and are expected to increase with the rising prevalence of diabetes. The eMERGE network, funded by the NHGRI, was established to support genomic research in the context of biobanks linked to electronic health records. Individuals were initially selected for one of five genome-wide association studies (GWAS); then secondary phenotypes were selected that reuse the existing genotyped samples. DR was one of the secondary phenotypes selected for GWAS. DR cases were defined by ICD-9 and procedure codes related to DR. Controls were individuals previously diagnosed with diabetes who had an eye exam in the previous two years and no diagnosis of diabetic retinopathy. DNA samples were genotyped at the Broad Institute and at the Center for Inherited Diseases Research at Johns Hopkins University using the Illumina 660w-quad and merged for QC following the eMERGE Genomics workgroup QC pipeline. A total of 701 cases (50.4% male; 19.7% African American) and 2373 controls (49.8% male; 16.1% African American) passed phenotype and genotype QC for analysis. In the GWAS analysis adjusted for smoking, sex, decade of birth, three principal components for ancestry, and site our top hits include 2 SNPs on chromosome 20 near GHRH (rs6018428 p<4.95x10-10 and rs6018432 p<2.00x10-7) and an additional hit on chromosome 20 near DTD1 (rs6081341 p<4.21x10-8). Antagonist analogs of growth hormone releasing hormone (GHRH) have been studied for treatment of DR. Thus, GHRH is an excellent candidate for association with DR. Isoform 1 of D-tyrosyl-tRNA(Tyr) deacylase 1 (DTD1) has been shown to exhibit proteomic differences in retina from diabetic mice and normal mice; thus it is another reasonable candidate. While these putative associations need to be replicated, we are encouraged by the biological plausibility of the results. Identification of genetic architecture of DR risk may lead to improved identification of patients at risk and better prevention and treatment strategies.

719T

Variants mapping to the *WNT16* locus are strongly associated with total body mineralization in children of different ethnic background and with total body BMD of elderly adults. *C. Medina- Gomez[1,3], J.P. Kemp[5,6], K. Estrada[1], D. Heppe[3], L. Herrera[1], L. Oei[1], A. Hofman[2], V.W. Jaddoe[3], P.H.C. Eilers[4], D.M. Evans[5,6], A.G. Uitterlinden[1,2], J.H. Tobias[7], F. Rivadeneira[1,2].* 1) Internal Medicine, Erasmus MC, Rotterdam, Netherlands; 2) Epidemiology, Erasmus MC, Rotterdam, The Netherlands; 3) The Generation R Study Group, Erasmus MC, Rotterdam, The Netherlands; 4) Biostatistics, Erasmus MC, Rotterdam, The Netherlands; 5) MRC Centre for Causal Analyses in Translational Epidemiology, University of Bristol, UK; 6) School of Social and Community Medicine, University of Bristol, Bristol, UK; 7) School of Clinical Science at North Bristol, University of Bristol, Bristol, UK.

Aim: Bone Mineral Density (BMD) is a highly heritable trait used to assess skeletal health showing strong ethnic variation. Multiple BMD loci have been identified in large studies of adult individuals from Northern European descent. We ran a genome-wide association study (GWAS) on total body (TB-)BMD to identify genetic determinants of bone accrual in a multiethnic children cohort; we replicated findings in British children of Northern-European origin and in 3 studies of mature and elderly Dutch individuals. **Methods**: The discovery set is part of the Generation R study, a birth cohort in Rotterdam, The Netherlands with >40% children of non-Northern European ancestry. We included 2,196 children (mean age=6.2,SD=0.28 yrs) with TB-DXA measurements and GWAS data (Illu660K). Replication was pursued in 5,434 children from the Avon Longitudinal Study of Parents and their Children (ALSPAC)(age=9.9,SD=0.32 yrs), and adults from the Rotterdam Study I (RS-I;n=2436), II (RS-II;n=749), and III (RS-III;n=668), age range 45-95. Replication cohorts were genotyped on Illu550K. Linear regression models were adjusted for age, sex, weight and population stratification. In the discovery cohort different numbers of genomic principal components (PC) were used (4-20) whereas for the North-European cohorts 4 PCs were incorporated. Inverse variance meta-analysis was performed. Genome-wide significance (GWS) was set at P<5x10-8. **Results**: In Generation R discovery set, genomic inflation factors were ~1 after PC correction regardless of the number of PCs used (λ=2.9 before correction). Two SNPs (MAF=0.27) in high LD mapping to 7q31 near the *WNT16* gene reached GWS after correction for 20 PCs, top-hit explaining 1.7% of the variation in TB-BMD (B=0.22;P=3.8x10-10). The association was replicated in ALSPAC (B=0.13; P=1.1x10-9) and all adult studies RS-I (B=0.13;P=6.3x10-5), RS-II (B= 0.21;P=5.2x10-4) and RS-III (B=0.14;P=0.02). Meta-analysis of all four studies was highly significant (B=0.15;P=2.1x10-24). **Conclusion**: We robustly show that variants in *WNT16* exert an effect on bone mineralization in children of different ethnicities and influences total body BMD variation in adulthood. *WNT16* is highly expressed in periochondrium and periosteum of developing long bones, in line with our results suggesting a role of Wnt factors on bone formation throughout life. These early effects of *WNT16* are likely to affect attainment of peak bone mass and impact the risk of osteoporosis in later life.

720T

Novel FDR approach identifies genes predictive of breast cancer survival. *J. Millstein[1], D. Volfson[2], E.E. Schadt[3], J. Bergh[4].* 1) Division of Biostatistics, University of Southern California, Los Angeles, CA; 2) iPierian Inc., South San Francisco, CA; 3) Mt. Sinai School of Medicine, New York, NY; 4) Karolinska Institutet and University Hospital, Sweden.

Permutation-based testing strategies have become broadly accepted and extremely useful in the analysis of genomic data, where parametric assumptions are usually difficult, if not impossible to meet across an overwhelming number of variables. However, the difficulty is that permutation-based approaches are computationally intensive. Permutation-based False Discovery Rate (FDR) methods are especially common in genomic studies that often include tens of thousands of tests or more. With such an essential role played in the interpretation of results, it is of paramount importance to understand the uncertainty associated with the FDR estimate. It is also important to know the number of permutations required to provide a reliable estimate. We describe fully non-parametric FDR point and confidence intervals estimators that do not require additional replicate randomly permuted data sets and are therefore computationally highly efficient. In fact, reliable FDR estimates are demonstrated using as few as 5 permutations. We applied this approach to gene expression micro-array data from poor prognosis breast cancer patients to identify genes predictive of breast cancer survival. We identified a statistically significant set of 53 genes including Single Minded 2 (SIM2) as well as other genes that otherwise might have been overlooked in a more standard analysis.

721T

Searching for genetic interactions in a genome-wide association study of psoriasis: a comparison of methods. *J.E. Mollon[1,2], D. Speed[3], K.K. Nicodemus[4], M.E. Weale[1,2].* 1) Medical and Molecular Genetics, King's College London, London, United Kingdom; 2) MRC Centre for Transplantation, King's College London; 3) UCL Genetics Institute, University College London; 4) Wellcome Trust Centre for Human Genetics, University of Oxford.

There is much interest in methods for analysing data from genome-wide association studies which go beyond traditional single-SNP methods. In particular it would be useful to have reliable methods that can identify epistatic interactions (here defined as departure from linearity in the additive genetic model). Several methods have been proposed for finding epistasis. A restriction on many of these is that they cannot handle genome-wide data, so SNPs are generally filtered first, often using single-SNP association p-values. Interactions that involve SNPs which have no main single-SNP association could be missed. Recently several new methods or adaptations of existing methods have made it possible to search through genome-wide association study data for interactions. In this study we investigate three promising methods - SNPHarvester, Sparse Partitioning and Random Jungle - on data from the WTCCC2 psoriasis GWAS. Random Jungle and SNPHarvester were run on genome-wide data and followed by a post-processing modelling step. Sparse Partitioning was run on genome-wide data in a single-step process. Outcomes of interest included common results from the three methods, any novel results replicated in a second GWAS, and identification of an interaction that was discovered in the WTCCC2 study.

722T

ROSELLA: Region Oriented Stringent Elastic-net Logistic Likelihood Analysis. *K.I. Morley[1,2], L. Jostins[1], J.A. Morris[1], D.G. Clayton[3], J.C. Barrett[1], The UK Inflammatory Bowel Disease Genetics Consortium.* 1) Statistical Genetics, Wellcome Trust Sanger Inst, Cambridge, United Kingdom; 2) Centre for Molecular, Environmental, Genetic, and Analytic Epidemiology, School of Population Health, The University of Melbourne, Melbourne, VIC 3010; 3) Juvenile Diabetes Research Foundation/Wellcome Trust Diabetes and Inflammation Laboratory, Cambridge Institute for Medical Research, Wellcome Trust/MRC Building, Addenbrooke's Hospital, Cambridge, CB2 0XY.

Until recently, data from genome-wide association studies (GWAS) were principally analyzed in a SNP-wise fashion, with statistical tests and associated p-values generated for each individual SNP. This approach, although successful, does not explicitly account for the correlated nature of SNP data, nor permit simultaneous consideration of multiple independent signals within a region. Addressing both analytical issues may increase capacity to identify association signals from GWAS data sets. Penalized regression methods offer a potential solution as they are suitable for identifying a small subset of the most important predictors from a large number of correlated variables. Some, such as the lasso, have previously been applied to GWAS data at a chromosomal or genome-wide level. However, broad use of these models has been hampered by difficulties in selecting appropriate values for penalization parameters and determining statistical significance. We have developed a gene-region oriented test for association that uses another penalized regression model, the elastic-net. Our test incorporates novel methods for identifying appropriate values for alpha (the mixing parameter for the elastic-net penalty) and lambda (the penalization parameter), as well as a test statistic for assessing the statistical significance of a region, based upon a mixture of chi-square distributions. The method is implemented in a software package, ROSELLA, which provides a user-friendly interface to region-based analyses on a genome-wide level. It is written in C++ and builds upon the implementation of an elastic-net estimation algorithm recently developed by Friedman et al. (2010) for the R statistical programming environment. Using simulated data sets, we demonstrated that the test implemented in ROSELLA has acceptable type-I error across genomic regions of variable size and SNP coverage, and greater power to detect regions of allelic heterogeneity than most currently available alternatives. We used ROSELLA to re-analyze GWAS data sets for Crohn's disease (WTCCC1) and ulcerative colitis (WTCCC2), using the GENCODE gene list. For both data sets we identified all loci found in the original study, and some additional loci that were subsequently identified in larger meta-analyses. For regions of known allelic heterogeneity (e.g. *NOD2* and *IL23R* in Crohn's disease) the significance of the region-oriented test surpassed that of any single SNP in the region by several orders of magnitude.

723T

SNPs near CCNL1 associated with infant adiposity in multi-ethnic mega analysis with strongest effect observed in European ancestry newborns. *J. Morrison[1], D.A. Scheftner[2], A. Pluzhnikov[1], L.P. Lowe[2], C. Ackerman[2], L. Armstrong[2], A.R. Dyer[2], H. Lee[2], M. Urbanek[2], C. McHugh[3], D. Mirel[4], B.E. Metzger[2], M.G. Hayes[2], N.J. Cox[1], W.L. Lowe[2], The HAPO Study Cooperative Research Group.* 1) The University of Chicago, Chicago, IL; 2) Northwestern University, Chicago, IL; 3) University of Washington, Seattle, WA; 4) The Broad Institute, Cambridge, MA.

We investigated genetic factors contributing to variation in infant size and adiposity in infants enrolled in the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) Study. To assess adiposity in newborns, caliper measurements of flank, tricep and sub-scapular skinfolds were taken in millimeters. We used the square-root of the sum of these three measurements [SQ_SSK] as our phenotype of interest. In analysis of 1303 infants of self-identified mothers of northern European ancestry from four study sites in Europe, North America and Australia, we found SNPs in a haplotype upstream of CCNL1 on chromosome 3 to be significantly associated with the SQ_SSK phenotype. The major T allele of rs17451107 was found to be associated with an increase in SQ_SSK of 0.074 per T-allele (se 0.013; p-value 1.71*10-8) which corresponds to an increase of 0.005 mm in summed skinfold measurements. When the Caucasian cohort was combined in mega-analysis with a data set of 874 Mexican-American infants and a set of 1284 Afro-Caribean infants, we estimated an effect size of 0.141 per T allele (se 0.023; p-value 8.97*10-10). SNPs in this haplotype have been previously found to be associated with infant birth weight and ponderal index, however, we did not find significant associations with any fetal phenotypes other than SQ_SSK. The SNPs in this haplotype did not reach genome-wide significance in either of the non-European ancestry data sets though we did observe moderately significant results with similar magnitude effect sizes in both cohorts. In Mexican-American infants we found an effect size of 0.192 per T allele (se 0.05; p-value 8.84*10-5) and in Afro-Carribean infants we found an effect size of 0.087 per T allele (se 0.04; p-value 0.042). The allele frequencies in the three cohorts are similar (0.35-0.42 minor allele frequency) and the distribution of the phenotype is also comparable between cohorts with the infants in the Mexican-American cohort having slightly higher SQ_SSK measures than the European ancestry infants and the Afro-Caribbean infants having slightly lower measures. This suggests that the different effects estimated in the three populations may result from an interaction with an undiscovered factor which differs between the groups.

724T

A genome wide association scan on the levels of inflammatory markers in Sardinia reveals associations with genes related with malaria resistance. *S. Naitza[1], E. Porcu[1], P. Scheet[2], J. Strait[3], X. Xiao[2], D.D. Taub[3], M. Dei[1], S. Lai[1], A. Mulas[1], F. Busonero[1], A. Maschio[1], G. Usala[1], M. Zoledziewska[4], C. Sidore[1,5], M.B. Whalen[6], D.L. Longo[3], E. Lakatta[3], A. Cao[1], G.R. Abecasis[5], D. Schlessinger[3], M. Uda[1], S. Sanna[1], F. Cucca[1,4].* 1) IRGB, CNR, Monserrato, Italy; 2) University of Texas, MD Anderson Cancer Center, Department of Epidemiology, Houston, Texas, USA; 3) Intramural Research Program, National Institute on Aging, 5600 Nathan Shock Drive, Baltimore, Maryland 21224, USA; 4) Dipartimento di Scienze Biomediche, Università di Sassari, Sassari, Italy; 5) Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, Michigan, USA; 6) Center for Advanced Studies, Research and Development in Sardinia (CRS4), AGCT Program, Parco Scientifico e tecnologico della Sardegna, Pula, Italy.

Identifying the genes that influence levels of pro-inflammatory molecules can help to elucidate the mechanisms underlying inflammation, and has implications, both positive and negative, about the co-evolution of host and parasites and diseases. We thus conducted a genome-wide association scan (GWAS) in a cohort of 4,694 individuals from the founder Sardinian population for key inflammatory biomarkers Interleukin-6 (IL-6), the general measure of inflammation erythrocyte sedimentation rate (ESR), monocyte chemotactic protein-1 (MCP-1) and high-sensitivity C-reactive protein (hsCRP). Overall, we typed 731,213 autosomal or X chromosome SNPs and imputed an additional ~1.9 million variants in the cohort. For IL-6, the strongest association was in the ABO locus, with a tag of the O allele (p= 2.69x10-21), providing a mechanistic clue for previous associations with the O blood group. For ESR, genome-wide significance was found for SNPs in CR1 (p=9.31x10-11). For MCP-1, strong confirmatory evidence was found for association with the DARC gene (p=1.68x10-30) and near the CCR2 receptor (p=8.49x10-08). For hsCRP, the strongest association signal was in the CRP gene (p=2.82x10-16) with a novel independent signal near the DARC gene (p=4.75x10-07). All the top signals were replicated in 1,392 individuals from an independent sample of Sardinians. Strikingly, three of the observed loci, ABO, DARC and, CR1, are known to be involved in resistance to malaria, which was endemic in Sardinia until a few decades ago. Indeed, by Fst analysis we showed that the frequency of the CR1 and ABO variants associated with ESR and IL-6, respectively, differs significantly in Sardinians when compared to other populations where malaria was not endemic and are more similar to that detected in malaria endemic populations. These results suggest that the genetic selection imposed by malaria may have contributed to increased levels of inflammation in this population.

725T

Genome wide linkage and family-based association analysis in musical aptitude. *J. Oikkonen[1,2], L. Ukkola-Vuoti[1], P. Onkamo[2], P. Raijas[3], K. Karma[4], I. Järvelä[1,5].* 1) Department of medical genetics, University of Helsinki, Helsinki, Finland; 2) Department of Biosciences, University of Helsinki, Helsinki, Finland; 3) DocMus Department, Sibelius Academy, Helsinki, Finland; 4) Department of Music Education, Sibelius Academy, Helsinki, Finland; 5) Laboratory of Molecular Genetics, Helsinki University Central Hospital, Helsinki, Finland.

Musical aptitude is a complex cognitive function of the human brain. It provides an excellent system for studying human behavior and brain functioning. Family and twin studies, brain imaging studies and molecular genetic studies suggest biological background contributing to musical aptitude. However, mechanisms underlying musical aptitude are largely uncharacterized. Our previous linkage study showed evidence for linkage for several loci. In this study we try to verify the previous results with a high-density marker panel of single nucleotide polymorphisms on extended family material. 96 extended families (N=1011) were collected from Finnish population. Musical aptitude was tested using auditory structuring ability test (Karma music test), and Seashore pitch and time discrimination subtests. DNA from 688 individuals was analyzed on the Illumina HumanOmniExpress 12 1.0v SNP chip. Preliminary results from family-based association analysis show association in several loci. One of these loci is located near corticotropin releasing hormone receptor 2. Interestingly we also detected an association near the best scoring area of the previous linkage study at chromosome 4q. We also carry out linkage analyses, the results of which will be presented in the meeting.

726T

Largest UK rheumatoid arthritis genome wide association study to date of 8,300 samples strengthens confirmed loci and highlights more potential RA genetic risk factors. *G. Orozco, S. Eyre, J. Bowes, E. Flynn, A. Barton, J. Worthington.* Arthritis Research UK Epidemiology Unit, University of Manchester, Manchester, United Kingdom.

The number of confirmed rheumatoid arthritis (RA) loci currently stands at 31 but many lines of evidence indicate this is unlikely to be the final number, and that additional, well powered genome wide association studies (GWAS) will still be required to develop a full picture of the genes involved in RA. The objective of this study was to extend our previous Wellcome Trust Case Consortium RA GWAS adding more independent cases and control samples, with the aim to increase power to confirm previously identify loci and to detect novel association signals for the susceptibility to RA. We had available 3223 UK RA cases and 5272 UK controls, which adds 1361 cases and 2334 controls to the original GWAS. All samples were genotyped using Affymetrix or Illumina arrays. The genotype data for all samples was imputed using IMPUTE2, with the 1000 Genomes Project and HapMap3 data as reference panels, to increase and unify the genomic coverage between samples. After a stringent QC was applied, we had 3034 cases and 5271 controls and 1831729 SNPs. Association analysis was performed using PLINK. We confirmed association to 22 of the 31 previously known RA susceptibility loci. For 15 of them, increased evidence of association was found when compared to the WTCCC GWAS. We also found association evidence for 2 loci that were at a suggestive significance level from the latest meta-analysis, *CD247* and *UBE2L3*. We did not find association with 5 of the known RA loci. However, these were not associated with RA in the original WTCCC GWAS either, so it is possible that they have a modest effect in RA predisposition in UK population. We could not find proxies for 4 of the known RA loci. 12 novel loci were associated with disease at genome-wide significance level. In addition, we found 10 novel RA loci in the next tier of significance ($P<10^{-5}$) that have been associated with other autoimmune diseases or have shown suggestive evidence of association with RA in previous meta-analysis of GWAS data. These 22 novel loci were selected for follow up. Preliminary validation data for 9 of these novel RA loci has confirmed association to 22q12, a region that has been associated with Crohn's disease and type 1 diabetes. In conclusion, we present preliminary results on the largest UK RA GWAS performed to date. Our initial findings suggest that there will be novel loci discovered in this study, but validation in a larger, independent UK cohort is required and is currently ongoing.

727T

Extremely low-coverage sequencing enables cost effective GWAS. *B. Pasaniuc[1,2,3], N. Rohland[3,4], H. Li[3], N. Zaitlen[1,2,3], P. McLaren[3,5], K. Garimella[3], L. Liang[1,2,3], P. deBakker[3,5], S. Sunyaev[3,5], N. Patterson[3], D. Reich[3,4], A.L. Price[1,2,3].* 1) Epidemiology Dept, Harvard School of Public Health, Boston, MA; 2) Biostatistics Dept, Harvard School of Public Health, Boston, MA; 3) Broad Institute of Harvard and MIT, Cambridge, MA; 4) Genetics Dept, Harvard Medical School, Boston, MA; 5) Division of Genetics, Brigham and Women's Hospital, Boston, MA.

Genome wide association studies (GWAS) have proven to be a powerful method for scanning the genome for common genetic variants contributing risk for disease. To date GWAS have been carried out using single nucleotide polymorphism (SNP) arrays. Here we show that extremely low-coverage sequencing (0.1-0.5x) is able to capture almost as much of the common (>5%) and low-frequency (1-5%) variation across the genome as SNP arrays. As an empirical demonstration of the power of this approach, we show that genome-wide SNP genotypes can be inferred at an r2 of 0.73 with genotyping arrays using off-target data (at an average of 0.5x) in an exome sequencing study of 127 samples of European ancestry from the International HIV Controllers Study, AIDS clinical trials group and the Swiss HIV Cohort Study. At known associated variants, imputation-aware association statistics are of similar magnitude as statistics computed using typed genotypes while properly controlling the false positive rate (genomic control (GC) of 1.03 for imputed as compared to 1.04 for typed genotypes). In light of recent reductions in sample preparation and sequencing costs, funds invested in ultra low-coverage sequencing are expected to yield several times the effective sample size of SNP-array GWAS for appropriately designed studies. Thus, for projects that wish to maximize statistical power as a function of cost, ultra-low coverage sequencing may be an attractive option.

728T

Unprogrammed presentation number

729T

A Bayesian Association Testing Approach that Accounts for Epigenetic Characteristics as well as Prior Molecular and Pathway Information. *C. Rakovski.* Mathematics, Chapman University, Orange, CA.

We propose a novel strategy applicable to association studies of all scales that advantageously addresses two important issues, accounting for epigenetic characteristics of the subjects and incorporation of important prior information regarding molecular characteristics of the proteins and putative pathway structure. We conduct a large scale simulation study to assess both the type I error rate behaviors of the new method as well as the power gains in comparison to classical association strategies under various scenarios that include genomewide/candidate gene data, complete/incomplete knowledge of the prior and the epigenetic variables as well as prior misspecification. Our results provide important details in understanding the benefits and risks of incorporating complex additional information to current genetic association studies.

730T

Genome-wide meta analyses of human anthropometric traits across 270,722 individuals reveal 6 new genetic associations with evidence for sexual dimorphism as well as evidence for sexual dimorphism at 8 previously reported overall genetic associations. *J.C. Randall[1,2], Z. Kutalik[3,4], T.W. Winkler[5], S.I. Berndt[6], A.U. Jackson[7], T.O. Kilpeläinen[8], K.L. Monda[9], L. Qi[10,11], T. Workalemahu[10], J. Czajkowski[12], F. Day[8], T. Esko[13], M.F. Feitosa[12], R. Mägi[1], I. Mathieson[1], V. Steinthorsdottir[14], G. Thorleifsson[14], I.B. Borecki[12], I.M. Heid[5,15], C.M. Lindgren[1], R.J.F. Loos[8], K.E. North[9,16] for the GIANT Consortium.* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, United Kingdom; 2) Department of Statistics, University of Oxford, 1 South Parks Road, Oxford, OX1 3TG, United Kingdom; 3) Department of Medical Genetics, University of Lausanne, Lausanne, Switzerland; 4) Swiss Institute of Bioinformatics, Lausanne, Switzerland; 5) Regensburg University Medical Center, Department of Epidemiology and Preventive Medicine, Regensburg, Germany; 6) Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA; 7) Department of Biostatistics, Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan 48109, USA; 8) Medical Research Council (MRC) Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, United Kingdom; 9) Department of Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; 10) Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts, USA; 11) Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA; 12) Division of Statistical Genomics, Department of Genetics, Washington University School of Medicine, St. Louis, Missouri 63108, USA; 13) Estonian Genome Centre, University of Tartu, Tartu, Estonia; 14) deCODE Genetics, Reykjavik, Iceland; 15) Institute of Epidemiology, Helmholtz Zentrum München-German Research Center for Environmental Health, Neuherberg, Germany; 16) Center for Genome Sciences, University of North Carolina, Chapel Hill, North Carolina 27587 USA.

Genome-wide association studies (GWAS) of complex traits have previously reported sexually dimorphic associations, but were limited to variants found in overall analyses, performing sex-specific analyses only in follow-up. To increase power to detect sexually dimorphic genetic variants with an effect on human anthropometric traits, we conducted sex-specific meta-analyses of 9 phenotypes: height (HT), weight (WT), body mass index (BMI), waist circumference (WC), hip circumference (HC), WC/HC ratio (WHR), WC adjusted for BMI (WCadjBMI), HC adjusted for BMI (HCadjBMI), and WHR adjusted for BMI (WHRadjBMI).

In the discovery stage, we performed sex-specific meta-analyses of 58 GWAS, comprising 60,586 men and 73,137 women. Each study used an additive model to test up to ~2.8M imputed SNPs for association with inverse-normal transformed phenotypes. We controlled for false discovery rate at 5% across all analyses, yielding a set of 619 independent loci taken forward into follow-up, which included 63 additional studies comprising 62,399 men and 74,660 women, many of which were genotyped on Metabochip, a custom Illumina iSelect array to which we submitted sex-specific SNPs.

We replicated sex-specific associations at 205 independent loci (combined $P<5x10^{-8}$ with consistent effect direction), and tested the difference in effect estimate between men and women, yielding 4 loci with significant ($P<0.05/205$), 14 loci with suggestive ($P\leq0.05$), and 186 loci with no ($P>0.05$) evidence for sexual dimorphism. Among the 18 loci showing evidence for sexual dimorphism, we discover new sex-specific associations with evidence of sexual dimorphism at 6 loci (1 WCadjBMI, 3 WHRadjBMI, 2 HT), confirm previously reported sexual dimorphism of WHRadjBMI at 4 loci (*GRB14*, *LYPLAL1*, *VEGFA*, and *ADAMTS9*), and find suggestive evidence for sexual dimorphism at 8 loci previously reported as overall associations (2 WT, 6 HT).

Novel associated loci include those implicated in breast cancer and testis development (*MAP3K1*, WCadjBMI sex-diff. $P=1.1x10^{-4}$), in peroxisomal fatty acid oxidation (*HSD17B4*, WHRadjBMI sex-diff. $P=3.3x10^{-3}$), and in the regulation of adipocyte differentiation (*PPARG*, WHRadjBMI sex-diff. $P=8.4x10^{-3}$). We conclude that sexually dimorphic genetic variants do exist for some anthropometric traits, while for other traits we may have been underpowered to detect them.

731T

An evaluation of several statistical approaches to detect rare variants in Genome Wide Association data of Parkinson's disease. *M. Saad[1,2], S. Lesage[3], A. Brice[4], M. Martinez[1,2].* 1) Inserm, UMR 1043, Toulouse, France; 2) Paul Sabatier University, Toulouse, France; 3) Inserm UMR_S975, Paris, France; 4) UMR_S975, AP-HP, Pitié-Salpêtrière Hospital, Paris, France.

The common disease-multiple rare variant hypothesis has recently received much attention. Different statistical methods have been developed for testing the hypothesis that collections of rare variants are associated with a disease in a case-control sampling design [Am J Hum Genet 2008, 311-21; PLoS Genet 2009, e1000384; Genet Epidemiol 2010,188-93; Am J Hum Genet 2010, 832-8]. The detection of rare polymorphisms requires high-quality whole-genome sequence data of a large number of cases and controls and remains expensive. Alternatively, recent studies showed that rare variants can be imputed into existing GWAS datasets from publicly available sequenced data, as the 1000 Genomes Project (i.e., pseudo-sequencing data). In addition, it has been reported that collapsing-based test of single-marker dosages in pseudo-sequencing data may have greater power than haplotype-based methods in genotyped data [Am J Hum Genet 2010,718-35]. Here, we evaluate such approaches in the genome-wide association data of Parkinson's disease [Hum Mol Genet 2011, 615-27]. Imputations were conducted with IMPUTE. We tested association at the gene level. The lowest rates of positive signals (exceeding a given significance threshold) are observed when the cumulative sum test is limited to the uncommon variants. We also show that depending on the approach, different sets of genes may be identified as being associated to the disease. Thereby, drastically different conclusions might be reached.

732T

Genome-wide association and interaction studies identify risk alleles for Congenital Heart Defects in Down syndrome. *M.R. Sailani[1,2], P. Makrythanasis[2], S. Deutsch[2], E. Migliavacca[2], A.J. Sharp[2], J.M. Delabar[3], L. Perez-Jurado[4], C. Serra-Juhe[4], G. Merla[5], S. Vicari[6], E. Kanavakis[7], R. Rabionet[8], X. Estivill[8], Y. Grattau[9], G. Dembour[10], A. Megarbane[9,11], R. Touraine[12].* 1) NCCR Frontiers in Genetics, University of Geneva; 2) University of Geneva, Geneva, Switzerland; 3) Université Paris Diderot, Paris, France; 4) Universitat Pompeu Fabra, Barcelona, Spain; 5) IRCCS, San Giovanni Rotondo, Italy; 6) Children's Hospital Bambino Gesù, Rome, Italy; 7) Laboratory of Medical Genetics, University of Athens Medical School, Athens, Greece; 8) Centre for Genomic Regulation, Barcelona, Spain; 9) Institut Jerome Lejeune, Paris, France; 10) Cliniques universitaires Saint-Luc, Bruxelles, Belgium; 11) Université Saint-Joseph, Beirut, Lebanon; 12) CHU de Saint-Etienne, hôpital Nord, Saint-Etienne Cedex 2, France.

Congenital heart defect (CHD) in Down syndrome (DS) is a common developmental defect occurring in 40% of these cases. Here, we show that genomic variation in concert with the trisomy 21 contributes to the risk for CHD in DS. We performed GWAS and Genome Wide Interaction Study (GWIS) in 192 DS with CHD (AVSD=72, ASD=53, VSD=67) as cases and 156 DS and 215 healthy individuals without CHD as controls. Single-locus association reveals rs2832616, rs1943950, rs2822392, and rs1785683 on chr21 as CHD risk alleles (Bonferroni P-values < 0.05). The first 2 of these SNPs are also cis-eQTLs for KRTAP7-1 gene. Also rs2822392 was identified as a risk factor for AVSD+VSD combined, while rs2183593 (cis-eQTL for the KRATP10-9, C21orf57, and C21orf123 genes) was identified as risk factor for ASD and AVSD+ASD combined. Non-chr21 GWAS reveals rs2066843 and rs2076756 ($P < 5 \times 10E-8$) both located in NOD2 gene as risk factors for CHD and VSD+ASD combined, both showing cis-eQTL effects for the HEATR3 gene. Furthermore, a 2-locus genomewide analysis shows significant interaction signals between loci on chr5 and chr6 ($P < 5 \times 10E-11$). Since DS is likely to be a disorder of gene expression, 2-locus interaction was applied for whole genome eQTLs as a subset of the SNPs space. Interestingly, eQTLs analyses reveal an interaction between eQTLs for CLCNKA on chr1 and USMG5 on chr10 ($P < 5 \times 10E-11$). We propose that the CHD risk of DS is determined by variations on chr21, variations on other chromosomes, and genome-wide interaction of specific alleles.

733T

Adjusting Rare Variant Association Tests for Population Stratification Using the Stratification Score. *G.A. Satten[1], Q. Ling[2], M.P. Epstein[3], A.S. Allen[4,5].* 1) Div Reprod Hlth, CDC, Atlanta, GA; 2) Dept of Biostats and Bioinf, Emory Univ, Atlanta GA; 3) Dept of Human Genet, Emory Univ, Atlanta GA; 4) Dept of Biostats and Bioinf, Duke Univ, Durham NC; 5) Duke Clinical Research Inst, Duke Univ, Durham NC.

Background: Association studies that collect sequence-level data can be used to compare the proportion of case and control participants that have rare variants in a region of the genome (or exome). As with SNP association studies, it is important that test findings reflect true association, not confounding due to population stratification. However, many such tests like the C(( ) test [Neale et al. PLoS Genet e1001322] are not regression-based, so principal-components-based adjustment for population stratification is problematic.

Methods: We show how the stratification score (Epstein, Allen & Satten ASHG 2007 80: 921-930) can be used to form strata such that stratified versions of any test statistic account for confounding. Alternatively, we reformulate tests like the C(( ) test as a comparison between the proportion of case and control participants having a rare variant at each locus, then standardize these proportions the over strata after Allen and Satten (Amer J Epid 2011 173:752-760). This gives the test statistic that we would have obtained if cases and controls each had the same population substructure. Significance can be assessed either asymptotically or by within-stratum permutation, which preserves population substructure but removes disease-variant associations.

Results: We study the size and power of the stratification-score-based C(( ) test using coalescent-based simulated data that mimics the African-American population. We also consider the effect of choice of standardizing distribution on the power of the test.

734T

Statistics for Global Scanning of Gene Sets using Genome Wide Association Studies. *D.J. Schaid, J.P. Sinnwell, S.K. McDonnell, G.D. Jenkins.* Dept Hlth Sci Res, Mayo Clinic Col Med, Rochester, MN.

Gene-set analyses have been widely used in gene expression studies, and some of the developed methods have been extended to genome wide association studies (GWAS). Yet, complications due to linkage disequilibrium (LD) among single nucleotide polymorphisms (SNPs), and variable numbers of SNPs per gene and genes per gene-set, have plagued current approaches, often leading to ad hoc "fixes". To overcome some of the current limitations, we developed a general approach to scan GWAS SNP data for both gene-level and gene-set analyses, building on score statistics for generalized linear models, and taking advantage of the directed acyclic graph structure of the gene ontology (GO) when creating gene-sets. However, other types of gene-set structures can be used, such as the popular Kyoto Encyclopedia of Genes and Genomes (KEGG). Our approach combines SNPs into genes, and genes into gene-sets, but assures that positive and negative effects of genes on a trait do not cancel. To control for multiple testing of many gene-sets, we use an efficient computational strategy that accounts for LD and provides accurate step-down adjusted p-values for each gene-set. Application of our methods to two different GWAS provide guidance on the potential strengths and weaknesses of our proposed gene-set analyses.

735T

Genome Wide Association Study Reveals Genetically Distinct Phenotypic Subtypes of Age-Related Macular Degeneration Distinct. *M. Schu[1], G. Jun[1,2,3], M.A. Morrison[6,7], J. Farrell[1], I.K. Kim[6], D.J. Morgan[7], G.S. Hageman[7], L.A. Farrer[1,2,3,4,5], M.M. DeAngelis[6,7].* 1) Dept Medicine (Biomedical Genetics), Boston University School of Medicine, Boston, MA; 2) Dept Ophthalmology, Boston University School of Medicine, Boston, MA; 3) Dept Biostatistics, Boston University School of Medicine, Boston, MA; 4) Dept Neurology, Boston University School of Medicine, Boston, MA; 5) Dept Epidemiology, Boston University School of Medicine, Boston, MA; 6) Dept Ophthalmology, Massachusetts Eye & Ear Infirmary, Boston, MA; 7) Ophthalmology & Visual Sciences, John A. Moran Eye Center, Center for Translational Medicine, University of Utah, Salt Lake City, UT.

Age related macular degeneration (AMD) affects over 10% of individuals older than age 40. The late stage of the disease has two main forms, geographic atrophy (GA) and neovascular "wet" AMD. GA is characterized by degradation of retinal pigment epithelial cells, whereas wet AMD is associated with growth of abnormal blood vessels which results in blood and fluid leakage into the macula. Both forms of the disease cause severe debilitating vision loss. Genome wide association studies (GWAS) have identified loci in two gene regions associated with increased risk for AMD, CFH and a haplotype spanning the HTRA1/ARMS2 region of chromosome 10. Previous studies have looked for associations with the collective late forms of AMD, however, in this study we performed genome-wide association tests using HapMap2 imputed SNPs for wet AMD and GA as distinct outcomes while accounting for two known genetic risk factors as well as age at exam and sex. The discovery sample comprised three Caucasian datasets including the Age Related Eye Disease Study (AREDS), the Michigan, Mayo, and Pennsylvania (MMAP) dataset, and the New England Sibling Cohort, yielding a total of 761 wet AMD cases, 394 GA cases, and 1369 controls. Each dataset was analyzed separately and then meta-analyzed using the inverse variance method implemented in METAL. The top-ranked result for both outcomes was obtained with CFH intronic SNP rs10737680 (Wet: $p=1.7x10^{-10}$, GA: $p=1.4x10^{-13}$) which is 20kb distal from the Y402H polymorphism (rs1061170). Because these two SNPs are modestly correlated ($R^2=0.28$) and the rs10737680 was significant after adjusting for rs1061170, this finding suggests there may be distinct multiple functional variants within the CFH region. Both end-stage forms of AMD were also associated with SKIV2L (Wet: $p=1.9 x10^{-10}$, GA: $p=1.7x10^{-7}$) and C3 (Wet: $p=1.1x10^{-7}$, GA: $p=3.4x10^{-9}$). Novel associations were observed for wet AMD with PRKD1 ($p=4.4x10^{-7}$), TCL1A ($p=6.5x10^{-7}$), and ADAM19 ($p=9.9 x10^{-7}$). Two loci were found to be associated primarily with GA, the ITGB8/ABCB5 region (Wet: p=0.02, GA: $p=2.1x10^{-7}$) and MIR2053 (Wet: p=0.0017, GA: $p=3.6x10^{-7}$). These results support the hypothesis that GA and wet AMD are likely distinct diseases that have both common and unique genetic risk factors. Replication of these results in independent cohorts is currently underway.

736T

Quality assessment of parallelization strategy for genome-wide imputation using beagle. *H. Sicotte, M. de Andrade, M.E. Matsumoto, N. Prodduturi, J.P. Kocher.* Health Sciences Research, Mayo Clinic, rochester, MN.

Genome wide imputation methods are rapidly becoming standard practice in genome wide association studies (GWAS) to enable merging of multiple datasets, to enable cross-platform comparison of results, and to find candidate functional variants in linkage disequilibrium (LD) with SNPs with significant GWAS association. Performing imputation on thousands of samples for millions of SNPs can take months of computer time with little parallelization possible using current software. Segmenting the genome in non-overlapping segments could lead to shorted run time on a computer cluster, but this naïve strategy might lead to lower quality imputation near segment edges since these edges would not benefit from the information provided by markers in LD with the imputed region. We have parallelized the BEAGLE imputation software by segmenting chromosomes in overlapping segments. To ensure that this strategy gave results as accurate as the whole chromosome imputation, each imputed segment was run using an extra buffer region. Each buffer regions contributed to the imputation of the segment region, but the imputed values in the buffer region were discarded. We studied the difference between the whole chromosome imputation and the buffered segment approach as a function of buffer size. To control for run to run variation, we also compared whole chromosome imputation run with different random seeds. There were no significant differences between whole chromosome imputation and the overlapping segmentation strategy for buffer sizes from 4MB down to 125kB. Differences between the two strategies were located in reproducible spots whose location was not related to the distance from the edges of segments. Some of those hotspots correlated with peaks of low estimated r^2 (the correlation between the true genotype and the imputed genotypes) while some hotspots were variable when running the whole chromosome algorithms with different random seeds, even though the estimated r^2 was excellent. Our results indicate that parallelizing by segmenting with a small buffer region (125Kb) provides as good imputation as imputating for an entire chromosome at a time. Our results also indicate that some SNPs have variable imputation that is not captured by the r^2 statistic. These variable SNPs can be detected by performing multiple runs of imputation. Although our results were derived using BEAGLE, they should apply to other imputation methods as well.

737T

Free Controls and Two-stage Designs: An opportunity to reduce cost and increase power. *A.D. Skol[1], S.A. Stanhope[2].* 1) Dept of Medicine, Sec of Genetic Medicine, Univ Chicago, Chicago, IL; 2) Dept of Human Genetics, Univ Chicago, Chicago, IL.

The two-stage genome-wide association study (2S-GWAS) was conceived as a way to maintain the power of a single stage GWAS while reducing its cost. In a 2S-GWAS, a proportion of the cases and controls are genotyped in stage 1 using a whole genome genotyping platform, and markers that demonstrate sufficient evidence of disease association are genotyped in stage 2 on the remaining cases and controls. Information from stages 1 and 2 are then combined and association between these markers and disease is evaluated. We have extend the 2S-GWAS to allow different proportions of cases and controls to be assigned to stage 1. Such flexibility is especially necessary when incorporating genome-wide genotype information from free controls (FCs). We demonstrate that allowing different proportions of cases and controls to be assigned to stage 1 can substantially improve the cost advantage of the standard 2S-GWAS. Further, we show that FCs can be used to either increase the power of a two-stage design while holding study costs constant or decrease the cost while maintaining the same power. Finally, we illustrate how 2S-GWAS designs that incorporate FCs can simultaneously yield both increased power and lower cost relative to those that do not use FCs. In summary, the use of more flexible 2S-GWAS that properly use FCs can further aid the GWAS community to identify disease predisposing variation. We have developed software, FreeConCaTS, that can be used to either calculate power for or determine the optimal design of a 2S-GWAS.

738T

Incorporating Genetic Ancestry into Risk Prediction Models. *N. Solovieff[1], C. Baldwin[2], M. Steinberg[3], T. Perls[3], P. Sebastiani[1].* 1) Department of Biostatistics, Boston University School of Public Health, Boston, MA; 2) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 3) Department of Medicine, Boston University School of Medicine, Boston, MA.

Genome-wide association studies have identified many disease associated variants for complex traits and have brought much attention to constructing genetic risk prediction models. Typically, investigators use a small number of genome-wide significant SNPs to construct a genetic risk model, either by including the individual SNPs as covariates in a regression model or by summarizing the risk alleles into a genetic risk score (GRS). However, these models do not account for varying SNP effects in subgroups of subjects, for example different ethnic groups, which may improve the discriminatory accuracy. For example, in a study of exceptional longevity the SNP rs405509 in APOE, a gene known to be associated with aging and dementia, has varying allele frequencies and odds ratios across Europe. Models that leverage this type of ancestral information may improve genetic risk prediction models. We present extensions to a Bayesian naïve classifier and to the traditional GRS that incorporate information about genetic ancestry. In practice, we often build a model using a training dataset and then want to use the model to make a prediction in a new individual or new set of data. However, if the ancestry of the new individual is unknown the typical prediction models cannot be used. We describe an extension to the Bayesian classifier that allows one to predict the phenotype in a new individual when ancestry information is not available. We compare these methods and show that incorporating genetic ancestry improves the accuracy of the prediction when SNPs with varying effects across ancestry are included. An improvement in accuracy is observed both in the Bayesian framework and in the GRS model; however, the accuracy is higher in the Bayesian framework. Finally, these models are not specific to ancestry and can incorporate any environmental factor that modifies the association between the SNP and the phenotype.

739T

Increasing Power of Groupwise Association Test with Likelihood Ratio Test. *J. Sul[1], B. Han[1], E. Eskin[1,2].* 1) Computer Sci, Univ California, Los Angeles, Los Angeles, CA; 2) Department of Human Genetics, Univ California, Los Angeles, Los Angeles, CA.

Sequencing studies have been discovering a numerous number of rare variants, allowing the identification of the effects of rare variants on disease susceptibility. As a method to increase the statistical power of studies on rare variants, several groupwise association tests that group rare variants in genes and detect associations between groups and diseases have been proposed. One major challenge in these methods is to determine which variants are causal in a group, and to overcome this challenge, previous methods used prior information that specifies how likely each variant is functional. Another source of information that can be used to determine causal variants is observation data because case individuals are likely to have more causal variants than control individuals. In this paper, we introduce a likelihood ratio test (LRT) that uses both data and prior information to infer which variants are causal and uses this finding to determine whether a group of variants is involved in a disease. We demonstrate through simulations that LRT achieves higher power than previous methods such as a variable-threshold approach by Price et al., a weighted sum statistic by Madsen and Browning, and an optimal weighted aggregate statistic by Sul et al. Unlike previous methods that use a sum or a linear combination of mutation counts as their statistics, LRT uses a nonlinear combination of mutation counts. We discuss how this difference influences the statistical power of groupwise methods by showing their decision boundaries. We show that the nonlinear combination of mutation counts yields a nonlinear decision boundary that allows LRT to achieve higher power than previous methods. In addition, we evaluate our method on mutation screening data of the susceptibility gene for ataxia telangiectasia, and show that LRT can detect an association in real data. To increase the computational speed of our method, we show how we can decompose the computation of LRT, and propose an efficient permutation test. With this optimization, we can efficiently compute an LRT statistic and its significance at a genome-wide level. The software for our method is publicly available at http://genetics.cs.ucla.edu/rarevariants.

740T

Association Testing on the X-Chromosome in Case-Control Samples with Related Individuals. *T. Thornton[1], M.S. McPeek[2,3].* 1) Biostatistics, University of Washington, Seattle, WA; 2) Statistics, University of Chicago, Chicago, Il; 3) Human Genetics, University of Chicago, Chicago, IL.

It is now common for association studies to have data for hundreds of thousands of markers across the entire genome. Despite the potential for complex traits to have X-linked causal genes, association methods have primarily been developed for the analysis of markers on the autosomal chromosomes, and significantly less attention has been given to analyzing X-linked markers. We consider the problem of case-control association testing with markers on the X-chromosome when some sampled individuals are related, with known relationships. We propose a method that appropriately adjusts for correlated alleles and genotypes among same and different gender relatives and also accounts for allele copy number differences in females and males. Some of the features of the method include: (1) it is applicable to completely general combinations of family and case-control designs, (2) it allows for both unaffected controls and controls of unknown phenotype to be included in the same analysis, (3) it can incorporate phenotype information on relatives with missing genotype data, and (4) it adjusts for sex-specific trait prevalence values. In simulation studies with related individuals, we demonstrate that the method has the correct type 1 error. We apply the method to X-chromosome data from the Collaborative Study of the Genetics of Alcoholism in a sample of moderate-size pedigrees of European descent, from Genetic Analysis Workshop 14. We detect a significant association, after Bonferroni correction, with alcohol dependence and a SNP located within the MAOA gene. The MAOA gene has previously been found to be associated with substance abuse and antisocial behavior. We also demonstrate the potential improvement of the test statistic when using a robust variance estimator over a variance estimator that assumes HWE at the marker.

741T

How much information is disclosed from the margins of high dimensional data? *A.L. Voorman[1], K.M. Rice[1], T. Lumley[2].* 1) Biostatistics, University of Washington, Seattle, WA; 2) Biostatistics, University of Auckland, New Zealand.

In recent years concern has arisen over the ability to identify confidential subject-specific trait information from publicly available GWAS results. However, the amount of information that `leaks' in this way has not been assessed, for realistic situations. Existing measures of leakage require and complete genetic data from the study subject, and genetic variants in linkage equilibrium. In truth, an attacker may have incomplete or noisy measurements of a subject's genotype, and information is still present from SNPs in LD.

In a novel approach, we formulate the leakage problem as one of regression and over-fitting, and present a closed form expression for the extent of information leakage in terms of the sample correlation between pairs of SNPs. In it, we see precisely how the R-squared for prediction of subjects' trait values depends on the number of SNPs measured, the sample size, and the extent of pairwise correlation between SNPs.

Using data from the Cardiovascular Health Study, we demonstrate the accuracy of our approach, when different amounts of SNP information are available, and for different sample sizes. While the presence of LD makes trait prediction substantially less accurate than would be expected if variants were assumed uncorrelated, we still achieve more leakage of information than the upper bounds based on using only independent SNPs. We also show that the amount of correlation between distant SNPs (i.e. the extent of population structure) is an important determinant of the amount of trait leakage.

742T

Selection of top SNPs for genome-wide association study using p values and magnitude of odds ratios. *J. Wang, S. Shete.* Dept Epidemiology, UT MD Anderson Cancer Ctr, Houston, TX.

Genome-wide association (GWA) study is a powerful approach for detecting genetic variants for common diseases. Generally, in GWA studies, the most significant SNPs associated with top-ranked p values are selected in stage one, with follow-up in stage two. However, when minor allele frequencies are relatively low, less-significant p values can still correspond to higher odds ratios (ORs), which might be more useful for prediction of disease status. Therefore, if SNPs are selected using an approach based only on significant p values, some important genetic variants might be missed. We proposed an approach for selecting SNPs from stage one of GWA study, based on both p values and ORs, and conducted a simulation study to demonstrate the performance of our approach. The simulation results showed that our selecting approach was more powerful than the existing ranked p value approach for identifying relatively less-common SNPs. Therefore, in GWA studies, SNPs should be considered for inclusion based not only on ranked p values but also on ranked ORs.

743T

Follow-up GWAS analysis and in silico candidate gene study for dental caries in permanent teeth. *X. Wang[1], A.R. Vieira[1], J.R. Shaffer[2], F. Begum[2], M. Lee[1], K.T. Cuenco[1], Z. Zheng[2], D.E. Polk[3], M.M. Barmada[2], J. Noel[1], I. Anjomshoaa[1], D.E. Weeks[2], E. Feingold[2], M.L. Marazita[1].* 1) Dept of Oral Biology, Univ Pittsburgh, Pittsburgh, PA; 2) Dept of Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 3) Dept of Dental Public Health, Univ Pittsburgh, Pittsburgh, PA.

Dental caries is a common chronic disease that causes pain and disability across all age groups. Untreated dental caries can lead to pain and infection, tooth loss, and edentulism (total tooth loss). Approximately 91% of dentate adults aged ⁄ 20 years have had caries (NHANES surveillance summaries on oral health, 2005). Genetic components make substantial contributions to caries susceptibility (heritability of up to 55% for permanent teeth in adults). Our previous GWAS analysis (based on over 2000 non-Hispanic whites aged ⁄ 17 from the GENEVA dental caries project) nominated 4 genomic regions that demonstrated suggestive significance (i.e. P<1.0*E-05) and harbored plausible biological candidate genes. Recently, we genotyped an additional 291 individuals with similar genetic backgrounds from the Univ. Pittsburgh Dental Registry and DNA Repository using the same Illumina Human610-Quad Beadchip. We repeated the GWAS analysis on the expanded cohort. Signals from two of the above four genomic regions (genes) were replicated in both the new cohort alone as well as in the combined cohort. In particular, p values from GWAS analysis became smaller in the combined cohort for the CNIH and ZNF98 genes on chromosomes 14 and 19 respectively, both of which are involved in tooth agenesis. In a separate analysis, we independently created a list of 98 caries candidate genes: genes involved in enamel and dentin formation, salivary composition and flow, tooth morphology and dietary and taste preference. We then used our GWAS data to perform an in silico candidate gene study by testing association between caries status in permanent teeth and the SNPs within and near the candidate genes. Our results showed several candidate genes (such as vitamin D receptor and taste receptor genes) and gene families (Matrix MetalloProteinase and calcium channel families) in which multiple SNPs from the same gene were observed to have P values of 0.001 or less. Of note, two SNPs (rs6445703 and rs3935714) from the CACNA2D3 and NFATC2IP genes respectively had P values less than 0.0005, significant under Bonferroni correction for multiple testing (i.e., 98 genes, (= 0.00051). Support: NIH U01DE018903, R01DE014899, R03DE021425, UL1RR024153.

744T

Genome-wide characterization of methyl-CpG-binding protein 2 (MECP2) recruitment in primary human CD4+ T cells. *M. Dozmorov[1], T. Hughes[1], R. Webb[2], J.D. Wren[1], A.H. Sawalha[1,3,4].* 1) Arthritis and Clinical Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) College of Pharmacy, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 3) Department of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 4) US Department of Veterans Affairs Medical Center, Oklahoma City, OK.

MECP2 is a dual transcriptional regulator that either activates or represses transcription. Here, we used chromatin immunoprecipitation followed by microarray hybridization to characterize genome-wide MECP2 binding sites in human primary CD4+ T cells. RNA polymerase II (Pol II) binding, an indication of active transcription, was similarly localized in the same sample. We identified 3,813 MECP2 binding sites (FRD<0.1) located within -5kb to +1kb from the transcription start site of 5,767 transcripts of known genes. Out of the genes that recruited MECP2 within their promoter region, 44% also recruited Pol II indicating active transcription. When MECP2 and Pol II are found at the same promoter region, they tend to co-localize. In these promoter regions, MECP2 is recruited closer to transcription start sites as compared with promoter regions that recruited MECP2 but not Pol II (P= 3.18X10-8). Within promoter sequences, only 13% of MECP2 binding sites occurred in methylated genomic regions. C+G nucleotide content in MECP2 binding sites were significantly lower when Pol II was co-recruited within the same promoter sequence compared to promoters with only MECP2 (P= 1.0X10-63). Together, our findings suggest that MECP2 preferentially binds unmethylated DNA and that the distance between MECP2 and the transcription start site and the DNA sequence directly recruiting MECP2 might play a role in dictating whether MECP2 will behave as a transcriptional activator or repressor.

745T

Genome-wide identification of new differentially methylated regions associated with imprinted genes. *C. Borel[1], F. Cheung[1], A. Guilmatre[1], B. Steiner[2], H. Brunner[3], D. Mackay[4], G. Perez de Nanclares[5], T. Eggermann[6], G. Gimelli[7], C. Schwartz[8], J. Vermeesch[9], P. Papenhausen[10], C. Ruivenkamp[11], A.J. Sharp[1].* 1) Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New-York; 2) Kinderspital Luzern, Switzerland; 3) University Medical Center Nijmegen, Netherlands; 4) Southampton University School of Medicine, UK; 5) Hospital de Cruces, Barakaldo, Spain; 6) Institut für Humangenetik, Aachen, Germany; 7) Istituto G. Gaslini, Genova, Italy; 8) Greenwood Genetic Center, SC; 9) Center for Human Genetics, Leuven, Belgium; 10) Laboratory Corporation of America, Research Triangle Park, NC; 11) Leiden University Medical Center, Netherlands.

Genomic imprinting is a mechanism in which alters the expression of genes depending on whether they are maternally or paternally inherited. Imprinting occurs through an epigenetic mechanism involving differential DNA methylation and histone modifications on the two parental alleles, with most imprinted genes marked by CpG-rich differentially methylated regions (DMRs). Approximately 60 human imprinted genes have been identified to date, and imprinted loci have been associated both with rare syndromes, and with common diseases including diabetes and cancer. Previously we showed that DNA methylation profiling in cases of uniparental disomy (UPD) provides a unique system that allows the isolated study of DNA derived from a single parent. We have now assembled an unprecedented collection of DNA from 113 patients with uniparental disomy for 18 different chromosomes, allowing the efficient detection of DMRs associated with imprinted genes for 84% of the human genome. We also included in our study samples obtained from androgenetic Complete Hydatidiform Moles and gynogenetic Ovarian Teratomas, allowing uniparental studies of the entire genome. We performed genome-wide DNA methylation profiling in this UPD cohort using Illumina Infinium Methylation BeadArrays that yield quantitative data on ~480,000 CpGs at single-nucleotide resolution. Imprinted DMRs were defined by sites at which the maternal and paternal methylation levels for multiple probes diverged significantly from the biparental average. Using stringent thresholds, we were able to detect 19 out of 20 (95%) previously described DMRs, validating our methodology for the detection of imprinted loci. Using these same criteria we identified a total of 93 DMRs genome wide. This included novel DMRs associated with known imprinted loci, and novel DMRs in regions not previously thought to be imprinted. Taking advantage of the highly quantitative nature of our data, we also observed much more subtle reciprocal methylation differences between the two parental alleles that extended across large chromosomal domains at known imprinted loci. We confirmed DMRs by bisulfite sequencing in UPD patients and biparental controls. These data provide a genome-wide map of imprinted sites in the human genome.

746T

Detection of epigenetic defects in Korean patients with Beckwith Wiedemann syndrome and Silver-Russell syndrome. *G.H. Kim[1], B.H. Lee[1,2], J.J. Lee[1], S.H. Choi[1], J.Y. Lee[1], H.W. Yoo[1,2].* 1) Medical Genetics Center, Asan Medical Center, Seoul, Korea; 2) Department of Pediatrics, Asan Medical Center children's Hospital, Seoul, Korea.

Beckwith-Wiedemann Syndrome (BWS) is overgrowth malformation syndrome, whereas Silver-Russell syndrome (SRS) is characterized by pre- or postnatal growth retardation. BWS and SRS share molecular genetic pathology of epigenic defects in chromosome 11p15. A small number of patients with SRS harbor maternal uniparental disomy of chromosome 7 as well. The aim of our study is to identify the epigenic detects in Korean patients with BWS and SRS. Fifteen BWS patients and 15 SRS patients were enrolled. Extensive genetic analyses comprising methylation specific (MS) PCR-RFLP, MS-MLPA, or MS-pyrosequencing analysis were performed. Ten out of 15 BWS patients (66.7%) showed imprinting detect as hypomethylation in imprinting center 2 (IC2). One BWS patient harbored hypermethylation in IC1 (6.7%). Two BWS patients had both IC and IC2 methylation defects, indicating paternal UPD (13.3%). Nine out of 15 SRS patients (60.0%) showed hypomethylation in IC1. One SRS patient (6.7%) had hypomethylation in GRB10 at 7p12. MS-pyrosequencing analysis revealed that methylation status was decreased at 9.5 ± 6.6 % (2.0 - 20.5 %) in 10 BWS with IC2 defects (normal range, 48.4 ± 8.7 %), whereas it was decreased at 21.6 ± 6.2 % (13.7% - 29.8%) in 9 SRS patients with IC1 defects (normal range, 56.4 ± 6.7 %). With extensive genetic analyses, we could identify genetic defects in 13 out of 15 BWS patients (86.7%) and 10 of 15 SRS patients (66.7%). These positive rates are higher than previously reported as 80% in BWS and 50% in SRS. In addition, with MS-pyrosequencing analyses, quantification of methylation defects was available, which could identify partial methylation defects that might not be revealed by MS-PCR-RFLP or MS-MLPA. The validity of MS-pyrosequencing method for the genetic diagnosis of BWS or SRS is needed to be investigated in a large patient cohort.

747T

The role of Ube3a-ATS in Ube3a imprinting and Angelman syndrome. *L. Meng, R. Person, A. Beaudet.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Angelman syndrome is a neurodevelopmental disorder mostly attributed to deficiency of *UBE3A* located at 15q11. Unlike ordinary autosomal genes, *UBE3A* is subject to genomic imprinting with expression only from maternal chromosome in neurons. It is unknown how the imprinting status of *UBE3A* is established, since no differential DNA methylation was found to be associated with its promoter. However, an imprinted antisense non-coding RNA named *UBE3A-ATS* overlapping *UBE3A* locus was identified and hypothesized to suppress *UBE3A in cis*. By studying a mouse model carrying 0.9kb genomic deletion of the paternal *Snrpn* major promoter, we found reduction of *Ube3a-ATS* associated with partial reactivation of paternal *Ube3a*. Such reactivation is not a result of imprinting defects because DNA methylation and expression of other imprinted genes remain normal, suggesting a direct role of *Ube3a-ATS* in mediating paternal *Ube3a* silencing. To better define the function of *Ube3a-ATS* in *Ube3a* imprinting and Angelman syndrome, knock-in mice expressing a truncated form of *Ube3a-ATS* were generated. Preliminary data show elevated *Ube3a* expression in this mouse model. In order to further understand the mechanism of *Ube3a* sense-antisense interaction, we performed strand-specific microarray analysis and found *Ube3a-ATS* RNA covers the entire locus of *Ube3a*, with its termination upstream of the *Ube3a* transcriptional start site. In addition, we found equal binding of transcription initiation complex at paternal and maternal *Ube3a* promoters by ChIP analysis, indicating silencing of paternal *Ube3a* might arise from defects of transcription elongation. Overall, our studies revealed a direct role of *Ube3a-ATS* in paternal *Ube3a* silencing. Further studies on the interaction between the sense and antisense transcripts may help us understand more about *Ube3a* imprinting and provide unique insights into therapeutic development for Angelman syndrome.

748T

High Resolution Methylation Map in Suicide Completers. *C. Nagy[1,6], M. Suderman[2,3,4], C. Ernst[1], M. Szyf[2,3], N. Mechawar[1,6], G. Turecki[1,5,6].* 1) McGill Group for Suicide Studies, Douglas Mental Health University Institute, McGill University, Verdun, Quebec, Canada; 2) Sackler Program for Epigenetics & Developmental Psychobiology, McGill University, Montreal, Quebec, Canada; 3) Department of Pharmacology and Therapeutics, McGill University , Montreal, Quebec, Canada; 4) McGill Centre for Bioinformatics, McGill University, Montreal, Quebec, Canada; 5) Department of Human Genetics, McGill University, Montreal,Quebec, Canada; 6) Department of Neurology and Neurosurgery, IPN, McGill University, Montreal,Quebec, Canada.

Background: There is mounting evidence to suggest that epigenetic modifications are the environment's means of regulating gene expression. Epigenetic mechanisms have been shown to be mediated by environment and are in turn, capable of mediating gene expression and potentially behaviour. This provides a compelling avenue for investigation of the altered gene expression described in the brains suicide completers Methods: A homogeneous subgroup of suicide completers (n=21) were selected based on a common genetic phenotype of low expression in astrocytic genes. These samples were matched to non-suicide, psychiatrically unaffected control samples, with no difference in age, PMI or pH (n=21). Each sample was enriched using MBD for methylated DNA isolating and sequenced on the Illumina GAII. For analysis the sequencing data was combined resulting ~250 million sequencing reads per group. Analysis: Sequencing was completed for all 42 samples but due to concerns of sequencing quality, the sequencing data from 17 suicide completers and 16 controls samples, were used to assess differentially methylated regions (DMRs). Reads were trimmed, duplicates removed and the resulting data was mapped to the human genome (hg19) using BWA. The genome was tiled using overlapping 500 bp windows at every 250 bp and reads were counted for each 500 bp interval. Using the DESeq software, a negative binomial test was implemented to obtain DMRs between suicides and controls. Read counts were used to estimate methylation levels. Results: We have identified 211 DMRs (FDR< 0.2) in the brains of a subgroup of suicide completers and controls. Chromosomes 1, 4, and 16 have the most regions of differential methylation. While of the some DMRs are in traditional gene regulatory regions, the use of next generation sequencing allowed us to identify DMRs in distal regions. Antibody/protein enrichment techniques are expected to pull down a large fraction of the genome reducing the power to identify methylation differences between samples, however our pooling by group technique allowed us to identify these differences. This method gives us a high resolution map of methylation differences, keeping the cost similar to microarray projects.

749T

Large Scale Genome-Wide Methylation Analysis of Schizophrenia. *K. Aberg[1], J. McClay[1], G. Rudolf[1], S. Nerrella[1], J. Bukszar[1], L. Xie[1], A. Hudson[1], A. Khachane[1], S. Vunck[1,2], S. Snider[2], P. Beardsley[1,2], C. Hultman[3], PKE. Magnusson[3], P. Sullivan[3,4], E. van den Oord[1].* 1) Center of Biomarker Research and Personalized Medicine, School of Pharmacy, Virginia Commonwealth University, Richmond, VA, USA; 2) Department of Pharmacology and Toxicology, School of Medicine, Virginia Commonwealth University, Richmond ,VA, USA; 3) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; 4) Department of Genetics, University of North Carolina at Chapel Hill, NC, USA.

DNA methylation studies represent a promising complement to traditional genetic studies. We performed a whole methylome association study in 1,575 schizophrenia (SCZ) case-control samples and then followed up the top findings in 1,600 independent case-control samples. The assays involved the use of MethylMiner to pull down the methylated part of the genome followed by next generation sequencing (NGS) of these enriched samples on SOLiD 4. Because procurement of human brain tissue is not feasible on a large scale and in living patients, the assays were performed with DNA from whole blood. To analyze this massive dataset we developed a pipeline that included alignment, quality control, novel estimators for calculating CpG coverage, association testing, and bioinformatics annotation. We obtained an average of 67 million reads per sample. Based on analysis of 75 duplicates we selected about 30% of the 30 million CpG sites for the association analyses as the methylation status of these sites varied across subjects making them potential disease biomarkers. To further prioritize marker selection for follow-up, we integrated our results with external data (e.g. a large scale replication study of 18 SCZ GWAS studies) using the MIND software. The most promising results are currently followed-up using a customized VeraCode GoldenGate assay and targeted PyroMark pyrosequencing. Using the same NGS approach as above, we also compared the methylation pattern in DNA from cortex, hippocampus and whole blood in mice. This investigation showed concordance rates between blood and brain of 0.3-0.5, suggesting that in addition to acting as biomarkers, methylation sites found in whole blood may point to relevant brain processes. The top findings from our studies represent one of the first sets of CpG sites identified through a large scale methylome wide approach that potentially can be used to improve treatment, diagnosis and disease etiology.

750T

Friedreich ataxia demonstrates increased levels of 5-hydroxymethylcytosine within the FXN gene. *S. Al-Mahdawi, C. Sandi, M. Pook.* Biosciences, Brunel University, Uxbridge, United Kingdom.

Friedreich ataxia (FRDA) is caused by a homozygous GAA repeat expansion mutation within intron 1 of the FXN gene, leading to reduced expression of frataxin protein. Evidence suggests that the mutation induces epigenetic changes and heterochromatin formation, thereby impeding gene transcription. Thus, studies using FRDA blood and lymphoblastoid cell lines have detected increased DNA methylation of specific CpG sites upstream of the GAA repeat, together with histone acetylation and methylation changes in regions flanking the GAA repeat. Our lab has previously reported that such epigenetic changes are also present in FRDA brain, cerebellum and heart tissues, which are the primary affected systems of the disorder. Bisulfite sequence analysis of the FXN flanking GAA regions revealed a shift in the FRDA DNA methylation profile, with upstream CpG sites becoming consistently hypermethylated and downstream CpG sites becoming consistently hypomethylated. We also identified differential DNA methylation at three specific CpG sites within the FXN promoter and one CpG site within exon 1. It has recently been shown that the modified base, 5-hydroxymethylcytosine (5-hmC), is present in mammalian DNA, and in approximately 20% of all CpGs in cerebellar Purkinje cell DNA. However, classical bisulfite sequence analysis cannot distinguish between 5-hmC and 5-mC residues. Therefore, we have now analysed the DNA methylation status at one of the upstream GAA CpG sites in 3 FRDA cerebellar DNA samples and 3 unaffected control samples using an approach that does allow distinction between 5-hmC and 5-mC. Our analysis has confirmed the previously identified increases in DNA methylation in FRDA samples, but now reveals that all of the modified CpG in both FRDA and unaffected DNA samples comprises 5-hmC. The potential role of such 5-hmC-modified CpG residues in normal functioning and in FRDA pathogenesis is yet to be determined.

751T

Genomic and Epigenomic Analysis of Primate Species-Specific DNA methylation Variation. *C.G. Bell, G.A. Wilson, L.M. Butcher, S. Beck.* Medical Genomics, UCL Cancer Institute, London, United Kingdom.

The assimilation of genomic, epigenomic and transcriptomic data will aid in the understanding of the effects of genetic variation, environmental perturbation and consequence outcomes on cell and tissue function. This integration will help unravel abnormal physiology in disease pathways. However, a detailed comprehension of each of these levels and particularly how they interface will be required to mesh these cogs of data together. In order to explore mutational mechanisms, time-invariant principles have been proposed to highlight potential commonality in somatic-, population- and species-specific change. Applying this concept with respect to the epigenetic mark of DNA methylation, significant somatic aberration in the cancer methylome is acknowledged, but only recently high resolution data has enabled the identification of dynamic tissue-dependent methylation changes in intermediate CpG density regions. Genomic CpG dinucleotides (CpGs) are distinct from other dinucleotides in that they have the ability to transmit both genetic and epigenetic information and this CpG methylation is vital in mammalian genomes in order to regulate transcription levels, isoform ratios and chromatin structure. Also compared to other dinucleotides, CpGs are substantially depleted (only ~25% of expected) due to >10-fold hypermutability in the germ-line caused by spontaneous deamination of methylated cytosines. Utilising comparative primate genomics focusing on the alignable Human, Chimpanzee, Gorilla, Orang-utan, Macaque and Marmoset genomes and subsequent epigenomic evaluation of DNA methylation variability with MeDIP-sequencing of Human, Chimpanzee and Macaque revealed unique sets of species-specific CpG clusters (Human genomic empirical $p = < 10^{-3}$). The creation of these clusters can be driven by neutral forces such as biased gene conversion, but were predominately not constitutively hypermethylated (Human 76.2%). This detailing of species-specific variation may in turn enable elucidation of intra-specific population epigenomic variation, with potential influence on disease susceptibility.

752T

Genome-wide MeDIP-sequencing profiles identify differentially methylated regions in monozygotic twins discordant for pain sensitivity. *J.T. Bell[1,2], A.K. Loomis[3], B. Zhang[3], C.L. Hyde[3], G.J. Brock[4], I.D. Harrow[5], L.M. Butcher[6], R. McEwen[5], J.M. Harris[1], F.M.K. Williams[1], S. Beck[6], S. Phillips[5], W. Jun[7], G. Burgess[5], S. John[3], T.D. Spector[1].* 1) Department of Twin Research, Kings College London, London, UK; 2) Wellcome Trust Centre for Human Genetics, Univ Oxford, Oxford, UK; 3) Pfizer Global Research & Development, Groton, CT, USA; 4) Biogen Idec, Cambridge, MA, USA; 5) Pfizer Global Research & Development, Sandwich, UK; 6) UCL Cancer Institute, UCL, London UK; 7) BGI-Shenzhen, Shenzhen, China.

Recent studies of DNA methylation differences in disease-discordant monozygotic (MZ) twins show that epigenetic regulation of gene expression can play a pivotal role in complex traits. We obtained DNA methylation sequencing profiles in 25 MZ twin-pairs discordant for objective pain-sensitivity determined experimentally via heat-induced pain, of at least 2°C twin-pair discordance. Whole blood DNA methylation was assayed using methylation capture followed by immuno-precipitation and sequencing (MeDIP-sequencing), resulting in 50 million paired-end reads per sample. We developed a computational and statistical pipeline to quantify MeDIP-seq reads into relative methylation levels across the genome in bins of size 1kb, and validated the resulting methylation levels using bisulfite sequencing in 8 selected genomic regions. To identify differentially methylated regions related to pain-sensitivity (pain-DMRs), we compared methylation to pain using a linear mixed effects model and non-parametric correlation. We obtained evidence for two pain-DMRs at a Bonferroni-adjusted genome-wide significance threshold of $P = 0.05$ (nominal $P = 2.9\times10^{-8}$), which are currently being validated using bisulfite sequencing. The pain-DMRs showed modest but consistent methylation changes across all twins. Annotation of the top 100 pain-DMRs demonstrated the following genomic distribution: intergenic (54%), intronic (32%), and exonic (10%), suggesting an enrichment of exonic regions. GO and Reactome analyses also highlighted genes involved in Rho GTPase signaling, components of which have been linked previously to nociceptive sensitization. Our results suggest that epigenetic studies of disease-discordant MZ twins are a potentially powerful tool for identifying functional genomic changes that may contribute to complex traits.

753T

DNA Methylation Alterations in CHARGE Patients with Heterozygous CHD7 Mutations. *D.T. Butcher[1], D. Grafodatskaya[1], T. Guha[1], W. Reardon[2], B. Gilbert-Dussardier[3], A. Verloes[4], F. Bilan[5], S. Bowdin[6,7], R. Mendoza-Londono[6], R. Weksberg[1,6,7].* 1) Genetics and Genome Biology, Sickkids Research Institute, Toronto, Ontario, Canada; 2) National Centre for Medical Genetics, Our Lady's Children's Hospital, Dublin, Ireland; 3) Service de Génétique, Centre de Référence Anomalies du Développement de l'Ouest, CHU Poitiers, France; 4) AP-HP, Groupe Hospitalier Pitié-Salpêtrière, UF de Génétique Clinique, Paris, France; 5) Institut de Physiologie et Biologie Cellulaires, Centre National de la Recherche Scientifique Unité Mixte de Recherche, Université de Poitiers, CHU Poitiers, France; 6) Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, Canada; 7) Department of Paediatrics, The University of Toronto, Toronto, Canada.

CHARGE association is a rare autosomal dominant genetic disorder, with an incidence of 1 in 8500-10000 births. Clinical diagnosis for CHARGE is based on non-random associations of the following congenital abnormalities: Coloboma of the eye, Heart defects, Atresia of the choanae, Retarded growth and development, Genital abnormalities, Ear abnormalities/deafness/ vestibular disorder. In the majority of cases, CHARGE association is the result of haploinsufficiency due to a nonsense, missense, or deletion in the gene encoding Chromodomain Helicase DNA-binding protein (CHD7). Mutational studies in Drosophila and mouse have found phenotypes similar to those found in human. In Drosophila reduced expression of kismet/CHD7 results in deficits in axonal pruning, guidance and extension as well as defects in memory and motor function. Normal mammalian growth and development depend on the correct epigenetic programming of the genome. Epigenetic patterns are established by mechanisms including DNA methylation and covalent modifications of histone proteins. In Drosophila, kismet the ortholog of human CHD7 has been demonstrated to regulate the repressive histone H3 methylation mark of lysine 27. In human cell lines, CHD7 has been shown to bind to chromatin regions that are active as demonstrated by histone H3 lysine 4 methylation and DNAse1 hypersensitivity of these binding sites. These epigenetic modifications of histone H3 are tightly linked to DNA methylation patterns. We hypothesized that specific DNA methylation alterations occur as a result of the heterozygous CDH7 mutations and could reveal critical downstream targets associated with CHARGE phenotypes. We have found both hyper and hypomethylation changes using the Illumina Human Methylation27 microarray and have validated them using pyrosequencing. We will look for correlations between phenotype variability in individuals with CHARGE association which could be explained by variable epigenetic alterations in patients with different CHD7 mutations.

754T

Methylation Profiling of Testicular Embryonal Carcinomas. *W.Y. Chan[1], O.M. Rennert[2], H.H. Cheung[2].* 1) School of Biomedical Sciences, Faculty of Medicine, the Chinese University of Hong Kong, Shatin, Hong Kong SAR, China; 2) Section on Clinical and Developmental Genomics, The Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA.

Testicular embryonic carcinoma (EC) is a major subtype of non-seminomatous germ cell tumors in males. ECs are pluripotent, undifferentiated germ cell tumors believed to be originated from primordial germ cell. Molecular event during testicular EC tumorigenesis resembles that of embryogenesis in many ways, including DNA methylation. To identify epigenetic alterations during testicular tumorigenesis, we profiled the DNA methylome of 6 ECs. These samples represent different stages (stage I and stage III) and different extent of invasiveness. Two normal testicular tissues were also included as normal control. Genome-wide methylation analysis by MeDIP-chip revealed a distinct methylation signature between normal and tumors. Differential methylated regions (DMRs) were identified in metastatic ECs. The majority of these DMRs are hypermethylation. A total of 1167 hypermethylated DMRs were found in the metastatic ECs. Mapping of hypermethylated DMRs to Refseq identified only 39 DMRs located in gene promoters. The majority of DMRs (~97%) were located in gene bodies or non-genic regions, consistent to our previous finding in testicular cancer cell lines. Interestingly, we found several sex-linked genes that were hypermethylated, including X-linked genes STAG2, SPANXD/SPANXE, MIR1184, Y-linked genes RBMY1A1/RBMY1B/RBMY1D, BTBD2, ZNF699 and FAM197Y2P. We selected 9 genes for qPCR analysis on the expression changes. Among the genes analyzed, expression of AGPAT3, SUCLG2, RBMY1A, SPANXD, RNF168, USP13, FAM197Y2P were significantly decreased in ECs. One of the Y-linked genes, RBMY1A, has been reported to be regulated by DNA methylation in another urological prostate cancer. The role of RBMY1A in male germ cell tumor is not well understood. Immunohistochemistry analysis on normal testis, EC and seminoma tissues indicate down-regulation of this protein in testicular tumor. Expression of RBMY1A is restricted to male germ cells, and disappeared in ECs and seminomas. Our genome-wide analysis identified methylation changes in several previously unknown genes for testicular ECs, which might provide insight into the crosstalk between normal germ cell development and carcinogenesis.

755T

DNA Methylation in Cerebellum of Bipolar Disorder, Schizophrenia and Depression Patients. *L. Cheng[1], C. Zhang[1], J. Badner[2], C. Chen[1], E. Gershon[2], C. Liu[1].* 1) Department of Psychiatry, University of Illinois at Chicago; 2) Department of Psychiatry and Behavioral Neuroscience, The University of Chicago.

DNA methylation of CpG dinucleotides is an epigenetic modification that plays an important role in regulating gene expression, X-chromosome inactivation, parental imprinting, development and complex diseases. Several studies have documented DNA methylation changes in the brains of patients with psychiatric diseases, as well as such changes induced by drug administration. However, genome-wide DNA methylation profile in brains of psychiatric patients remains poorly understood. Using the Illumina Infinium HumanMethylation27 BeadChip, we assayed 27,578 highly informative CpG sites spanning 14,495 genes on 153 human cerebellum samples from the Stanley Medical Research Institute. These samples included 45 schizophrenia (SZ), 46 bipolar disorder (BD) and15 depression (Dep) patients, and 47 unaffected controls. After ANCOVA analysis, using variables of age, gender, affection status, brain pH, PMI, smoking and use of antipsychotics, only age, gender and depression were associated with significant differential methylation (FDR $q < 0.05$). Of 83 genes showing depression-related differential methylation, 82 genes showed lower methylation in depression relative to controls, and only six of those genes showed differential expression, indicated that dominant down regulation of DNA methylation. Age-related differential methylation was observed in 355 genes, which included ten CpG sites which have been reported significantly changed with age in a previous study. Of 1448 genes showing gender-related differential methylation, 68% were on the X chromosome, while the remaining 465 genes were on autosomes. Methylation of CpG sites in 15 genes showed significant age-depression interactions. Methylation of two genes, OTUD5 and CETN2, were associated with gender-effects and depression. Validation of DNA methylation in depression is ongoing.

756T

Differential DNA methylation associated with anti-dsDNA autoantibody production in systemic lupus erythematosus. *S.A. Chung[1], K.E. Taylor[1], H.L. Quach[2], L.F. Barcellos[2], L.A. Criswell[1].* 1) Department of Medicine, University of California, San Francisco, San Francisco, CA; 2) School of Public Health, University of California, Berkeley, Berkeley, CA.

Purpose: Aberrant DNA methylation has been implicated in the pathogenesis of systemic lupus erythematosus (SLE), with less DNA methylation observed in SLE patients compared to healthy controls. We conducted this study to identify differences in DNA methylation across the genome associated with anti-dsDNA autoantibody production, a clinically relevant autoantibody associated with kidney damage and more severe disease in SLE. **Methods**: Genomic DNA from peripheral blood leukocytes was isolated from 104 pairs of SLE cases (n=208 cases total). All SLE cases were female, of European descent, and had never smoked. Case pairs were discordant for anti-dsDNA autoantibody production status (positive vs. negative), and were matched (within 5 years) on age at DNA sample collection and SLE disease duration. Using the Illumina HumanMethylation27 Beadchip, the methylation status of 27,568 CpG sites across the genome were interrogated in all SLE cases. Paired t-tests were used to identify site-specific methylation differences associated with anti-dsDNA autoantibody production. P-values less than 1.8×10^{-6} (Bonferroni corrected) were considered statistically significant. **Results**: Overall, less methylation was observed in the anti-dsDNA positive group. Mean methylation levels were decreased in the anti-dsDNA positive cases when compared to anti-dsDNA negative cases for 63% of the CpG sites investigated (n=17,286). Increased methylation of 2 CpG sites in *PRIC285*, a transcriptional co-activator for nuclear receptors, was significantly associated with anti-dsDNA autoantibody production ($p= 1.9 \times 10^{-7}$ and 3.4×10^{-7}). Decreased methylation of a CpG site near *SOCS2* ($p=3.5 \times 10^{-7}$), an inhibitor of the STAT family of transcription factors, was also associated with anti-dsDNA autoantibody production. No evidence of interaction ($p>0.05$) was found between methylation of the associated *SOCS2* CpG site and rs7574865 of *STAT4*, a known SLE susceptibility and anti-dsDNA propensity locus. **Conclusions**: Similar to comparisons between SLE cases and controls, DNA from autoantibody positive SLE cases appears to be less methylated than autoantibody negative SLE cases. Abnormal methylation of specific genes such as *PRIC285* and *SOCS2* may contribute to autoantibody production in SLE. Lastly, studies of DNA methylation and other epigenetic modifications may identify additional biologic mechanisms involved in the pathogenesis of SLE.

757T

Effect of folic acid supplementation on DNA methylation among reproductive age women in Honduras. *K.S. Crider[1], J. Rosenthal[1], D.R. Maneval[2], G.P.A. Kauwell[2], L.B. Bailey[2].* 1) CDC, National Center on Birth Defects and Developmental Disabilities, Division of Birth Defects and Developmental Disabilities, Pediatric Genetics Team; 2) Food Science and Human Nutrition Department, University of Florida Gainesville, FL.

Folic acid supplementation has been shown to reduce the risk of birth defects. As a result of the role of folic acid in one-carbon metabolism, it has been hypothesized that there may be additional positive and potentially negative unintended consequences. In order to determine if supplementation with folic acid changes global DNA methylation, we utilized samples collected as part of a 12 week double-blind randomized trial of two doses of folic acid, 5 mg once per week, and 1 mg once per day. Participants were reproductive age women (mean age 28 SD+/- 5.5 years; range 20-47 years). Global DNA methylation level was determined by liquid chromatography tandem mass spectrometry (% methylation= the ratio of methylated cytosine to total cytosine). The study included 108 subjects (n= 50 1mg/day and n = 58 5mg/week) and % DNA methylation was assessed at baseline, 6, and 12 weeks of folic acid supplementation. At baseline, DNA methylation level was 3.9% +/- 0.19 (min. 3.5% max. 4.3%) and there was no association of global DNA methylation level with age, BMI, or treatment group. Subjects were genotyped for the *methylenetetrahydrofolate reductase (MTHFR)* 677 C to T transition (23% CC, 49%CT, 28% TT). At baseline *MTHFR* genotype was not associated with DNA methylation level. No significant changes in global DNA methylation after six or 12 weeks of folic acid supplementation in either folic acid dosage group were detected using a general linear model for repeated measures including age, BMI, and *MTHFR* genotype. At completion of the 12 week trial, DNA methylation level was 3.9 95% Confidence Interval (CI) 3.89-4.00 in the 1 mg/day dose group and 3.86 95%CI 3.80-3.93 in the 5 mg/week dose group). In conclusion, we found no association of folic acid supplementation with changes in DNA methylation level in this double-blind randomized trial of folic acid supplementation in women of reproductive age.

758T

Genomic Analysis of Site-specific DNA Methylation Patterns in Primary Epithelial Ovarian Cancers and Endometrial Metastases to the Ovary. *L. Elnitski[1], D.L. Kolbe[1], T.C. Krivak[2], J.A. DeLoia[3], L.C. Brody[1].* 1) National Human Genome Research Institute, Rockville, MD; 2) University of Pittsburgh Medical School, Pittsburgh PA; 3) Georgetown University, Washington DC.

More than 20,000 new cases of epithelial ovarian cancer are diagnosed in the U.S. each year. Despite improved outcomes in the past 30 years, less than half of all women diagnosed with ovarian cancer remain alive 5 years after their diagnosis. Although typically classified as a single disease, EOC includes several distinct histological subtypes grouped by their resemblance to different gynecological tissues (such as fallopian tube, endometrium, and others). However, clinical treatment is not tailored to each subtype, even though these distinct pathophenotypes are likely to result from different genetic and epigenetic changes. In this study, we addressed questions regarding the site of origin and the pathways involved in ovarian tumorigenesis by comparing normal fallopian tube against papillary serous and endometrioid ovarian tumor subtypes. We profiled the methylation status of 27,578 CpG dinucleotides occurring in or near promoter regions of 14,495 genes. We used these molecular data, in a blinded fashion, to classify tumors based on methylation patterns alone. Our data show that classification of tumors via methylation patterns strongly recapitulates the histopathologic classifications and identifies outlier tumors with discordant methylation patterns. As potential biomarkers for serous tumors, thirty-six sites showed significant and consistent increases or decreases in methylation. The data are considered in pathway analyses that correspond to known mechanisms of disease in ovarian epithelial cancer. Comparisons among primary endometrioid tumors of the ovary and uterus with metastatic endometrioid tumors (from uterus to ovary) confirm that methylation profiles correlate strongly with histopathology regardless of tissue location and provide a quantitative molecular approach to discriminating conventional and atypical tumors that cannot be distinguished by a pathologist. We identify known and novel ovarian cancer genes that can be targeted by approved therapeutic compounds, suggesting alternative intervention strategies for specific subtypes of ovarian cancer.

759T

Silver-Russell Syndrome : Phenotypic comparison between patients with *H19*-DMR epimutations and those with upd(7)mat, and 3 unique cases of SRS. *T. FUKE-SATO[1,2], K. YAMAZAWA[3], K. MATSUBARA[1], M. KAGAMI[1], K. NAKABAYASHI[4], K. HATA[4], T. OGATA[5].* 1) Department of Molecular Endocrinology, National Research Institute for Child Health and Development, Tokyo, Japan; 2) Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan; 3) Department of Physiology, Development and Neuroscience, University of Cambridge, UK; 4) Department of Maternal-Fetal Biology, National Research Institute for Child Health and Development, Tokyo, Japan; 5) Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Japan.

BACKGROUND : Silver-Russell syndrome (SRS) is a congenital developmental disorder characterized by intrauterine and/or postnatal growth retardation as well as associated somatic features, especially relative macrocephaly. To date, two major epigenetic and genetic abnormalities have been identified for SRS, i.e., maternal uniparental disomy for chromosome 7 (upd(7)mat) that accounts for 5-10% of SRS patients and epimutations of the *H19*-DMR on 11p15 that accounts for 30-60% of them. Here, we report on the phenotypic comparison between two groups and 3 unique cases of SRS that were identified through our analyses. EPIMUTATIONS OF THE *H19*-DMR AND UPD(7)MAT : We studied 38 patients with epimutations of the *H19*-DMR and 8 patients with upd(7)mat. These patients were identified through methylation analyses of the *H19*-DMR on 11p15 and the *MEST*-DMR on 7q32.2 and microsatellite marker analyses in 118 SRS patients. Some somatic features were significantly more frequent in patients with *H19*-DMR epimutations than in those with upd(7)mat, whereas speech delay were significantly more frequent in patients with upd(7)mat than in those with *H19*-DMR epimutations. CASE 1 : Parthenogenetic chimaerism/mosaicism with SRS-like phenotype. CASE 2 : Monozygotic female twins discordant for the SRS and hypomethylation of the *H19*-DMR. CASE 3 : A mosaic upd(7)mat case of SRS. CONCLUSION : The results imply that clinical phenotypes are somewhat different between patients with upd(7)mat and those with the *H19*-DMR epimutations. And 3 unique cases of SRS indicates the variety of pathogenic mechanism of SRS.

760T

Identification of candidate epimutations in schizophrenia and autism brain samples. *J. Ge, K. Mohan, J. Wiszniewska, A. Beaudet.* Molecular and Human Genetics, Baylor College of Medicine, BCM MS:225, 1 Baylor Plaza, Houston, TX.

Epigenetic changes in DNA cytosine methylation could be associated with alternations in RNA expression, and may play a role in the etiology of psychological diseases. Specifically, monoallelic DNA methylation changes could result in functional haploinsufficiency equivalent to loss-of-function mutations that are known associated with schizophrenia and autism. The authors hypothesized that epimutaions may contibute to the pathogeny of some fraction of schizophrenia and autism. The Aim of this study is to discover candidate epimutations associated with schizophrenia and autism. A Genome-wide analysis of DNA cytosine methylation was performed in the Illumina Infinium human 27 methylation bead chip platform. For schizophrenia, frontal cortex samples (Broadmann Area 8, or BA8) of 22 patients and 30 controls were examined; and for autism, cerebellum samples of 13 patients and 23 controls were examined. The CpG island at the promoter region of LOC652276, a non-coding RNA gene, was found hypermethylated in one schizophrenia patient but not in any control; similarly, the CpG island at the promoter region of SERHL was found hypermethylated in one autism patient but not in controls. Bisulfite treatment of DNA followed by PCR and sequencing demonstrated the heterozygous methylation of both cases, and dramatic decreasing in RNA expression for both cases were observed in the real time RT-PCR assay. We also identified other hyper- and hypomethylation loci for both schizophrenia and autism cases. This work is being extended using the Illumina Human Methylation 450 DNA Analysis BeadChip and additional patient samples. This work is supported by the Simons Foundation Autism Research Initiative (SFARI).

761T

DNA methylation and gene expression changes in monozygotic twins discordant for psoriasis: identification of functionally important genes involved in immune response. *K. Gervin[1], G. Gilfillan[1], M.D. Vigeland[1], M. Mattingsdal[2,3], M. Hammerø[1], H. Nygård[1], A.O. Olsen[4], I. Brandt[5], J.R. Harris[5], D.E. Undlien[1], R. Lyle[1].* 1) Department of Medical Genetics, Oslo University Hospital, Oslo, Norway; 2) Research Unit, Sørlandet Hospital, Kristiansand, Norway; 3) Institute of Psychiatry, University of Oslo, Oslo, Norway; 4) Department of Dermatology, Oslo University Hospital, Oslo, Norway; 5) Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway.

Psoriasis is a common chronic inflammatory disease affecting the skin, scalp, nails and joints. Psoriasis has a strong genetic component with ~20 susceptibility loci, but epigenetic changes are involved since concordance rates among monozygotic (MZ) twins are 35-70%. Here we used MZ twins (n=20 pairs) discordant for psoriasis to explore genome-wide differences in DNA methylation and gene expression. The study of discordant MZ twins is an attractive model to investigate epigenetic mechanisms in disease. Discordance in this context can be interpreted as a result of external factors that shape the epigenetic profile and thereby the susceptibility for disease through altered gene expression. We isolated different lymphocyte subpopulations and studied single-cell types (CD4+ and CD8+) to overcome the issue of epigenetic heterogeneity in whole blood. In order to detect gene specific DNA methylation differences, we used the 27K Infinium methylation assay (Illumina). To integrate the analysis of global methylation status and gene expression of approximately all associated genes we used HumanHT gene expression assays (Illumina). Analysis of these data identified genes where differences in DNA methylation between twins were correlated with gene expression in CD4+ cells, thus identification of genes where DNA methylation has a functional role in development of psoriasis. We also present preliminary data on genome-wide DNA methylation (RRBS) and ChIP. To our knowledge this is the first study using MZ twins discordant for psoriasis in order to reveal epigenetic alterations which potentially contributes to the development of disease.

762T

Role of epigenetic dysregulation in neurodevelopmental syndromes: lessons from *KDM5C* mutations. *D. Grafodatskaya[1], B.H.Y. Chung[1,2], D.T. Butcher[1], S. Goodman[1], S. Choufani[1], Y. Lou[1], C. Zhao[1], R. Rajendram[1], F.E. Abidi[3], C. Skinner[3], J. Hamilton[4], S.W. Scherer[1,5], C.E. Schwartz[3], R. Weksberg[1,2].* 1) Gen & Genome Biol, Hosp Sick Children, Toronto, ON, Canada; 2) Division of Clinical and Metabolic Genetics, Hosp Sick Children, Toronto, ON, Canada; 3) J.C. Self Research Institute, Greenwood Genetic Center,Greenwood, South Carolina, USA; 4) Division of Endocrinology, Hosp Sick Children, Toronto, ON, Canada; 5) The Centre for Applied Genomics, Hosp Sick Children, Toronto, ON, Canada.

Genes encoding proteins that function in epigenetic modification play an important role in normal neurodevelopment. However, the molecular mechanisms that underpin hierarchical epigenetic marks and drive normal development are not well understood. Human diseases caused by mutations in genes that modify epigenetic marks provide a unique opportunity to study the pathophysiology of epigenetic dysregulation. Mutations in the X-linked gene, *KDM5C*, encoding an lysine (K) specific demethylase 5C are implicated in intellectual disability and autism. Experiments in model organisms show that, in early embryonic development, Histone H3 lysine 4 methylation protects DNA from de novo methylation. Therefore, we hypothesized that the loss of function mutations in *KDM5C* result in loss of DNA methylation at specific genomic targets. To identify critical downstream DNA methylation targets, a genome-wide approach was used. DNA methylation profiles from white blood cells of 13 male patients with known *KDM5C* mutations were compared to 13 male controls using the Illumina Human Methylation27 microarray covering 27,578 CpG sites. Differential methylation analysis identified 13 genes with loss of DNA methylation in patients with *KDM5C* mutations. Three genes, *FBXL5*, *SCMH1*, and *CACYBP* with the most significant loss of DNA methylation are implicated in ubiquitin-mediated protein degradation. Loss of DNA methylation at these three genes was validated by bisulfite pyrosequencing. Since *KDM5C* escapes X-inactivation and has the Y-linked homologue *KDM5D*, we have evaluated the DNA methylation status of *FBXL5* in the context of various sex chromosome constitutions. We found significant differences in DNA methylation dependent on *KDM5C* and *KDM5D* dosage in XX females, XY males and X0 females, suggesting that gene targets regulated by KDM5C could be involved in neurophenotypes of females with Turner syndrome, as well as normal sex differences in brain function. Overall, these data suggest that DNA methylation alterations identified in blood could serve as a biomarker for epigenetic alterations occurring early in embryonic development. Further, the dysregulated epigenetic targets identified in this study could be relevant to the molecular pathophysiology of idiopathic forms of intellectual disability and autism.

763T

Whole DNA methylome profiling and correlative gene expression analysis in the temporal cortex tissue of 28 autism cases and controls. *S.G. Gregory[1], G. Meredith[2], J.K. Beaver[1], C. Lintas[3,4], K. Garbett[5,6], K. Mirnics[5,6], J. Klizer[2], A.M. Persico[3,4], G. Marnellow[2], M. Landers[2].* 1) Duke Center for Human Genetics, Duke University Medical Center, Durham NC 27710, USA; 2) Life Technologies, Carlsbad CA 92009; 3) Child Neuropsychiatry Unit, Laboratory of Molecular Psychiatry and Neurogenetics, University "Campus Bio-Medico", Rome, Italy; 4) Department of Experimental Neurosciences, I.R.C.C.S. "Fondazione Santa Lucia", Rome, Italy; 5) Department of Psychiatry, Vanderbilt University, Nashville, TN, USA; 6) Kennedy Center for Research on Human Development, Vanderbilt University, Nashville, TN, USA.

Autism comprises a spectrum of behavioral and cognitive disturbances of childhood development that includes deficits in social interaction, language development and patterns of repetitive behaviors and/or restricted interests. Autism spectrum disorders (ASDs) have been shown to be highly heritable but are exceedingly heterogeneous at clinical presentation. Although the identification of ASD associated genetic variants and genomic rearrangements have contributed significantly to our understanding of the genetic architecture of ASDs, it is apparent they alone do not explain the developmental complexity of the disorder. Recent evidence supports a role for epigenetic regulatory mechanisms in the pathogenesis of ASDs. Suggestive links arise from duplications of an imprinted region of 15q11-13 where DNA methylation has been linked to Prader-Willi syndrome and Angelman syndrome; mutation within methyl-DNA-binding protein (MECP2) that causes Rett syndrome and is associated with ASDs; and our own data identifying differential methylation of the oxytocin receptor (OXTR) in peripheral blood and the temporal cortex of individuals with autism. In this study we report whole DNA methylome profiling of temporal cortex tissue from 28 individuals (15 autism cases and 13 controls from the Autism Tissue Program) using MethylMiner enrichment prior to next generation sequencing using Life Technologies SOLiD sequencing platform (MBD-Seq). Whole methylome profiles were correlated with expression data from 6 cases and 6 controls from the same dataset, as well transcriptome data of temporal cortex tissue of individuals with autism from recent literature. Initial analysis of the aggregate autism cases and controls identified an expected global hypomethylation in autism cases. MBD-Seq peaks (> 10 reads) that were enriched either within cases or controls were correlated with 353 known autism candidate genes and predicted CpG islands (CGI) in the UCSC genome browser. Forty three of the 353 genes that showed MBD-Seq/CGI correlation were processed in DAVID and showed post correction enrichment for postsynaptic membrane (p=0.00013) and synapse (p=0.0011) GO terms. These data provide further support for epigenetic mechanisms underlying the development of autism in an etiologically relevant tissue; identifies possibly epigenetically labile pathways of autism development; and pinpoints putatively important gene regions necessary for the regulation of autism candidate genes.

764T

Genome-wide and Allele-Specific Differences in DNA Methylation in Mexican-American Children. *N. Holland, P. Yousefi, K. Harley, R. Aguilar, K. Huen, S. Venkat, L. Barcellos, B. Eskenazi.* School of Public Health, CERCH, University of California, Berkeley, CA, 94720.

Children's susceptibility to environmental toxicants may depend on their genotype, levels of exposure and their interactions. In the longitudinal birth cohort study of low-income Latino farmworker families in California (CHAMACOS) we found that trans-placental exposures to several pollutants were associated with birth outcomes and neurodevelopment at different ages (*Eskenazi et al, 2004, 2007, 2010; Young et al, 2005; Bouchard et al, 2011*). We also demonstrated that these health effects depend on phenotype and genotype of paraoxonase (PON1), a multifunctional enzyme involved in detoxification of pesticides and oxidative stress (*Holland et al, 2006; Huen et al, 2009*). Further, we observed a modification of effects of exposure on these health outcomes by PON1 (*Eskenazi et al, 2010; Harley et al, 2011*). Epigenetic mechanisms may explain how early life exposures may lead to diseases during childhood, adolescence and effects may be trans-generational, as postulated by the hypothesis of "fetal origin of human diseases". DNA methylation is one of the main types of epigenetic markers. Little is known about ontogenetic changes of DNA methylation and other epigenetic markers in children, and whether they mediate health effects remains largely unknown.

Genome-wide and site-specific DNA methylation was analyzed in samples of the same CHAMACOS children at birth and age 9 using Infinium Illumina 450K BeadChips to interrogate 485,577 CpG sites. After adjusting for multiple testing by controlling for the False Discovery Rate (FDR), we identified over 2,500 CpG sites that were differentially methylated by age and >70 CpG sites displaying sex-specific differences. Pathway analysis identified CpG sites associated with obesity that were significantly enriched with clusters of genes related to adipogenesis, insulin sensitivity, and Type II Diabetes. Furthermore, DNA methylation appeared to be allele-specific in some genes, including PON1, which has previously been associated with susceptibility to pesticides and health outcomes (*Huen et al, 2009; Eskenazi et al, 2010*). These data suggest that changes in site-specific DNA methylation, which can be age, sex, and allele-specific, may be associated with children's health and development.

765T

Genome-wide hypermethylation and hypomethylation in renal cell carcinoma. *C.L. Hsiao[1], C.S.J. Fann[1], W.H. Weng[2], C.K. Chuang[3], B.T. Teh[4,5], S.T. Pang[3,*].* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Department of Chemical Engineering and Biotechnology, Graduate Institute of Biotechnology, National Taipei University of Technology, Taipei, Taiwan; 3) Department of Urology, Chang Gung University College of Medicine and Memorial Hospital, Tao-Yuan, Taiwan; 4) NCS-VARI Translational Cancer Research Laboratory, National Cancer Centre, Singapore; 5) Laboratory of Cancer Genetics, Van Andel Research Institute, Grand Rapids, MI, USA.

In the past decade, DNA methylation is under intense investigation in a variety of tumors. Such epigenetic alterations in DNA methylation has been proved to play an important role in regulating gene expression and in development of cancer, either by hypermethylation of the promoter regions on tumor suppressor genes or hypomethylation on highly repetitive sequences. We have conducted a genome-wide methylation analysis on 32 renal cell carcinoma (RCC) patients by using methylated CpG island recovery assay (MIRA) method. To discover methylation markers of carcinoma, the MIRA-enriched tumor sample and its matching normal sample (normal-tumor paired design) were co-hybridized to a NimbleGen 385K RefSeq promoter array. Our results showed that 35 and 70 CpG islands were identified as recurrent hyper- and hypomethylation in 35 RCCs, respectively. The methylation and demethylation frequencies of those CpG islands in 35 RCCs were in a rage from 0.4 to 0.7 with median 0.45 and from 0.15 to 0.4 with median 0.25, respectively. The functional and validation analyses confirmed the results by using gene-expression microarray and qPCR. A better understanding of the mechanisms of methylation in oncogenesis can help with the early diagnosis and revolution for treating RCC.

766T

Allele-specific Methylation - Epigenetics, GWAS and Autoimmune Disease. *J.N. Hutchinson[1], J. Fagerness[2], A. Gimelbrant[3], A. Zak[3], M.J. Daly[2], J.M. Seddon[4], A. Chess[5], R.M. Plenge[1].* 1) Division of Rheumatology, Immunology & Allergy, Brigham and Women's Hospital, Boston, MA, USA; 2) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA; 3) Department of Pathology, Harvard Medical School, Boston, MA, USA; 4) Ophthalmic Epidemiology and Genetics Service, New England Eye Center, Tufts Medical Center, Boston, MA, USA; 5) Department of Developmental and Regenerative Biology and Fishberg Department of Neuroscience, Mount Sinai School of Medicine, New York, NY , USA.

DNA methylation can control gene expression and is implicated in the pathogenesis of multiple diseases. Aside from the differentially methylated regions associated with imprinted genes and found on the X-chromosome, maternal and paternal DNA methylation patterns have often been assumed to be identical. However, recent studies have described the widespread occurrence of non-imprinted autosomal allele specific methylation (ASM). ASM is an example of the complex interaction of genetics and epigenetics, as many of these ostensibly epigenetic events are under at least partial genetic control. The strength of this genetic control falls on a continuum, with some ASM being neither purely sequence influenced (i.e. methylation is linked to a particular allele) or stochastic (i.e. either allele can be associated with methylation) but instead showing an intermediate, or skewed association of allele with methylation.

The examination of this process has great potential to maximize and extend genome wide association studies. ASM may explain the relationship between noncoding SNPs and disease in certain GWAS results; stochastic ASM, where putative disease-causing ASM is not be linked to particular allele, would not be well detected by traditional GWAS approaches.

Using Affymetrix 6.0 SNP arrays and methyl-sensitive restriction enzyme digests we have uncovered extensive amounts of ASM encompassing tens of thousands of autosomal regions. Our results indicate ASM to also have some classically epigenetic properties, as it varies in its penetrance between genetically identical monozygotic twins, providing a potential basis for any observed phenotypic discordances between twins. Further examination of these ASM regions reveals that some are associated with eQTLs in peripheral blood monocytes and have been implicated by GWAS in autoimmune diseases such as Crohn's disease and rheumatoid arthritis. Our results may indicate the biological mechanism underlying association of these GWAS derived SNPs with disease.

767T

Neuronal methylome mapping in bipolar disorder and schizophrenia. *K. Iwamoto[1], M. Bundo[1], J. Ueda[2], T. Asai[1,3], T. Miyauchi[2], A. Komori-Kokubo[2], K. Kasai[3], T. Kato[2].* 1) Department of Molecular Psychiatry, Graduate School of Medicine, University of Tokyo, Bunkyo-ku, Japan; 2) Laboratory for Molecular Dynamics of Mental Disorders, RIKEN Brain Science Institute; 3) Department of Neuropsychiatry, Graduate School of Medicine, University of Tokyo.

Epigenetic factors such as DNA methylation and histone modification are believed to be important for the pathophysiology of major psychiatric diseases such as schizophrenia, and bipolar disorder. We have previously established the method for the separation of neuronal and non-neuronal nuclei from the fresh-frozen postmortem brain, and have successfully performed genome-wide DNA methylation analyses. Here we performed a large-scale neuronal and non-neuronal methylation analyses using brains of patients with schizophrenia (N = 35), bipolar disorder (N = 35) as well as controls (N = 35) with promoter tiling arrays. Postmortem prefrontal samples were obtained from the Stanley Medical Research Institute. In both psychiatric diseases, we identified several significant DNA methylation differences compared to control subjects. Interestingly, some differences were only found in neuronal nuclei or non-neuronal nuclei. In addition, to assess the effect of medication, we have profiled the DNA methylation pattern of the human neuroblastoma cell line that was cultured with various treatment drugs. Their DNA methylation profiles were used for identifying the possible treatment-dependent or -independent epigenetic changes. The treatment-independent epigenetic changes may have greater importance in the pathophysiology of schizophrenia or bipolar disorder.

768T

Genome-wide epigenetic regulation by early-life trauma. *B. Labonte[1,2], M. Suderman[3,5], G. Maussion[1], Y. Volodymyr[1], I. Mahar[1,2], A. Bureau[6], N. Mechawar[1,2,4], M. Szyf[5], M.J. Meaney[2,4,7], G. Turecki[1,2,4].* 1) McGill group for suicide studies, Douglas Mental Health University Institute, McGill University, Montreal, Quebec, Canada; 2) Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada; 3) McGill Centre for Bioinformatics, McGill University, Montreal, Quebec, Canada; 4) Department of Psychiatry, McGill University, Montreal, Quebec, Canada; 5) Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada; 6) Centre de recherche Robert-Giffard, Université Laval, Quebec City, Quebec, Canada; 7) Singapore Institute for Clinical Sciences, Singapore.

Our genome adapts to environmental influences in part through epigenetic mechanisms, including DNA methylation. Variations in the quality of the early environment associates with alterations in DNA methylation in rodents and recent data suggest similar processes in humans in response to early life adversity. Here we report a genome-wide study of promoter methylation in individuals with severe abuse during childhood. Promoter DNA methylation levels were profiled using MeDIP followed by microarray hybridization in hippocampal tissue from 41 French-Canadian men (25 with a history of severe childhood abuse and 16 controls. Methylation profiles were compared to corresponding genome-wide gene expression profiles obtained by mRNA microarrays. Methylation differences between groups were validated on neuronal and non-neuronal DNA fractions isolated by fluorescence-assisted cell sorting (FACS). We identified 362 differentially methylated promoters in individuals with a history of abuse compared to controls. Among these promoters, 248 showed hypermethylation and 114 hypomethylation. Validation and site-specific quantification of DNA methylation in the most differentially methylated gene promoters indicated that methylation differences occurred mainly in the neuronal cellular fraction. Genes involved in cellular/neuronal plasticity were among the most significantly differentially methylated. Amyotrophic lateral sclerosis 2 (ALS2) was the most significant finding. Methylated ALS2 constructs mimicking the methylation state in samples from abused suicide completers showed decreased promoter transcriptional activity associated with decreased hippocampal expression of ALS2 variants. Our results suggest that childhood adversity associates with epigenetic alterations in the promoters of several genes in hippocampal neurons.

769T

DNA methylation analysis of IPSCs(induced pluripotent stem cells) using methyl-CpG binding domain (MBD) protein captured sequencing. *D.S. Lee[1,2,5], J.Y. Shin[1,4], Y.S. Ju[1,2,3,5], J.I. Kim[1,2,4,5], A. Nagy[6,7], J.S. Seo[1,2,3,5].* 1) GMI, Seoul national university, Seoul, Seoul, Korea; 2) Department of Biomedical Sciences, Seoul National University Graduate School, Seoul 110-799, Korea; 3) Macrogen Inc., Seoul 153-023, Korea; 4) Psoma Therapeutics Inc., Seoul 110-799, Korea; 5) Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine, Seoul 110-799, Korea; 6) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario M5G 1X5, Canada; 7) Department of Molecular Genetics, University of Toronto, Toronto M5S 1A8, Canada.

Induced pluripotent stem cells (iPSCs) offer immense potential for regenerative medicine and studies of disease and development. Somatic cell reprogramming involves epigenomic reconfiguration, conferring iPSCs with characteristics similar to embryonic stem (ES) cells. However, it remains unknown how complete the reestablishment of ES-cell-like DNA methylation patterns is throughout the genome, and how the methylation pattern changes throughout the reprogramming process. Here we report the profiles of DNA methylation in ES cell, primary iPSC reprogrammed from MEFs by piggybac transposition, MEF redifferentiated from primary iPSC, secondary iPSC induced from this MEFs, and 7 cells, under reprogroming process of day 2, 5, 8, 11, 16, and 18, using methyl-CpG binding domain (MBD) protein captured sequencing. iPSCs show significant reprogramming variability, including somatic memory and aberrant reprogramming of DNA methylation.

770T

The epigenetic modification of FLI1 in the colorectal cancer. *SK. Lee[1], JW. Moon[1], JO. Lee[1], JH. Kim[1], GY. You[1], J. Kim[2], HS. Kim[1], SH. Park[1].* 1) Dept Anatomy, Colledge of Medicine, KOREA University, Seoul, Korea; 2) Department of General Surgery, Korea University Medical Center, Seoul, Korea.

Epigenetic DNA methylation is an alternative mechanism to genetic events lead to the inactivation of gene expression. The promoter hypermethylation of gene has been frequently observed during the pathogenesis of colorectal cancer (CRC). However, the precise information regarding the colorectal cancer mechanism is still not sufficient even though a lot of ongoing research. We analyzed the methylation profile of 27,578 CpG sites spanning more than 14,000 genes in 20 CRC and 20 adjacent non-tumor containing tissues with methylation bead chip array technology. Four-hundreds genes were revealed significantly hypermethylated in CRC. Friend leukemia integration 1 transcription factor (FLI1) is one of the significantly hypermethylated genes at the promoter CpG island region in CRC tissues. FLI-1 has been described to be a useful marker for Ewing sarcoma as a fusion protein form with EWS to be an aberrant transcription factor. However, the mechanism for the cancer development is unclear. To prove the epigenetic mechanism of FLI1 in CRC, we investigated the expression levels both CRC tissues and cell lines using reverse transcription polymerase chain reaction (RT-PCR). The expression level of FLI1 was suppressed in CRC tissues compared with adjacent non-tumor containing tissues (8/10, 53.3%). The down-regulated expression level of FLI1 was also observed both mRNA and protein levels in CRC cell line, DLD-1. Moreover, the suppressed expression was recovered by treatment of 5-Aza-2'-deoxycytidine, a DNA methyl-transferase inhibitor. These results show that promoter methylation of FLI1 gene is frequently occurred and gene silencing may play an important role in colon carcinogenesis.

771T

Differential DNA methylation in TrkB gene in frontal cortex of suicide completers. *G. Maussion, C. Ernst, J. Yang, N. Mechawar, G. Turecki.* Department of Psychiatry, Mental Health University Institute , Douglas Hospital, Montreal, QC, Canada.

TrkB gene codes for a receptor of BDNF.Previous studies by our group indicate that a subgroup of suicide completers have low expression levels of TrkB-T1, an isoform which has no tyrosine kinase domain and is highly expressed in astrocytes. In previous studies, we observed epigenetic modifications in the TrkB promoter, which partially explained TrkB-T1 low expression level in brain tissue from suicide completers. The general aim of this study was to investigate DNA methylation throughout the TrkB-T1 gene sequence and adjacent genomic regions which could be responsible for the significant deregulation in TrkB-T1. Twelve low TrkB-T1 expressor suicides and 12 normal TrkB-T1 expressor controls were investigated. DNA was extracted from BA8/9, treated with sodium bisulfite and submitted to whole genome amplification. Microarray studies were performed using custom-made Agilent arrays tiling the whole TrkB-T1 gene and adjacent genomic sequence. Statistical correction was applied for multiple testing. We used Sequenom's EpiTYPER method to validate sequences shown as differentially methylated according the microarray study. After statistical correction for multiple testing, 9 DNA sequences located in TrkB gene were identified hypermethylated in frontal cortex of suicide completers. Four out of nine probes matched with intronic regions of TrkB gene whereas the five remaining probes corresponded to TrkB-T1 3'UTR sequence. These data are in agreement with the hypothesis of a transcript-specific deregulation through DNA methylation. Intergenic and extragenic regions present differential methylation between groups. Additional studies need to assess the functional significance of these findings.

772T

The promoter hypermethylation of ADHFE1 gene in the colorectal cancer. *JW. MOON[1], SK. LEE[1], JH. KIM[1], GY. YOU[1], JO. LEE[1], J. KIM[2], HS. KIM[1], SH. PARK[1].* 1) Department of Anatomy, College of Medicine, Korea University, Seoul, Korea; 2) Department of General Surgery, Korea University Medical Center, Seoul, Korea.

The colorectal cancer (CRC) is one of the most common cancer in the Korea, and constantly being increased. Aberrant methylation of gene promoters and corresponding loss of gene expression has been frequently observed during initiation and progression of CRC. These aberrantly methylated genes are attractive predictive markers for molecular diagnostics, early detection, and targeted therapies. In this study, we analyzed the methylation profile of 27,578 CpG sites spanning more than 14,000 genes in 20 CRC tissues and in 20 adjacent non-tumor containing tissues with methylation bead chip array-based technology. We screened 739 CpG sites as aberrant methylation CpG sites ($p<0.005$), and identified 125 CpG sites located in promoter CpG islands that were strongly hypermethylated in CRC compared to adjacent normal tissue ($p<0.005$). Among these genes, alcohol dehydrogenase, iron containing, 1 (ADHFE1) was methylated highly in 20 CRC tissues compared with 20 adjacent non-tumor containing tissues ($p<0.005$). We identified that expression level of ADHFE1 was decreased in CRC tissues compared with in the adjacent non-tumor containing tissues (10/14, 72%) using a reverse transcription PCR (RT-PCR). In addition, we identified of the promoter methylation status of ADHFE1 gene in two normal colon cell lines and in two CRC cell lines using methylation-specific polymerase chain reaction (MSP). As a results, the methylation of ADHFE1 was detected in two CRC cell lines but not detected in two normal colon cell lines. The expression of ADHFE1 was confirmed using RT-PCR and immunoblot analysis in four cell lines. Gene expression was silenced or down-regulated in HT-29 cells with methylated-ADHFE1, but was restored by treatment with 5-aza-2'-deoxycytidine, a DNA methyl-transferase inhibitor. This study suggests that the promoter hypermethylation of ADHFE1 is frequently present in colon cancer and gene silencing may play an important role in colorectal carcinogenesis.

773T

Phosphodiesterase 11A (Pde11a) expression in mouse tissues and characterization of a Pde11a mouse knock-out model. *I. LEVY[1], M. STAROST[2], E. BALL[1], F. FAUCZ[1], S. KOLIAVASILLIS[1], A. HORVATH[1], K. TSANG[1], K. NEDELLA[1], M. ALATSATIANOS[1], M. NESTEROVA[1], C. STRATAKIS[1].* 1) Program in Developmental Endocrinology and Genetics. NICHD / National Institutes of Health. Bethesda, Maryland, USA; 2) Division of Veterinary Resources. National Institutes of Health. Bethesda, Maryland, USA.

Phosphodiesterases catalyze the hydrolysis of cyclic nucleotides and maintain physiologic levels of intracellular concentrations of cAMP and cGMP. Increased cAMP signaling has been associated with genetics disorders that lead to adrenocortical tumors and Cushing syndrome. Genetics defects in the phosphodiesterase 11A are associated with increased levels of cAMP and bilateral adrenal hyperplasia; they also contribute to the development of adrenocortical, prostate, and testicular tumors in the general population. The aim of the present work is to study the expression of Pde11a in mouse tissues, as well as to understand better the Pde11a defect in a previously published Pde11a-/- knockout(KO) mouse model.A colony of Pde11a-/-(KO), Pde11a+/-(Het) and wild type(wt) mice was studied in a complete phenotypical gross and microscopy pathological study. Molecular, immunohistochemical and biochemical studies were done to determine the function and expression of Pde11a in the tissues of normal and mutant mice. In normal mice higher PDE11A immunoreactivity(PDE11A-IR) was found in small intestine villi, thyroid follicular cells, prostate and spermatocytes; moderate PDE11A-IR in brain, liver hepatocytes, and exocrine pancreas and little, if any, in the pituitary gland. In mutant mice RNA studies showed a reduced Pde11a expression in steroidogenic tissues (adrenals and testis), lung, brain, and liver while prostate and kidney presented comparable expression with normal mice. PDE11A protein expression and activity assays did not show differences among three groups. Pathological studies showed differences: Adrenal subcapsular hyperplasia and foamy cells were more frequent in Het(37.7% and 30.2% respectively, $p<0.05$) than in wt group (26.3% and 15.8% respectively); Spinal epidermoid cyst in Het (15%) and wt mice (10%); kidney-pelvis interstitial lymphocytes only in KO mice (10.7%, $P<0.05$). KO mice showed increased absolute and organ specific weight. This study show a clear profile of Pde11a protein expression in endocrine and other mouse tissues, as well as convincing evidence of only partial Pde11a suppression in the previously published KO mouse. These mice developed adrenal hyperplasia and other abnormalities like humans with heterozygote PDE11A defects and partial inactivation of the enzyme. These data support the involvement of PDE11A in the pathogenesis of adrenocortical hyperplasia, but also provide significant insight into a previously reported mouse model.

774T

PHYLOGENOMICS STUDDIES AND STRUCTURALIST MODEL OF INFORMATION. *P. Grigoriu, Bogota.* Medical Departament, Kinio S.A., Bogota, Colombia.

My previous research focused on the interaction studies of different repair mechanisms in Drosophila melanogaster. It was found that pre-replication repair mechanisms, which are specific for each type of lesion and which faithfully reestablish the information present before DNA damage, interact additionally to maintain cellular viability. In contrast, post-replication repair mechanisms (non-homologous recombination and translesion synthesis), which do not always recover genetic information, interact synergistically to reestablish cellular viability. Consequently I proposed the following: (1) Post-replication repair mechanisms are archaic repair mechanisms which function principally to reestablish cell viability by adding repetitive sequences, which can result in loss of genetic information; (2) Pre-replication repair mechanisms, which have appeared more recently in evolution, have the function of correctly recovering genetic information; (3) A structuralist model of genetic information which identifies two principal elements of information systems: (1) a repetitive structure or carrier wave and (2) information as alterations of the repetitive structure. The human genetic project data allow for phylogenetic studies which can support the structuralist model of information and the DNA repair evolution model. Following these models: (1)ligase 4, which operates in non homologous recombination and considered as an archaic repair mechanism, is present in both prokaryotes and eukaryotes. The same occurs with the translesion synthesis enzymes, which also appear the two karyotes groups; (2)In contrast enzymes operating in nucleotide excision repair are different in the two karyotes groups. The same occurs with base excision repair mechanisms It is expected that additional phylogenetic studies will further support the information presented here.

775T

Replacement of the myotonic dystrophy type 1 CTG repeat with `non-CTG repeat' insertions in specific tissues. *M.M. Axford[1,2], A. Lopez-Castel[1,3], M. Nakamori[4], C.A. Thornton[4], C.E. Pearson[1,2].* 1) Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 3) Valentia Biopharma, Parque Cienti fico de la Universidad de Valencia, Paterna, Spain; 4) Department of Neurology, University of Rochester School of Medicine and Dentistry, Rochester, New York, USA.

Recently, curious mutations have been reported to occur within the (CTG)n repeat tract of the myotonic dystrophy type 1 (DM1) locus. For example, the repeat, long presumed to be a pure repeat sequence, has now been revealed to often contain interruption motifs in a proportion of cases with expansions. Similarly, a few de novo somatic CTG expansions have been reported to arise from non-expanded DM1 alleles with 5-37 units, thought to be genetically stable. This study has characterised a novel mutation configuration at the DM1 CTG repeat that arose as somatic mosaicism in a juvenile onset DM1 patient with a non-expanded allele of (CTG)12 and tissue specific expansions ranging from (CTG)1100 to 6000. The mutation configuration replaced the CTG tract with a non-CTG repeat insertion of 43 or 60 nucleotides, precisely placed in the position of the CTG tract with proper flanking sequences. The inserts appeared to arise from a longer human sequence on chromosome 4q12, and may have arisen through DNA structure mediated somatic inter-gene recombination or replication fork template switching errors. De novo insertions were detected in cerebral cortex and skeletal muscle, but not in heart or liver. Repeat tracts with -1 or -2 CTG units were also detected in cerebellum, which may have arisen by contractions of the short (CTG)12 allele. This non-CTG configuration expands current understanding of the sequence variations that can arise at this hypermutable site.

776T

Blood platelet as a new model to study the interactions of the fragile X mental retardation protein with the cytoskeleton. *A.J. Meunier, F. Corbin.* Département de biochimie, Université de Sherbrooke, Sherbrooke, Quebec, Canada.

Fragile X syndrome is the most common form of inherited intellectual disability. This disease results from the expansion of CGG repeats in the *FMR1* gene leading to its methylation and thus, its transcriptional silencing. The absence of the corresponding product, FMRP (Fragile X Mental Retardation Protein), is responsible for the clinical features. FMRP possesses RNA-binding motifs essential to its interaction with the mRNA of polyribosomes and would modulate protein synthesis. However, additional functions have also been demonstrated in other cellular models. Uncovering novel observations in a simpler human biological system, will allow us to better understand the real contribution of those suggested functions. In fact, we confirm in blood platelets, characterized by low rates of translation, the presence of FMRP in soluble form. In contrast, in most of the other cell types studied, including neurons, where this protein was rather found in the polyribosome's fraction. Since platelet activation triggers many intracellular processes including cytoskeleton's reorganisation and protein synthesis, we therefore investigated the behavior of FMRP upon platelet activation. Human platelets were activated by means of different agonists and subjected to cell fractionation protocols in order to determine the subcellular localization of FMRP. We observed a shift of FMRP from the soluble to the cytoskeletal fraction, supporting that platelet activation promotes the interaction of FMRP with certain components of this fraction. Moreover, this shift was proportional to the percentage of platelet aggregation and independent of the stimulation pathway employed. Interactions of FMRP during platelet activation were also assessed in the presence of various chemical agents that influence different cellular processes. Agents affecting actin network polymerization modified FMRP's behavior, suggesting that FMRP might interact with components of the microfilaments. The resultant redistribution of FMRP, although consistent with some models described by other groups, suggests a new function for this protein in connection with the platelet cytoskeletal reorganization upon activation. Since this process can easily be modulated in blood platelets, its has the potential to be a very promising model for studying FMRP. This new human cell model will not only allow us to deepen our knowledge of FMRP functions, but also to improve our understanding of the fragile X syndrome.

777T

The role of AGG interruptions in the *FMR1* CGG repeat during transmission in a clinical sample. *C.M. Yrigollen[1], B. Durbin-Johnson[2], R.J. Hagerman[3,4], F. Tassone[1,3].* 1) Biochemistry Molecular Medicine, University of California Davis, Sacramento, CA; 2) Department of Public Health Sciences University of California Davis, School of Medicine, Davis, California; 3) M.I.N.D. Institute, University of California Davis Medical Center, Davis, California; 4) Department of Pediatrics, University of California Davis Medical Center, Davis, California.

Background: The most common cause of Fragile X syndrome is the expansion of a trinucleotide repeat (>200 CGG) in the *FMR1* promoter. This expansion occurs during transmission in cases where a mother is a premutation carrier. Many *FMR1* CGG repeat alleles contain AGG sequences interspersed among the CGG repeats. These AGG "interruptions" are thought to confer DNA stability and reduce the risk of expansion. It has been theorized that pure CGG repeat length is the underlying factor. The smallest premutation alleles documented to expand to full mutations have lacked AGG interruptions. **Methods:** We used a novel CGG Repeat Primed PCR-based approach to identify AGG interruptions in 275 mothers of children with an expanded allele (>44 CGG). Twelve mothers had an intermediate range allele and 263 had a premutation range allele that was transmitted to the child. Additionally, we looked for an association between the presence of AGG interruptions and *FMR1* transcription levels, measured by qRT-PCR, within the mothers. **Results:** Modeling the probability of transmission, we found the optimal cutoff for total CGG length as a predictor of transmission status to be 75 while the pure stretch was determined to be 65. The risk for expansion increased as a function of 3'-continuous CGG repeat length. No association between presence of AGG interruptions and transcriptional levels was observed. **Conclusion:** We have used a novel approach to assess the AGG structure in 275 premutation women, representing 381 transmissions. Our findings point to a relevant role of AGG interruptions in the stability of the CGG repeats in mothers. The risk of expansion in equally sized alleles is determined by pure CGG stretch. Knowledge of the AGG structure in intermediate and premutation alleles is of great importance in determining risk stratification for women and improving genetic counseling.

778T

Differential functional analyses of (-actinin isoforms. *C.P. Hsu, B. Moghadaszadeh, B.E. Rider, A.H. Beggs.* Division of Genetics and Program in Genomics, The Manton Center for Orphan Disease Research, Children's Hospital Boston, Harvard Medical School, Boston, MA.

The (-actinin proteins are a highly conserved family of actin crosslinkers that mediate interactions between a host of cytoskeletal and sarcomeric proteins: (-Actn1 and (-Actn4 crosslink actin filaments in the cytoskeleton, while (-Actn2 and (-Actn3 serve a crucial role in anchoring actin filaments to the sarcomeric Z-line. In an effort to better understand the protein-protein interactions of each (-actinin isoform, as well as perform further structure/function analysis of the (-actinin family, we are utilizing Fluorescence Recovery After Photobleaching (FRAP) to investigate their association dynamics at the Z-line. These FRAP studies are performed using primary mouse *flexor digitorum brevis* cultures, which we have determined to all express (-Actn2, with a subset (66%) co-expressing (-Actn3. Using an adenoviral transduction system, we are expressing GFP-tagged (-Actn1, (-Actn2, (-Actn3, and (-Actn4 in these cultures and quantitating their fluorescence recoveries after photobleaching. We have found that the recovery kinetics of these proteins follow one of three distinct patterns, with (-Actn2/(-Actn3 recovering the least, (-Actn1 recovering to an intermediate degree, and (-Actn4 recovering most fully. This suggests that different (-actinin isoforms have unique association kinetics at the Z-line. Currently, we are creating chimeric constructs to map the interactive domains, and we plan to use this methodology to further investigate the specific sequences and binding partners of each isoform responsible for these functional differences. Although we have not detected any significant difference in protein dynamics between (-Actn2 and (-Actn3, there nevertheless remains the question of whether they are fully compensatory for one another in skeletal muscle (as they share 90% identity and 80% similarity). Previously, we have found that a lack of (-Actn2 causes embryonic lethality. Since it has been shown that (-Actn2 can compensate for (-Actn3, we investigated whether the converse would be true, using a murine knock-in approach to replace the endogenous *Actn2* gene with *Actn3* (Actn3-KI). Remarkably, we find that Actn3-KI homozygotes die during late embryogenesis. Thus, although expression of (-Actn2 clearly compensates for absence of (-Actn3, the converse does not appear to be true, suggesting that (-Actn2 serves unique physiological functions.

779T

Genome-wide Expression Profiling Implicates a *MAST3* Controlled Gene Set in Colonic Mucosal Inflammation of Ulcerative Colitis Patients. *C. Labbé[1, 2], G. Boucher[1], S. Foisy[1], A. Alikashani[1], H. Nkwimi[1], G. David[1], M. Beaudoin[1], P. Goyette[1], G. Charron[1], J.D. Rioux[1, 2].* 1) Montreal Heart Institute, Montréal, Qc, Canada; 2) Département de médecine, Université de Montréal, Montréal, Qc, Canada.

Inflammatory bowel diseases (IBD) refer to Crohn's disease and ulcerative colitis (UC), two common autoimmune diseases of the gastrointestinal tract, most prevalent in urban areas of North America and Europe. Genome-wide association studies have already identified about 100 loci responsible for the susceptibility to IBD. Estimates of heritability suggest that many more are still to be discovered. The next challenge is to explain the individual role of each of these modest effect loci in the disease state. We have previously identified *MAST3* as an IBD gene through association mapping of the 19p linkage region and shown that MAST3 modulates the activity of inflammation master switch NF-2B. In this follow-up study, we wish to further characterize the role of MAST3 in the cell using an unbiased genome-wide survey of MAST3 controlled gene expression. More specifically, we looked at differential gene expression resulting from overexpression and knockdown of the *MAST3* gene in HEK293 and THP1 cells, respectively. The overexpression model resulted in 28 genes being significantly overexpressed by 2-fold or greater. Expression from genome-wide microarrays was validated by qPCR in the overexpression model and further confirmed in the knockdown model. The activity of NF-2B was found to correlate to differential gene expression. Additionally, expression of the MAST3 controlled gene set was measured in biopsies of control and UC patient's inflamed and non-inflamed regions of the colon. Expression was found to be enriched in patients inflamed regions compared to non-inflamed regions. The MAST3 controlled genes are heavily involved in the immune response. Among them are pro-inflammatory cytokines (*CCL20*, *IL8*, etc), regulators of NF-2B (*TNFAIP*3, *LY96*, *NFKBIA*, etc), genes involved in interferon induced defence against pathogen invasion (*IFIT1*, *ISG15*, etc), and genes involved in cell adhesion/or migration (*CD44*, *TMOD1*, etc). Taken together these results confirm MAST3 as a modulator of the NF-2B pathway and suggest its control over the expression of a set of immune genes directly involved in the onset of inflammation typical of IBD.

780T

BAC-Based Small-Molecule Screen to Investigate Regulation of the Macular Degeneration Candidate Gene HTRA1. *J.D. Hoffman, P.C. Mayo, N.C Schnetz-Boutaud, D.P. Mortlock, J.L. Haines.* Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Age-Related Macular Degeneration (AMD) is one of the most common causes of visual impairment among the aging Caucasian population in the United States. Recent genome-wide association studies have implicated HtrA serine peptidase 1 (HTRA1) and age-related maculopathy susceptibility 2 (ARMS2) as being associated with AMD. Although these genes are located in close proximity to one another on chromosome 10, their individual roles in the pathogenesis of AMD are the subject of debate. We present a high-throughput small-molecule screen to characterize expression at the HTRA1 locus. Screening was carried out using a bacterial artificial chromosome (BAC) due to the ability of BACs to capture the local and distant cis-regulatory elements that are necessary to recapitulate the endogenous cis-regulatory architecture. To measure expression from the HTRA1 locus in response to small molecules, a luciferase reporter cassette was inserted at the HTRA1 translational start site within the RP11-72B24 BAC clone. The BAC was transfected into HeLA cells and these were screened against a small molecule library of 2,000 compounds that have a mixture of known and unknown pharmacological effects. 167 compounds gave a significant response as measured by greater than 3 standard deviations from the luciferase expression measured in a vehicle-only control, and were followed up with 4-point dose-response curve tests for further validation. At present, 7 of 16 compounds have been validated for repression of endogenous HTRA1 expression in wild-type HeLa cells using quantitative-PCR. Three compounds that repress HTRA1 mRNA include podophyllotoxin acetate, an antimitotic agent that inhibits tubulin polymerization; propargite, a pesticide with ATP-synthase inhibitory activity; and monensin sodium, a monovalent cation inhibitor. Menadione was also marginally able to repress HTRA1 transcripts. Menadione has vitamin-K-like properties, stimulates the ERK/MAPK pathway and can regulate connexins via interaction with EGFR signaling. We also confirmed previous reports that the proteasome inhibitor MG132 represses HTRA1 mRNA. Analysis of the preliminary results is promising but requires further investigation and optimization.

781T

Determination of SNPs in miR-9 genes related regions in alcoholism using COGA samples. *Y. Wang, O. Anees, N. Kinstlinger, A. Pietrzykowski.* Rutgers University, Department of Animal Sciences, 67 Poultry Farm Lane, New Brunswick, NJ 08901.

Alcoholism has a strong genetic component, however the exact genetic and molecular underpinnings of this debilitating disease are yet to be determined. Work of others and us have recently indicated that expression of miR-9, a member of non-coding gene expression regulators (microRNA) is modulated by alcohol. We observed that exposure of rodent neurons to alcohol leads to the development of tolerance to this drug via upregulation of the mature form of miR-9 in these cells. Typically, one microRNA can regulate expression of hundreds of targets, therefore microRNAs are very important master regulators of gene expression. Mature form of miR-9 is a product of three different miR-9 genes located on three different chromosomes. We observed that, in humans, each miR-9 gene has a different architecture, particularly differing in the promoter region. Interestingly, two out of three miR-9 genes are located within loci of high susceptibility to alcoholism. We focused on one of these genes, miR-9-3, located on chromosome 15 to search for single nucleotide polymorphisms (SNPs) associated with alcoholism. We used COGA samples and performed a bidirectional direct sequencing using high fidelity Taq polymerase of nested PCR products covering miR-9-3. We discovered several new SNPs in the stem, loop and mature miR-9 regions, including the seed region. These SNPs can change the secondary structure of miR-9, its biogenesis and affinity to targets.

782T

Deciphering of the molecular events governing hMSH4 protein homeostasis in human cells. *Y. Xu, C. Her.* School of Molecular Biosciences, College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

The ability to maintain proper levels of protein factors involved in DNA repair and damage response is critical for sustaining genomic stability. Recent evidence suggests that deviations from homeostatic levels of many proteins can be as detrimental as mutations to cells. The levels of the human MutS family protein hMSH4 are strictly regulated in such a way that its presence in mitotic cells is near detection limits, whereas its expression rises during the onset of meiotic recombination and coincides with the generation of DNA double-strand breaks. To this end, we are intrigued by the physical interaction between hMSH4 and the von Hippel-Lindau binding protein 1 (VBP1), of which the latter is a binding partner of the VHL ubiquitin E3 ligase as well as a subunit of the prefoldin complex. Here, we demonstrate that VBP1 promotes hMSH4 polyubiquitination and therefore proteasome-mediated degradation. Knockdown of VBP1 significantly increases the hMSH4 protein levels by flow cytometry analysis, while overexpression of VBP1 decreases hMSH4 protein levels. In addition to proteasome inhibitor MG-132, hMSH4 degradation could also be slightly inhibited by leupeptin or 3-MA, suggesting the potential involvement of other pathways. Furthermore, RNAi-mediated silencing of VBP1 or VHL leads to a decrease in polyubuiquitination of hMSH4 by an in vivo ubiquitination assay, suggesting that VBP1-mediated degradation of hMSH4 is accomplished through the CUL2/VHL E3 ligase pathway. Together, our studies provide an insight into the regulation of hMSH4 by VBP1 and imply that this mechanism might be important in maintaining the low levels of hMSH4 protein in mitotic cells. It is known that ectopic recombination between repetitive sequences is a major source for genomic instability in cancer cells, thus this study will also provide a foundation for a better understanding of the molecular mechanisms controlling recombination and genomic stability in human cells.

783T

Clinical and genetic characteristics of patients with X-linked deafness. *R. Birkenhager, E. Prera, N. Lueblinghoff, S. Arndt, A. Aschendorff, R. Laszig.* Otorhinolaryngology H&N Surg, Univ Freiburg, Freiburg, Germany.

Hearing impairment is the most common sensory disorder in humans, affecting approximately one to three in 1000 newborns, with 50 % due to genetic causes. The majority of these cases (70 %) are non-syndromic, about 2 % of these are X-linked. So far five different X-linked loci have been mapped, but the causative gene POU3F4 (MIM 300039) has been identified only for the Locus DFNX2. Clinical characteristics of DFNX2 include, in most cases, a mixed type of hearing loss. This hearing loss is often progressive, with temporal bone abnormalities and stapes fixation. Temporal bone anomalies include dilation of the fundus of the internal acoustic canal (IAC) (3), that can increase the risk of CSF gusher during stapes or cochlear implant surgery. During inner ear development, the homologous mouse Pou3f4 gene is exclusively detected in the mesenchymal tissue adjacent to the otic epithelium. Knockout Pou3f4 mice have abnormalities in mesenchyme derived structures including the scala tympani, spiral limbus and spiral ligament fibrocytes of the cochlea as well as variances in the temporal bone. POU3F4 belongs to a sub-family of transcription factors, which are characterized by two conserved DNA binding domains, a POU and a HOX domain, both helix-turn-helix (HTH) structural DNA binding motifs. Several molecular genetic studies of gusher patients have identified mutations in the POU3F4 gene, including partial or complete deletions of the gene, as well as deletions, inversions, and duplications of the DFNX2 genomic region not encompassing the POU3F4 coding sequence. Little is known about how such mutations affect the normal function of the POU3F4 protein, which leads to characteristic inner ear malformations and hearing impairment. Here we present the clinical characteristics of patients of four independent German families. Additional analyses were performed in the POU3F4 gene. Mutations analysis in the POU3F4 gene was brought by direct sequencing of the coding exons including the intron transitions. Due to the radiological findings, mutation analyses of the POU3F4 gene were performed. Sequence analyses in one family revealed a novel missense mutation at nucleotide position 973, thymine to adenine (c.973 T>A, p.W325R) and in the second patient a missense mutation at nucleotide position (c.902C>T, p.P301L). Until now two novel strongly conserved mutations in the HOX-domain of the POU3F4 transcription factor were identified leading to an effect in the protein function.

784T

Identification of protein sub-networks implicated in Autism Spectrum Disorders. *C. Correia[1,2], Y. Diekmann[1], J.B. Pereira-Leal[1], A.M. Vicente[1,2], Autism Genome Project Consortium.* 1) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 2) Instituto Nacional de Saúde Dr.Ricardo Jorge, Lisboa, Portugal.

Autism Spectrum Disorders (ASDs) represent a group of childhood neurodevelopmental disorders characterized by three primary areas of impairment: social interaction, communication, and restricted and repetitive patterns of interest or behavior. Although autism is one of the most heritable neuropsychiatric disorders, most of the known genetic risk has been traced to rare variants. Genome-wide association studies (GWAS) have thus far met limited success in the identification of common risk variants, suggesting that ASD may result from the interaction of many variants with low or moderate individual risk, which cannot be detected in current GWAS in a single SNP analysis framework. Recently, molecular interaction networks have been integrated with high-throughput expression data, and the success of this application has been demonstrated through the identification of biologically meaningful subnetwork markers that are more reproducible and with a higher prediction performance. To identify subnetworks implicated in autism and with predictive value for autism diagnosis we have applied a network-based approach to the Autism Genome project consortium GWAS. We have integrated family- based association data from 2588 ASD families genotyped for 1 million single-nucleotide polymorphisms (SNPs) with a Human Protein-Protein interaction (PPI) network. We show, in line with observations in other complex diseases, that the proteins encoded by top genes (genes including one or more SNPs with a Transmission Disequilibrium Test $P<0.01$ or 0.005) are significantly closer to each other in a PPI network, suggesting that they are functionally related. Furthermore, these proteins were found to preferentially directly interact with each other, and were connected in a significantly larger component than random expectation, indicating that they are involved in a small number of interconnected biological processes. Having validated our initial assumption that autism-associated genes are confined to a limited number of biological processes, we searched for subnetworks that locally maximize the proportion of genes with low *P*-values in the GWAS dataset. Validation of the results in an independent GWAS and determination of prediction value of these subnetworks are underway. With this approach, we expect to identify biological processes associated with increased susceptibility to ASD, and eventually to derive clinically useful predictive markers.

785T

TAS2R38 variation and its impact on bitter and thermal tasting in humans. *A.B. Bering[1], G. Pickering[1,2,3], P. Liang[1].* 1) Biological Sciences, Brock University, St. Catharines, Ontario, Canada; 2) Cool Climate Oenology and Viticulture Institute, Brock University, St. Catharines, Ontario, Canada; 3) Department of Psychology, Brock University, St. Catharines, Ontario, Canada.

TAS2R38 is a bitter taste receptor known to be associated with PROP (6-n-propylthiouracil) taster status. PROP tasting is a proxy for general taste responsiveness, and has been linked with diet-related behavior and health outcomes, including alcohol consumption, alcoholism, body mass index and cardiovascular disease risk. Ability to taste PROP allows classification of individuals into 3 phenotypes: super-tasters (pST), medium-tasters (pMT) or non-tasters (pNT). Recently, another form of taste phenotype, also serving as a proxy for general taste responsiveness, was discovered and known as thermal taster status, in which the heating and cooling of a region of one's tongue evokes a phantom taste response in approximately 20-50% of individuals. The mechanism behind thermal taste is not yet known. In this study, we sought to investigate the relationship between the TAS2R38 sequence variation with both PROP and thermal taster status. DNA extractions from buccal cells obtained from 56 individuals via mouthwashes were performed, and analysis of TAS2R38 variation was conducted through PCR amplification and sequence analysis. Among a total of 15 SNPs identified, a statistically significant correlation between PROP taster status and three TAS2R38 SNPs was evident as previously reported, with SNP site 1 having the strongest correlation compared to the other 2 known SNPs. However, not all PROP phenotype differences can be explained by variation at these 3 SNP sites, suggesting the involvement of additional sites either in TAS2R38 or other TAS2R genes. There was no association between thermal taster status and the TAS2R38 genotype suggesting the involvement of different genes in this phenotype. Our research provides the first genetic evidence to support previous phenotype-based observation that there is no association between PROP and thermal taster status by excluding the involvement of TAS2R38 in thermal tasting.

786T

Genetic Regulation of ABC Transporter Expression in Human Liver and Kidney. *A. Chhibber, SW. Yee, C. Wen, RJ. Eclov, H. Fukushima, KM. Giacomini, DL. Kroetz.* Dept of Bioengineering & Therapeutic Sciences, Univ of California, San Francisco.

ATP -Binding Cassette (ABC) transporters are responsible for the transport of endogenous compounds, xenobiotics, and pharmaceutical agents across cell membranes. Inter-individual variation in expression of these transporters can explain differences in drug efficacy and toxicity, or susceptibility to a variety of conditions caused by a disruption in homeostasis of endogenous compounds. While several large studies have been conducted linking genetic variation to changes in gene expression (eQTLs) in lymphoblastoid cell lines, variation in expression caused by genetic differences is often highly tissue specific. For many ABC transporters, the liver and kidney are key sites of function. In this study we identify both tissue specific and cis and trans eQTLs that regulate ABC transporter expression in healthy human liver and kidney tissue. Gene expression for 48 ABC transporters was evaluated in 58 kidney and 60 liver samples from surgical resection or postmortem collections in Caucasian males and females using the Biotrove Open Array™ qPCR platform. Of these samples, 58 kidney samples and 34 liver samples were successfully genotyped on the Affymetrix Axiom genotyping platform using the Axiom Genome-Wide EUR Array Plate. After initial QC, 52 kidney samples and 34 liver samples were included in subsequent analyses. We identify associations between variants and expression levels by linear regression, and use the co-expression patterns between genes to propose possible regulatory pathways for ABC Transporters. Cis-eQTLs within 50 kb of transcription start and stop sites were identified for nine ABC transporters, each with one to three independent eQTLs; additional eQTLs were identified for 35 transporters within 500 kb of each gene. Trans-eQTLs were identified for 31 transporters, including several putative “hotspots” that may regulate expression of multiple genes. Both tissue-specific and cross-tissue QTLs were identified. This study includes the first genotype-expression study in healthy human kidney tissue, and the results may elucidate new clinically relevant sources of inter-individual variation. Further, this data will provide insights on the pathways regulating expression of ABC transporters. Functional validation of selected hits is ongoing. (This work was funded by NIH grant GM61390.).

787T

Identification of putative causal genes for IBD in the 1q32 region: from genetics to biological mechanism. *G. David[1,2], M. Budarf[2], G. Charron[2], S. Foisy[2], A. Gardet[3], R.J.W. Heath[3], R.J. Xavier[3], J.D. Rioux[1,2].* 1) Medicine, Universite de Montreal, Montreal Heart Institute, Montreal, Quebec, Canada; 2) Montreal Heart Institute, Montreal, Quebec, Canada; 3) Center for computational and integrative biology, Massachusetts General Hospital, Harvard University.

Crohn's disease (CD) is an inflammatory bowel disease (IBD) characterized by chronic inflammation. This complex disease appears to be the result of an immune system dysregulation. Genome-wide association studies have identified 99 loci that contribute to IBD susceptibility. Chromosome 1q32 has been identified as a CD (rs 11584383, p=1,43x10e-11) and UC locus (p= 5,71x10e-7). This region contains four genes: Chromosome 1 open reading frame 106 (*C1orf106*), Kinesin family member 21B (*KIF21B*), Calcium channel, voltage-dependant, L type, alpha 1S subunit (*CACNA1S*) and Chromosome 1 open reading frame 81 (*C1orf81*). The goal of the present study is to place these genes in a biological context and to determine their possible involvement in IBD. First, using quantitative PCR, we established expression profile of the 1q32 genes in murine tissues and human cell lines. We determined that only *KIF21B* and *C1orf106* were expressed in immune and gastrointestinal tissues. Next, we tested the involvement of KIF21B and C1orf106 in biological pathways, more specifically NF-2B activation and ER stress that are both involved in pro-inflammatory processes. We found that an overexpression of KIF21B, in HEK293T cells, decreased ER stress response as well as the activation of NF-2B pathway, whereas C1orf106 increased ER stress response and slightly upregulated the NF-2B activation. Taken together our results suggest that KIF21B and C1orf106 are good candidate causal genes for IBD based on functional studies of the 1q32 region. Additional investigations are needed to fully explain the mechanism of KIF21 and C1orf106 activity in IBD pathogenesis.

788T

Mutations in *ZBTB24* are associated with immunodeficiency, centromeric instability and facial anomalies (ICF) syndrome type 2. *J. Wang[1,17], J.C. de Greef[1,17], J. Balog[1], J.T. den Dunnen[1], R.R. Frants[1], K.R. Straasheijm[1], C. Aytekin[2], M. van der Burg[3], L. Duprez[4], A. Ferster[5], A.R. Gennery[6], G. Gimelli[7], I. Reisli[8], C. Schuetz[9], A. Schulz[9], D.F.C.M. Smeets[10], Y. Sznajer[11], C. Wijmenga[12], M.C. van Eggermond[13], M.M. van Ostaijen-ten Dam[14], A.C. Lankester[14], M.J.D. van Tol[14], P.J. van den Elsen[13,15], C.M. Weemaes[16], S.M. van der Maarel[1].* 1) Dept. of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands; 2) Department of Pediatric Immunology, Dr. Sami Ulus Children's Health and Diseases Training and Research Center, Ankara, Turkey; 3) Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands; 4) Department of Medical Genetics, Hôpital Erasme-ULB, Brussels, Belgium; 5) Department of Paediatrics, Haemato-Oncology Unit, Hôpital Universitaire des Enfants Reine Fabiola-ULB, Brussels, Belgium; 6) Department of Paediatric Immunology, Newcastle Upon Tyne Hospital, NHS Foundation Trust, Newcastle Upon Tyne, United Kingdom and Institute of Cellular Medicine, Newcastle University, Newcastle Upon Tyne, United Kingdom; 7) Laboratorio di Citogenetica, Instituto G. Gaslini, Genove, Italy; 8) Department of Pediatric Immunology and Allergy, Selcuk University, Konya, Turkey; 9) Department of Pediatrics and Adolescent Medicine, University Hospital Ulm, Ulm, Germany; 10) Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; 11) Pediatric Clinical Genetics and Center for Human Genetics, Hôpital Universitaire des Enfants Reine Fabiola (HUDERF), Brussels, Belgium; 12) Genetics Department, University Medical Center and Groningen University, Groningen, the Netherlands; 13) Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, the Netherlands; 14) Department of Pediatrics, Leiden University Medical Center, Leiden, the Netherlands; 15) Department of Pathology, VU University Medical Center, Amsterdam, the Netherlands; 16) Department of Paediatric Infectious Diseases and Immunology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; 17) These authors contributed equally to this work.

Immunodeficiency, centromeric instability and facial anomalies syndrome (ICF; MIM 242860) is a rare autosomal recessive primary immune deficiency. It is mainly characterized by recurrent, often fatal, respiratory and gastrointestinal infections due to greatly reduced serum immunoglobulin levels. About half of ICF patients (ICF1) have mutations in the DNA methyltransferase 3B (*DNMT3B*) gene, resulting in reduced enzymatic activity and hypomethylation of specific repetitive DNA sequences in their genome. The remaining ICF patients carry unknown genetic defects (ICF2) but share with the ICF1 patients largely identical immunological and epigenetic features, including hypogammaglobulinemia and hypomethylation of juxtacentromeric heterochromatic DNA. By a combination of homozygosity mapping, whole exome sequencing and Sanger sequencing we identified loss-of-function mutations in the zinc-finger- and BTB (bric-a-bric, tramtrack, broad complex)-domain-containing 24 (*ZBTB24*) gene in the majority of ICF2 cases.

ZBTB24 is a member of a family of transcriptional repressors, such as BCL-6 (also known as ZBTB27) and PATZ1 (ZBTB19), with prominent regulatory roles in hematopoietic development and malignancy. Fluorescence activated cell sorting (FACS) followed by quantitative RT-PCR analysis showed that ZBTB24 is ubiquitously expressed with highest mRNA levels in human peripheral blood B lymphocytes, especially in naive B cell-subpopulation. Moreover, expression of ZBTB24 seemed to be co-regulated with DNMT3B during B cell development. Together, these data suggest that ZBTB24 is involved in the methylation of juxtacentromeric heterochromatic DNA and in the regulation of B-cell development and/or function. Current efforts focus on the elucidation of the molecular function of ZBTB24 in B-cell development and on the identification of the gene defect in the remaining ICF cases.

789T

The Asthma Susceptibility Gene, *DENND1B* Regulates TNF(Signaling. *M.E. March, P.M.A. Sleiman, C. Hou, J. Bradfield, C.E. Kim, E.C. Frackleton, J.T. Glessner, H. Hakonarson.* Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA.

Asthma is a chronic inflammatory condition of the lungs, characterized by excessive responsiveness of the lungs to stimuli, in the forms of infections, allergens, and environmental irritants. Currently, 22.9 million Americans suffer from asthma, and the prevalence has increased dramatically since 1980. Asthma is the leading chronic illness in U.S. children, with 6.8 million affected in 2006. Twin studies have shown that there is a genetic element to asthma susceptibility (with heritability of the condition estimated at between 0.36 and 0.77), but only a fraction of the heritability is explained with previously identified loci. In a genome wide association screen (GWAS), we identified *DENND1B* as a novel asthma susceptibility locus in populations of both European and African descent. The single nucleotide polymorphisms (SNPs) that marked DENND1B were all noncoding (either intergenic or intronic), suggesting that factors such as mRNA stability, alternative splicing, and expression level may be involved. Expression of DENND1B was determined in EBV transformed B cells from patients using Affymetrix arrays, and the expression was analyzed based on the individuals' genotypes at the most statistically significant SNP (rs2786098) identified in the GWAS. The minor allele at this SNP, which was more prevalent in non-asthmatic individuals of European descent, correlated with lower expression of DENND1B. DENND1B is a guanine nucleotide exchange factor for Rab35, and associates with the clathrin-mediated endocytosis machinery. It is therefore possible that it influences the surface expression, endocytosis, or turnover of cell-surface receptors, thereby altering signaling. Through overexpression of various splice isoforms of DENND1B, we show that the molecule has the potential to regulate signaling through the TNF(receptor, a pathway important in asthma pathogenesis. The longer isoform enhances the TNF(response, while the shorter isoform reduces it. We are currently determining which of the multiple splice isoforms are expressed in different cell types, and if splicing is altered in individuals with either asthma-susceptible or -resistant genotypes. Additionally, studies on intracellular localization and the effect of DENND1B on receptor expression and intracellular trafficking are currently underway.

790T

Functional characterization of the role that components of the retrograde transport machinery play in early HIV events. *S. Liu[1], M. Dominska[1], D. Dykxhoorn[1,2].* 1) John P. Hussman Institute for Human Genomics, Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL; 2) Department of Microbiology and Immunology University of Miami Miller School of Medicine, Miami, FL.

Human Immunodeficiency Virus (HIV) is a highly lethal lentivirus which is responsible for a tremendous amount of suffering and death in the United States and throughout the world. By attacking the host's immune system, HIV leaves the infected individual susceptible to a wide variety of opportunistic infections. HIV, like all viruses, relies on host cell factors for successful infection, replication and release of progeny virus. Previously, a large-scale functional genomic screen identified over 230 novel factors whose silencing inhibited viral replication in cultured cells. A more detailed examination of these HIV-dependency factors (HDFs) showed a significant enrichment for factors involved in the trans-Golgi network (TGN), a pathway which had not been previously implicated in HIV replication. Initial characterization of several of these factors suggested that their silencing resulted in an early defect in HIV replication prior to integration of the viral genetic material into the host genome. Once integrated, the viral DNA is maintained and replicated with the host genome. Anti-retroviral drugs can be used to suppress new viral production but they can't eliminate the integrated virus. Therefore, the identification of HDFs that inhibit viral integration could have tremendous therapeutic potential. To examine the role that the TGN-HDFs play in the HIV-1 life cycle, a series of cell lines were developed which are silenced for the various factors and submitted to biochemical and cell biological analysis. Our results indicate that many TGN genes (Rab5, Rab6, SNX1, SNX2, VPS26, VPS53) play important roles in early HIV events that precede gag translation, either viral fusion with the host cell, reverse transcription, intracellular trafficking, or preintegration, and silencing of them can effectively inhibit viral transmission.

791T

***Mycobacterium leprae*-dependent antigen presentation in primary human macrophages with *PARK2* gene mutations.** *R.H.M. Sindeaux[1], V.S. Sotomaior[1], D.E. Costa[1], A.C Senegaglia[2], M.T. Mira[1].* 1) Advantage Core of Molecular Investigation, Pontifical Catholic University of Paraná, Curitiba, Paraná, Brazil; 2) Laboratory of Experimental Cell Culture, Pontifical Catholic University of Paraná, Curitiba, Paraná, Brazil.

Genetic control of human susceptibility to leprosy is widely accepted; however, the biological basis of this genetic effect is far from understood. A series of previous studies resulted in the identification of genetic variants shared by two genes, *PARK2* and *PACRG*, as major risk factors for susceptibility to leprosy “per se” in three distinct ethnic populations. Interestingly, severe mutations of the *PARK2* gene, that encodes Parkin, an E3 ubiquitin-protein ligase, have been implicated as causative of an autosomal recessive early-onset form of Parkinson's disease (AR-EOPD). The aim of this initiative was to use monocyte-derived macrophages (MDM) from AR-EOPD patients with *PARK2* spontaneous mutations as a model to investigate several aspects of host cellular response against *M. leprae*. Here, we applied the model to investigate antigen-presentation capability. Primary peripheral blood mononuclear cells isolated from three AR-EOPD patients with *PARK2* mutations and three healthy controls matched by age were cultured *in vitro* in 24-well culture plates for 5 days to obtain MDM (approximately 1.5-2.0x10^5 cells/ well). The cells were treated with irradiated *M. leprae* (multiplicity of infection bacilli to monocyte of 50:1) for 24 hours; MDM were incubated with anti-HLA-ABC, HLA-DR, CD80, CD86 and ICAM-I and the percentage of positive cells before and after challenge with *M. leprae* was scored by flow cytometry - FACScan. Basal and post-challenge *in vitro* expression of HLA-ABC, HLA-DR, CD80 and ICAM-I did not differ between AR-EOPD and control cells. However, *PARK2* mutant macrophages expressed higher levels of co-estimulatory molecule CD86 as compared to control cells, independently on *M. leprae* challenge. This result indicates promising directions for further experiments, as well as validates our model as suitable for investigation of the molecular basis of leprosy pathogenesis.

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792T

Delineating causal variation and the regulatory landscape at *TNFSF4* in systemic autoimmunity. *H. Manku[1], R. Torres[2], C. Langefeld[3], S. Guerra[1], T. Malik[4], G. Gilkeson[5], J. Edberg[6], R. Kimberley[6], S. Nath[7], B. Tsao[8], R. Festenstein[2], T. Vyse[1], LLAS2, SLEGEN.* 1) Division of Genetics and Molecular Medicine, Kings College London, London, United Kingdom; 2) Division of Medicine, Imperial College London, London, United Kingdom; 3) Wake Forest School of Medicine, Winston-Salem, NC, USA; 4) Division of Immunology and Inflammation, Imperial College, London, United Kingdom; 5) Division of Rheumatology and Immunology, Medical University of South Carolina, Charleston, SC, USA; 6) Division of Clinical Immunology and Rheumatology, Department of Medicine, University of Alabama at Birmingham, Alabama, USA; 7) Arthritis and Clinical Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, USA; 8) Division of Rheumatology, Department of Medicine, David Geffen School of Medicine at UCLA, LA, USA.

Introduction: The TNF superfamily member *TNFSF4* operates at the T-cell-APC interface as a late-stage lymphocyte costimulator. This gene is an established SLE risk locus in European and Asian populations. Genetic polymorphisms in the 5' region of *TNFSF4* predispose to SLE and form an overtransmitted 100Kb haplotype (P_p<10^{-12}, *OR=1.6 [CI=1.27-1.89]*) associated with disease risk. However, causal variants have remained elusive due to strong linkage disequilibrium exhibited by alleles at *TNFSF4* in these populations. *Objectives:* Delineation of causal variation and the regulatory landscape at *TNFSF4*. *Methods*: We used massively parallel 454 sequencing to define causal variation at the 200Kb *TNFSF4* locus (n=100) and inferred fine-scale recombination maps in 2 ancestral and 2 admixed populations (n=6000). We fine-mapped the locus in a multi-ethnic SLE-control cohort of 17,900 individuals by Illumina Beadexpress. Putative causal variants were then profiled by ChiP (n=30) against H3K27me1, H3K27me3 and transcription factors identified by our bioinformatic scan. Chromosome conformation capture (3C) was used to characterise long range cis-interaction between risk variants and a novel upstream SLE susceptibility locus (n=20). *Results:* We find strong association of *TNFSF4* risk alleles in all groups tested; the novel African-American (AA) association replicates in AA Gullah and increased recombination in AAs refines the associated haplotype to 10Kb. *rs2205960-T* ($_UP_{META}$=7.1x10^{-32}, *OR=1.41[1.25-1.59]*) explains the risk in all four cohorts and our ChIP data suggest risk variant individuals have altered chromatin patterns at this location. Specifically, *rs2205960-T* alters a PU1 transcription factor binding site in LCLs and we have suggestive replication in B lymphocytes from *rs2205960-T* homozygotes. Overall results of our ChIP and 3C experiments are in analysis. Our phenotypic data demonstrate strong association of *rs2205960-T* with autoantibody production in AAs, Europeans and Hispanics ($_UP_{META}$=6.8x10^{-35}, *OR=2.2[1.78-2.5]*). *Conclusion:* In this study, increased haplotypic diversity in AAs reveals the association of *rs2205960-T* with SLE. Presence of this marker alters transcription factor binding. Our phenotype scan in multiple groups specifically associates *rs2205960-T* with autoantibody production to suggest a role for *TNFSF4* in lymphocyte dysregulation during SLE pathogenesis. These data confirm the validity of transancestral mapping in complex traits.

793T

The protection roles of phosphorylated Heat Shock Protein 27 in human cells harboring MERRF A8344G DNA Mutation. *M. Hsieh[1], H.F. Chen[1], C.Y. Chen[1], Y.S. Ma[2], Y.H. Wei[2].* 1) Deptartment of Life Science, Tunghai Univ, Taichung, Taiwan; 2) Department of Biochemistry and Molecular Biology, School of Life Sciences, National Yang-Ming Univ, Taipei, Taiwan.

Mitochondria play an important role in the life and death of cells. They have their own DNAs which encode subunits of mitochondrial electron transport chain complexes. Mitochondrial DNA (mtDNA) mutations are associated with a large number of neurological and muscular diseases. Myoclonus Epilepsy with Ragged Red Fibers (MERRF) is a maternal inheritance mitochondrial disease. The most common mutation in MERRF disease, A8344G, is associated with severe defects in mitochondrial protein synthesis, which impaires electron transport chain assembly and respiratory chain activity. Our previous findings showed a decreased level of heat shock protein 27 (HSP27) and phosphorylated HSP27 (p-HSP27) in MERRF cybrids, compared with the control cybrids. Over-expressed wild type HSP27 in mutated cybrids resulted in a significant decrease of activated caspase 3 under staurosporine (STS) treatment, suggesting a protective function of HSP27 in cells harboring MERRF mutation. In the present study, we further examined the protective function of p-HSP27 in the disease cellular model. Even though the steady-state level of p-HSP27 was reduced in mutant cybrids, normal phosphorylation and dephosphorylation responses were observed upon stresses, indicating normal kinase and phosphatase activities. To explore the roles that p-HSP27 may play, transfection experiments with HSP27 mutants, in which three specific serine residues were substituted with alanines or aspartic acids, showed that the phosphomimicking-HSP27 desensitized mutant cybrids to apoptotic stress induced by STS. After heat shock stress, p-HSP27 entered the nucleus immediately after heat shock. With prolonged recovery intervals after heat shock, p-HSP27 returned to the cytoplasm in normal cybrids but not in mutant cybrids and this translocation is correlated to cell viability, as evidenced by the increased apoptotic cells after p-HSP27 returning to the cytoplasm. In summary, our results demonstrate that p-HSP27 provides significant protective functions when cells are under different stresses in the MERRF disease cellular model. Therapeutic strategies against anomalous HSP27 phosphorylation might be a potential treatment for the disease.

794T

Advances in understanding the molecular mechanism of Goldberg-Shprintzen syndrome. *L. Drévillon[1,2], A. Megarbane[3], D. Gaillard[4], P. Benit[5], C. Matar[1,6], A. Briand-Suleau[1,2], J. Ghoumid[1,2], M. Nasser[1,2], V. Bodereau[1], M. Conti[1,6], M. Doco-Fenzy[4], P. Rustin[5], M. Goossens[1,2], I. Giurgea[1,2].* 1) Service de Génétique, Hopital Henri Mondor, CRETEIL, France; 2) INSERM, Unité U955, Equipe 11, and Université Paris-Est, Créteil, France; 3) Laboratory of Molecular Biology and Cytogenetics, Faculty of Medicine, University Saint-Joseph, Lebanon; 4) Service de Génétique, CHU, UFR de médecine, IFR53, Reims, France; 5) INSERM U676, Hôpital Robert Debré, Paris, France; 6) INSERM, Unité U955, Equipe 07, and Université Paris-Est, Créteil, France.

Goldberg-Shprintzen syndrome (GOSHS, MIM #609460) is an autosomal recessive disorder associating severe intellectual disability, typical facial gestalt and Hirschsprung disease. *KIAA1279* was identified as the disease causing gene with homozygous nonsense mutations. The encoded protein, named KBP (KIF-binding protein) due to its related interaction with kinesin-like protein, was initially claimed to be located at the mitochondria, a subcellular localization not confirmed by a recent study. KBP was implicated in axonal in structure, outgrowth and maintenance in zebrafish, however its underling mechanism remains unknown.

The aim of this study was to assess the functional consequences of two novel *KIAA1279* mutations (c.599C>A, p.Ser200X and c.604-605delAG, p.Arg202IlefsX2) on the mitochondrial function and to advance in the understanding of the molecular mechanism of Goldberg-Shprintzen Syndrome. In parallel, we detailed the phenotype of four patients from two different families carrying theses mutations. In fibroblasts of patients with truncating homozygous mutation, *KIAA1279* mRNA was barely detectable and in heterozygous carriers it was two-fold decreased, suggesting a NMD (nonsense mediated decay) mechanism. The subcellular localization of KBP by immunocytochemistry did not show mitochondrial localization in human fibroblasts. The activities of respiratory chain complexes by spectrophotometry were normal in fibroblasts of patients with *KIAA1279* homozygous nonsense mutations.

In conclusion, we report for the first time a *KIAA1279* NMD as the molecular mechanism of GOSHS. The absence of normal KBP protein did not influence mitochondrial respiratory chain complex activity in human fibroblasts.

795T

Genetic and functional characterization of the RNF114 psoriasis susceptibility gene implicates dysregulation of innate antiviral responses in disease pathogenesis. *F. Capon[1], A. Onoufriadis[1], S. Kanneganti[1], A.D. Burden[2], J.N. Barker[1], M.J. Bijlmakers[3], R.C. Trembath[1].* 1) Division of Genetics and Molecular Medicine, King's College London, London, United Kingdom; 2) Glasgow Western Infirmary, Glasgow, United Kingdom; 3) Division of Immunology, Infection and Inflammatory Disease, King's College London, London, United Kingdom.

Psoriasis is a chronic inflammatory skin disorder that is inherited as a complex genetic trait. Genome-wide association scans (GWASs) have successfully identified more than 20 disease susceptibility genes, highlighting the pathogenic role of pathways related to antigen presentation, IL-23 and NF-2B signalling. GWASs have also uncovered a number of genes of unknown function, which could provide novel insights into disease processes. Here we have investigated the role of RNF114, which was first identified as a psoriasis susceptibility gene in a GWAS carried out by our group (Capon et al., Hum Mol Genet 2008 17:1938-45). Homology searches indicate that RNF114 is a paralogue of RNF125, an E3 ubiquitin ligase which regulates the innate antiviral response (i.e. the production of IFN(/)) driven by the RIG-I and MDA5 receptors. To investigate the possibility that RNF114 may have a similar function, we carried out a range of biochemical assays, which indicated that RNF114 has ubiquitin ligase activity in-vitro. We next showed that RNF114 is up-regulated by IFN(and poly (I:C), a synthetic RIG-I/MDA5 ligand. Finally, we demonstrated that RNF114 overexpression results in increased IFN) reporter activity in response to poly (I:C) stimulation. These data suggest the existence of a positive feedback loop, whereby the IFN(/) produced upon viral infection up-regulates RNF114 expression, which in turn drives the release of more IFN(/) . To investigate the mechanisms whereby disruption of this pathway may contribute to disease pathogenesis, we re-sequenced RNF114 in 171 psoriatic patients, looking for rare functional variants. We did not detect any changes in the gene coding sequence, but identified two novel promoter substitutions, which were not found in 842 controls analysed in house or in the 1,094 individuals sequenced by the 1,000 genome project. Functional assays demonstrated that both variants affect Sp1 binding and result in reduced promoter activity. Taken together, these data point to dysregulation of gene expression as the mechanism underlying association signals at the RNF114 locus and pave the way for further studies into the alteration of innate immune responses in psoriasis.

796T

Secretion-defective genetic variants of Decoy Receptor 3 in pediatric-onset Crohn's disease. *C.J. Cardinale[1], S. Panossian[1], F. Wang[1], E.C. Frackelton[1], C.E. Kim[1], F.D. Mentch[1], R.M. Chiavacci[1], K. Kachelries[2], R. Pandey[1], S.F.A. Grant[1], R.N. Baldassano[2], H. Hakonarson[1].* 1) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA., USA; 2) Division of Gastroenterology, Children's Hospital of Philadelphia, Philadelphia, PA., USA.

Decoy Receptor 3 (TNF Receptor Superfamily 6B) is a secreted protein which binds to and neutralizes tumor necrosis factor superfamily ligands TL1A, Fas ligand, and LIGHT. Based on the results of genome-wide association studies, we expect DcR3/TNFRSF6B to play a role in early-onset Crohn's disease and ulcerative colitis (Kugathasan et al., 2009). We have sought to identify nonsynonymous amino acid variants of DcR3/TNFRSF6B that may underlie the GWAS signal by Sanger sequencing of the three exons comprising the gene in 532 pediatric Crohn's disease cases and 169 pediatric ulcerative colitis cases. We identified 12 novel nonsynonymous variants of which 11 are unique to cases and absent from public databases or 1100 genotyped controls. Six of these variants are defective in secretion from HEK 293T cells when expressed from plasmids. Investigation of the pedigrees of mutation carriers showed that the rare secretion-defective variants are present in parents with and without a phenotype. Further investigation of extended family members carrying these mutations will help elucidate whether the defective variants contribute to early-onset pediatric inflammatory bowel disease.

797T

The Role of Pyrin On Cell Migaration and The Effect of Colchicine In The Process. *A. Cetinkaya[1], E. Taskiran[2], Y. Akkaya[2], B. Peynircioglu[2], M. Alikasifoglu[1], E. Yilmaz[1,2].* 1) Medical Genetics, Hacettepe University, Ankara, Turkey; 2) Medical Biology, Hacettepe University, Ankara, Turkey.

Familial Mediterranean Fever is an autoinflammatory autosomal recesively inherited disease characterized by inflammatory episodes. The disease is as common as 1:1000 in Mediterranean countries. The drug of choice for the disease is colchicine which both relieves the patient from episodes and prevents late complications like amyloidosis. The abberantly acting protein responsible for the disease is pyrin, shown to have several roles in inflamation control, apoptosis and cytokine secretion. Furthermore pyrin was suggested to have several roles in cell migration through its interaction with well-known regulators of cell migration PSTPIP1 and actin. Appropriate highest concentration of colchicine that would mimic the in vivo colchicine treatment was determined in COS-7 cells through LDH activity measurement, morphological analysis of cells staining of actin-tubulin cytoskeleton and endoplasmic reticulum - Golgi Aparatus. Wound healing assay was used to show the effect of pyrin in transiently transfected HeLa cells. Boyden chamber assays were used to determine the role of pyrin, 3 of its common disease-causing mutants, namely M694V, M680I, V726A and colchicine in transiently transfected COS-7 cells migrating against an insulin gradient. The highest concentration that would mimic colchicine treatment for COS-7 cells was determined as 10ng/ml. In Boyden chamber assays, a significant relationship between wild type pyrin overexpression and increased cell migration was shown, which was not observed in either control group or 3 common disease causing mutants of pyrin. In addition, colchicine treatment would only decrease cell migration in pyrin overexpression group. In wound healing assays, we noted an increased number of pyrin overexpressing HeLa cells neighboring the wound border compared to control group. The data presented here shows direct involvement of pyrin in control of cell migration. This maybe either through pyrin protein's interaction through PSTPIP1 or actin, or a different yet unknown mechanism. Colchicine's cell migration abolorating effects appearing only in the presence of wild type pyrin is also another interesting point to note. Further research in this subject may lead to discovery of novel mechanisms controlling cell migration in inflammatory cells and would result in novel treatment options for inflammatory diseases in general. This study was supported by TUBITAK-109S297 and TUBITAK-109S364.

798T

The Effect of Colchicine on LPS induced MEFV Gene Expression. *E. Yilmaz[1,2], E. Taskiran[1], A. Cetinkaya[2], Y. Akkaya[1], B. Peynircioglu[1].* 1) Hacettepe University, Faculty of Medicine, Department of Medical Biology,Sihhiye, Ankara, Turkey; 2) Hacettepe University, Faculty of Medicine, Department of Medical Genetics,Sihhiye, Ankara, Turkey.

Familial Mediterranean Fever (FMF) is the most common auto-inflammatory disease. It is characterized by recurrent attacks of fever and inflammation. It is caused by mutations in the MEFV (MEditerranean FeVer) gene. The product of MEFV gene, Pyrin, appears to be a regulator of inflammation, though the nature of this regulatory activity remains to be identified. Prophylactic treatment with colchicine, a microtubule toxin, has had a remarkable effect on disease progression and outcome. It has been thought that, inhibition of microtubule polymerization is the main mechanism of action of colchicine. But, the exact cellular mechanism explaining the efficacy of colchicine in suppressing FMF attacks is still unclear. Given the ability of colchicine treatment to be considered as a differential diagnosis criteria of FMF, we hypothesized that colchicine may have a specific effect on gene expression level of MEFV. To test our hypothesis, differentiated THP-1 cells were administered with microtubule non-disrupting concentrations of colchicine and MEFV gene expression was analyzed on both RNA and protein level. Quantitative real-time PCR studies revealed that colchicine caused a dramatic (~6 fold) decrease in MEFV levels compared to DMSO treated control cell. The results of Western blot analysis were similar to real-time PCR data. When differentiated THP-1 cells were treated with 10ng/ml LPS, MEFV expression was up-regulated (~2,5 fold), but in THP-1 cells treated with colchicine containing medium, MEFV levels was not up-regulated by LPS. We observed increasing number of actin stress fibers in THP-1 cells in 200ng/ml and 250ng/ml colchicine containing mediums. We further noted that filopodia was decreased by means of both the average length and the number of filopodia in differentiated THP-1 cells after colchicine treatment. These changes indicate that colchicine causes actin re-organization while it does not disrupt microtubules. Pyrin is an actin-binding protein that specifically localizes with polymerizing actin filaments. Thus, MEFV expression might be affected by re-organization of actin cytoskeleton. The data presented here reveal a potentially important connection between colchicine and MEFV gene which might explain the remarkable efficacy of colchicine in preventing FMF attacks by reducing the mutant protein level in FMF patients. Therefore, our data provide evidence for a pro-inflammatory role of Pyrin. Supported by H.U.BAB-0601101025 and TUBITAK-105S364.

799T

Alternative splicing of MBNL2 transcripts upon C2C12 differentiation. *M. Fardaei, N. Farajzadeh.* Genetics, Shiraz University of Medical Sciences, Shiraz, Iran.

Muscleblind-like protein 2 (MBNL2) has a specific binding affinity for CUG or CCUG expanded repeat transcripts in a length dependent manner. The same as other 2 muscleblind proteins (MBNL1 and MBNL3), MBNL2 protein was also sequestered by nuclear foci of expanded repeats in adult muscle and cultured cells from myotonic dystrophy (DM1 and DM2) patients. This is well accepted that these proteins were diluted from their function in the regulation of pre-mRNA alternative splicing. Previous studies including both knockout mice and zebrafish knockdown model strongly suggested the role of MBNL2 in myotonic dystrophy patients. Alternative splicing of MBNL exons are conserved during embryonic muscle development. In this study we investigated splicing variation of MBNL2 during C2C12 differentiation and in completely differentiated muscle and brain cells in mice. Using RT-PCR, we identified a new splicing form of MBNL2, GQ906534, which encodes a protein with different amino acid sequences at the carboxy terminal end. Also, according to our results the splicing of MBNL2 transcript is changed during C2C12 differentiation, while MBNL2 transcript spliced in one form before differentiation, a bigger isoform also spliced after differentiation in C2C12, muscle and brain cells of mice.

800T

Disease alleles in the general population: HGMD-DM variants in the 1000 Genomes Pilot samples. *Y. Xue[1], Y. Chen[1], N. Huang[1], E.V Ball[2], M. Mort[2], A.D Phillips[2], P.D Stenson[2], D.N Copper[2], C. Tyler-Smith[1], 1000 Genomes Project.* 1) Wellcome Trust Sanger Institute, Cambridge, UK; 2) Institute of Medical Genetics, Cardiff University, Heath Park, Cardiff, UK.

What is the genetic disease allele burden in the general population? In the 1000 Genomes Pilot paper [1], we identified 578 base substitutions from the HGMD database [2] classified as DM (the most severe category) among the 179 individuals sequenced. 72% of these variants showed a disease allele frequency >5%, ~32% were found in the homozygous state in one or more individual, and each person carried 57-80 disease alleles, 9-15 homozygous. We have now investigated further the likely disease implications of these alleles. In some cases, additional literature information was available and indicated that some HGMD-DM alleles were unlikely to be causative, but such information was not available for most variants. However, predictions of the functional consequences of the substitutions were possible for most alleles. We therefore used SIFT [3] and PolyPhen2 [4] to predict the consequences of the ns variants found in (1) the 1000G data only, (2) the HGMD dataset only, and (3) the overlapping set. Assuming that alleles found in set (1) were predominantly “healthy variants”, and that alleles in set (2) were predominantly “disease variants”, we used the distribution of the combined SIFT+PolyPhen scores in “disease variants” and “healthy variants” to calculate a Bayesian posterior probability that each variant caused disease. The majority of overlap set (3) variants had a low posterior probability of being causative, and only 185 variants had >95% probability. In this reduced set, disease alleles were rarer (only 14% >5%), fewer were seen in a homozygous state (22%) and on average each individual carried 15-20 disease alleles, 2-5 homozygous. We also investigated the HGMD-DM indels present in the 1000G data, and found 19. Again, manual curation suggested that the majority were unlikely to be causative, but a single base deletion in PRF1, linked to familial Haemophagocytic lymphohistiocytosis [OMIM: 603553], in the heterozygous state in a single YRI, was perhaps causative. In conclusion, the majority of apparent disease alleles found in the general population are probably not causative and reflect limitations in the original literature reports incorporated into databases. Better disease annotation and functional prediction tools are needed to identify true causative alleles. 1. 1000 Genomes Project Consortium (2010) Nature 467: 1061 2. Stenson et al (2009) Genome Med 1: 13 3. Prateek Kumar et al (2009) Nat Prot 4: 1073 4. Adzhubei et al (2010) Nat Meth 7: 248.

801T

Genomewide Gene Expression Profiling of Monozygotic Twins Discordant for Autism Spectrum Disorder. *E.L. Meaburn[1, 2], C. Wong[1], J. Mill[1], A. Ronald[2], T. Price[1], L.C. Schalkwyk[1], R. Plomin[1].* 1) Psychological Sciences, Birkbeck University, London, United Kingdom; 2) Institute of Psychiatry, King's College London, Denmark Hill, London, SE5 8AF.

Autism Spectrum Disorders (ASDs) are a heterogeneous group of neurodevelopmental disorders that affect approximately 1% of the population. Delineating the etiological basis of ASD will aid diagnosis, prediction, and help develop better treatment and intervention strategies. It is widely recognized that ASD is highly heritable, with identical twin (monozygotic; MZ) concordance rates of about 60%;. However, MZ concordance is far from 100%, illustrating that there is an important - but often overlooked - non-shared environmental component that contributes to the development of ASD. To-date, molecular genetic investigation of ASD has primarily focused on identification of specific genetic risk variants. We sought to interrogate the molecular basis of non-shared environmental influence on the development of ASD. Within the Twins' Early Development study (TEDS) of 6,000 twin pairs, we identified a cohort of MZ twins discordant for ASD traits (N=36 twin pairs), concordant for high-ASD scores (N= 6 twin pairs) and concordant for low-ASD scores (N=11 twin pairs). The selection of MZ twin pairs was based on parental ratings on the Childhood Autism Symptom Test (CAST) when the twins were age 8 years. RNA was extracted from peripheral blood samples and genomewide gene expression was quantified using the Affymetrix GeneChip Human Gene 1.0 ST array platform. Transcripts detected as being differentially expressed (empirical Bayes paired t test) between ASD discordant MZ twins, which did not differ significantly between ASD-concordant or low-ASD concordant control pairs were highlighted as potential candidates under the influence of non-shared environment factors. In order to gain insight into biological themes and enriched processes and pathways, evaluation of the differentially expressed transcript list for previously reported genetic and methylomic ASD risk factors has been undertaken, and gene ontology analyses performed.

802T

The genetics of idiopathic membranous nephropathy elucidated by GWAS. *H. Stanescu[1], M. Arcos-Burgos[2], A. Medlar[1], A. Kottgen[3], D. Bockenhauer[1], S.H. Powis[1], J.M. Hofstra[4], P. Brenchley[5], J. Feehally[6], A.J. Rees[7], H. Debiec[8], J.F.M. Wetzels[4], P. Ronco[8], P.W. Mathieson[9], R. Kleta[1].* 1) University College London, London, United Kingdom; 2) NHGRI, NIH, Bethesda, MD, USA; 3) University Hospital Freiburg, Freiburg, Germany; 4) Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 5) University of Manchester, Manchester, UK; 6) University Hospitals of Leicester, Leicester, UK; 7) Medical University of Vienna, Vienna, Austria; 8) INSERM UMR S702, UPMC Univ-Paris 6, Paris, France; 9) University of Bristol, Bristol, UK.

Idiopathic membranous nephropathy (IMN) is a major cause of nephrotic syndrome and kidney failure, the etiology of which is not fully understood. We investigated the genetic basis of biopsy-proven cases of IMN in Caucasians. Three independent genome-wide association studies (GWAS) utilizing single nucleotide polymorphisms (SNPs) were performed in patients from Caucasian populations (75 French, 146 Dutch, 335 British cases of IMN). Cases were compared with ethnically matched controls; population stratification, quality controls, and statistics were carried out according to standard criteria. In a joint analysis from the 556 cases studied, we identified significant alleles at two genomic loci associated with IMN. Chromosome 2q24 contains the M-type phospholipase A2 receptor gene PLA2R1 (SNP rs4664308, p= 8.6x10-29) shown previously to be the target of an autoimmune response. Chromosome 6p21 contains the human leukocyte antigen complex (HLA) class II gene HLA-DQA1 (SNP rs2187668, p=8.0x10-93). The association to HLA-DQA1 was significant in all three populations (p=1.8x10-9, 5.6x10-27 and 5.2x10-36 in French, Dutch and British, respectively). The odds ratio for IMN with homozygosity for both risk alleles is 79 (35-178 (95% confidence interval)). We sequenced both loci to ascertain the basis for our GWAS findings. An HLA-DQA1 allele on chromosome 6p21 is most significantly associated with IMN in Caucasians. This allele may facilitate an autoimmune response against targets, such as variants of PLA2R1. Our findings, including sequencing of both loci, suggest a basis for the understanding of IMN and illuminate how powerful GWAS can be for the study of a rare disease.

803T

Whole genome gene expression profile of omental adipose tissue in obese Type 2 diabetic African Americans. *A.P. Doumatey, H. Xu, A. Adeyemo, A. Elkahloun, H. Huang, C.N. Rotimi.* Center for Research in Genomics and Global Health, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Type 2 diabetes (T2D) is the most common co-morbidity associated with obesity. Adipose tissue especially omental adipose tissue (OAT) has been shown to play an important role in the pathophysiology of many obesity-related diseases including T2D by oversecreting adipokines and other active biomolecules that are involved in glucose homeostasis and low grade inflammation. Although, a number of studies have explored the differential gene expression profiles in obese T2D, they have focused on: 1) selected set of genes based on a priori biological knowledge and 2) populations of European ancestry. To further understand the genomics of T2D in African Americans (AA), we performed a whole genome gene expression on OAT attained from 20 obese AA. The study consisted of 14 obese subjects with T2D and 6 obese without T2D. Gene expression profiling was done using the whole human genome expression array, U133 Plus 2.0 (Affymetrix), which evaluates the relative expression level of more than 47,000 transcripts and variants, including more than 38,500 well characterized genes and UniGenes. One sample was determined as outlier during quality control and was excluded from subsequent analysis. Analysis of covariance was performed to identify genes differentially regulated between T2D subjects and controls after controlling for the effect of gender. The mean age of the 20 participants was 41.3 ±7.9 years. At a fold-change (FC) threshold >1.5 and false discovery rate <0.05, 130 transcripts were differentially expressed in obese T2D compared to controls. Among them,9 transcripts were upregulated notably HINT3 (FC 2.4),TBRG1 (FC 2.1),COL4A2 (FC 1.7),PPP2R2D (FC 1.51), and the remaining 121 genes were downregulated in obese T2D particularly ABCA9 (FC -2.9),ING3 (FC -2.9) and SEMA4C (FC -1.9).In pathway analysis using Ingenuity database, inflammatory signaling pathways were significantly enriched in the genes differentially regulated between obese T2D and controls, including the actin-based mobility by Rho pathway, Antigen presentation pathway and the Fc. receptor mediated phagocytosis in macrophages and monocytes pathway. In this study, we showed for the first time in a population of African ancestry, significant differential gene expression profiles in OAT of T2D patients, the most active endocrine organ implicated in obesity related diseases. Investigating the biological roles of the identified genes is likely to provide new insight into the patho-biology of T2D.

804T

Adiponectin Q gene harbours risk variants for susceptibility traits of metabolic syndrome. *S. Majid[1], M. Al-Najai[1], E. Andres[1], S. Elhawari[1], M. Vigilla[1], D. Gueco[1], P. Muiya[1], M. Al-shahid[2], N. Dzimiri[1].* 1) Dept. of Genetics, King Faisal Specialist Hosp, Riyadh, Saudi Arabia; 2) King Faisal Heart Institute, King Faisal Specialist Hosp, Riyadh, Saudi Arabia.

Metabolic syndrome (MS) relates to a cluster of risk factors for atherosclerosis and type 2 diabetes mellitus (T2DM) comprising obesity (OB), insulin resistance, hypertension (HTN) and dyslipidemia. Adiponectin (ADIPOQ) is a hormone that modules several metabolic processes and is thought to play an important role in the suppression of the metabolic derangements that may lead to MS. In a preliminary genome-wide linkage study of early onset CAD involving a Saudi family of 11 individuals with predominant heterozygous familial hypercholesterolemia using the Affymetrix Gene Chip 250 sty1 mapping array, we identified several loci, among others, in chromosome 3 region which harbours the ADIPOQ gene. We then pursued a population-based association study for 8 ADIPOQ SNPs with MS risk factors in 4049 Saudi individuals (1584 OB cases versus 2450 controls) using the Applied Biosystems real-time PCR procedure. The rs6773957A>G [Odds ratio(95% Confidence Interval = 1.10(1.01-1.21); p=0.033) and the rs4686804A>G [1.11(1.02-1.22; p=0.021) conferred risk for OB, while the rs2241766G>T [0.88(0.79-0.99; p=0.032) was protective. These associations followed a recessive mode of inheritance. One variant, rs9842733A>T was implicated in both HTN [1.38(0.97-1.95); p=0.070] and T2DM [1.29(0.99-1.68); p=0.058), while another rs1063539 (p=0.07) was further implicated in HTN. These results point to sharing of susceptibility variants by risk traits for the metabolic syndrome.

805T

Surprisingly Low Frequency of Deep Heteroplasmy in Human Mitochondrial DNA (mtDNA); However, Old People Tend to Have more Heteroplasmies but at Lower Levels in Blood. *S. Sommer[1], C. Buzin[1], R. Boles[2], C. Boysen[1], W. Scaringe[1].* 1) MEDomics, LLC, Azusa, CA; 2) Childrens Hospital Los Angeles.

BACKGROUND: Maternal inheritance characterizes the mitochondrial genome mutations that can underlie mitochondrial genome diseases. Mitochondrial genome diseases do not fit the general paradigm of genetic diseases due to the absence of centromeres and the hundreds to thousands of mitochondrial genome copies within a cell. While nuclear mutations at a given position are quantized (generally 2, 1, or 0 per individual), the percentage of mutant DNA, i.e. heteroplasmy level, of mitochondrial genome mutations varies from tissue to tissue and from sib to sib depending on the stochastic and selective factors. Mitochondrial DNA (mtDNA) has a high rate of mutation due to increased free radical exposure and decreased effectiveness of DNA repair, compared to nuclear DNA. New mutations coexist with wild-type mtDNA in the same cells (heteroplasmy). Previous evidence strongly suggested that mtDNA has a very high rate of mutation due to high free radical exposure and little or no available DNA repair mechanisms, predicting that deep heteroplasmy could be common in normal individuals and significantly confound clinical interpretation of mtDNA heteroplasmic variants found in patients with potential mitochondrial disease. METHODS: The entire mtDNA was NextGen sequenced thousands of fold using an Applied Biosystems SOLiD platform in predominantly leukoctye samples (blood or saliva) of 77 subjects. This method has about a 30-fold enhanced sensitivity relative to capillary sequencing. RESULTS: Of 77 patients reveals the majority (39) have zero heteroplasmies at 1% or greater. Within the age categories of 0-12, 13-25, and 25-50 the remaining individuals had 1 or 2 heteroplasmies, while 5 out of 15 of those individuals older than 50 had 3 or more heteroplasmies (p=0.0002). Older people may tend to have a higher number of heteroplasmic variants, but the frequency of those heteroplasmies tends to be lower. For example, 24% (8 of 34) of heteroplasmic variants found in individuals aged 0 to 50 had a heteroplasmic frequency of 10% or greater while 4% (1 of 27) of the heteroplasmic variants found in older people had a heteroplasmic frequency of 10% or more (p=0.03). CONCLUSIONS: The number of heteroplasmic variants at levels of 1% or more within the 16.5 kb genome is low. While more data are desirable, it seems that older individuals tend to have a larger number of heteroplasmic variants, but high frequency heteroplasmic variants are less common.

806T

Integrated transcriptome and enhancer networks during inner ear hair cell regeneration by Next Gen sequence analysis. *Y. Ku, N. Renaud, R. Veile, C. Helms, M. Warchol, M. Lovett.* Genetics, Washington University in Saint Louis, Saint Louis, MO.

Loss of inner ear hair cells is the leading cause of human deafness and balance disorders. Unlike mammals, lower vertebrates such as birds are capable of regenerating new sensory hair cells. We used Next Generation Sequencing technology to derive a comprehensive description of all mRNAs and miRNAs over a 7 day regenerative time course in vitro, following chemical ablation of hair cells within avian inner ear sensory epithelia (SE). We also identified putative enhancer elements activated during regeneration by ChIP-Seq, using an antibody to p300. From mRNA-seq, we detected ~15,000 genes and ~1800 transcripts that reproducibly changed by >2-fold during regeneration, compared to controls. These include previously described components of NOTCH, WNT, FGF, PAX and SOX signaling. We are systematically testing the phenotypic relevance of these and many additional genes by high throughput screening of RNAi knockdowns in utricle supporting cells. Each of these knockdowns is also assessed by RNA-seq to determine downstream transcriptional effects and to computationally build interaction networks for sensory epithelial regeneration. By applying these genomic and bioinformatic approaches we have been able to link together many genes in the regenerative network, including specific components of WNT and NOTCH signaling that influence regeneration. Seventy one avian miRNAs show >2-fold changes in expression during regeneration. We have tested several of these miRNAs by knockdowns and over expression to assess their effects upon regenerative events. For example, a knockdown of miR-34c significantly increased regenerative proliferation, whereas over expression decreased proliferation. Components of the NOTCH signalling pathway are predicted targets of this miRNA and the levels of several of these mRNAs correlate with changes in miR-34c expression. In our enhancer dataset there are many examples of temporal changes in activity. We have built an integrated map of transcription related to flanking putative enhancers/insulators. We are comparing our regeneration data to RNA-seq data from mouse and chicken SE induced to transdifferentiate by gamma secretase inhibitors, to identify similarities and differences in regenerative programs between birds and mammals. The long term goal of our studies is to identify promising therapeutic targets for hair cell replacement and hearing restoration in humans.

807T

Genome-wide gene expression study of peripheral blood monocytes for osteoporosis. *H. Shen[1,2], Y. Chen[2], T. Xu[1,2], C.H. Yi[1], Y.Z. Liu[1,2], H.W. Deng[1,2].* 1) Center for Bioinformatics and Genomics, School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA 70112, USA; 2) School of Medicine, University of Missouri-Kansas City, Kansas City, MO 64108, USA.

Osteoporosis is a disease of bone fragility, with an increased risk of low trauma fracture. The disease has significant impact to the health of the elderly, especially old women. Bone mineral density (BMD) is a key parameter to characterize osteoporosis and genetics plays an important role in BMD variation. An important mechanism for osteoporosis is an excess of bone resorption by osteoclasts over bone formation by osteoblasts. Peripheral blood monocytes (PBMs) are precursors of osteoclasts and play an essential role in osteoclastogenesis by producing cytokines, such as TNF and IL-1. Here we performed a genome-wide gene expression profiling analysis of PBMs in 69 middle-aged Caucasian females (age of 47-53) with extremely high (n=41) or low (n=28) hip BMD. By using Affymetrix Human Exon 1.0 ST arrays, we identified a number of genes showing significant evidence of differential expression between high vs. low BMD subjects. Among these differentially expressed genes, some were previously known to be important factors for osteoclast differentiation (e.g, CTNNBIP1, p= 2.89E-4, fold-change =1.45 between low vs. high BMD subjects) and some have been associated with BMD variation (e.g., C1orf61, p=3.59E-5, fold-change=0.61, and NAMPT, p=6.13E-4, fold-change=1.58). Interestingly, we also identified a number of novel candidate genes for BMD variation, such as CCDC62 (p=2.95E-3, fold-change=0.83), which may act on monocyte-mediated postmenopausal bone loss through estrogen receptor regulation. Our findings, together with the previous evidence, suggested a list of interesting candidate genes for BMD regulation and risk of osteoporosis, which are worth further pursuing with molecular and functional studies.

808T

Admixture mapping of fat mass in African American women identifies region on 3q13.31. *L. Preus[1], L.E. Sucheston[2], J. Wactawski-Wende[1], N. Johnson[3], F. Zakharia[3], H. Tang[3], N. Risch[4], C. Ambrosone[2], C. Carlson[5], C. Carty[5], Z. Chen[6], C. Hutter[5], R. Kaplan[7], C. Kooperberg[5], S. Liu[1], M. Neuhouser[5], U. Peters[5], C. Thompson[8], H.M. Ochs-Balcom[1].* 1) University at Buffalo, Buffalo, NY; 2) Roswell Park Cancer Institute, Buffalo, NY; 3) Stanford University, Stanford, CA; 4) University of California, San Francisco, San Francisco, CA; 5) Fred Hutchinson Cancer Research Center, Seattle, WA; 6) University of Arizona, Tucson, AZ; 7) Albert Einstein College of Medicine, Bronx, NY; 8) Case Western Reserve University, Cleveland, OH.

Obesity is well recognized as a complex phenotype. Body composition phenotypes are particularly appealing for admixture mapping, given the differences in rates of obesity in African American and European American populations. Several studies have shown correlation between global ancestry and body mass index, which is the impetus for our study of more specific adiposity phenotypes. Using dual energy X-ray absorptiometry (DXA) scans available from 842 African American women in the WHI SNP Health Association Resource (SHARe) sample, we performed admixture mapping for the following DXA fat mass phenotypes: total body fat mass, trunk fat mass, ratio of trunk to total body fat mass and whole body percent fat mass. We derived global and local ancestry estimates for each individual from Affymetrix 6.0 data using four reference samples (African, Northern and Western European, East Asian, and Native American). We measured the correlation of DXA phenotypes with African ancestry and used proportion of Northern and Western European ancestry to adjust for population substructure. For each phenotype, we used linear regression to assess the significance of 570,282 SABER estimates of local ancestry (number of African ancestral alleles at each marker) in additive models with adjustment for MET-hrs/week walking, height, weight, smoking, duration of hormone therapy and geographic region. Analyses were performed with and without women who reported a diabetes diagnosis. We found statistically significant covariate-adjusted correlations of whole body fat mass ($p<0.001$) and trunk fat mass ($p<0.04$) with proportion of African ancestry. Genome-wide (admixture) significance ($p<5.5x10-6$) for trunk fat mass was achieved across a 3.3 Mb region on 3q13.31. The five most significant SNPs at this locus showed an inverse association of trunk fat mass and African ancestry ($p=9.4x10-7$). When women with a diabetes history were excluded, we observed a significant inverse association of percent fat mass and African ancestry on 3q24 ($p=4.1x10-6$). After an additional adjustment of the 3q13.31 and 3q24 regions for global ancestry (and multiple testing), both signals were attenuated, with p-values of 2.9x10-4 for trunk fat mass and 1.5x10-4 for percent fat mass. This is the first admixture mapping study of DXA-derived fat mass phenotypes performed to date. Our results suggest that genomic regions in postmenopausal African Americans that contribute to variance in adiposity exist.

809T

A genome-wide association study for primary open-angle glaucoma: Results from the NEIGHBOR and GLAUGEN consortia. *B.L. Yaspan[1], J.L. Wiggs[2], M.A. Hauser[3], J.H. Kang[4], L.M. Olson[5], R.R. Allingham[6], D.L. Budenz[7], H. Chin[8], D.S. Friedman[9], D. Gaasterland[10], T. Gaasterland[11], C. Laurie[12], R.K. Lee[7], P. Lichter[13], S. Loomis[2], Y. Liu[6], S. Moroi[13], A. Realini[14], J.E. Richards[13], J.S. Schuman[15], K. Singh[16], D. Vollrath[17], R. Weinreb[18], G. Wollstein[15], D. Zach[9], K. Zhang[18], M.A. Pericak-Vance[19], L.R. Pasquale[2], J.L. Haines[5].* 1) Center for Human Genetics Research, Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN; 2) Department of Ophthalmology, Harvard Medical School, Boston, MA; 3) Departments of Medicine and Ophthalmology Duke University School of Medicine, Durham, NC; 4) Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 5) Center for Human Genetics Research, Vanderbilt University School of Medicine, Nashville, TN; 6) Department of Ophthalmology Duke University Medical School, Durham, NC; 7) Department of Ophthalmology University of Miami Medical School, Miami, FL; 8) National Eye Institute, NIH, Bethesda, MD; 9) Department of Ophthalmology Johns Hopkins University School of Medicine, Baltimore, MD; 10) Eye Doctors of Washington DC; 11) Bioinformatics and Systems Biology Program, University of California at San Diego, La Jolla, CA; 12) Department of Biostatistics, University of Washington, Seattle, WA; 13) Department of Ophthalmology University of Michigan School of Medicine, Ann Arbor, MI; 14) Department of Ophthalmology University of West Virginia School of Medicine, Morgantown, WV; 15) Department of Ophthalmology University of Pittsburgh School of Medicine, Pittsburgh, PA; 16) Department of Ophthalmology Stanford University School of Medicine, Palo Alto, CA; 17) Department of Genetics Stanford University School of Medicine, Palo Alto, CA; 18) Department of Ophthalmology University of California at San Diego, La Jolla, CA; 19) Institute for Human Genomics, University of Miami School of Medicine, Miami, FL.

Primary open-angle glaucoma (POAG) is a common, genetically complex degenerative optic neuropathy that is a leading cause of blindness worldwide. Positive family history of POAG is a strong risk factor, indicating a genetic component to the disease. The NEIGHBOR (NEI Glaucoma Human genetics collaBORation) consortium is a collaborative effort involving 12 institutions throughout the United States, contributing 2,170 cases and 2,347 controls, and constituting the largest collection of POAG cases to date. A genome-wide association study (GWAS) revealed 17 single nucleotide polymorphisms (SNPs) of genome-wide significance. Sixteen are within the *CDKN2BAS* region at chr9p21.3 with a top hit at rs4977756 ($p=7.34x10-16$; OR=0.66). The remaining SNP, rs10483727, is 40 kb 5' from *SIX1* at chr14q23.1 ($p=3.10x10-8$; OR=1.13). Since POAG occurs across a continuum of intraocular pressure (IOP), we stratified our cases by ocular tension status (pretreatment, when available), defining 2 subsets: a normal tension glaucoma (NTG) group with IOP <22 mmHg at or before diagnosis and a high tension glaucoma (HTG) group with IOP ⁄ 22 mmHg. We found the association with *CDKN2BAS* was stronger in our NTG cases (rs4977756; $p= 7.31x10-7$; OR=0.59) than HTG cases ($p=6.61x10-4$; OR=0.78) despite significantly smaller sample size in the former subset (398 NTG; 971 HTG). These data suggest *CDKN2B* SNPs significantly influence susceptibility to glaucomatous optic neuropathy even in the absence of elevated IOP. In addition, the NEIGHBOR consortium has harmonized clinical definitions and genotyping platforms with the Glaucoma Genes and Environment (GLAUGEN) POAG GWAS, which is part of the GENEVA (Gene Environment Association studies) consortium. Combined, the two studies include 3,146 cases and 3,487 controls. A meta-analysis identified a new locus at 8q23 of genome-wide significance in NTG cases (top hit rs284489; $p=8.88x10-10$; OR=1.62). *CDKN2B* is a part of the transforming growth factor-beta signaling pathway (TGFB-p). Furthermore, evidence suggests the SNPs identified at 8q23 influence the expression of *TSC22*, which itself modulates signaling within the TGFB-p. Using the PARIS pathway analysis algorithm, we found the TGFB-p (Kyoto Encyclopedia of Genes and Genomes) associated with POAG in our combined NTG dataset ($p=0.021$). The identification of genetic variation predisposing to POAG is an important step toward understanding its pathophysiology and eventual design of gene-based treatment.

810T

Triplet domain hyper-amplification drove human-lineage specific DUF1220 domain expansion. *M. O'Bleness[1], L. Dumas[1], H. Kehrer-Sawatzki[2], G. Wyckoff[3], J. Sikela[1].* 1) Biochemistry and Molecular Genetics, Univ Colorado Denver AMC, Aurora, CO; 2) Institute of Human Genetics, University of Ulm Albert-Einstein-Allee, Ulm, Germany; 3) Molecular Biology and Biochemistry, University of Missouri, Kansas City, Missouri, USA.

DUF1220 protein domains show the largest human lineage-specific (HLS) increase in copy number of any protein coding sequence in the human genome. From >260 copies in human DUF1220 copy number markedly decreases as a function of a species evolutionary distance with human (125 in chimp; 1 in mouse/rat). Most HLS DUF1220 copies map to 1q21.1 and recently 1q21.1 CNVs that either encompass or flank DUF1220 domains have been implicated in numerous human diseases, including microcephaly, macrocephaly, autism, schizophrenia, mental retardation and heart disease, suggesting a link between this dramatic HLS DUF1220 copy number amplification and the etiology of these disorders. Here we analyze 40 animal genomes and present the most complete portrait to date of the evolutionary history of DUF1220 domains and the genes that encode them (NBPF family). We describe several key events related to DUF1220 evolution, the most striking of which is that the dramatic HLS increase in DUF1220 copy number : from 102 in our last common ancestor with chimp/bonobo to 272 in human (an average of ~28 copies added to the human genome every million years) was driven almost exclusively by domain hyper-amplification involving a 4.7kb, tandemly-repeated three DUF1220 domain unit we have named the HLS DUF1220 triplet. The triplet amplifications lie completely within a unique, recently evolved genomic landscape flanked by the human-specific, large heterochromatic C-band at 1q12, and within an HLS pericentromeric inversion, features which are likely to be key to the sustained rapid expansion of the DUF1220 domain within the human lineage. The data presented here support the view that while the HLS DUF1220 triplet hyper-amplification is among the most striking changes found in the human genome, it has come at an expensive price, having been largely responsible for creating and maintaining one of the most unstable and disease-prone regions of the human genome.

811T

Identification of non-coding elements as candidate sequences associated with diseases. *A. Henrion[1], M. Naville[2], M. Girard[1], C. Mugnier[1], S. Bandiera[1], A. Munnich[1], M. Le Merrer[1], S. Lyonnet[1], H. Roest Crollius[2].* 1) UMR781 INSERM, Paris, France; 2) Dyogen Group, CNRS UMR8197-INSERM U1024, Institut de Biologie de l'Ecole Normale Supérieure (IBENS), Paris, France.

Over three percent of the human genome consists of conserved non-coding elements (CNEs). Those elements are either non-transcribed or transcribed, for instance as microRNAs. In the present work, we considered three groups of genetic diseases: diseases with mapped loci but no known genes (group I), diseases with cloned genes (group II), and diseases with recurrent chromosomal rearrangement (group III). To address the interest of studying non-coding elements in disease-causing genes for which mutations are already known and characterized, we first plotted group II together with the location of microRNAs. Interestingly, we could identify 274 diseases with cloned genes that may still involve microRNAs. In particular, we were able to identify a notable enrichment in monogenic diseases with clinical heterogeneity in this category. By comparative genomics alignments across multiple vertebrate species we then selected possibly functional CNEs. Our aim was to establish functional linkages between these CNEs and their likely target gene by replacing them in an ancestral mammalian genome, and by postulating the maintenance of CNE/target gene physical proximity in ancestral and modern genomes. This approach led to identify 72 disease-causing genes putatively controlled by CNEs and involved in diseases that are listed herein. To increase the specificity and the sensitivity of the analysis we increased the number of species and used the ancestral Boreoeutheria genome as reference. We compiled an atlas of the X-linked diseases that may involve CNEs. Our results provide a source of non-coding elements associated with diseases worthwile interest to sequence in those patients with no mutation previously identified. It also paves the way to unravel sets of CNEs aiming to the concerted phenotypic-specific regulation of a disease-causing gene, otherwise involved in numerous developmental fields.

812T

Development of a novel multiplex assay for major Y haplogroup assignment. *T.D. Parrish[1], A.E. Justice[2], S.M. Johnson[2], K.G. Beaty[2], C. Phillips-Krawczak[3], P. Williams[1], M.H. Crawford[2].* 1) Evogen, Inc, Kansas City, MO; 2) Department of Anthropology, Lab of Biological Anthropology, University of Kansas; 3) Genetics Program, Department of Molecular Biosciences, University of Kansas.

Uni-parentally inherited markers, such as those found on the Y chromosome, are of interest for their utility in determining the ancestry of individuals and reconstructing population history. To accurately assign a region of origin for Y chromosome ancestry it is important to use regionally specific Single Nucleotide Polymorphisms (SNPs). Typically this requires costly and time consuming sequencing of multiple markers or AFLP with expensive kits for haplogroup assignment. This study aims to provide a cost and time efficient means of accurately typing Informative SNPs for Y haplogroup ancestral assignment in a hierarchical fashion. Hybeacons® Probes (Evogen, Inc.) were designed in three multiplex reactions containing a total of 15 SNPs for major Y haplogroup/macrohaplogroup assignment (A, B, C, D/E, F, G, H, I/J, K, L, M, N/O, Q, R, and R1). Hybeacons® Probes use a single primer pair per SNP to amplify the region of interest, normal PCR reagents, and fluorescently-labeled probes in a single PCR reaction. For multiplexing, HyBeacons® were labeled with different fluorescence and staggered by melting temperatures for easy identification of positive SNPs. The difference among melt curves allows the researcher to differentiate among the wild type and the polymorphic SNPs. The multiplexes were used to type descendant and admixed populations from Europe, Africa, Asia, North America, Central America and South America (Peru). Typing was confirmed by sequencing a subset of the samples from each haplogroup/macrohaplogroup and comparing SNP typing to Y STR profiles. The SNP multiplex was successfully employed to obtain ancestral information for paternal lineage identification. The hierarchical fashion of the multiplexes decreased the number of reactions and thus reagent required to complete haplogroup assignment. The SNP multiplex provides a low-cost, high throughput method for typing of Y chromosome haplogroups that will prove useful in the fields of forensics and anthropology.

813T

Alzheimer's disease susceptibility loci: evidence for natural selection and altered gene expression. *T. Raj[1,2,3,4], J.M. Shulman[1,3,4], B.T Keenan[1,3,4], L.B. Chibnik[1,3,4], D.A. Evans[5,6], D.A. Bennett[5,6], B.E. Stranger[2,3,4], P.L. De Jager[1,3,4].* 1) Program in Translational NeuroPsychiatric Genomics, Department of Neurology, Brigham and Women's Hospital, 77 Avenue Louis Pasteur, Boston, MA 02115; 2) Department of Genetics, Brigham and Women's Hospital, 77 Avenue Louis Pasteur, Boston, MA 02115; 3) Harvard Medical School, Boston, MA 02115; 4) Program in Medical and Population Genetics, Broad Institute, 7 Cambridge Center, Cambridge, MA 02142; 5) Departments of Internal Medicine and Neurological Science, Rush University Medical Center, 600 S Paulina St., Chicago, IL 60612; 6) Rush Alzheimer's Disease Center, Rush University Medical Center, 600 S Paulina St., Chicago, IL 60612.

Genome-wide association (GWA) studies have identified a number of susceptibility loci Alzheimer's disease (AD). Many of these loci include genes involved in cholesterol metabolism, immune functions and cell membrane processes, but the contribution of these genes to AD pathophysiology is largely unknown. To dissect the functional consequences of the associated genetic variants, we explored large-scale data sets interrogating the human genome for evidence of positive natural selection and of effects on gene expression in each associated locus (cis-regulatory effects). Using a long-range haplotype test for positive selection, we find that putative AD susceptibility loci including CD2AP, PICALM, EPHA1, MS4A2, CD33 and CR1 harbor haplotypes with evidence of recent selection. For example, the haplotype carrying the AD risk variant rs561655 in PICALM shows strong evidence of positive selection after correction for genome-wide testing. Our results further show that multiple physically interacting proteins (based on protein-protein interaction network) including the genes PICALM, CD2AP, BIN1 and EPHA1 show significant evidence for selection, indicating a possible co-evolution of genetic variants underlying AD pathology, perhaps in response to a single evolutionary pressure. To support the hypothesis that AD susceptibility alleles may influence the function of immune cells, we analyzed gene expression data from monocytes and peripheral blood and identified 4 cis-regulatory effects (PICALM, MS4A2, BIN1 and CD33) in the 10 tested loci, including the rs3826656 variant which is strongly associated with the RNA expression of CD33 in monocytes ($p = 4.07 \times 10^{-17}$) and in peripheral blood ($p = 3.94 \times 10^{-4}$), suggesting that AD susceptibility may act, in some cases, through altered gene expression in immune cells. We have confirmed this effect at the protein level in monocytes: CD33 expression is upregulated on the cell surface relative to the risk allele. Our results provide significant evidence for signatures of recent positive selection acting on several haplotypes carrying AD susceptibility alleles, some of which have cis-regulatory effects on a nearby gene. Taken together, our data suggest several different genes implicated in AD may have worked together in another context to enhance survival over the course of recent human evolutionary history.

814T

Co-existence of Hemoglobin D and IVSII-5 in the same allele of Beta globin gene in Iranian population. *N. Bayat, F. Daneshimehr, S. Jalilnejad, N. Faramarzi, H. Imanian, A. Azarkeivan, H. Najmabadi.* Molecular genetics, Kariminejad-Najmabadi genetic center, Tehran, Tehran, Iran.

The Beta Thalassemia, is one of the most common hemoglobinopathies is Iran as well as Hemoglobin type D (Cd121 G>C). The IVSII-5 beta thalassemia mutation was reported before in a family with Chinese background. And the hemoglobin D Punjab (Also known as Hb D-Los Angeles) has been reported in different places of the world. In this study we have identified these two mutations located in the same allele of Beta globin. The family showing the phenotype of beta thalassemia (maternal side), the HbD phenotype with a little raise of HbA2 in hemoglobin electrophoresis (paternal side) was referred to our lab in case of prenatal diagnosis investigation. They have also had a child with a same phenotype as his father. The DNA was extracted from peripheral blood leucocytes, the beta globin gene was amplified by PCR, and the DNA sequencing was performed to identify the beta mutations. We have determined that the paternal side has two mutations in beta globin gene (HbD and IVSII-5) and the maternal side has only one Beta thalassemia mutation (IVSII-1). Since the maternal and paternal mutations were identified, we would logically look for the same mutations in the child, but the surprising result was that the child has inherited both mutations from his father. In this study we have identified the rare IVSII-5 mutation in Iranian population accompanying the HbD mutation. Also we have shown that these two mutations are located in the same allele, this data is so important for any future pregnancy of this family because they are in a risk of having a child with three beta globin mutations. Also we realized that the Beta globin gene DNA sequencing should be performed for all suspicious cases as well as family study, to identify whether the mutation is located in the same allele and is the person carrying only one mutation or more in the same allele.

815T

Vitamin D Sufficiency Modulates the Effect of Polymorphisms on Bronchodilator Response in Asthmatics. *A.C. Wu[1,2], B.E. Himes[3,4,5,6], K. Tantisira[6], S.T. Weiss[5,6], A. Litonjua[6].* 1) Population Medicine, Harvard Medical School, Boston, MA; 2) Children's Hospital, Boston Division of eneral Pediatrics, Boston, MA; 3) Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA; 4) Children's Hospital Informatics Program, Boston, MA; 5) Harvard Partners Center for Genetics and Genomics, Boston, MA; 6) Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

Low vitamin D levels appear to be associated with asthma, asthma symptoms, and decreased airway responsiveness. Single nucleotide polymorphisms (SNPs) influence bronchodilator response. Thus, we hypothesized that vitamin D could modulate the effect of multiple SNPs on bronchodilator response. We assessed whether vitamin D sufficiency modulates the measured association of SNPs with bronchodilator response in a population of subjects with asthma using a genome-wide association approach. Our primary population is composed of subjects from the Childhood Asthma Management Program (CAMP), a clinical trial that followed asthmatic children for four years. Genome-wide SNP genotyping for 561 Caucasian CAMP subjects and their families was performed on Illumina's HumanHap550 Genotyping BeadChip (Illumina, Inc., San. After excluding SNPs with missing data in more than 5% of subjects, having minor allele frequency (MAF) less than 1%, and having Hardy-Weinberg equilibrium p-values among controls less than 0.001, there were 512,284 SNPs. We conducted a gene by environment analysis in PLINK with vitamin D sufficiency versus insufficiency as the environmental exposure and bronchodilator response as the outcome measure. We found that the SNPs that are most significantly associated with bronchodilator response while accounting for vitamin D sufficiency are in chromosomes 1, 2, 3 and 13. The top 8 SNPs had a gene by environment interaction p-value of <1E-05. Thus, we have identified SNPs that appear to modify bronchodilator response differently depending on Vitamin D levels in the CAMP population. We are replicating these associations in an independent population. This work was supported by.

816T

Keeping it in the Family. *E. Bruford, L. Daugherty, M. Lush, R. Seal, M. Wright.* HGNC, EMBL-EBI, Hinxton, United Kingdom.

The HUGO Gene Nomenclature Committee (HGNC) has assigned unique approved gene symbols and names to over 31,000 human loci to date. Over 19,000 of these are protein coding genes, but we also name pseudogenes, phenotypic loci, genomic features and non-coding RNAs. Our recently updated website, genenames.org, is a searchable repository of HGNC-approved gene nomenclature and associated resources, including nomenclature guidelines and the HGNC Comparison of Orthology Predictions (HCOP) tool. Each locus has an individual “gene symbol report” which can include links to genomic, proteomic and phenotypic information. Approved gene symbols are based on names describing structure, function or homology, and where possible the HGNC also organise these into gene groupings and families, many of which have specialist advisors who are experts in that particular area of biology. HGNC also create web pages for specific gene families, and we are currently in the process of generating more of these pages and expanding their scope using data curated by the HGNC combined with external resources that focus on particular families. These new gene family pages will be sorted into meaningful categories which could include function, structure and homology. We also now provide links from our individual gene symbol reports to our improved gene family resources. If you know of a gene family that you think should be represented please contact us via hgnc@genenames.org or talk to us during this meeting. The work of the HGNC is supported by the NHGRI and the Wellcome Trust.

817T

Genetic risk factors associated with systemic lupus erythematosus (SLE) in reactive intermediate genes vary across African ancestries. *J. Oates[1], D. Kamen[1], P. Gaffney[2], C. Langefeld[3], J. Kelly[2], K. Kaufman[2], R. Kimberly[4], T. Niewold[5], C. Jacob[6], B. Tsao[7], E. Brown[8], M. Petri[9], R. Ramsey-Goldman[10], J. Reveille[11], L. Vila[12], J. James[2], J. Guthridge[2], J. Merrill[13], S. Boackle[14], B. Freedman[15], H. Scofield[2], A. Stevens[16], T. Vyse[17], L. Criswell[18], K. Moser[2], M. Alarcon-Riquelme[2,19], J. Harley[20], G. Gilkeson[1], P. Ramos[3].* 1) Department of Medicine, Medical University of South Carolina, Charleston, SC; 2) Arthritis and Clinical Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK; 3) Wake Forest School of Medicine and Center for Public Health Genomics, Winston-Salem, NC; 4) Department of Medicine, University of Alabama at Birmingham, Birmingham, AL; 5) Section of Rheumatology and Gwen Knapp Center for Lupus and Immunology Research, University of Chicago, Chicago, IL; 6) Keck School of Medicine, University of Southern California, Los Angeles, CA; 7) David Geffen School of Medicine, UCLA, Los Angeles, CA; 8) Department of Epidemiology, University of Alabama at Birmingham, Birmingham, AL; 9) Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 10) Division of Rheumatology, Northwestern University Feinberg School of Medicine, Chicago, IL; 11) Rheumatology and Clinical Immunogenetics, University of Texas Health Science Center at Houston, Houston, TX; 12) Department of Medicine, Division of Rheumatology, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico; 13) Clinical Pharmacology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK; 14) Division of Rheumatology, University of Colorado Denver, Aurora, Colorado; 15) Department of Internal Medicine, Wake Forest School of Medicine, Winston-Salem, NC; 16) Division of Rheumatology, Department of Pediatrics, University of Washington, Seattle, WA; 17) Division of Genetics and Molecular Medicine, King's College, London, United Kingdom; 18) Rosalind Russell Medical Research Center for Arthritis, University of California, San Francisco, CA; 19) Center for Genomic and Oncological Research, Universidad de Granada, Granada, Spain; 20) Division of Rheumatology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Very little is known about the genetic etiology of systemic lupus erythematosus (SLE) in individuals of African ancestry - in spite of its higher prevalence, incidence, disease severity, and mortality rates in African Americans (AA). Overproduction of nitric oxide (NO) has been implicated in its pathogenesis and correlated with disease severity, making NO synthases and other reactive intermediate genes biological candidates for disease susceptibility. Here, we report a comprehensive analysis of reactive intermediate genes for their association with SLE in populations of African ancestry. One such population are the Gullah of the Sea Islands of South Carolina: a population isolate with limited and well defined ancestral diversity. Such reduced genetic heterogeneity may increase the power to detect associations in this population. In addition, their higher familial disease prevalence might reflect a stronger genetic component to the disease. We analyzed 279 SNPs from 55 regions in 133 Gullah cases and 112 Gullah controls, as well as in other 1432 AA cases and 1575 AA controls. These and approximately 300 additional ancestry informative markers were genotyped on an Illumina custom array; principal components analysis and admixture estimates were computed and adjusted for in association analyses. While the glutathione reductase GSR (rs2253409, P=0.0014) and paraoxonase PON3 (rs17879114, P=0.0016) were the most significant single-SNP associations in AA, in the Gullah the NADH dehydrogenase NDUFS4 (rs381575, P= 0.0065) and nitric oxide synthase NOS1 (rs561712, P=0.0072) were the most strongly associated with SLE. When analyzed together, GSR remained the most significant effect (rs2253409, P=0.00072). Haplotype analyses revealed a significant 3-SNP haplotype in NOS1 in AA (rs3741476-rs10774909, P=0.00029), as well as in the joint cohorts together (P= 0.00074). Two-loci interaction analysis uncovered significant interactions between NDUFS2 (rs4656993) and the minichromosome maintenance complex component MCM5 (rs4645794) in AA (P=9.74E-05), between the ring finger protein RNF157 (rs11099897) and the toll-like receptor TLR7 (rs5741880) in the Gullah (P=6.61E-05), and between PON3 (rs13226149) and TLR7 (rs1731479) in the joint cohort (P=0.0003). These results suggest that different variants in reactive intermediate genes in different African ancestries may be associated with SLE.

818T

Tailored pathway-based analysis identifies significant enrichment of apoptosis-related genes associated with cardiac manifestations of neonatal lupus. *P. Ramos[1], M. Marion[1], C. Langefeld[1], J. Buyon[2], R. Clancy[2].* 1) Department of Biostatistical Sciences and Center for Public Health Genomics, Wake Forest School of Medicine, Winston-Salem, NC; 2) New York University Langone School of Medicine, New York, NY.

Cardiac manifestations of neonatal lupus, comprising atrioventricular conduction defects and cardiomyopathy, occur in fetuses exposed to anti-Ro/SSA antibodies, and carry substantial mortality. Increased recurrence rates, concordance in a third of monozygotic twins, and results from the first published genome-wide association study (GWAS) support a fetal genetic risk. Although several significant associations were identified in individual genes, we hypothesize that there may be a global enrichment of specific pathways that may be overlooked if only the most significant associations are considered. We used the results from this admixture adjusted GWAS (inflation factor 1.026) to search for a potential enrichment of associations in specific pathway-related genes. Based on the finding of exaggerated apoptosis in autopsy studies from affected hearts, we posit that apoptotic responses may promote cardiac injury in these children. We used Ingenuity Pathway Analysis to compile a list with all genes with functions in apoptosis (n=2,283 genes). We then tested for an enrichment of admixture and genomic control adjusted significant P-values in SNPs that met quality control criteria (at alpha=0.05, 0.01, and 0.001). We observed a strong enrichment of significant SNPs (P<1x10-07) at all three significance levels; the enrichment existed whether including (n=16,031 SNPs) and excluding (n=15,935 SNPs) the extended HLA region. These data exhibit highly significant enrichment of association in apoptosis-related genes with cardiac neonatal lupus. We conclude that common genetic variation in genes within this pathway is involved in predisposing to cardiac manifestations of neonatal lupus.

819T

Identification of IL6R and chromosome 11q13.5 as risk loci for asthma. *M.A.R. Ferreira[1], M.C. Matheson[2], D.L. Duffy[1], G.B. Marks[3], J. Hui[4,5,6,7], P. Le Souef[8], S. Baltic[10], G. Willemsen[11], W. Ang[12], M. Kuokkanen[13], J. Beilby[4,6,7], F. Cheah[10], A. Ramasamy[14], S. Vedantam[15,16], P.A. Madden[17], A.C. Heath[17], J.L. Hopper[2], M-R. Jarvelin[19], D.I. Boomsma[11], J. Hirschhorn[15,16,20], H. Walters[21], N.G. Martin[1], A. James[7,18,22], G. Jones[23], M.J. Abramson[24], C.F. Robertson[25], S.C. Dharmage[2], M.A. Brown[9], G.W. Montgomery[1,26], P.J. Thompson[10,26] for the Australian Asthma Genetics Consortium.* 1) Queensland Institute of Medical Research, Brisbane, Australia; 2) Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, University of Melbourne, Melbourne, Australia; 3) Woolcock Institute of Medical Research, University of Sydney, Sydney, Australia; 4) PathWest Laboratory Medicine of Western Australia (WA), Nedlands, Australia; 5) School of Population Health, The University of WA, Nedlands, Australia; 6) School of Pathology and Laboratory Medicine, The University of WA, Nedlands, Australia; 7) Busselton Population Medical Research Foundation, Sir Charles Gairdner Hospital, Perth, Australia; 8) School of Paediatrics and Child Health, Princess Margaret Hospital for Children, Perth, Australia; 9) University of Queensland Diamantina Institute, Princess Alexandra Hospital, Brisbane, Australia; 10) Lung Institute of WA and Centre for Asthma, Allergy and Respiratory Research, University of WA, Perth, Australia; 11) Netherlands Twin Register, EMGO & NCA Institute, Department of Biological Psychology, VU University, Amsterdam, The Netherlands; 12) Telethon Institute for Child Health Research, Centre for Child Health Research, University of WA, Perth, Australia; 13) Department of Chronic Disease Prevention, National Institute for Health and Welfare, Finland; 14) Respiratory Epidemiology and Public Health, Imperial College, London, United Kingdom; 15) Divisions of Genetics and Endocrinology, Children's Hospital, Boston, United States; 16) Broad Institute, Cambridge, United States; 17) Washington University School of Medicine, St Louis, United States; 18) School of Medicine and Pharmacology, University of WA, Nedlands, Australia; 19) Department of Epidemiology and Biostatistics, Imperial College, London, United Kingdom; 20) Department of Genetics, Harvard Medical School, Boston, United States; 21) Menzies Research Institute, Hobart, Australia; 22) Department of Pulmonary Physiology, West Australian Sleep Disorders Research Institute, Nedlands, Australia; 23) University of Western Sydney, Penrith, Australia; 24) Department of Epidemiology& Preventive Medicine, Monash University, Melbourne, Australia; 25) Respiratory Medicine, Murdoch Children's Research Institute, Melbourne, Australia; 26) These authors contributed equally to this work.

Background. We aimed to identify novel genetic variants affecting asthma risk, as these may provide novel insights into molecular mechanisms underlying asthma.

Methods. We performed a genome-wide association study (GWAS) in 2,669 physician-diagnosed asthmatics and 4,528 controls from Australia. Seven loci were prioritised for replication after combining our results with those from the GABRIEL consortium (N=26,475), and these were tested in an additional 25,358 independent samples from four in-silico cohorts. Quantitative multi-SNP scores of genetic load were constructed based on results from the GABRIEL study and tested for association with asthma in our Australian GWAS dataset.

Results. Two loci were confirmed to associate with asthma risk in the replication cohorts and reached genome-wide significance in the combined analysis of all available studies (n=57,800): rs4129267 (OR=1.09, combined $P=2.4x10^{-8}$) in the interleukin-6 receptor gene (IL6R) and rs7130588 (OR= 1.09, $P=1.8x10^{-8}$) on chromosome 11q13.5 near the leucine rich repeat containing 32 gene (LRRC32, also known as GARP). The latter was significantly associated with atopic status among asthmatics (OR = 1.33, P = $7x10^{-4}$), suggesting that it is a risk factor for allergic- but not non-allergic asthma. Multi-SNP association results are consistent with a highly polygenic contribution to asthma risk, including loci with weak effects that may be shared with other immune-related diseases, such as NDFIP1, HLA-B, LPP and BACH2.

Conclusions. The IL6R association further supports the hypothesis that cytokine signalling dysregulation affects asthma risk, and identifies a novel target for biological therapies. Results for the 11q13.5 locus suggest that it directly increases the risk of allergic sensitization which, in turn, increases the risk of subsequently developing asthma. Larger or more functionally focused studies are needed to characterise the many loci with weak effects that remain to be identified for asthma.

820T

Association of polymorphisms of the TNIP1 gene with celiac disease. *R. McManus[1], A.W. Ryan[1], M.P. Sperandeo[2], C. Coleman[1], L. Greco[2], G. Turner[1].* 1) Department of Medicine and Institute of Molecular Medicine, Trinity College, St James Hospital, Dublin, Ireland; 2) Department of Pediatrics and European Laboratory for the Investigation of Food-Induced Diseases, University Federico II, Naples, Italy.

Background. Recent whole genome analysis studies have indicated association with celiac disease and numerous regions containing genes whose products act as immune mediators, including T-Cell regulators, and mediators of the inflammatory response, such as TNFAIP3 and C-Rel. TNFAIP3's gene product acts in conjunction with TNIP1 to repress NFkB mediated inflammation. The gene coding for TNIP1 lies within the 5q region, a broad genomic area which has previously shown association with celiac disease in several European populations. We investigated 9 SNPs in the region of TNIP3 selected on a number of criteria, including previous association with inflammatory diseases and potential for regulatory effects due to their potential to disrupt transcription factor binding sites (TFBS) in evolutionary conserved regions (using ECR Browser and rVista software). Six SNPs were situated in conserved TFBSs, two (rs2233292 and rs2233289) were non-synonymous coding sequence polymorphisms and one (rs17728338) had previously been associated with psoriasis. Methods and Results. All 9 selected SNPs were analyzed for allelic, genotypic and carrier status and deviations from HWE. Both of the selected coding SNPs proved to be invariant in the Irish population, as did 3 of the suspected regulatory SNPs. Of the four remaining SNPs, two showed significant association with celiac disease (N>570 cases, >930 controls). rs8177834 was associated with the disease at the allelic level using Fisher's exact test (P=0.0037; OR=1.49 [95%CI, 1.1-2.2), the genotypic level (P=0.009) and dominant/recessive models, with homozygosity for the G allele particularly associated (P= 0.0097; OR=1.45 [95%CI, 1.1-1.9]). rs2017638 was not significantly associated (P=0.067). We replicated these SNPs in an Italian population (N>350 cases, 420 controls) which trended towards, but did not reach, significance. The Cochran-Mantel-Haenszel test was used to test for significance in the combined populations. rs8177834 was significantly associated (P=0.00082; OR=1.4, 95%CI, 1.15-1.71). rs2017638 was weakly associated in the combined populations (P=0.02). Conclusion.The TNIP1 SNP rs8177834 shows evidence of association to coeliac disease in combined Irish and Italian populations. The presence of the major allele completes a consensus sequence for a c-MYB transcription factor binding site which is conserved between mouse and human genomes. We are currently testing this site for function.

821T

Genetic Determinants and Racial Differences in Childhood Asthma. *T. Mersha[1], M. Butsch Kovacic[1], J. Biagini Myers[1], L. Martin[1], M. Lindsey[1], T. Patterson[1], H. He[1], M. Ericksen[1], J. Gupta[1], A. Tsoras[1], A. Lindsley[1], M. Rothenberg[1], M. Wills-Karp[1], N. Eissa[2], L. Borish[3], G. Hershey[1].* 1) pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) Department of Medicine, Baylor College of Medicine, Houston, TX; 3) Department of Medicine, University of Virginia, Charlottesville, VA.

RATIONALE Candidate gene case-control studies have identified several single nucleotide polymorphisms (SNPs) that are associated with asthma susceptibility. Most of these studies have been restricted to evaluations of specific SNPs within a single gene and within populations from European ancestry. Recently, there is increasing interest in understanding racial differences in genetic risk associated with childhood asthma. Our aim was to compare association patterns of asthma candidate genes between children of European and African ancestry. METHOD Using a custom-designed Illumina SNP array, we genotyped 1,485 children within the Greater Cincinnati Pediatric Clinic Repository and Cincinnati Genomic Control Cohort for 259 SNPs in 28 genes and evaluated their associations with asthma. RESULTS Fourteen SNPs located in six genes were significantly associated (p-values <0.05) with childhood asthma in African Americans. Among Caucasians, 13 SNPs in 5 genes were associated with childhood asthma. Two SNPs in IL4 were associated with asthma in both races (p-values <0.05). Gene-gene interaction studies identified race specific sets of genes that best discriminate between asthmatic children and non-allergic controls. CONCLUSION For the first time, we identified IL4 as having a role in asthma susceptibility in both African American and Caucasian children. However, while IL4 SNPs were associated with asthma in children with European and African ancestry, the relative contributions of the most replicated asthma-associated SNPs varied by ancestry. These data provide valuable insights into race specific pathways that may predispose to asthma in individuals with European vs. African ancestry.

822T

Whole Genome Sequencing and Rare Variant Analyses of a Multiplex Bipolar Pedigree. *J. Xing[1], C.D. Huff[1], B. Moore[1], R. Robinson[2], J. Ross[3], M.D. Yandell[1], J.A. Badner[4], W. Byerley[3].* 1) Department of Human Genetics, University of Utah, Salt Lake City, UT; 2) Department of Psychiatry, University of Utah, Salt Lake City, UT; 3) Department of Psychiatry, University of California San Francisco, San Francisco, CA; 4) Department of Psychiatry, University of Chicago, Chicago, IL.

Bipolar disorder (MIM 125480) is a complex genetic disorder affecting ~1% of the population. Genome-wide association studies have identified a few common alleles for this disorder, but they all have small effects and together explain no more than 1% of the genetic variance. In contrast to schizophrenia, large CNVs do not appear to play a role in disease etiology. Taken together, these data suggest that a number of large-effect rare variants, many nominal-effect common variants, or both explain a large fraction of disease susceptibility. Large multiplex pedigrees - which are more likely to contain rare variants with large effect sizes - will be a valuable resource for identifying rare variants and distinguish these possibilities. We selected a large multiplex pedigree containing 6 Bipolar Type 1 (BPI) cases. This pedigree was used in a previous linkage study that identified two suggestive signals on chromosomes 4 and 10. Whole genome sequencing of 3 BPI cases from this pedigree was carried out at Complete Genomics. We used a novel analytic tool, VAAST, to characterize variants and prioritize genes. VAAST candidate gene prioritization analysis was performed under both the dominant and recessive inheritance models, assuming an expected allele frequency lower than 0.1% (dominant model) or 1% (recessive model) for the causal variant in the general population, respectively. Genome-wide VAAST analysis identified ~100 rare non-synonymous exonic SNPs including one in the chromosome 4 linkage region (*SORBS2*) and two on the chromosome 10 region (*NEBL* and *CHAT*). We are currently testing for co-segregation of the exonic variants identified in the linkage regions in the pedigree. Because it is not yet financially feasible to perform large-scale case/control whole genome sequencing to map the full range of rare variant loci, whole genome sequencing of pedigrees previously used in linkage studies could be a cost-effective method for mapping rare variants for bipolar disorder.

823T

Genetic variation on inducible gene expression. *H.J. French, K. Hardy, M.F. Shannon, R.B.H. Williams.* Genome Biology, The Australian National University, Canberra, Australian Capital Territory, Australia.

Because activation of the immune response is dependent on extensive changes in gene expression, it is likely that a major component of inter-individual variation in the immune response is ultimately mediated at the level of gene regulation. Here, we examine the influence of genetic variation on inducible gene expression in the murine immune response. We extracted primary CD4+ splenocytes from inbred strains A/J, C57BL/6J, BALB/c, DBA/2J, and 129x1/SvJ (>3 animals/strain) and measured mRNA transcript levels using microarrays in both basal state and four hours after stimulation with PMA/Ionomycin. We defined an expression change occurring during activation as the difference in measured expression intensity between stimulated and basal conditions. We identified genetically influenced genes using a gene-wise single-factor (strain) ANOVA (B-H corrected P<0.05). We identified 2607, 1145 and 506 transcripts whose expression levels are under potential genetic influence in basal state, stimulated state and during activation, respectively. Using previously published data in CHX-treated mouse EL4 cells studied using the same protocol, we confirmed that mRNA levels of both primary response (e.g. Fos, Tnfaip3) and secondary response (e.g. Irf4) genes were subject to genetic influence across activation. These differentially activated genes are ideal candidates for further study into the influence of genetic variation on the mechanisms of gene induction, and provide mechanistic insight into inter-individual variation in the host response to infection.

824T

Genetic variation of FGF-23 gene associates with phosphate homeostasis and bone health in Finnish children and adolescents. *M. Pekkinen[1], H.T Viljakainen[2], R. Mäkitie[1], C. Laine[1], O. Mäkitie[1,2].* 1) Folkhälsan Institute of Genetics, Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 2) Children's Hospital, Helsinki University Central Hospital, Helsinki, Finland.

Fibroblast growth factor 23 (FGF-23), synthesized by osteoblasts and osteocytes, is part of hormonal bone-parathyroid-kidney axis, which is modulated by PTH, 1,25(OH)2-vitamin D, diet and serum phosphorus levels. Several hereditary disorders with inappropriately high serum FGF-23 levels are associated with phosphate wasting and impaired bone mineralization. In the present study we investigated the genetic variation in the FGF-23 gene and its association with parameters of phosphate homeostasis and bone health in Finnish children and adolescents. This school-based study included 183 healthy children and adolescents (110 girls, 73 boys) aged 7-19 years, who were assessed for bone health and its determinants. The genetic variation was detected by direct sequencing of FGF-23 coding exons with flanking intronic regions. The concentrations of plasma/serum Ca, Pi, 25-hydroxyvitamin D (25-OHD), PTH, FGF-23 and the bone formation and resorption markers PINP and ICTP, as well as urine Pi and creatinine concentrations were determined. Bone mineral density (BMD) was assessed with DXA (Hologic Discovery A), and volumetric BMD was measured from non-dominant radius with pQCT (XCT-2000; Stratec). Statistical analyses were performed with PASW18. We detected three different FGF-23 polymorphisms: c.212-37insC (rs3832879) in intron 1 and two SNPs in exon 3, rs7955866 (p.T239M) and rs11063112 (p.P241P). Genotypes among the 183 subjects were for c.212-37insC -/- (78%) and -/C (22%); for rs7955866 G/G (80%) and G/A (20%); and for rs11063112: A/A (47%), A/T (45%) and T/T (8%). Nine different haplotypes were observed: 1 (31.7%), 2 (0.5%), 3 (25.7%), 4 (13.7%), 5 (4.9%), 6 (12%), 7 (2.7%), 8 (3.3)% and 9 (4.9 %). The variation in FGF-23 gene was significantly associated with P-Pi (p= 0.045), total BMD (p=0.038) and cortical volumetric BMD z-scores (p=0.049), but not with stress and strain index z-score (SSI). In boys, but not in girls, FGF-23 gene variation associated with U-Pi/U-Crea (p=0.014). The observed associations between FGF23 haplotypes and plasma and urine Pi, volumetric total and cortical BMD suggests that genetic variation in the FGF23 gene may play a role in mineral homeostasis and bone remodeling.

825T

Known bone mineral density (BMD) associated SNP allele frequencies reflect trends in average population BMD across European, Asian and African populations. *M. Ramsay[1,2], A. Choudhury[1,2].* 1) Division of Human Genetics, NHLS & School of Pathology, University of the Witwatersrand, Johannesburg, Gauteng, South Africa; 2) Wits Bioinformatics, University of the Witwatersrand, Johannesburg, Gauteng, South Africa.

Low bone mineral density (BMD) is correlated with fracture risk and susceptibility to osteoporosis. In GWA studies, 76 SNPs have been significantly associated with BMD in populations of European origin. Sixty four of these are in or near 50 different genes and 12 are intergenic. Many of the genes are represented in the RANKL/OPG, estrogen endocrine and WNT signaling pathways. GWA studies related to bone mineralization phenotypes have not been performed on non-admixed African populations. Average BMD distributions vary widely among populations, being significantly higher in Africans when compared to Europeans, and with intermediate levels in Asians. The aim of this study was to determine which previously associated SNPs have allele frequencies that reflect a cline related to population BMD phenotype and may, therefore, be expected to contribute to BMD in different populations. We used HapMap data to examine this trend. Eight populations were selected from three geographic regions: Africa (YRI, LWK, MKK); Europe (CEU, TSI); and Asia (CHB, CHD and JPT). The 76 SNPs were clustered according to allele frequencies among populations from the three geographic regions and separated into 6 clusters. One cluster showed the expected cline (risk allele for low BMI with the highest frequency in Europeans and the lowest in Africans), and contained 18% of the SNPs. Four of the SNPs in this group showed at least moderate population differentiation (Fst above 0.05) and included one BMD-associated SNP close to the *OPG* gene; rs2062377, with frequencies of 0.47 (European), 0.29 (Asian) and 0.07 (African). Interestingly, 25% of the SNPs showed the opposite cline, including the other four *OPG* associated SNPs. LD structure in and around the *OPG* gene is complex in Europeans and, as expected, is associated with smaller haplotype blocks in Africans (Haploview). The challenge remains to identify the causative variants in LD with the associated SNPs and studies in African populations may provide additional insight. In cases where there are distinct population differences in the prevalence of complex phenotypic traits, genome analysis of data in the public domain (HapMap and 1000 Genomes) may help to identify likely causative genetic determinants across populations.

826T

Combined haplotype sharing analysis and QTL mapping for identification of disease risk alleles. *H. Sadeghi, S. Müller, A. Recke, S. Möller, U. Samavedam, D. Zillikens, SM. Ibrahim, RJ. Ludwig.* Dermatology, UK-SH Universitätsklinikum Schleswig-Holstein, Luebeck, Schleswig-Holstein, Germany.

Epidermolysis bullosa acquisita (EBA) is an autoimmune blistering skin disease, characterized by antibodies to type VII collagen (COL7). Currently, little is known about the genetic basis of EBA. Therefore, we used a multi-step approach to identify genetic loci controlling autoantibody-induced tissue injury (effector phase) in experimental EBA, in mice. First, 16 genetically well characterized inbred strains were tested for EBA susceptibility in an antibody transfer model. A genome-wide association study was then performed using 600,000 SNPs, which are available on public sources. This lead to the identification of 9 quantitative trait loci (QTL) controlling autoantibody-induced tissue injury in experimental EBA. These QTLs were located on chromosomes 1, 6, 8, 9, 11, 12, 15 and 16. For confirmation, antibody-transfer EBA was induced in mice from an advanced 4-way intercross line generated by breeding of 4 different strains (NZM, MRL, BXD2, Cast). Two QTLs located on chromosomes 9 and 12 were confirmed by genotyping the respective single-nucleotide polymorphisms in the mice from the intercross line. Using haplotype-sharing analysis, we then fine mapped QTLs on chromosome 9 and 12 down to 6 Mb (65-71) and 2Mb (92-94) respectively. Overall, we demonstrate that this combined approach allows fast identification of risk alleles.

827T

Integrative Analysis of Genotype and Gene Expression Data from Blood and Sputum Samples Identifies Potential Functional Mechanisms for COPD Genetic Risk Loci. *P. Castaldi[1,3], W. Qiu[1], M. Cho[1,2], J. Riley[4], W. Anderson[5], D. Singh[6], P. Bakke[7], A. Gulsvik[7], A. Litonjua[1,3], D. Lomas[8], J. Crapo[9], T. Beaty[10], B. Celli[2], S. Renard[11], R. Tal-Singer[12], S. Fox[4], E. Silverman[1,2], C. Hersh[1,2].* 1) Channing Laboratory, Boston, MA, USA; 2) Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA; 3) Institute for Clinical Research and Health Policy Studies, Tufts Medical Center, Boston, MA, USA; 4) GlaxoSmithKline, Uxbridge, UK; 5) GlaxoSmithKline, Research Triangle Park, NC, USA; 6) Medicines Evaluation Unit, University of Manchester, Manchester, UK; 7) Department of Thoracic Medicine, Haukeland University Hospital and Institute of Medicine, University of Bergen, Bergen, Norway; 8) Cambridge Institute for Medical Research, University of Cambridge, Cambridge, UK; 9) Department of Medicine, National Jewish Health, Denver, CO, USA; 10) Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA; 11) Division of Pulmonary and Critical Care Medicine, University of Nebraska Medical Center, Omaha, NE, USA; 12) GlaxoSmithKline, King of Prussia, PA, USA.

Background: COPD is a major cause of morbidity and mortality. The 4q24 locus has been associated with pulmonary function and COPD susceptibility in genome-wide association studies in multiple population-based and case-control cohorts. However, it is unclear which gene or genes in this region are driving these association signals. Using genome-wide SNP data and gene expression data from blood and sputum from 131 individuals with COPD, we performed an unbiased screen to identify cis eQTL SNPs in whole blood and induced sputum of individuals with COPD. Based on these results, we further interrogated the 4q24 locus to identify potential candidate genes related to COPD in this region. **Methods:** Gene expression profiles from whole blood and induced sputum samples were generated for 131 subjects with COPD from the ECLIPSE study using the Affymetrix HG Plus 2.0 GeneChip. Genome-wide identification of cis eQTLs was performed using a window of +- 50kb from the transcription start site to define potential cis-acting eQTL SNPs. Multiple comparison adjustment was performed by controlling the false discovery rate at 0.05. Identified eQTL SNPs were then tested for association with COPD susceptibility in 4 COPD case-control studies (NETT, ECLIPSE, Norway Genkols, and in first 1000 subjects of COPDGene). eQTL tests were performed using linear regression adjusting for age, sex, and genetic ancestry, and tests of association using logistic regression adjusting for age, pack-years of cigarette smoking, and genetic ancestry were carried out. **Results:** Cis eQTL analysis in COPD cases identified 10,732 eQTL SNPs in whole blood, and 3,298 eQTL SNPs in induced sputum samples. Fifty-eight percent (1900) of the sputum eQTL SNPs were also cis eQTLs in whole blood. When these eQTL SNPs were tested for association with COPD, rs2656069 and rs1051730 were significantly associated with COPD susceptibility as well as transcript levels for IREB2 and CHRNA3/5 in whole blood and sputum. SNP rs4235415, which has previously been associated with lung function measures, was significantly associated with transcript levels for GSTCD in blood, but not sputum. **Conclusions:** Genetic variants near IREB, CHRNA3/5, and GSTCD have been previously associated with COPD susceptibility and/or pulmonary function. We have demonstrated these same genetic variants are associated with expression levels of these genes, suggesting these loci may affect COPD susceptibility through regulatory mechanisms.

828T

Genome-wide Association Study for Central Adiposity in African Americans. *C.-T. Liu[1], K.L. Monda[2], K.C. Taylor[2], L. Lange[3], A. Adeyemo[4], M. Allison[5], D.M. Becker[6], L.F. Bielak[7], I. Borecki[8], G. Burke[9], C. Carlson[10], M.K. Evans[11], J.N. Hirschhorn[12], E.K. Kabagambe[13], Y. Liu[14], C. Palmer[15], G. Papanicolaou[16], S. Paterl[17], P. Schreiner[18], H. Taylor[19], L.R. Yanek[6], E.W. Demerath[20], L.A. Cupples[1], K.E. North[21], C.S. Fox[22] on behalf of African American Central Adiposity Consortium and the CARe Consortium.* 1) Biostatistics, Boston University SPH, Boston, MA; 2) Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC; 3) Department of Genetics, University of North Caroline, Chapel Hill, NC; 4) Center for Research on Genomics and Global Health, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 5) Family and Preventive Medicine, UCSD La Jolla CA; 6) Department of Medicine, Johns Hopkins School of Medicine, Baltimore MD; 7) Department of Epidemiolgy, School of Public Health, University of Michigan, Ann Arbor, MI; 8) Division of Statistical Genomics Washington University School of Medicine, St Louise MO; 9) School of Medicine Wake Forest University, Winston-Salem, NC; 10) Division of Public Health Sciences, Fred Hutchinson Cancer Research Center,Seattle, WA; 11) Health Disparities Research Section, Clinical Research Branch, National Institute on Aging, National Institutes of Health, Baltimore Maryland; 12) Harvard University, Cambridge MA; 13) Department of Epidemiology, UAB, Birmingham AL; 14) Department of Epidemiology & Prevention, Public Health Sciences, Wake Forest School of Medicine, Winston-Salem, NC; 15) Division of Nephrology University of California, San Francisco Medical School and San Francisco VA Medical Center, San Francisco CA; 16) NHLBI, Bethesda, MD; 17) Case Western Reserve University, Cleveland, Ohio; 18) Division of Epidemiology & Community Health, Minneapolis, MN; 19) University of Mississippi Medical Center, MS; 20) School of Public Health University of Minnesota, Minneapolis MN; 21) Department of Epidemiology and Carolina Center for Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC; 22) NHLBI's Framingham Heart Study and the Center for Population Studies, Framingham, MA.

Background: Central obesity, measured by waist circumference (WC) or waist-to-hip ratio (WHR), is a marker of body fat distribution and a risk factor for type 2 diabetes, hypertension and cardiovascular disease. Although obesity disproportionately affects minority populations, few studies have reported genome-wide association study (GWAS) results in individuals of predominantly African ancestry. **Methods:** We performed gender-specific meta-analyses of GWA data in up to 5975 men and 17580 women of African-American ancestry, derived from 14 cohorts, seeking to identify loci associated with: a) WC and b) WHR-adjusted for BMI. Approximately 2.5 million typed and/or imputed SNPs across the genome were modeled additively using multivariable linear regression models adjusting for age, age2 and data collection center (within each cohort where appropriate) for both traits. Inverse variance weighted fixed effects meta-analysis was used to aggregate results across studies. We also interrogated SNPs within the ±250kb flanking region of previously reported waist-related loci in populations of European ancestry in our WHR-adjusted-BMI meta-analysis results to compare genetic signals across different populations. **Results:** We detected two genome-wide significant associations with WC in men at PCSK1 (chr2, p=3.8 x10-8) and IL1RL2 (chr2, p=4.0x10-8) and one genome-wide significant signal with WHR in men at MYH11 (chr16, p=3.9 x10-8). None of these three loci were nominally significant in women (all p>0.09). Mutations in PCSK1 have been previously reported to cause monogenic obesity. SNPs in IL1R12 have been demonstrated in association with osteoarthritis, a sequela of obesity. Finally, MYH11 has been previously associated with smooth muscle function and familial thoracic aortic aneurysm. Seven out of fourteen previously reported WHR loci, including GRB14, ADAMTS9, and LY86, were significant in women only, after accounting for the number of interrogated independent SNPs. **Conclusion:** In 23555 samples of African-American ancestry, we identified three genome-wide significant loci in men only. While replication efforts are still underway, these findings demonstrate the similarity of the genetic architecture for WC and WHR among populations of European and African-American ancestry.

829T

A unifying framework for analyzing rare variant quantitative trait associations in selected samples: application to sequence data. *D.J. Liu[1,2], R.C Banuelos[1,2], S.M. Leal[1,2].* 1) Dept Statistics, Rice Univ, Houston, TX; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

A large number of exome sequencing studies are currently being performed to understand the etiology of quantitative traits (QT) due to rare variants. In order to reduce sequencing cost and improve power, sequencing individuals with extreme QT is often applied. In addition, many publically available well-phenotyped cohorts (e.g. NHLBI ESP-the exome sequencing project) can be used and jointly analyzed to improve power. Although a number of methods have been developed for analyzing rare variants, they have a variety of limitations: 1.) Most of the methods do not have a valid likelihood model. Therefore it is not possible to adjust for complicated sampling schemes, which are crucial for making powerful inferences, estimating genetic parameters and interpreting identified associations. 2.) Most methods were developed exclusively for the analysis of binary traits. Although QT in selected samples can be dichotomized, ignoring QT values can result in a loss of power. 3.) Some leading methods e.g. the weighted sum statistics (WSS) do not allow for the control of confounders, such as sequence coverage depth and population substructure. Failure to control for confounders will lead to spurious associations which cannot be eliminated by permutations. To overcome these limitations, a unifying method was developed for mapping and interpreting QT associations (UNI-QTL) which can be applied to any studies with known sampling designs. Using a rigorous regression model based likelihood framework, UNI-QTL extends most existing rare variant analysis methods. Testing of associations can be performed using efficient score statistics, which are statistically optimal and numerically stable. Properties of UNI-QTL and the power for different extended rare variant tests were extensively evaluated using datasets generated under realistic population genetic and complex trait models. A sequence dataset from the Dallas Heart Study was also analyzed. For the analysis of QT we show that the power of almost all rare variant tests can be consistently improved, when they are extended in UNI-QTL and applied to analyze full QT information. Although there does not exist a uniformly most powerful test, the extended WSS, variable threshold (VT), and kernel based adaptive cluster (KBAC) methods perform well under most scenarios. With large scale implementation of next generation sequencing, UNI-QTL will be extremely important for mapping rare variant/QT associations using sequence data.

830T

Million Veteran Progam (MVP): Department of Veterans Affairs (VA) Longitudinal Cohort for Genomic and Epidemiologic Research. *S. Muralidhar[1], R. Przygodzki[1], M.J. Gaziano[2,3], J. Concato[4,5], L. D'Avolio[2,7], L. Fiore[2,6], M. Brophy[2,6], T.J. O'Leary[1].* 1) Office of Research & Development, Department of Veterans Affairs, Washington, DC; 2) VA Boston Healthcare System, Boston, MA; 3) Harvard Medical School, Boston, MA; 4) VA Connecticut Healthcare System, West Haven, CT; 5) Yale University School of Medicine, Yale, CT; 6) Boston University, Boston, MA; 7) Brigham and Women's Hospital, Boston, MA.

Background: The goal of the Million Veteran Program (MVP) is to develop a longitudinal cohort of Veterans to study the role of genes, environment, lifestyle and military experience on health. This landmark undertaking will enroll as many as 1 million users of the Veterans Healthcare Administration (VHA) over the next 5-7 years. VHA is the largest integrated healthcare and research system in the United States, with an electronic medical record system and about 7 million enrollees. Veterans who volunteer to participate are asked to: provide a blood sample for future, unspecified research; complete health-related surveys; provide ongoing access to their electronic medical record; and allow for future contact.

Methods: The primary recruitment method is a centralized model; Veterans are invited to participate in MVP via mailings. If interested, they are asked to complete an enclosed Baseline Survey on health, medical history, family history, lifestyle and military experience. Upon receipt of the completed survey, a study visit is scheduled at the local VA hospital, during which the informed consent process, HIPAA authorization, and a blood draw takes place. Participants also receive an optional, more detailed Lifestyle Survey. Veterans can also enroll by calling the MVP Information Center and scheduling a study visit, or as walk-ins at the hospitals. The blood samples are shipped to the VA Central Biorepository and processed to extract 10-15 μg of DNA and to store two 1ml aliquots of plasma and one aliquot of buffy coat; samples are stored at -80 °C. Phenotype and biosample data as well as analysis results are stored in the Genomic Information System for Integrated Science (GenISIS) and will be made available to researchers in a secure, scientific computing environment.

Results: The pilot phase was launched in January 2011 at nine Vanguard sites. An additional eighteen sites will begin enrollment in June 2011. Plans are underway to expand to 50 VA medical centers within a year. Recruitment rate at the Vanguard sites is 19%. Results of the recruitment strategies and the first application for a genomic study will be discussed.

Conclusion: The early recruitment rate indicates feasibility of meeting the enrollment target and developing one of the largest cohorts/databases of its kind in the world for genomic and epidemiologic research.

831T

Determining the genomic factors of sickle cell disease severity among West African children. *J. Quinlan[1], Y. Idaghdour[1], E. Gbeha[1], A. Sanni[2], M.C. Rahimy[3], P. Awadalla[1].* 1) Ste-Justine Research Center, University of Montreal, Montreal, Quebec, Canada; 2) Biochimie et Biologie Moleculaire, Universite d'Abomey-Calavi, Cotonou, Benin; 3) National Sickle Cell Disease Center, Centre National Hospitalier et Universitaire de Cotonou, Universite d'Abomey-Calavi, Cotonou, Benin.

Although sickle cell disease (SCD) is classically thought to be a simple mendelian disease, there is considerable heterogeneity in the clinical expression of the disease, with little understanding on the mechanisms underlying this. By integrating quantitative data from many biological sources, we took a systems biology approach to investigate the factors that explain variation in disease severity and clinical status of 400 SCD patients aged 6 months to 5 years with different degrees of disease severity from a cohort of 2000 children at the National Sickle Cell Center in Cotonou, Benin. We recruited children diagnosed with various SCD severities, plus ethnically matched controls, and obtained peripheral blood samples in order to perform gene expression profiling, genome-wide genotyping, and hematological profiling. Gene expression profiles were compared using various statistical approaches that take into account disease status and various clinical and biochemical covariates, including complete blood count and hemoglobin levels. Unsupervised clustering analysis of the data identified distinct groups of individuals with similar profiles. Principal component (PC) analyses of the transcriptomes indicates that approximately 40% of the variation in the data is contained in the first three PCs. Variance component analysis shows that SCD phenotype and clinical state explain the majority of the observed variance. An analysis of covariance identified several hundred differentially expressed transcripts that are associated with disease status. Out of the differentially expressed transcripts that were significant, we identified enrichment in biological pathways associated with clinical categories. These differentially expressed transcripts were used in a stepwise linear discriminant analysis in order to identify transcripts that can accurately identify each category. “Leave one out” cross validation confirmed these results. Finally, given our large cohort, and coupled with data from a control sample in the same region, we performed the first genome wide association of gene expression traits for SCD and identified several hundred expression single nucleotide polymorphisms (eSNPs). Our study identifies specific transcripts associated with SCD status and demonstrates the power of joint analysis of gene expression and genotyping data in systems biology.

832T

Who will be the biggest loser? GWAS reveals clues about response to gastric bypass. *E. Rinella[1], C. Still[2], Y. Shao[3], G.C. Wood[2], X. Chu[2], G. Gerhard[2], H. Ostrer[1].* 1) Human Genetics Program, New York University Langone Medical Center, New York, NY; 2) Geisinger Obesity Research Institute, Geisinger Clinic, Danville, PA; 3) Division of Biostatistics, New York University Langone Medical Center, New York, NY.

Purpose: Bariatric surgery offers an effective treatment for obesity and obesity-related complications when other interventions fail. However, despite its success, many patients do not achieve and/or maintain sufficient weight loss following bariatric surgery. Roux-en-Y Gastric Bypass (RYGB) is one of the most effective bariatric procedures but does have potentially significant short and long-term risks. Our ultimate goals are to identify clinical and genetic factors that are associated with favorable and unfavorable outcomes following bariatric surgery. In order to better understand the long-term response to RYGB we designed a case-control genome-wide association study (GWAS) to examine the genetic variation between those that lost the least and those that lost the most weight as a result of RYGB. **Method:** We studied a discovery cohort of 175 patients receiving RYGB intervention for obesity (BMI > 35). 86 cases and 89 controls were selected based on those who had the least excess body weight loss (< 50% EBWL-regainers) and those who lost the most (>90% EBWL-losers), respectively, at 2 years post-surgery. DNA from whole blood was used for genotyping using the Affymetrix 6.0 SNP array. Significant SNPs were tested in a replication cohort of 333 RYGB patients, 164 regainers and 169 losers. **Results:** Confirming previous studies, we found that pre-surgery BMI was strongly associated with response to RYGB, those at <50 kg/m^2 losing the most weight and those at >50 kg/m^2 losing the least amount of excess body weight. We also identified 111 SNPs in the discovery cohort, many clustering within the same genes, whose frequencies were significantly different between regainers and losers (χ^2-test p<10-4). Linear regression of percent excess body weight remaining at 2 years post-surgery revealed 14 SNPs that were validated in the replication cohort (p<0.05). These SNPs cluster in or near several genes with potential biological relevance including a gene previously shown to be associated with antipsychotic drug related weight gain, appetite, adiposity and energy balance. **Conclusions:** We conclude that there are genetic components associated with response to RYGB and that further studies are needed to reveal the function of these SNPs and their predictability in determining who should undergo such procedures.

833T

Does Predisposition to Unhealthy Eating Behavior Share Genetic Variants with Genes Influencing Obesity? A Genome-Wide Association Study on Eating Behavior and Obesity Indices. *Y. Yang[1], D.H. Lee[1], M. Lee[1], Y.M. Song[2], J. Sung[1].* 1) Department of Epidemiology, School of Public Health and Institute of Health and Environment, Seoul National University, Seoul, South Korea; 2) Department of Family Medicine, Samsung Medical Center, and Center for Clinical Research, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, 50 Ilwondong, Gangnamgu, Seoul 135-710, South Korea.

Backgrounds: Growing evidence indicates that eating behavior may be a predictor of obesity independent of dietary measurements. Both behavior and body mass index (BMI) have genetic influences, and recent genome-wide association (GWA) studies identified a list of genetic variants related to obesity. The specific hypothesis of the study is that some of the single nucleotide polymorphisms (SNPs) influencing obesity may be mediated by eating behavior. The authors attempted to test the hypothesis to explore shared genetic variants, by comparing the GWA studies on DEBQ measures and obesity traits. **Method:** This study involves 3077 adult individuals (1217 men) who have participated in the Healthy Twin study Korea. DEBQ measured three domains of eating behavior; restrained eating(RE), emotional eating(EmE) and external eating(ExE). Obesity level was measured by; Body Mass Index (BMI), waist circumference (WC), total body fat (toFat%) and trunk fat (trFat%) proportion measured by Dual-energy X-ray absorptiometry (DXA) . The Affymetrix GeneChip Array version 6 (1 million SNPs) platform was used for generating genetic marker information. The GWA study was conducted by combining p-values of transmission test (FBAT) and population mean effects using founders (PLINK). We listed obesity-related genes from HuGE Navigator website (www.hugenavigator.net), and compared the GWA findings for DEBQ and obesity indices to the list. **Results;** Each of DEBQ domains and obesity index showed significant genetic influences, with heritability ranging from 0.17(EmE) to 0.30(RE) for DEBQ and from 0.53(WC) to 0.65(BMI) for obesity indices. From GWA analysis on DEBQ measures several candidate loci were identified; “RE” 11q23.3, p=1.27E-6; “EmE” 18q23, p=8.33E-7; and “ExE” 9p24.1, 15q22, p=7.42E-6. Candidate loci associated with obesity indices were; “BMI” 10p14, p=1.52E-7; “toFat%” 18q11.2, p=2.75E-6; “trFat%” CAP2 on chromosome 13, p=1.72E-6; and “WC” 15q24, p=1.03E-7. We compared associated genes of the most significant SNPs of DEBQ measures to obesity-related genes list; several genes were shared; “RE” APOB (p=4.8E-5) and RGS6(p=8.6E-5), “EmE” FAAH(p=1.3E-5), “ExE” ADRA2A (p=1.5E-5). **Conclusion:** GWA study on DEBQ and obesity indices suggested that a part of genetic variants are shared by certain unhealthy eating behavior and obesity.

834T

A Genome-wide Screen of Gene-Gene Interactions for Osteoporosis Susceptibility. *F.P. Zhao[1], T.L. Yang[2], Y. Guo[2], H. Shen[1], Q. Tian[1], H.W. Deng[1].* 1) Center for Bioinformatics and Genomics, Department of Biostatistics and Bioinformatics, School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA 70112, USA; 2) Xi'an Jiaotong University, Xi'an 710049, P. R. China.

Osteoporosis is a typical complex disease determined by both actions and interactions of multiple genes. Bone mineral density (BMD) is the most important risk phenotype for osteoporosis. Recent genome-wide association studies (GWAS) identified several genes for their independent effects on osteoporosis. However, no studies have been performed to identify gene-gene interactions in GWAS analyses. Here, we performed a genome-wide gene-gene interaction analysis for osteoporosis susceptibility in two independent US Caucasian populations. Sample 1 consisted of 916 unrelated subjects with extreme hip BMD values (458 high and 458 low) selected from a cohort of 2286 subjects. Sample 2 consisted of 400 unrelated subjects with extreme hip BMD values (200 high and 200 low) selected from 1000 subjects. Combing results from these two samples, we detected one SNP-interaction pair (rs8047431 and rs1975460) significantly associated with hip BMD after FDR adjustment for multiple testing, with the original P value of 1.88E-10. SNP rs8047431 is located at WW domain-containing oxidoreductase (WWOX) gene, which encodes a tumor suppressor. WWOX functionally suppressed RUNX2 transactivation ability in osteoblasts, and Wwox knockout mice exhibited a delay in bone formation and developed metabolic bone disease. The evidence suggested that WWOX might be a potential candidate gene for BMD variation. SNP rs1975460 is located at 16q23 and the nearest gene is ribosomal protein L36a pseudogene (RPL36AP46). In addition, we detected some other interaction pairs of SNPs showing suggestive association with osteoporosis susceptibility (P = 3.77E-8 to 4.85E-9), such as SNPs in LNX1 and TMEM132D, and SNPs in SOX6 and HAUS6. In summary, our findings suggest that gene-gene interactions may play a role in osteoporosis susceptibility.

835T

Genome-wide Association Study of Early-Onset Myasthenia Gravis: Identification of TNIP1 and evidence for multiple autoimmune loci. *R. Kosoy[1], M.F. Seldin[1], A. Lee[2], J. Lamb[3], N. Wilcox[4], F. Piehl[5], R. Pirskanen[5], J.J.G.M. Verschuuren[6], M. Titulaer[6], J. Sussman[3,7], D. McKee[3,7], A. Maniaol[8], A. Elsais[8], C. Tallaksen[8], B.A. Lie[8], H.F. Harbo[8], B. Tackenberg[9], M. Pütz[9], H-J. Garchon[10], A. Melms[11], L. Hammarstrom[5], P.K. Gregersen[2], Myasthenia Gravis Genetics Consortium.* 1) Dept Biological Chemistry, Univ California, Davis, Davis, CA; 2) The Robert S. Boas Center for Genomics and Human Genetics, Feinstein Institute for Medical Research, North Shore LIJ Health System, Manhasset, NY; 3) Centre for Integrated Genomic Medical Research, Manchester Academic Health Science Centre, University of Manchester, Manchester, UK; 4) University of Oxford, Oxford, UK; 5) Karolinska Institutet, Stockholm, Sweden; 6) Leiden University Medical Center, Leiden, Netherlands; 7) Greater Manchester Neuroscience Centre, Salford Royal Hospital; 8) Departments of Neurology and Medical Genetics, Oslo University Hospital, Oslo, Norway; 9) Klinik für Neurologie, Philipps-Universität, Marburg, Germany; 10) INSERM U1016, University Paris Descartes, Paris, France; 11) Tübingen University Medical Center, Tubingen, Germany.

We report the first genome-wide association study for myasthenia gravis (MG). MG is a relatively uncommon disease (prevalence 1-2/10,000) characterized by antibodies directed against the muscle motor end-plate and results in severe muscle weakness. Previous studies have reported associations with HLA and PTPN22. To reduce potential genetic heterogeneity our study focused on early-onset MG (EOMG) in European patients meeting restricted entry criteria (anti-AChR antibody +, onset age >10 and <40 without known thymoma or < 45 with thymic histology showing hyperplasia without thymoma). The study was conducted in two phases using EOMG matched 1:4 with population controls selected from >8900 subjects using principle components analyses (discovery, 400 EOMG cases, 1600 controls; replication, 249 cases, 996 controls). Association tests (adjusted for residual substructure) for HLA, PTPN22 and TNIP1 met replication criterion with the following combined results for the strongest associated SNPs (HLA, rs7750641, p = 1E-92, OR = 6.3; PTPN22, rs2476601, p = 4E-10, OR = 1.71; TNIP1, rs4958881, p = 3E-10, OR = 1.73). For TNIP1 (TNFAIP3-interacting protein 1), a novel association for EOMG, multiple SNPs achieved thresholds for association (p < 5E-08) and each associated alleles was found on a common haplotype (p values > 0.05 after conditioning on rs4958881). The TNIP1 OR is the largest reported for any autoimmune disease and the haplotype was the same as that for SLE and disparate for psoriasis. For HLA, most of the association was also due to a single haplotype (peak near MHC Class 1 region, and part of HLA B 8.1 haplotype). However, after conditioning on rs7750641 a second associated haplotype was uncovered (including rs1265109, p =0.49 prior to conditioning, p = 4.31E-10 after conditioning). The peak association for this haplotype was near HLA class I and suggested definition of a more limited region of ~150 kb. Other autoimmune disease susceptibility loci were also over-represented in SNPs with suggestive evidence for association (7 of 151 loci with p values < 5E-04). Of 71 autoimmune loci examined, STAT4, IKZF1, IRF5, NKX2-3, ORMDL3, CD226 and PTTG1 met Bonferroni corrected criterion (p < 7E-04). A SNP for TRAF3, a gene critical to multiple B and T cell signaling pathways also showed suggestive association (rs4906269, p = 2.25E-05). Together these data suggest that a unique combination of autoimmune loci and haplotypes are critical to the etiopathogenesis of EOMG.

836T

Association of SLE specific susceptibility alleles with ANA production or transition to disease. *P. RAJ[1], DR. KARP[1], QZ. LI[1], P. DOSHI[1], N. OLSEN[1], K. MOSER[2], JA. KELLY[2], EK. WAKELAND[1].* 1) IMMUNOLOGY, UT SOUTH WESTERN MEDICAL CENTER, UNIVERSITY OF TEXAS, DALLAS, TX; 2) Oklahoma Medical Research Foundation, 825 North East 13th Street, Oklahoma City, Oklahoma 73104, USA.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of IgG autoantibodies directed towards nuclear antigens. The production of IgG anti-nuclear autoantibodies (ANA) is virtually uniform in SLE patients, and found in about 5% of the normal population. Genetic predisposition is a strong component of SLE susceptibility, and more than 30 SLE susceptibility loci have identified and replicated. Recently, a consortium of investigators produced a SNP array consisting of 196,524 SNPs that provide a dense coverage of susceptibility loci associated with a variety of autoimmune diseases, including SLE. To assess the role of individual SLE susceptibility loci in ANA production versus the transition to disease pathology, we assessed the association of SLE susceptibility loci in 372 European ANA negative normal controls (ANA-), 206 ANA positive normal controls (ANA+), and 134 SLE patients (SLE). These samples were obtained from the DRADR collection at UT Southwestern and the LRR collection at the Oklahoma Medical Research Foundation. Association analysis comparing ANA- and SLE patients identified 141 SNPs in 14 SLE-associated genes with chi-square p value ≤10-5. Association analyses were performed which compared these SNPs between ANA- and ANA+ controls, and separately between ANA+ and SLE patients. The results indicated that genetic variations in genes predominantly affecting pathways in adaptive immunity, such as BLK, LYN, and TNFSF4, may predispose to autoantibody production, and hence may be involved in the loss in immune tolerance that mediates the transition of normal individuals from ANA- to ANA+. On the other hand, variations in TNIP1, XKR6 and UBE2L3 are significantly associated with SLE in comparison to either ANA+ or ANA- individuals, which suggests that these variations may be functional drivers of the transition from ANA positive to full SLE phenotype. Other genes i.e. STAT4, VDR, IRAK1 and MECP2 appeared to be involved in both, ANA production as well as progression to SLE. We are currently expanding this analysis to ultimately encompass > 4000 patients and controls. These results, though performed on a small set of samples, indicate that individual susceptibility alleles contributing to SLE may specifically influence the initial autoantibody production or the subsequent transition from high ANA to full SLE disease.

837T

Direct assessment and validation of allele-specific transcription factor binding in the human genome. *A. Schiavi[1,2], V. Adoue[1,2], S. Busche[1,2], B. Ge[2], T. Kwan[1,2], T. Pastinen[1,2].* 1) Human Genetics, McGill University, Montreal, Quebec, Canada; 2) McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, Canada.

Characterization of human genetic variation, which effects gene expression, has focused on expression quantitative trait loci (eQTL) mapping; however, direct assessment of *cis*-regulatory variation necessitates allele-specific approaches. Measuring allelic expression (AE) on a genome-wide scale is more powerful as environmental and trans-acting influences are minimized. Results indicate that allele-specific differences among transcripts within an individual can affect up to 30% of loci. These variants can be identified by mapping differences in AE on Illumina Omni-1M/2.5M BeadChips. Over 50% of population variance in AE is explained by mapped *cis*-rSNPs. Studies show that these *cis*-rSNPs have been implicated in differences in transcription factor binding, suggesting a strong genetic component that needs to be further investigated. Combination of multiple GWAS datasets, eQTLs in osteoblasts, AE in primary fibroblasts and DHS-seq from mesenchymal stem cell lineage (MSC) allowed determination of a single SNP >200kb upstream of the *WNT4* gene. Consistent with a cell type restricted chromatin signal, we observed the regulatory association only in fibroblasts, indicating that the SNP alters gene regulation in MSC lineage and therefore is directly relevant to bone disease. Numerous loci contributing to bone mineral density and osteoporosis risk have been described by GWAS; however, the underlying biological effect of many of these variants remains unknown. Bioinformatic analysis of the *cis*-rSNP indicates that it alters a *SNAI1* binding site. We have shown in vitro allele-specific EMSA signals in nuclear extracts from MSC lineage and are now pursuing in vivo validation of *SNAI1* binding in living cells by carrying out ChIP with allele-specific readouts, as well as, *SNAI1* knockdown by RNAi with monitoring of its consequences in *WNT4* allelic expression phenotype. Consistent inhibition (85%) of *SNAI1* using RNAi in transfection studies has been observed. We are pursuing allelic expression imbalance assessment of *WNT4* in cells heterozygous for this *cis*-rSNP and treated with efficient *SNAI1* RNAi. These approaches will be generically extended to other allele-specific transcription factor binding and the consequences of these gene knockdowns will be monitored in a genome-wide manner. In progress is work on the *NF2B* transcription factor that has been shown to be involved in the immune response and where the *NF2B* motif is enriched in lymphoblastoid cell lines.

838T

Evaluation of oral samples and tracheal fluid collection from premature newborns as a source of DNA for genotyping. *J. Niles[1], V. Biran[2], R.M. Iwasiow[1].* 1) DNA Genotek Inc., Ottawa, Ontario, Canada; 2) Service de Pédiatrie et Réanimation Néonatales Hôpital Robert Debré, APHP, Université Paris 7, France.

Newborn screening is an important procedure in which newborn infants are screened for a list of treatable diseases. The standard procedure is to sample whole blood from the heel of the infant and blot it on a specially designed filter paper. Recently it has been suggested that screening dried blood spots for some infections, such as congenital cytomegalovirus (CMV), may not be suitable and that other specimen types, such as oral sample and tracheal fluids, should be explored. In the current study oral sample and tracheal fluid, both non-invasive collection methods, were evaluated for suitability of DNA collection from premature newborns for genotyping purposes. The oral samples were collected using sterile rayon swabs to collect sample from the mouth of the premature newborn and cutting the heads of the swab into an Oragene.DNA device containing preservation solution. Paired tracheal fluid was also collected by rinsing a tracheal tube with saline (between 300 and 500 μL) and added to the Oragene.DNA collection devices. The samples were taken from premature newborns as young as 24 weeks. Oragene.DNA is a non-invasive collection device that is intended for collection and stabilization of DNA from saliva for extended periods at room temperature, thus enabling transport through regular mail. The performance of the extracted gDNA was assessed by evaluating different markers using PCR based genotyping methods. The results indicate good quality and quantity of DNA can be extracted from both tracheal fluids and oral samples collected into Oragene.DNA devices. The oral samples had an average concentration of 8.90 ng/μL ranging between 4.52 and 23.23 ng/μL and tracheal fluid average concentration was 10.23 ng/μL ranging between 0.99 and 19.02 ng/μL. The average total yields for these samples were 1.78 μg (oral) with a range of 0.90 to 4.65 μg and 2.49 μg (tracheal fluid) with a range of 0.23 to 4.26 μg. Each sample demonstrated 100% genotyping call rates with a 100% concordance of results between oral and tracheal fluid samples.

839T

Rare functional variants in CFH, LIPC, and TLR3 segregate with severe bilateral AMD in multiplex families negative for known risk alleles. *J. Ayala-Haedo[1], A.G. Martinez[1], W. Cade[1], I. Konidari[1], A. Agarwal[2], S.G. Schwartz[3], J.L. Kovach[3], G. Wang[1], W.K. Scott[1], J.L. Haines[4], M.A Pericak-Vance[1].* 1) Hussman Institute Human Genomics, Univ Miami Miller Sch Med, MIAMI, FL; 2) Vanderbilt Eye Institute, Vanderbilt University Medical Center, Nashville, TN; 3) Bascom Palmer Eye Institute, University of Miami School of Medicine, Miami, FL; 4) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN.

Age-related macular degeneration(AMD)has multiple associated genetic risk factors. Of these, gene variants in the CFH and ARMS2 loci are the most prevalent and strongest risk factors. However, some individuals negative for susceptibility variants in CFH and ARMS2 still develop severe disease (bilateral grade 5 or neovascular AMD). The purpose of this study is to identify new, potentially damaging and rare variants in loci previously associated with AMD using next generation whole-exome sequencing of families with multiple AMD cases. We selected 4 multiplex families for our study. Each family was composed of at least two affected siblings; families had two, three or five affected individuals. At least one affected individual with bilateral neovascular AMD in each family was negative for both the CFH402H and ARMS69S risk alleles. In addition, two families had one individual with the known genetic risk factors in CFH and ARMS2 without disease manifestation. Exome capture was performed using Agilent SureSelect and sequencing done on the Illumina HiSeq 2000 platform. Sequence alignment and variant calling were carried out using MAQ and variants were annotated with SeattleSeq. We focused primarily on missense, splice junction, frameshift and nonsense mutations in 48 loci previously reported as associated with AMD. Variants were filtered using dbSNP and Hapmap databases assuming these multiplex families have novel and possibly family-specific variants that follow Mendelian inheritance. We found 6,137 novel and rare variants overall and 11 rare and probably damaging variants in 8 genes previously associated with AMD that were only present in the 12 cases and absent in the 3 controls. We confirmed by Sanger sequencing two rare variants in CFI, LIPC and four variants in CFH, HCMN1, CX3CR1,TLR3 predicted to probably be damaging by the Polyphen program that may potentially have an influence on disease pathogenesis. Of particular interest is the potentially damaging gene variant in the CFH gene found in all affected individuals of one family that are negative for the primary CFH genetic risk factor and the variants in LIPC and TLR3 that were shared by multiple families. By applying whole exome sequencing to our four multiplex families, we identified several potential damaging and rare variants in previously reported loci offering an interesting application of next generation sequencing (NGS) in discovering additional genetic factors contributing to AMD pathogenesis.

840T

Using Genomic Markers to Stratify Risk for Prostate Cancer: A Case Study and Literature Review. *J. Davies[1], A. Lionel[2, 3], L. Velsher[1], R. Singal[1, 4], J. Aw[1].* 1) Medcan Clinic, Toronto, Ontario, Canada; 2) University of Toronto, Toronto, Ontario, Canada; 3) The Hospital for Sick Children MaRS Centre, Toronto, Ontario, Canada; 4) Toronto East General Hospital, Toronto, Ontario, Canada.

Current guidelines for prostate cancer screening are controversial and largely inconsistent across different countries. This is mainly due to suboptimal value of PSA alone as a screening tool as well as the indolent nature of prostate cancer in many men. There are also inconsistencies in criteria used for prostate cancer biopsies. Recent GWAS studies have implicated several SNP loci in prostate cancer risk. Patient genotypes at these loci could potentially provide predictive information beyond that of PSA and could thus be clinically relevant in helping classify patients into different levels of cancer risk. Here, we review previous studies of the clinical utility of SNP genotype data in prostate cancer screening, and go on to further investigate this issue in a new clinical dataset. The dataset comprises 400 men over the age of 35 from the Medcan Clinic, who underwent Personal Genome Testing, which includes a detailed review of their family history, results from Navigenics' genome-wide SNP microarray test (including 9 specific SNP markers associated with prostate cancer risk) and in-person genetic counseling. In addition to genomic and family history data, we also have access to phenotype data from each patient's annual health assessment at Medcan, including PSA, review of genitourinary symptoms and digital rectal examination (DRE) results. We will analyze this unique combination of clinical, genomic and family history data, particularly in the context of individuals who are not known to have prostate cancer. Medcan uses a comprehensive screening algorithm for prostate cancer prediction (nomogram), but this does not currently use SNP genotype data. By incorporating this data into the nomogram, we hypothesize that we will obtain additional predictive value for categorizing patients into low/high risk screening groups. To test this hypothesis, we will systematically compare the relative contribution of the SNP genotypes with those of risk factors currently part of the nomogram, such as PSA, family history and DRE. This study will help to assess the clinical utility of using SNP genotype information as an early screening tool for prostate cancer to determine eligibility for additional surveillance and/or biopsy. This information is particularly useful as the cost of whole genome sequencing continues to decline, thereby increasing feasibility of using genetic markers for risk prediction on a population screening level.

841T

Genome-wide association study for the personality trait in Korean women. *H. Kim[1], S. Kim[1], C. Hong[2], E. Lee[2], Y. Sung[3], H. Chung[4], J. Lee[2], H. Kim[1].* 1) Dept Biochem, Sch Med, Ewha Womans Univ, Seoul, Korea; 2) Center for Genome Science, Korea National Institute of Health, Korea Centers for Disease Control and Prevention, Seoul, Korea; 3) Dept Internal Medicine, Sch Med, Ewha Womans Univ, Seoul, Korea; 4) Dept Obstetrics & Gynecology, Sch Med, Ewha Womans Univ, Seoul, Korea.

Personality traits are heritable components, influence major life outcomes and strongly link to psychiatric disorders. To identify genetic factors influencing personality traits, we performed a genome wide association study in 1,119 individuals from the Young Women cohort constructed with the PCOS study in Korea. Participants were between the ages of 17 to 40 years old. Personality traits were measured by the Korean version of the Revised NEO Personality Inventory (NEO-PI-R), items measure of the five dimensions of personality (Big5 personality). The Korean version of NEO-PI-R showed good psychometric properties: The factor structure replicated the American normative structure; internal consistency reliabilities ranged from 0.75 to 0.86. Participants' scores on Neuroticism, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness were tested for genome-wide association with 636,870 autosomal SNPs after Quality Control. Four newly identified loci were strongly associated with variation in Openness (P value from 9×10^{-8} to 9×10^{-9}). The newly identified loci, along with several additional loci with suggestive associations, were involved in important neuronal processes. Although none of the SNPs for the other dimensions except Openness to Experience did not achieve genome-wide significance (P value < 7.85×10^{-8}), several strong signals within or near genes previously implicated in psychiatric disorders or behavior disorders were found. Our results are the first significantly positive report of genome-wide association study for Big5 personality traits and meaningful because the interval of age is narrow, though the small sample size. These variants can be expanded the insight for influence of genetic factors on human personality as well as personality disorder, psychiatric disorder and other behaviors. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0026606) and Center for Genome Science, Korea, National Institute of Health research contract (4845-301).

842T

Genome-wide Association Study of Systemic Lupus Erythematosus Specific Autoantibodies to Sm B', Sm D1 and 60 kD Ro Peptide Epitope in European Americans. *C.P. Lin[1], I. Adrianto[1], J.A. Kelly[1], K.M. Kaufman[1,2,3], J. Anderson[1], L. Curley[1], S.B. Glenn[1], J.B. Harley[4,5], P.M. Gaffney[1], K.L. Moser[1,2], J.A. James[1,2], C.G. Montgomery[1].* 1) Arthritis and Immunology, Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) College of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 3) US Department of Veterans Affairs Medical Center, Oklahoma City; 4) Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 5) U.S. Department of Veterans Affairs Medical Center Cincinnati, Cincinnati, OH.

Systemic lupus erythematosus (SLE) is a prototypic systematic autoimmune disease characterized by multi-organ-system involvement and the production of autoantibodies that can lead to tissue inflammation, destruction and even end-organ damage. Further, studies of SLE support the contribution of environmental exposure to the development of disease. Multiple studies have found the presence of temporal sequence of specific immune targets of autoantibodies years prior to clinical diagnosis and in the early years following diagnosis. Curiously, these autoantibodies are closely related to the antibodies that arise in some lupus patients to viral infection, specifically Epstein-Barr Virus (EBV) nuclear antigen 1 (EBNA-1). To better determine how genes influence the production of these early autoimmune structures and how that response might be related to infection, we conducted a genome-wide association study (GWAS) of the three initial antigenic epitopes of anti-Sm B' (PPPGMRPP), anti-Sm D1 (a Gly-Arg repeat) and anti-60kD Ro (amino acids 169-180) in a study population of 703 independent SLE patients of European descent. Tests of association between the quantitative traits and the genetic variants were performed using linear regression under an additive model adjusted for gender and global ancestry. We identified several single-nucleotide polymorphisms (SNPs) exceeding suggestive significance ($p<1x10^{-4}$) for each of the three antigenic epitopes, with the most significant SNP at rs7531957 ($p=1.95x10^{-6}$), rs7576105 ($p=1.06x10^{-5}$) and rs9882901 ($p=1.72x10^{-6}$) for anti-Sm B', ant-Sm D1 and anti-60kD epitopes respectively. SNPs concentrated in previously implicated SLE genes were also evident in the findings, emphasizing the potential power gained by examining more precise phenotypes. Overall, our study presents the first evidence of genetic associations to early lupus autoimmunity. It highlights the importance of examining sub-phenotypes of a complex disease and hence defines the involvement of gene by environment interaction toward initiating specific lupus autoimmune responses.

843T

Rapid Identification of Mutations Responsible for Mendelian Disorders. *J. Majewski, J. Schwartzentruber, E. Lalonde, R. Koenekoop, E. Shoubridge, N. Jabado, D. Rosenblatt, RaDiCAL.* Dept Human Genetics, McGill Univ, Montreal, PQ, Canada.

Exome sequencing has ushered in a new era in Mendelian genetics. In contrast to linkage and positional cloning approaches, which typically involved a large number of individuals and took years to complete, we can now routinely sequence the coding exons of even single individuals affected with rare recessive disorders and identify the causative mutations. In many cases, the process takes around two weeks, starting from sample reception at a sequencing centre, through data analysis, to producing a short candidate list, or a single causative mutation. A number of international and Canadian initiatives now aim at large scale investigation of a variety of Mendelian disorders. At McGill University, we have formed the RADICAL (Rare Disease Consortium for Autosomal Loci), bringing together local clinicians and international collaborators who provide phenotypically well characterized DNA samples from patients affected with rare recessive disorders, and a technology and bioinformatics analysis support group. Our goal is to sequence the exomes of single individuals, small families, or multiple unrelated patients, and identify as many new disease genes as possible. To date, we have sequenced over 50 disorders, and have achieved about 25% success rate in identifying the causative genes using this approach. Here, we describe our detailed approach and successes including finding genes responsible for blindness, mitochondrial disease, methylmalonic acid metabolism, and immune deficiency.

844T

Admixture analyses identifies a region on 19q13 associated with femoral neck bone mineral density in African American women from the Women's Health Initiative-SNP Health Association Resource (WHI-SHARe). *L. Sucheston[1], H. Ochs-Balcom[2], L. Preus[3], N. Johnson[4], F. Zakharia[4], H. Tang[4], N. Risch[5], C. Carty[6], Z. Chen[7], C.B. Ambrosone[1,2], J. Nie[2], M. Neuhouser[6], S. Liu[3], M. Seldin[8], C. Carlson[6], C. Hutter[6], C.L. Thompson[9], R. Jackson[10], C. Kooperberg[6], U. Peters[6], J. Wactawski-Wende[2].* 1) Department of Cancer Prevention and Control, Roswell Park Cancer Institute, Buffalo, NY; 2) Department of Social and Preventive Medicine, State University of New York at Buffalo, Buffalo, NY; 3) Department of Biostatistics, State University of New York at Buffalo, Buffalo, NY; 4) Department of Genetics, Stanford University, Stanford, CA; 5) Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA; 6) Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 7) College of Public Health, The University of Arizona, Tucson, AZ; 8) Department of Biological Chemistry and Medicine, Rowe Program in Human Genetics, University of California, Davis, Davis, CA; 9) Department of Family Medicine, Case Western Reserve University, Cleveland, OH; 10) Division of Endocrinology, Diabetes and Metabolism, College of Medicine, The Ohio State University, Columbus, OH.

STATEMENT OF PURPOSE: With aging, decline in bone mineral density (BMD) places individuals at greater risk for diseases such as osteoporosis. Both genes and environment have been implicated in determining BMD in individuals of European ancestry, however few studies have been conducted among other races or ethnicities, and even fewer in older individuals. In this report, we present results from the first genome-wide admixture scan, to date, of dual-energy X-ray absorptiometry (DXA) derived BMD measurements in African American (AA) post-menopausal women. METHODS: DXA scans were performed on 842 AA women in the WHI-SHARe cohort to obtain BMD measurements of the total body, hip (total, trochanter, femoral neck, inter-trochanter) and spine (L2-L4). The Affymetrix 6.0 platform was used for genotyping with appropriate quality control to remove poor quality samples and SNPs. Four populations, capturing African, Northern and Western European, East Asian, and Native American ancestry, were used as reference groups to estimate global ancestry proportions for each individual. Locus-specific ancestry estimates for 570,282 markers were obtained using an extension of the method implemented in SABER software. This new approach accounts for haplotype structure and long range SNP correlations. Each local estimate of ancestry was included in crude and covariate adjusted genome-wide regression models of BMD phenotypes. Regression was also performed on a set of 3731 highly informative SABER derived local ancestry estimates. RESULTS: For both panels, genome-wide (admixture) significance (p<6e-6) for femoral neck (FN) BMD was achieved on 5.2 Mb region on 19q13. The most significant markers showed a direct association between an increasing number of African alleles and higher FN BMD (p=5.3x10e-7). A direct association between greater hip BMD and increasing copies of African alleles was also seen on 14q21 using the reduced SNP panel. Possible candidate genes in 19q13 include CKM, CYP2A and TGFB1 and ESR2 in 14q21. CONCLUSIONS: CKM/19q13 was previously associated with BMD in a Hispanic population and ESR2/14q21 was associated with BMD in European-American (EA) cohorts. TGFB1 variants have shown no association with BMD in a previous meta-analysis of EAs, however SNPs in this gene have not been tested for association in women with predominantly African ancestry. Our results suggest that there are genomic regions in postmenopausal AA women that contribute to variance in BMD.

845T

DNA methylation in early-stage non-small cell lung cancer. *N. Tonisson[1,8], K. Kirotar[1], T. Vooder[1,2], R. Kolde[3], K. Välk[1], R. Roosipuu[4], L. Milani[5,6], K. Fischer[5,6], M. Solovjova[1], T. Annilo[1], A. Metspalu[1,5,6,7].* 1) IMCB, University of Tartu, Tartu, Estonia; 2) Lung Clinic, Tartu University Hospital, Estonia; 3) Institute of Computer Science, University of Tartu, Estonia; 4) Dept. of Pathology, Tartu University Hospital, Estonia; 5) Estonian Genome Center, University of Tartu; 6) Center for Translational Genomics, University of Tartu, Estonia; 7) Estonian Biocentre, Tartu; 8) United Laboratories, Tartu University Hospital, Estonia.

Background: Despite of intense research, there is still a lack of biomarkers for the reliable detection of early-stage malignant tumours, including non-small cell lung cancer (NSCLC). DNA methylation changes are common in various cancers, and might be used as diagnostic or prognostic biomarkers. Methods: We performed DNA methylation profiling of samples from 48 patients with stage I NSCLC and 18 matching cancer-free lung samples using HumanMethylation 27 microarrays (illumina, Inc.) that cover the promoter regions of more than 14,500 genes. The DNA methylation changes discovered were correlated with gene expression levels and survival analysis performed. Results: We observed hypermethylation of 496 CpGs in 379 genes and hypomethylation of 373 CpGs in 335 genes in NSCLC. Methylation differences were also present in two major histological types of NSCLC, adenocarcinoma and squamous cell carcinoma. A majority of these CpG sites showed the expected inverse correlation between CpG site methylation and gene expression levels. As a result of a survival analysis, we found 10 CpG sites in 10 genes, in which the methylation level differs in different survival groups. Conclusions: We have identified a set of genes with altered methylation in NSCLC and descibed their correlation with gene expression levels. We also found a number of genes that associated with the survival of the patients. These newly-identified marker candidates will need further analysis in order to determine their clinical utility.

846T

Whole exome sequencing of populations of African ancestry. *A. Adeyemo[1], D. Hernandez[2], J.R. Gibbs[2], A. Doumatey[1], H. Huang[1], D. Shriner[1], J. Adeleye[3], W. Balogun[3], J. Zhou[1], O. Fasanmade[4], T. Johnson[4], J. Oli[5], G. Okafor[5], A. Amoah[6], B. Eghan[7], K. Agyenim-Boateng[7], J. Acheampong[7], C. Adebamowo[8], A. Singleton[2], C. Rotimi[1].* 1) CRGGH, NHGRI/NIH, Bethesda, MD; 2) Laboratory of Neurogenetics, NIA//NIH, Nethesda, MD; 3) University of Ibadan, Ibadan, Nigeria; 4) University of Lagos, Lagos, Nigeria; 5) University of Nigeria, Enugu; 6) University of Ghana Medical School, Accra, Ghana; 7) University of Science and Technology, Kumasi, Ghana; 8) University of Maryland, Batimore, MD & Institute of Virology, Abuja, Nigeria.

The application of high throughput sequencing approaches to human exomes and whole genomes promises to provide new insights into population variation, disease mapping and evolutionary biology. African populations play a unique role by virtue of belonging to some of the oldest human lineages and exhibiting the greatest genetic diversity of any continental population. In the present study, we conducted whole exome sequencing of ten individuals of African ancestry, comprising Ghanaians (three Akan, two Gaa-Adangbe), Nigerians (two Yoruba and one Igbo) and African Americans (two individuals from the Washington DC region). To our knowledge, this is the first time that individuals from three of the ethnic groups (Akan, Gaa-Adangbe, Igbo) are undergoing whole exome sequencing (WES). Sequence enrichment was done using Nimblegen SeqCap EZ Exome v2.0 which targets a total of ~30,000 coding genes (~300,000 exons, total size 36.5 Mb, 44.1 Mb target region). Sequencing was done on an Illumina HiSeq 2000. Reads were mapped and aligned, then aligned data files were converted, sorted, and indexed using Samtools and Picard. The sequence quality scores were recalibrated with the Genome Analysis Toolkit (GATK). After identification and removal of duplicate reads, variants were identified with GATK's UnifiedGenotyper and DINDEL tools. The single-nucleotide variants were then filtered for the removal of low-quality variant calls with GATK's VariantFiltration walker. Mean coverage per sample was ~88X. The number of sites in the covered regions that differed from the NCBI Build 36 (hg18) reference ranged from 25,911 to 35,394 per individual. The number of novel SNPs fell from ~5500 per person with reference to dbSNP129 (the last pre-1000 Genomes version) to ~1600 per person (with reference to dbSNP132). This represents a ~70% reduction in the number of novel variants, demonstrating the enormous contribution of the 1000 Genomes Project and other large-scale sequencing projects. The individuals represented by the newly sequenced groups (Akan, Gaa-Adangbe, Igbo) had variants that were absent from the 1000 Genomes YRI reference. This suggests that more samples from more African populations would prove useful in characterizing genetic variation and utilizing this variation to map disease genes. New and ongoing genomic initiatives in Africa (e.g., H3Africa and MalariaGen) will benefit from strategic integration of whole exome and whole genome sequencing technologies.

847T

Combined Comprehensive Exome Sequencing and Exon-Resolution CGH for Identification of Germ Line Alleles in Inherited Cancer Susceptibility Syndromes. *T. Albert[1], H. Ji[2, 3], S. Garcia[3], D. Newburger[4], D. Burgess[1], K. Kingham[2], M. D'ascenzo[1], T. Richmond[1], R. Selzer[1], X. Zhang[1], J.M. Bell[3], J.M. Ford[2].* 1) Roche NimbleGen, 504 S. Rosa Rd., Madison WI 53719; 2) Stanford University, Division of Oncology, Department of Medicine, 269 Campus Dr., Stanford CA 94305; 3) Stanford Genome Technology Center, 855 California Ave. Palo Alto CA 94304; 4) Biomedical Informatics Training Program, Department of Medicine, Stanford University School of Medicine, Stanford, CA, 94305.

Exome sequencing is rapidly becoming a standard technique for identification of alleles responsible for inherited genetic disorders. Despite several early successes, there are a number of examples of inherited diseases for which likely alleles have not been elucidated using existing exome sequencing technologies. To increase the probability of allele identification, we have developed two technologies which can be utilized in combination to comprehensively characterize the coding portion of the human genome for a fraction of the cost, time, and data analysis requirements of whole genome sequencing. The first is a comprehensive exome capture technology that targets 62Mb of exonic and other functional content that includes the nearly complete coding exons of the RefSeq, CCDS, Vega, and Gencode, databases, ACESscan predictions that overlap with UCSC genes, as well as mirRBase v16, and additional miRNAs, snoRNAs and miRNA binding sites from USCS. Secondly, copy number variation of coding exons is also an important contributor to alleleic variation, but has proven difficult to measure at the exon level using exome sequence. We have developed a new 4.2 million probe whole genome comparative genomic hybridization (CGH) array that allows copy number to be determined at ~1 Kb resolution, sufficient to identify deletions and amplifications of individual exons. We are applying these technologies to identify the mutation alleles responsible for a number of hereditary cancer syndromes which have been clinically evaluated and current genetic diagnostics have not revealed a mutation in a known cancer susceptibility gene. This includes gastric and colorectal cancer syndromes which have so far eluded a specific genetic diagnosis. The resulting data from extended pedigrees will be used to identify loci and the specific mutation that appropriately segregate with affected family members.

848T

Patterns of indel variation in 202 drug target genes from >14,000 individuals. *N. Bing[1], K.C. Huang[2], E.Y. Liu[3], J. Li[4], D. Fraser[1], J. Aponte[1], X. Liu[5], H. Zhang[5], A. Slater[1], P. Woollard[1], S.L. Chissoe[1], J.C. Whittaker[1], V.E. Mooser[1], M.G. Ehm[1], S. Zöllner[6], J. Novembre[7], Y. Li[8, 2], M.R. Nelson[1].* 1) Quantitative Science, GlaxoSmithKline, RTP, NC, USA; Upper Merion, PA, USA; and Stevenage, UK; 2) Biostatistics, University of North Carolina, Chapel Hill, NC, USA; 3) Computer Science, University of North Carolina, Chapel Hill, NC, USA; 4) Human Genetics, University of Michigan, Ann Arbor, MI, USA; 5) BGI, Shenzhen, China; 6) Biostatistics, Psychiatry, University of Michigan, Ann Arbor, MI, USA; 7) Ecology and Evolutionary Biology, University of California, Los Angeles, CA, USA; 8) Genetics, University of North Carolina, Chapel Hill, NC, USA.

Short insertion and deletion variants (indels) are an important and yet poorly studied fraction of genetic variation. With the advancement of high-throughput sequencing technology and improvement of indel calling algorithms, it is increasingly feasible to systemically measure indels, describe their variation within and among populations, and assess their influence on human traits. We have investigated the quality and patterns of indel variation within the exons of 202 drug target genes sequenced in over 14,000 individuals, predominantly of European origin. Indels were called from the short read data using Dindel, GATK, and a novel method developed at BGI. Among these methods, Dindel had the highest duplicate heterozygous concordance: 83% for uncommon (minor allele frequency [MAF] < 5%), 89% for common variants. Genotype validation of all indels with MAF > 0.5% from all three methods and capillary sequence validation for a sample of rare indels is ongoing. Analysis of the Dindel genotypes identified 4,529 unique indels, 42% consisting of a single base and only 17% with a length of more than 5 bases. Most indels were rare: 66% were singletons and doubletons, and 88% had MAF < 0.1%. Deletions were enriched among rare and insertions were enriched among common indels. The indel density varied substantially across gene regions, with 1.6/kb in coding regions, compared to 9.0 and 9.8/kb in introns and UTRs, respectively. The bias against coding indels was evident even amongst singletons (1.1/kb versus ~5/kb outside coding regions). Amongst coding indels, we observed approximately two frameshift indels for every in-frame variant within singletons and doubletons, but a ratio less than one for more frequent variants. There were no frame-shift indels with MAF > 1%. Our results demonstrate the strong effect of purifying selection on protein coding indels. This study estimates the accuracy of indel calls with current algorithms, presents the previously undescribed pattern of rare indel variation, and provides insight into the selective pressures on short insertions and deletions.

849T

Whole-exome and transcriptome sequencing of families with Primary Immunodeficiencies. *F. Casals[1,2], Y. Idaghdour[1,2,4], I. Fernández[1,3], V. Bruat[1], T. de Malliard[1], E. Haddad[1,3], F. Le Deist[1,3], P. Awadalla[1,2,4].* 1) Centre de Recherche, Université de Montréal, Montréal, Québec, Canada; 2) Département de Pédiatire, Université de Montréal, Montréal, Québec, Canada; 3) Département de Microbiologie et Immunologie, Université de Montréal, Montréal, Québec, Canada; 4) CARTaGENE, Montréal, Québec, Canada.

Primary immunodeficiencies (PID) constitute a heterogeneous group of disorders with defects in one or more components of the immune system. Although these diseases have a wide spectrum of clinical manifestations, they are mainly characterized by unusually increased susceptibility to infections, autoimmunity and malignancies. Although PIDs are mostly rare, cumulatively they have an overall incidence of 1 in 5,000 newborns, and this number is increasing with the improvement of diagnostic tools. However, the molecular basis for many of these immune deficiencies remains unknown. To understand the molecular mechanisms underlying these immunodeficiencies we have integrated full exome sequencing, RNAsequencing of leukocytes and clinical data from over 15 families with different uncharacterized immunodeficiencies. These families include both sporadic as well as cases having some family history for the PID. SNPs and indels calling were performed in the context of pedigrees, where each variant discovered in patients can be validated with the information obtained in its relatives. We obtained an initial list with less than fifty candidate genes for each patient, according to a compound heterozygote model for the disease. Functional classifications coupled with an in-house algorithm for prioritizing mutations generated a shorter list of candidate genes for each patient. We also used RNASeq analysis to identifiy candidate genes with significant differences in expression levels between patients and healthy individuals and to characterize variation in allelic expression and RNA editing. We estimated the frequencies for the different RNA editing changes using the comparison of whole exome and full transcriptome sequencing in the same individuals. Integrative statistical analysis of sequencing, comparison to our cohort of French-canadian controls, gene expression and clinical data helped narrow down the list of candidate causal genes and mutations, to a list of few candidate genes that might be involved in the etiology of the disease. Functional validation of candidate genes will be performed to test their role in the molecular origin of the disease.

850T

A Comprehensive Comparison of Whole Human Genome and Exome Sequencing Technologies. *M.J. Clark[1], R. Chen[1], H. Lam[1], M. O'Huallachain[1], K. Karczewski[1], G. Euskirchen[1,2], P. Lacroute[1,2], M. Snyder[1,2].* 1) Department of Genetics, School of Medicine, Stanford University, Stanford, California, U.S.A; 2) Center for Genomics and Personalized Medicine, Stanford University, Stanford, California, U.S.A.

Whole human genome (WGS) and exome sequencing (exome-seq) has become a reality in research and disease diagnosis. As the per-base cost of sequencing has decreased, the demand for sequencing in research, medicine and personalized genomics has increased. There are numerous approaches for WGS and exome-seq in competition with one another. Here we present a comprehensive comparison of genome and exome sequencing technologies. We have sequenced the whole genome of a single individual using both Illumina HiSeq and Complete Genomics Inc. (CGI). We analyzed the Illumina sequence data with an academic pipeline developed by our lab and compared it to the processed data reported by CGI. Using our pipeline to process Illumina data, we detected 3.64 million SNPs, and with CGI we detected 3.4 million SNPs. We found that the majority of SNPs (>3.3Mb) were detected by both platforms, but a number of platform-specific SNPs were found. We have investigated the nature of these differences. We also found differences in copy number and structural variation calls generated by the two pipelines. CGI detected 1,573 SVs, but our Illumina pipeline detected 44,781 SVs. These findings demonstrate significant differences in variants detected by each technology. We have also performed exome-seq on the same sample using three commercial exome enrichment platforms (Agilent SureSelect 50M, Nimblegen EZ Exome 2.0 and Illumina TruSeq Exome) sequenced on the HiSeq. Each of these platforms targets and enriches specific (sometimes different) parts of the exome. They also take different approaches to tiling (Nimblegen—overlapping baits, Agilent—adjacent baits, Illumina—spaced baits). The Nimblegen has >2.1M baits and targets only 44Mb while the Agilent has 656k baits and targets 51.5Mb and the Illumina has 340k baits and targets 62Mb. We found that denser tiling improves targeting efficiency. However, this occurs at the expense of target size. The higher density platforms capture fewer total bases and therefore fewer variants. We found high concordance between variants called by each platform, but we also noted a significant subset of variants uniquely called by each platform, suggesting biases related to bait density and target choice. We also find differences between calls in overlapping regions covered by exome and whole genome sequencing. We present these findings to inform the genomic research and diagnosis communities about the benefits and drawbacks of each of these technologies.

851T

Whole Genome Analysis of Clinical Samples using Omicia's Genome Annotation System. *E. Coonrod[1], R. Margraf[1], P. Ridge[1,2], S. Guthery[3], J. Durtschi[1], E. Lyon[1,4], L. Jorde[5], M. Yandell[5], A. Russell[6], S. Chervitz[6], E. Kiruluta[6], M.G. Reese[6], K.V. Voelkerding[1,4].* 1) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; 2) Department of Biology, Brigham Young University, Provo, UT; 3) Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, UT; 4) Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT; 5) Department of Human Genetics, Eccles Institute of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT; 6) Omicia Inc., Emeryville, CA.

Whole genome sequencing (WGS) for clinical research and diagnostics is expanding as technical complexity and costs decline. It is now possible to diagnose inherited disorders based on WGS of affected and unaffected relatives. To facilitate the use of WGS as a diagnostic tool for identifying genetic causes of disease, novel informatics tools are needed that are capable of fast and accurate clinical annotation of detected variants. In this study, we describe the performance of Omicia's Genome Annotation System (GAS), a new software tool for variant annotation and analysis. A family of four comprised of a male proband with autoimmune enteropathy of unknown but suspected genetic origin, an unaffected brother, and both parents, were studied. WGS was performed on each family member using an Illumina HiSeq 2000 with 100 bp paired-end reads. Reads were aligned using BWA and variant detection was performed with SAMTools. Variants were annotated using an early-access beta-testing program with the GAS software pipeline which cross-correlates the variants with several databases including dbSNP, OMIM, LSDB, PharmGKB and HGMD. From the 3.5 million variants called with SAMTools, the GAS software annotated approximately 1.3 million variants in introns, exons, and 1kb upstream regions for each genome. From this list, variants of interest were further prioritized to as few as 35 and at most 42 per individual genome by filtering out variants that are common in the population and keeping only non-synonymous variants with a potentially pathogenic association. Other filtering options include showing only homozygous changes, stop gain/loss, insertion/deletion variants, or variants with OMIM evidence or other known potentially pathogenic associations. Features of high utility for diagnostic purposes include disease and drug filters and a viewer that displays the individual's variant location within the gene compared to known variants as well as cDNA and amino acid changes. Accuracy is also important for WGS analysis, so we searched for known exonic variants in the RET gene in these individuals and found that all variants queried were accurately classified by the GAS software. Omicia's GAS software is a fast and easy to use method for annotating WGS data. Inherent multiple filtering options are useful to limit lists of candidate genes and ongoing analyses are focused on using the GAS software to compile a list of potentially disease-causing variants.

852T

Comparison of Library Construction Methods and Evaluation of Whole Exome Capture Products for Next-Gen Sequencing. *B. Craig, K. Hetrick, B. Marosy, J. Romm, H. Ling, E.W. Pugh, K.F. Doheny.* Center for Inherited Disease Research (CIDR), Johns Hopkins University, Baltimore, MD.

The Center for Inherited Disease Research (CIDR) provides high quality next-gen sequencing (NGS), genotyping and statistical genetics consultation to investigators working to discover genes that contribute to disease. As the demand for custom targeted and whole exome capture sequencing increases, multiple technologies have emerged designed to produce viable library constructions and capture these regions of interest. We evaluated the Agilent© SureSelectXT library preparation and 50Mb Human All Exon capture products and the Illumina® TruSeq™ DNA sample preparation and 62 Mb Exome Enrichment products. There are a number of differences between the 2 products (Agilent vs. Illumina respectively) that are of interest to compare and determine the impact, if any, on quality of the sequencing data and use in a production scale service. Input DNA requirements (3ug vs. 1ug) and average shearing size range (150-200 vs. 200-300 base pairs) differ as well as master mix creation type (manual vs. pre-made), point of sample pooling (post vs. pre capture), bait type (RNA vs. DNA), hybridization time (One 24hr vs. Two 24hr capture events) and sequencing run length recommended for each platform (75bp vs. 100bp paired-end on the HiSeq 2000). Analysis of these data will show if there is any impact on library complexity, capture efficiency or SNV detection as well as any benefit to lab workflow time or automation and effect on the quality, cost and throughput of the sequencing data. We processed 4 HapMap samples using the most recent protocols at the time for each of the platforms except for the size selection in the Illumina protocol. Here, we replaced gel purification with a modified SPRI bead cleanup to select for the 300-400 bp size range. The samples were sequenced with the recommended run length parameters and repeated using the parameters recommended for the other platform. Experiments were analyzed, in brief, with BWA and SAMtools (refer to the CIDRSeqSuite abstract). Comparing the Agilent and Illumina products resulted in, respectively; 16%|28% duplicate molecules, 69%|55% capture efficiency, 99.7%|99.8% concordance with a genotyping array, 3.1|3.0 Transition/Transversion ratio on exon, 32,210 (on bait)| 43,310 SNVs (on target), 94%|94% SNVs in dbSNP 131 and 3.5%|0.6% targeted exonic regions with no coverage.

853T

Whole-genome medical annotation of admixed genomes reveals ancestry-related ascertainment biases in variants of clinical significance. *F. De La Vega[1,2], A. Moreno-Estrada[1], A. Russell[2], J.K. Byrnes[1], J.M. Kidd[1], S. Gravel[1], M.G. Reese[2], C.D. Bustamante[1].* 1) Department of Genetics, Stanford University Medical School, Stanford, CA; 2) Omicia, Inc., Emeryville, CA.

Due to colonialism, slave trade, and more recently, the easy access to modern means of transportation, populations of recent mixed genetic ancestry are widespread throughout the world. Understanding how to analyze and interpret admixed genomes will be critical for enabling trans and multi-ethnic medical genetic studies. Here, we analyzed the genomes of admixed individuals from North America previously genotyped by the International HapMap 3 project: five of African-American descent (ASW), five of Mexican-American descent (MXL), and two of Puerto Rican decent (PUR). Two of these genomes where sequenced with the SOLiD™ System, and the rest are part of a set released to the public domain by Complete Genomics. To infer continental ancestry for each individual across the genome, we utilized a PCA-based admixture deconvolution approach that assigned segments of the paternal and maternal haplotypes to three potential ancestral populations: West African, European, or Native American. Intersection of the variants from each genome with the OMIM, HGMD, PharmGKB, NHGRI GWAS, and locus specific databases using the Omicia Genome Analysis System identified an average of ~70 non-synonymous coding alleles per genome which are annotated to have an effect on human phenotypes. We show that in spite of lower heterozygosity expected for the segments of Native American ancestry, there is an under-annotation of alleles of medical significance in these as compared with European descent segments. In addition, we observe variants that appear in admixed genomes that otherwise are rare in the origin population fall within segments derived from a different ancestral population (e.g. an allele that confers susceptibility to immunoglobulin A nephropathy which is rare in Europeans and Hispanics, but common in Africa appears in an African-ancestry segment in a MXL sample). This demonstrates that, on an individual level, the average proportion of the genome derived from a given ancestry is less informative a metric than the specific ancestry assignment at a given genomic position. Our results also underscore the need to consider local genomic ancestry in interpreting medical genetic studies, and makes it critical that efforts to map the genetic basis of common disease undertake variant discovery and association mapping in individuals of diverse ancestries relevant to the population of medical genetic interest.

854T

Phased whole genome genetic risk in a family quartet. *F.E. Dewey[1], R. Chen[1], S.P. Cordero[1], K.E. Ormond[1], C.C. Caleshu[1], K.J. Karczewski[1], M.W. Carrillo[1], M.T. Wheeler[1], J.T. Dudley[1], J.K. Byrnes[1], O.E. Cornejo[1], J.W. Knowles[1], M. Woon[1], K. Sangkuhl[1], L. Gong[1], C.F. Thorn[1], J.M. Hebert[1], E. Capriotti[1], S.P. David[1], A. Pavlovic[1], A. West[2], J.S. West[3], C.D. Bustamante[1], M.P. Snyder[1], R.B. Altman[1], T.E. Klein[1], A.J. Butte[1], E.A. Ashley[1].* 1) Stanford University, Stanford, CA; 2) The Harker School, San Jose, CA; 3) ViaCyte, Inc., San Diego, CA.

Whole genome sequencing harbors unprecedented potential for characterization of individual and family genetic variation. Here, we develop a novel synthetic human reference sequence that is ethnically concordant and use it for the analysis of genomes from a nuclear family of four with history of familial thrombophilia. In doing so we demonstrate that prior population mutation rate estimates are biased upwards by the reference sequence, and estimate a Watterson's theta of 7.8 x 10-4. We infer recombination sites to a median resolution of less than 1000 base pairs, show equal male and female recombination frequency, consistent with RNF212 haplotypes, and high recombination hotspot usage, consistent with PRDM9 haplotypes. We use family inheritance state analysis to control sequencing error and inform family-wide haplotype phasing, allowing quantification for the first time of genome-wide compound heterozygous risk alleles and reduction of genotype error rates by ~90 percent. We develop a sequence-based methodology for Human Leukocyte Antigen typing that utilizes long range phased haplotypes from short read sequencing. Finally, we advance methods for analysis of disease and pharmacogenomic risk across the coding and non-coding genome that incorporate phased variant data. We show these methods capable of identifying multigenic risk for inherited thrombophilia in the family quartet involving the genes F5, MTHFR, and HABP2, and drug dosing predictions for the anticoagulant warfarin that are consistent with steady state dosing derived empirically. These ethnicity-specific, family-based approaches to interpretation of genetic variation represent the next generation of genetic risk assessment using whole genome sequencing.

855T

Evaluation of Mitochondrial DNA Alignment Data in Illumina HiSeq Whole Genome Sequencing for Quality Control Monitoring. *J.D. Durtschi[1], R.L. Margraf[1], P.G. Ridge[1,2], K.V. Voelkerding[1,3].* 1) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT 84108; 2) Department of Biology, Brigham Young University, Provo, UT 84602; 3) Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT 84108.

Massively parallel sequencing with the Illumina HiSeq 2000 instrument allows whole genome sequencing (WGS) for research and clinical applications. Sequencing data quality must be monitored to insure the accuracy of resulting variant identification. Error and bias enter the sequencing process from initial library preparation through data post processing and lead to increased variant miscalls in the complex and moderate coverage alignments of WGS. Metrics generated with sequencing, alignment and variant call data can indicate data quality. A standard DNA sample such as phiX is typically spiked into a sample library or sequenced in a separate lane to monitor base call error rate. The goal of the current study was to evaluate whether mitochondrial DNA (MT) sequences, inherently generated during WGS, could be used as an alternative to phiX. Six whole genome libraries were prepared using Covaris fragmentation followed by Illumina library generation on the Beckman SPRI-TE platform. WGS was performed using an Illumina HiSeq 2000 with 100 bp paired-end reads. Alignment to the whole genome including the MT reference followed by variant identification was performed with BWA and SAMtools. QC metrics were generated with tools including the Illumina HiSeq RTA software, FastQC and the GATK suit. All sequence data sets had between 86 and 91% of reads passing the Illumina filter. PhiX aligned error rates ranged from 0.37 to 0.8%. Average alignment coverage for each of the six full genomes was approximately 30 while average coverage of only the MT sequence regions ranged from 1250 to 1600. After filtering MT alignments to remove homozygous variants and those due to apparent misalignments, MT average error rates for five of the six genomes ranged from 0.29 to 0.7%, similar to phiX. In one of the six genomic samples, average MT error rates were 3.7% and the source of this higher error rate is under investigation. Trends observed in other QC parameters including base quality, read mapping quality, coverage variability and ti/tv ratio appeared unrelated to MT error rates. MT sequencing error data appears to be a potentially useful QC tool to complement spiked control samples such as phiX as the MT data is integral to the sample experiencing similar storage and processing steps.

856T

Rapid detection of the ACMG/ACOG recommended 23 CFTR mutations using Ion Torrent semiconductor sequencing. *A. Elliott, J. Radecki, B. Moghis, X. Li, A. Kammesheidt.* Dept R&D, Ambry Genetics, Aliso Viejo, CA.

Cystic fibrosis (CF) is one of the most commonly diagnosed autosomal recessive diseases in the Caucasian population. To date over 1500 causative mutations have been detected in the cystic fibrosis transmembrane conductance regulator (CFTR) gene responsible for CF; however, the majority of these account for ≤ 0.1% of CF cases. For general population CF carrier screening The American College of Medical Genetics (ACMG)/American College of Obstetricians and Gynecologists (ACOG) have recommended a core panel of 23 mutations that will identify ~88% of carriers in Caucasians. Using a genotyping technology that can rapidly identify disease causing mutations is important for high-throughput general population carrier screening, confirming clinical diagnosis, determining treatment options, and prenatal diagnosis. Here we describe a proof of principle study to determine if the IonTorrent Personal Genome Machine™ Sequencer can reliably identify all ACMG/ACOG-23 CFTR mutations. DNA specimens representing all 23 CFTR mutations, which had previously been verified by Sanger and Illumina next generation sequencing, were selected and Ion Torrent specific fusion primer libraries were constructed. Amplicons representing the mutations of interest were sequenced bi-directionally on the Ion Torrent 314 chip, and data was analyzed using a custom script. We were able to correctly identify all the selected mutations except c.2184delA. An intrinsic limitation of flow-based sequencing is that the signal strength in homopolymers is only linear up until ~5-6 nucleotides. The mutation c.2184delA lies in a homopolymer stretch of 7 adenines and therefore cannot be accurately detected due to homopolymer length sequencing errors. Based on our study, we believe Ion Torrent sequencing may be a suitable method for identifying CFTR mutations in the future. However, due to the high rate of base calling errors within homopolymer stretches, mutations within such regions may need to be sequenced using an alternative method.

857T

Exome Sequencing and Unrelated Findings in the Context of Complex Disease Research: Ethical and Clinical Implications. *E.C. Frackelton[1], G.J. Lyon[1, 2, 3], H. Hakonarson[1], T. Jiang[4, 5], R. Van Wijk[7], W. Wang[9], P. Bodily[11], J. Xing[6], L. Tian[1], R. Robison[2], M. Clement[11], L. Yang[4], P. Zhang[4], Y. Liu[4, 5], B. Moore[6], J. Glessner[1], J. Elia[1], F. Reimherr[2], W. Van Solinge[7], M. Yandell[6], J. Wang[4, 5, 8], W.E. Johnson[10], Z. Wei[9], K. Wang[1].* 1) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Psychiatry, 30 North 1900 East, University of Utah, Salt Lake City, UT 84132; 3) NYU Child Study Center, New York, NY 10016; 4) BGI-Shenzhen 518083, China; 5) Genome Research Institute, Shenzhen University Medical School, Shenzhen 518060, China; 6) Eccles Institute of Human Genetics, University of Utah, Salt Lake City, UT 84132; 7) Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, Netherlands; 8) Department of Biology, University of Copenhagen, Copenhagen, Denmark; 9) Department of Computer Science, New Jersey Institute of Technology, Newark, NJ 07102, USA; 10) Department of Statistics, Brigham Young University, Provo, Utah, USA; 11) Department of Computer Science, Brigham Young University, Provo, Utah, USA.

Exome sequencing has identified the causes of several Mendelian diseases, although it has rarely been used in a clinical setting to diagnose the genetic cause of a disorder in a single patient. We performed exome sequencing on a pedigree with several members affected with attention deficit/hyperactivity disorder (ADHD), in an effort to identify candidate variants predisposing to this complex disease. While we did identify some rare variants that might predispose to ADHD, we could not prove causality for any of them as of yet. However, over the course of the study one subject was discovered to have idiopathic hemolytic anemia (IHA), which was suspected to be genetic in origin. Analysis of this subject's exome readily identified two rare non-synonymous mutations in PKLR as the most likely cause of the IHA, although these two mutations had not been documented before in a single individual. We therefore confirmed the deficiency by functional biochemical testing, consistent with a diagnosis of red blood cell pyruvate kinase deficiency. Our study implies that exome and genome sequencing will certainly reveal additional rare variation causative for disease even in already well-studied classical Mendelian diseases, while also revealing variants that might play a role in complex diseases. Furthermore, our study has clinical and ethical implications for exome and genome sequencing in a research setting: how to handle unrelated findings of clinical significance, in the context of originally planned complex disease research, remains a largely uncharted area for clinicians and researchers.

858T

Title: Identifying potentially life-threatening variants in an unscreened population using whole exome sequencing. *S.G. Gonsalves[1], D. Ng[1], J.J. Johnston[1], F.M. Facio[1], S.L. Ruppert[1], C. Krause[1], J.K. Teer[1,2], J.C. Mullikin[1,2,3], L.G. Biesecker[1,2], National Institutes of Health Intramural Sequencing Center, NIH, Bethesda, MD.* 1) Genetic Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 2) NIH Intramural Sequencing Center, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 3) Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Background: Malignant hyperthermia (MH) is a potentially lethal, genetically heterogeneous disorder of skeletal muscle calcium metabolism, triggered by anesthetics and depolarizing muscle relaxants. Whole exome sequence (WES) from an unselected *ClinSeq™* cohort was screened for MH susceptibility variants. Methods: WES was performed on 401 *ClinSeq™* participants. Variants in RYR1 and CACNA1S were annotated using an algorithm that filtered results based on mutation type, frequency, and information in locus-specific mutation databases (LSDB). Variants with low genotype quality were scored 0; the remainder were scored on a pathogenicity scale of 1-5 based on published criteria (Plon et al., 2008). Medical histories and pedigrees were reviewed for MH and related disorders. Clinically relevant results will be returned to participants for medical management. Results: We identified 53 RYR1 and 41 CACNA1S sequence variants in 401 exomes. Variants with a heterozygous frequency >1% or a most probable genotype (MPG) score/coverage value of <0.5 were excluded. Forty-seven RYR1 and 32 CACNA1S variants passed the quality/frequency metrics and were further analyzed. One CACNA1S variant p.Arg498His was listed in LSDB as pathogenic without supporting literature and defined as a variant of uncertain significance (score 3). Eight RYR1 missense variants were listed in the Human Gene Mutation Database as disease causing variants (MH, central core disease, and multi-minicore disease). RYR1 p.Arg614Cys, and p.Asn2342Ser were found in two participants without medical or family histories of MH. One *ClinSeq™* participant with an unpublished missense variant p.Arg3498Gly was found to have a three-generation family history of MH. Several affected relatives in the pedigree had positive in-vitro muscle contracture tests consistent with MH. Conclusions: We found 3 out of 401 (0.0075) participants in *ClinSeq™* carrying an RYR1 variant associated with MH susceptibility or an MH related disorder. This is an extraordinarily high frequency and exceeds the highest published incidence of MH (1/2000). Possible explanations include: 1) penetrance of the disease is lower than previous estimates; 2) self-selection in the *ClinSeq™* cohort; 3) some of the disease associated RYR1 variants are actually common, benign polymorphisms. Identified participants will be further evaluated with a focused family history, genotyping of relatives for co-segregation studies, and muscle contracture testing.

859T

Leveraging off-target sequence reads for more precise inference of local ancestry and haplotypes from exome sequence data. *Y. Hu, Y. Lo, G. Abecasis, H. Kang, 1000 Genomes Consortium.* Department of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, MI.

Deep sequencing of exomes allows us to comprehensively understand the profile of common and rare coding variations potentially causative to Mendelian disorders and complex diseases. When analyzing exome sequences of admixed samples, local ancestry information around the locus of interest allows us to decompose the between- and within-population genetic variations to better understand the genetic architecture of the disease traits. However, typical analyses of exome sequence data focus only on protein-coding regions, which represent only a few percent of the genome. The local ancestry inference only from sparse coding variants is likely less precise than the inference using genome-wide data.

We are motivated by the fact that a substantial amount of exome sequence data comes from off-target regions of the genome due to imperfect capturing of targets in current technologies. While off-target reads are uninformative for the purpose of understanding coding variations, they can increase the precision of local ancestry mapping by providing shallow but wide coverage across the genome. For example, only 2.4% of OMNI 2.5M SNP chip sites reside in on-target region of exome data in the 1000 genomes project. However, > 54% of Omni SNPs have coverage in a typical exome sequence sample, mostly at shallow depths supported by the off-target reads.

We propose a sequence-based Hidden Markov Model (HMM) approach to precisely estimate the local ancestry from both on- and off-target exome sequence reads. Our method utilizes sequence reads at a wide range of known variant sites to model the likelihood of sequence reads given the HMM state. We apply our method to the exome sequence of admixed individuals in the 1000 Genomes project. Compared to the approach using only on-target sequence reads, we demonstrate that the proposed method provides more precise inference of local ancestry which is more concordant to independent inferences from whole-genome sequence data or high-density genotype data of the same sample. Finally, we also evaluate how much the off-target reads are informative in inferring haplotypes, especially when genome-wide array or sequence data is not readily available.

860T

Multi-pronged Approach to Highly Accurate, Highly Comprehensive Complete Human Genomes. *S. Jacobs, J. Baccash, C. Tian, S. Ghosh, G. Nilsen, K.P. Pant.* Complete Genomics, Mountain View, CA.

Complete human genome sequencing studies offer the promise of elucidating the genetic architecture of a wide spectrum of simple and complex diseases. Such studies require a very low error rate: billions of nucleotides are assembled for each genome, and the number of errors needs to be small compared to the number of causal variations within any class of interest. Complete Genomics offers a service for complete human genome sequencing using DNA nanoarrays and a non-sequential, unchained read technology that generates 70-base paired end reads. The reads are assembled using methods that have recently been optimized for more sensitive detection of variants of low allelic fraction, as would occur in aneuploid or heterogeneous tumor genomes. Approaches adopted to ensure a highly accurate and sensitive representation of each genome sequenced will be described. A new and comprehensive analysis of the error rate in the genomes of an extended pedigree has also been performed. This analysis, which uses both Mendelian inheritance consistency and phasing within the pedigree to identify errors, will also be discussed.

861T

Development of an automated sample preparation and quality control pipeline for high throughput sequencing applications. *J. Lambert, A. Barry, S. Cohen.* Caliper Life Sciences, Inc., Hopkinton, MA.

Recent advances in data generation capacity for high throughput sequencing technologies has resulted in new bottlenecks for upstream sample preparation and downstream data processing. Leveraging recent advancements in microfluidic and automation technologies, an automated pipeline has been developed that integrates sample preparation, quality assessment, and quantification to improve overall yields, sample to sample consistency, and reduce the need for amplification. The pipeline also incorporates sample tracking and LIMS compatibility. The system includes methods for DNA library preparation, RNA-Seq, and exome capture protocols, including Roche Nimblegen's SeqCap, Illumina's TrueSeq, and Agilent's SureSelect, and can process up to 96 samples in parallel. Detailed workflows and data demonstrating improved yields from optimization will be presented.

862T

Exome sequencing identifies mutations in *PDE4D*, encoding phosphodiesterase 4D, in acrodysostosis. *H. Lee[1], J.M. Graham[2], D.L. Rimoin[1,3,4,7,8], R.S. Lachman[2], S.F. Nelson[1,9], D. Krakow[1,5], D.H. Cohn[1,5,6].* 1) Human Genetics, Univ California, Los Angeles, Los Angeles, CA; 2) Clinical Genetics and Dysmorphology, Cedars-Sinai Medical Center, Los Angeles, CA; 3) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 4) Pediatrics, Cedars-Sinai Medical Center, Los Angeles, CA; 5) Orthopaedic Surgery, Univ California, Los Angeles, CA; 6) Molecular, Cell and Developmental Biology, Univ California, Los Angeles, CA; 7) Pediatrics, Univ California, Los Angeles, CA; 8) Medicine, Univ California, Los Angeles, CA; 9) Psychiatry, Univ California, Los Angeles, CA.

Acrodysostosis is a dominantly-inherited, multisystem disorder with neurological, endocrine and skeletal abnormalities. Mental retardation is a consistent but variable feature, and endocrine abnormalities include hypogonadism, hypothyroidism and irregular menses. Skeletal features include brachycephaly, midface hypoplasia with a small upturned nose, brachydactyly, spinal stenosis and osteoporosis. To identify the molecular basis of acrodysostosis, exome sequencing was carried out in four genetically independent cases. Exome sequences were also determined for the unaffected parents in two of the cases, facilitating filtering the variants to identify new mutations. Mean oversampling of bases was 107X and at least 93.9% of the ~33.4 Mb of protein coding sequence defined by RefSeq was read to at least 10X depth. A single plausible mutational target was identified genome-wide. Distinct missense mutations in *PDE4D*, which encodes cAMP-specific phosphodiesterase 4D, were identified in three of the cases. The parental exome sequences demonstrated that two of the mutations had occurred de novo, providing strong genetic evidence of causation. Each missense change altered an evolutionarily conserved residue in the encoded protein, further suggesting that the mutations are pathogenic. The acrodysostosis-like phenotype of the *Pde4d* knockout mouse which, in the homozygous state, exhibits neurological abnormalities, infertility and poor growth, imply that the mutations lead to reduced PDE4D-mediated phosphodiesterase activity. *PDE4D* is the human orthologue of the *Drosophila* gene *dunce*, which has been shown to play a role in learning and memory in flies. Thus an evolutionarily conserved neurological pathway is disrupted by PDE4D deficiency. Many clinical findings in acrodysostosis are similar to the phenotype of Albright's hereditary osteodystrophy (pseudohypoparathyroidism type IA), which also results from defects in cyclic nucleotide metabolism and results in resistance to parathyroid hormone (PTH). Our data therefore implicate PDE4D as a component of the PTH signaling pathway. We conclude that acrodysostosis can result from missense mutations in *PDE4D*, and that the wide variety of phenotypic consequences of PDE4D deficiency underscores the exquisite sensitivity of many tissues to alterations in cAMP homeostasis.

863T

Low DNA input optimization using Agilent SureSelect XT Library Prep and Whole Exome Selection for Next Generation Sequencing. *B. Marosy, B. Craig, K. Hetrick, KF. Doheny.* Center for Inherited Disease Research (CIDR), Johns Hopkins University, Baltimore, MD.

The Center for Inherited Disease Research (CIDR) provides high quality next-gen sequencing (NGS), genotyping and statistical genetics consultation to investigators working to discover genes that contribute to disease. CIDR has developed a NGS lab using the Illumina® HiSeq™2000 platform in conjunction with the Agilent© SureSelect™XT library prep and target enrichment kits. Historically, sample requirements for a variety of targeted selection and NGS platforms have been a limiting factor for investigators. Recently, the Broad institute published automation and optimization methods for library prep and solution hybrid selection capture, including a reduction in the DNA input. CIDR has adapted these optimizations for use with the Agilent SureSelectXT library prep and target enrichment kit. Two HapMap samples with DNA inputs of 3ug, 1ug and 100ng were processed in parallel using 1) the standard XT protocol adapted for plate processing; and 2) the optimized low input protocol adapted for the XT reagents, which includes the `with bead' clean up. Further comparisons were made between samples in which the library preps were constructed using the optimized low input protocol (2) and subsequently processed post capture in parallel using 3) the standard Agilent capture protocol adapted for plate processing; and 4) the optimized post capture protocol which includes the `off Bead catch'. Samples were hybridized using 500ng of constructed library and the SureSelect 50Mb Human All Exon library for 24hrs. Samples were then clustered for sequencing using the Illumina cBOT Cluster Generation system. Seventy-six bp paired-end sequencing was performed on the HiSeq2000. Estimated total DNA, using the Agilent 2100 BioAnalyzer, after the pre-capture PCR yielded >1.5ug for the 3ug input samples using the standard methods (1). By comparison, the 1ug and 100ng samples had estimated total DNA yield of >2ug and ~1ug, respectively using the optimized library prep method (2). Preliminary sequencing data analysis of the 100ng input experiment using the optimized library prep and post capture methods (4) yielded 91.4% of the baited bases covered >= 8X depth, an estimated selection library size of 208 million, and a molecular duplication rate of 15%. This should be compared to 81.8%, 123 million and 13.2% respectively for the optimized library prep with standard post capture methods (3).

864T

Whole-exome sequencing in multiplex families with multiple sclerosis. *J.L. McCauley[1], A.H. Beecham[1], A. Hadjixenofontos[1], P.L. Whitehead[1], I. Konidari[1], A. Aviram[1], Y. Pasco[1], S.L. Hauser[2], J.R. Oksenberg[2], D.J. Hedges[1], J.M. Vance[1], J.L. Haines[3], M.A. Pericak-Vance[1].* 1) John P. Hussman Institute for Human Genomics, Miller School of Medicine, University of Miami, Miami, FL, USA; 2) Department of Neurology, School of Medicine, University of California, San Francisco, CA, USA; 3) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN, USA.

Multiple sclerosis is a common neurodegenerative disease affecting more than 1.3 million individuals worldwide. Since the first genome-wide association study (GWAS) of MS was published in 2007, over 20 genetic loci have demonstrated undisputable evidence for association to this disease. Moreover, comprehensive follow-up, meta-analyses, and second generation GWAS continue to identify additional loci. Despite this progress toward understanding the genetics of MS, the genes identified thus far represent only a fraction of the inherited susceptibility. The importance and success of the GWAS approach to identifying loci underlying MS cannot be overlooked. However, alternative hypotheses concerning the genetic architecture of MS must be explored, including the multiple rare variant (MRV) hypothesis. The MRV hypothesis states that susceptibility to common diseases may be determined in part by a large number of rare variants with higher penetrance. Given the number of multiplex families found in MS, it is plausible that rare variants may be found to directly contribute to MS in these families. To test this hypothesis, we have employed next-generation sequencing technologies to scan multiplex families for evidence of rare variants involved in MS. We have used a targeted-enrichment capture approach (50Mb Agilent exome enrichment kit) to selectively capture and subsequently sequence (Illumina HiSeq2000) the exomes of 110 individuals spanning 27 multiplex MS families. Using BWA, 85% of the target is covered at a read depth of 8X or greater. At this stage of our analysis, these data are filtered on quality (GQ⁄ 30), and non-synonymous coding variations. Additional filters include conservation scores as well as polyphen prediction of damaging versus benign amino acid changes. As an example, the sequencing of one multiplex family, containing 8 individuals (2 unaffected parents; 4 affected and 2 unaffected children), found 11,107 non-synonymous coding variants. Thirty-one variants followed an autosomal recessive model; three of these (found in *LIPA*, *FAM35A*, and *SMYD4*) show strong conservation, are predicted to be damaging, are not found in the unaffected siblings, and have not previously been associated with MS. In general, these results will help to address whether rare variants of strong main effect are present within MS. Moreover, our whole-exome approach will allow us to examine both established and novel MS loci and to explore putative pathway analyses.

865T

A nucleic acid fractionation solution for high throughput sequencing applications. *I. Meek[1], E. Wong-Ho[2], J. Molho[2].* 1) Caliper Life Sciences, Inc., Hopkinton, MA; 2) Caliper Life Sciences, Inc., Alameda, CA.

The LabChip XTe provides rapid (<30 minutes), reproducible, and economical fractionation and isolation of nucleic acids. Recent assay and software improvements have expanded the platforms use into different sequencing chemistries and application areas, including ChIP-seq, microRNA-seq, and targeted resequencing. Data will be presented to demonstrate how this microfluidics-based platform increases average read lengths and ensures diversity with higher sample recovery.

866T

Multiplex exome enrichment with a new system from pooled barcoded libraries yields efficient variant detection on the SOLiD™ System. *G. Meredith, G. Bee, M. Dudas, L. Pickle, C. Adams, W. Zhang, M. Allen, J. Bishop, Y. Sun, G. Marnellos, M. Storm, J. Ichikawa, R. Bennett.* R & D, Life Technologies Corp, Carlsbad, CA.

The identification of genetic variation associated with human disease requires the development of a robust and cost-effective approach for systematic resequencing of candidate regions in the human genome. Even though the cost of sequencing a human genome continues to drop, the demand for high sample throughput continues to increase. Higher sample throughput is considered necessary to enable larger patient cohort studies which hold the key to identifying rare disease-related alleles. Thus, scalable and automatable workflows for target enrichment and sequencing are needed to facilitate cancer and other genetic disease research. Described here is a targeted resequencing workflow that employs pooled barcoded fragment libraries, multiplexed exome enrichment and multiplexed sequencing on Applied Biosystems® SOLiD™ 4.0 and 5500xl Systems. To validate the performance of this multiplexed workflow, barcoded fragment libraries were automatically constructed, using an AB Library Builder™ System, from either HapMap sample NA12878 or HuRef genomic DNA. Resulting libraries were then pooled in multiples of 4 for exome capture with the TargetSeq™ Exome Enrichment System. This new system for solution-phase hybridization-based exome enrichment targets exons from VEGA, CCDS, RefSeq, and miRBase and covers > 20,000 gene models within a 45.1 Mb probe footprint. The design was developed in conjunction with a team from the Human Genome Sequencing Center at the Baylor College of Medicine (manuscript submitted). As an example, data obtained from 5.8 Gb of raw SOLiD sequencing of the NA12878 exome yielded an average depth of coverage over the targets of 49X with an on-target rate of 68.5%. For this same dataset, only 5.4% of bases were not covered, 91.3% were covered at 5X, 87.0% at 10X and 75.1% at 20X. Likewise for this set, more than 42,900 SNP calls (26,240 homo and 16,703 het) were made with the BioScope™ 1.3 targeted resequencing analysis pipeline with agreement to dbSNP of 94.2%. Overall, good barcode balance, similar mapping efficiencies and similar SNP/indel calls are observed for 4-plex exome capture samples. Data will also be presented on single-tube exome enrichment of DNA from patient-matched breast tumor vs normal tissue obtained with the 5500xl SOLiD™ System. The combination of multiplexed exome enrichment and SOLiD™ Systems provides an efficient and economical solution for the high-throughput detection of genetic variation in multiple human genomes.

867T

Characterization of non-unique insertion content in the mouse genome using next-generation paired-end sequencing. *N. Parrish, F. Hormozdiari, E. Eskin.* Department of Computer Science, UCLA, Los Angeles, CA., USA.

Genome resequencing techniques have proven effective in characterizing single-nucleotide polymorphisms (SNPs) in an individual genome. However, large-scale structural variations (SVs) are difficult to characterize using resequencing. While SVs have been shown to play an important role in genetic disorders, few methods have been developed to determine the content of the SVs within an individual. Previous methods to determine the content of large inserted sequences have used paired-end sequencing information and de-novo assembly tools, but focused solely on novel sequences. Our recently developed method utilizes additional information gathered during resequencing to assemble both novel and copied insertion content. By looking for patterns in both the overall depth of coverage and the distance between read pairs, our method is able to identify reads which were likely sampled from an inserted region. These reads, along with unmapped reads, are then assembled into contigs using a custom assembly technique referred to as segment extension. Segments of the genome bordering identified insertion locations are progressively extended using the identified reads and mate-pair information. Building on our previous paper, we apply our method to the 129S1/SvImJ and DBA/2J strains of the mouse genome data from the Sanger Institute, and report our findings of assembled insertions common between the two strains and an analysis of the differences.

868T

Optimized automation of next-generation, multiplexed DNA sequencing. *E.A. Phillips, S.R. Austin, A.N. Gracien, M.R. Annable, C.E. Igartua, B.P. Munson, M.B. Mynsberg, K.M. Sawatzki, M.L. Tackett, L.A. Urquhart, B.W. Paeper, J.D. Smith, E.H. Turner, M.J. Rieder, D.A. Nickerson.* Genome Sciences, University of Washington, Seattle, WA.

We have adapted our automated, 96-well library preparation method to pool libraries into a composite, multiplexed sample for sequencing without the need for manual sample handling. While exome sequencing has proven a cost-effective method for sequencing and analyzing the coding regions of the human genome, recent improvements in sequencing technology and methods for custom target capture of smaller regions of the genome make running individual samples inefficient. With our current library preparation and exome capture methods (Roche/Nimblegen EZCap v2 - 36 Mb), a single lane of paired-end, 50bp sequencing on an Illumina HiSeq2000 using the latest V3 chemistry and flowcells with provides an average of 90M unique read pairs (~82% passing >Q30 filter) for a single lane of sequencing (~200X coverage for a single sample). This level of data production necessitates the implementation of pooling or multiplexing strategies to improve the per sample cost of sequencing. We have developed automated approaches to process samples in a 96-well format using a liquid handling robot. 8-base barcodes are added to the samples during the library preparation phase prior to capture. Post capture, the libraries are normalized to 10nM using qPCR, and pooled into a single tube for sequencing. An initial flowcell is run on the pool, and automated QC analysis is performed to provide information on individual library quality and complexity as well as balance of library representation within the pool. Based on this information, libraries are the automatically re-queued within our LIMS system, regrouped and repooled, to eliminate libraries that show unbalanced representation across the target area or exhibit poor capture efficiency. These newly balanced pools can then be sequenced to completion. This strategy allows us to complete 2-4 exomes per lane of sequence run, or even larger multiplexed sample configurations for smaller capture targets, thereby increasing overall efficiency and lowering costs per exome. By adopting multiplexed DNA sequencing as our primary workflow we have decreased costs and optimized high throughput next generation sequencing.

869T

The Totalome ,Towards a Complete Description of a Sample. *M. Rhodes[1], M. Storm[1], Y. Sun[1], N. Fong[1], J. Ichikawa[1], G. Meredith[2], C. Adams[2], B. Nutter[1], A. Tobler[1].* 1) Life Technologies, Foster City, CA; 2) Life Technologies, Carlsbad, CA.

It is now over 25 years since the advent of routine automated sequencing, and 10 years since the sequencing of the Human Genome. Since then there has been a radical change in the number and types of sequencing technologies, with rapid platform and chemistry development driven by the desire to increase throughput and decrease costs. The ability to generate in excess of 50 gigabases of sequence data in a single run has enabled the analysis of both the genome and transcriptome. It is now possible to contemplate collecting all the information on a sample in a single run on a 5500xl Genetic Analyzer. In order to validate this a sample consisting of MCF7 DNA and RNA was run on 2 slides on a 5500 Genetic Analyzer. RNA was analyzed via a whole transcriptome poly A+ selected library as well as a small RNA library. Methylation was analyzed by carrying out a pulldown of the methylated regions using MethyMiner™ Kit and two different salt concentrations. Protein binding was analyzed via a series of ChIP-seq experiments in which a range of Protein DNA binding regions were captured with a variety of antibodies (targets included POL1). In order to increase efficiency the smRNA and ChIP-seq, samples were barcoded so that they could be loaded into the same lane. The whole genome was analyzed by paired end sequencing (the two ends of the same molecule are sequenced , the distance between the two tag sequences being ~250 bases , in order to overcome the challenges of repeat regions Mate Pair sequencing was carried out with 3 kilobases between the two sequenced Tags The true power of the totalome is achieved during analysis, although each technique provides valuable information on its own by combining data will enable significant improvements in analysis. This ranges from the simple, by knowing the SNP present in the genomic sequence, it becomes possible to more efficiently observe allelic bias in the Transcriptome data. More complicated data analysis enabled us to correlate the transcription levels to the binding of RNA polymerase as well as methylation. As well as showing the results of this experiment data for a “mix and match” approach will be presented, this will allow a user to tune the experiment so that only the needed information is collected, for example by choosing the number of transcriptome reads the user can choose the sensitivity required, the same can be done for ChIP-seq, methylation, genome sequencing, thus a custom experiment can be designed.

870T

Combining short (Illumina) and long (PacBio) NGS reads to improve de novo genome assemblies of rice and Saccharomyces cerevisiae genomes. *M. Schatz, M. delaBastide, M. Muller, L. Gelley, E. Antoniou, R. McCombie.* Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY.

Several large genomes have been sequenced using next generation sequencers. However, the completeness of these genome assemblies is limited by the short size of the sequencing reads. The goal of this project is to assess the potential improvement in genome assemblies when using a mix of short (Illumina HiSeq) and long reads (PacBio RS). We used SOAPdenovo to assemble sequences produced by the Illumina HiSeq and BLASR to align PacBio reads. The analysis described below is preliminary and will evolve as the coverage of long reads increases and we learn how to best process this type of sequencing data. We generated over 200x coverage of the yeast genome in 2x100bp with 275bp inserts (Illumina). The PacBio RS was used to get the following coverage; 38x if every read is included, 33x for reads > 500bp, 17x for reads > 1kbp, 5.7x for reads > 2kbp, and 1.7x for reads > 3kbp. The max read length is 8,495bp. The average accuracy is ~83% relative to the reference, with errors dominated by insertions errors (11.5%), deletions (3.4%) and substitutions (1.4%). Using only short reads, the scaffold N50 is 81kbp and the contig N50 is 64kbp. There are 151 scaffolds pairs linked by at least 2 PacBio reads. Using all reads, the scaffold N50 is improved by 54% to 125kbp, and the number of scaffolds (> 1kbp in size) is reduced by 28% to only 210. For the rice genome, we sequenced a mix of 2x100 PE reads (134bp and 270bp inserts), plus 2x100 mate reads (1.8kbp inserts) for a total coverage over 100x. The scaffold N50 is 28.8kbp and the contig N50 is 5.2kbp. PacBio coverage again depends on read length; 10.8x considering every read, 9x for reads > 500bp, 5.6x for reads >1kbp, 2x for reads >2kbp and .6x for reads >3kbp. The longest read is 10,008bp.There are 14,890 scaffold pairs linked by PacBio reads. The hybrid genome assembly is ongoing. We also started a project to investigate the usefulness of the PacBio RS to validate SNPs detected by Illumina reads. The PacBio RS can sequence short inserts multiple times to improve accuracy and the cost would be well below the cost of Sanger sequencing the same number of fragments. The mean insert size of our initial library is 182bp and the mean mapped read length is 2,241bp. Initial evaluation using the SMRT view browser shows very few instances of the same sequencing error present on multiple reads when coverage is <10 sequences/position. Additional analyses are required to assess to accuracy of the consensus sequences.

871T

An updated assembly model for the human reference genome. *V.A. Schneider[1], P. Flicek[2], T. Graves[3], T. Hubbard[4], D. Church[1] for the Genome Reference Consortium and the NCBI Genome Annotation Team.* 1) NCBI, Bethesda, MD 28092; 2) EBI, Hinxton, Cambridge, CB10 1SD, U.K; 3) The Genome Institute at Washington University, St. Louis, MO 63108; 4) The Wellcome Trust Sanger Institute, Hinxton, Cambridge, CB10 1SA, U.K.

From the time of its draft release a decade ago, the human reference assembly has played critical roles in basic research and clinical advances. However, many of the discoveries this resource enabled now demonstrate that the assembly model initially used to represent the genome is insufficient in its ability to successfully capture the extent of human genomic variation and to represent the more complex regions of the genome. The Genome Reference Consortium (GRC), responsible for updates and improvements to the human reference genome since 2007, has developed a new assembly model, used for the current reference version (GRCh37), to address these issues. Alternate representations for variant regions of the genome are represented on scaffolds known as alternate loci, which exist as distinct sequences from the reference chromosomes. The GRC provides alignments of these scaffolds to the reference chromosomes, enabling users to place alternate loci in chromosome context. We will show how inclusion of alternate loci improves alignments of short read sequences by reducing mapping errors, thus curtailing false variant predictions. The new assembly model also provides users with timely access to assembly updates without the disruptive coordinate changes that occur in full assembly releases. The GRC releases updates as patches, which like the alternate loci, are standalone scaffold sequences with alignments to the reference chromosomes. Fix patches reflect changes in the existing assembly sequence, and will be incorporated into the chromosomes at the next full assembly release. Novel patches add new sequences, such as allelic variants, and will become alternate loci in the next assembly version. To date, quarterly patch releases to GRCh37 have included 37 fix patches and 55 novel patches, many of which include corrections or additions to clinically relevant genomic regions. We will show how these patches have added a significant amount of the sequence declared as “novel” in several individual genomes to the reference assembly. NCBI provides bioinformatics support for the GRC and annotates the human assembly, including alternate loci and patches, as part of its genome annotation pipeline. Additionally, NCBI supports mapping of features from one assembly to another, or from an assembly to clinical references such as RefSeqGene. We will present data from the NCBI annotation of the most recent GRCh37 patch release.

872T

Extensive genomic diversity identified through massively parallel sequencing of eighteen Korean individuals. *J. Seo[1,2], Y. Ju[1,2], J. Kim[1], S. Kim[1], D. Hong[1], S. Lee[1], H. Park[3], C. Lee[3].* 1) Genomic Med Inst, Medical Research Center, Seoul National Univ, Seoul, Korea; 2) Macrogen, Inc., Seoul, Korea; 3) Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115, USA.

Massively parallel sequencing technologies have identified broad spectrum of diversity of human genome. Here, we sequenced 18 genomes of Korean individuals to an average of 26.1 fold coverage using Illumina GA IIx platform. The majority of the short-reads were sequenced from both ends with 76 to 151 bp read lengths. We aligned the short reads to the NCBI human reference genome build 36.3. Upon applying a set of bioinformatic filter conditions, we identified 3.45 million - 3.73 million SNPs from each whole-genome sequenced. The SNPs clustered into a non-redundant set of 8.37 million SNPs, 21.9 % (1.83 million) of which were considered to be novel when compared to dbSNP131. Specifically, of these 1.83 million novel SNPs, 73.9 % (1.36 million) were found as singletons among the 10 individuals studied, suggesting a large proportion of these novel variants are rare. Each individual genome has approximately 130,000 of these putative rare SNPs, consistent with the understanding that many rare variants still remain to be identified. We identified 28,179 genomic variants influencing protein sequences. Of these, 8,130 (28.6%) are novel. A subset of the nsSNPs showed remarkably high allele frequencies among the Koreans studied, compared to other populations, including Europeans and West Africans represented in the HapMap project. For example, the rs4961 SNP in the ADD1 gene, the rs17822931 SNP in the ABCC11 gene and the rs3827760 SNP in the EDAR gene are known to be associated with common salt-sensitive hypertension, dry type ear wax and thick hair morphology, respectively. A subset of genes was found to be highly enriched for nsSNPs, herein called super nsSNP genes. Among the 86 super nsSNP genes, 49 (57.0 %) are associated with sensory and immunological function, such as olfactory receptors and HLA related genes. The deep sequencing of Korean genomes also enabled us to assess the relationship between Korean common nsSNPs (allele frequency > ~10%) and previously known SNPs. Indeed, 53.4% of novel but common Korean nsSNPs showed insufficient LD (r2 <0.8) with currently known SNP variants within a distance of 20kb. This finding suggests that current GWA studies, based on common tagging SNPs observed in other ethnic groups (e.g. Caucasians) may have fundamental limitations for detecting common nsSNPs in other populations, that may be capable of having a functional role in the etiology of complex human diseases.

873T

Resequencing of lipid and glucose associated genes in two large Finnish cohorts. *S.K. Service[1], T.M. Teslovich[2], D. Koboldt[3], C. Fuchsberger[2], V. Ramensky[1], M. McLellan[3], Q. Zhang[3], R.P. Welch[2,4], H.M. Stringham[2], W. Schierding[3], C. Sabatti[5], V. Magrini[3], R. Fulton[3], L. Ding[3], L. Peltonen[6], M.R. Jarvelin[7,8,9], M.I. McCarthy[10,11,12], F. Collins[13], R.N. Bergman[14], K.L. Mohlke[15], J. Tuomilehto[9], R. Wilson[3], M. Boehnke[2], N.B. Freimer[1].* 1) Center for Neurobehavioral Genetics, UCLA, Los Angeles CA 90095; 2) Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor 48109; 3) The Genome Institute at Washington University, 4444 Forest Park Ave.St. Louis MO 63108; 4) Bioinformatics Graduate Program, The University of Michigan Medical School, Ann Arbor, MI 48109; 5) Department of Health and Research Policy, Stanford University, Stanford, CA 94305; 6) Institute for Molecular Medicine (FIMM), P.O. Box 20, FIN-00014 University of Helsinki, Finland; 7) Institute of Health Sciences and Biocenter Oulu, University of Oulu, Oulu Finland; 8) Department of Biostatistics and Epidemiology, School of Public Health, Imperial College, Faculty of Medicine, London, UK; 9) National Institute of Health and Welfare, Finland; 10) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Churchill Hospital, Old Road, Headington, Oxford OX3 7LJ, UK; 11) Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Headington, Oxford, UK OX3 7BN; 12) Oxford NIHR Biomedical Research Centre, Churchill Hospital, Old Road, Headington, Oxford, UK OX3 7LJ; 13) National Institutes of Health, Bethesda, MD 20892; 14) Keck School of Medicine, University of Southern California, Los Angeles CA 90089; 15) Department of Genetics, University of North Carolina, Chapel Hill, NC 27599.

Elevated lipid and glucose levels together constitute part of the spectrum of “metabolic syndrome” and are a risk factor for cardiovascular disease and type 2 diabetes (T2D). Recent genome-wide association studies have unequivocally implicated multiple loci associated to lipid/glucose levels. To identify variants that may directly affect levels of these quantitative phenotypes, we performed resequencing of 79 genes at 17 loci previously implicated as associated with lipid or glucose levels, in over 5,000 persons from the 1966 Northern Finnish Birth Cohort (NFBC) and 919 T2D cases and 919 normal glucose tolerant controls from the Finland-United States Investigation of NIDDM Genetics (FUSION) study. Exons and splice sites comprising 270kb of target were captured using the WU-cap method developed at The Genome Institute at Washington University and were sequenced using paired-end Illumina sequencing. The first data release for 1,941 NFBC subjects identified 895 variant sites in target regions, 32% of which are present in dbSNP. Newly identified variants include 15 nonsense and 208 missense variants that were predicted to be deleterious by PolyPhen-2. Variants associated with lipid and glucose levels in these samples are candidates for statistical and functional follow-up, and additional analyses are underway. At the time of abstract submission, sequencing is complete for nearly 5,500 samples, and we anticipate the sequencing of 79 genes in the full set of 6,965 samples will be complete by the end of summer 2011.

874T

TARGETED RESEQUENCING OF SLE SUSCEPTIBLE LOCI. *E.K. Wakeland[1], E. Rai[1], B. Wakeland[1], C. Liang[1], N. Olsen[1], D. Karp[1], G. Wiley[2], K. Kaufman[2], J. Harley[2], P. Gaffney[2].* 1) Department of Immunology, The University of Texas Southwestern Medical Center, dallas, TX, USA; 2) Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA.

Susceptibility to systemic lupus erythematosus (SLE) is impacted by both genetic and environmental factors. Six SLE GWAS reported >30 SLE susceptible loci, however, the causal variants responsible for these associations are largely unknown. We have developed a high throughput sequencing technology to perform targeted resequencing of 90 SLE susceptible LD segments (~10.4 Mb genomic sequence) implicated by association mapping or/and play roles in developing murine lupus. 335 Caucasian (184 SLE cases and 151 controls) were sequenced using combined method of SureSelect array capture (Agilent) and Paired End sequencing protocol from Illumina. High quality non redundant reads were assembled and variations were called using GATK and VCFtools. Of the targeted bases, an average of ~99% bases was captured by at least one non-redundant read and ~94.5% bases by at least eight non-redundant reads, yielding average fold coverage of ~60 fold. We have identified >90,000 variations (SNPs and Indels), of which ~35% were novel (not in dbSNP 132). A significant proportion of our novel variations were rare. Of the total variations, ~20% were localized to gene segments that potentially impacted function. The distribution of potentially functional variations constituted ~10% non-synonymous; ~11% synonymous; ~23% UTR; ~2% deleterious (including nonsense, frameshift, probably/possibly damaging and essential splice); ~2% splice and the rest were regulatory and conserved. Most of the non-synonymous and deleterious variations were rare, suggesting that either they are newly evolved or have been subjected to purifying selection. Interestingly, we have seen a high accumulation of novel potentially functional variations in the loci reported to have major effect on SLE susceptibility or informative in animal models (C1Q; TREX1; TLR9 etc). To explore the degree of functional variation that was associated with alleles tagged by SNPs strongly associated with SLE, phylogenic networks were drawn using haplotypes formed by potentially functional SNPs in complete LD with SLE associated SNPs. This analysis identified a specific CLADE of alleles containing multiple SLE associated SNPs in ITGAM and identified extensive variations in functional SNPs among families of alleles that carried disease associated SNPs for many other genes. These results suggest that many disease-associated SNPs actually identify a cluster of functional variants, rather than a single disease allele.

875T

Exome Sequencing in Autism Spectrum Disorder. *S. Walker[1], A. Prasad[1], C.R. Marshall[1, 2], S.L. Pereira[1], L. Lau[1], J. Foong[1], E. Cheran[1], J. Howe[1], O. Buske[3], M. Dzamba[3], M. Fiume[3], B. Thiruvahindrapuram[1], M. Brudno[3], S.W. Scherer[1, 2].* 1) The Centre for Applied Genomics and Program in Genetics and Genome Biology, Research Institute, The Hospital for Sick Children, Toronto, Canada; 2) McLaughlin Centre, Faculty of Medicine, University of Toronto, Canada; 3) Department of Computer Science, University of Toronto, Toronto, Canada.

Autism Spectrum Disorder (ASD) is neurological condition characterised by limited communication skills, impaired social interaction and repetitive behaviours. There is a strong but complex genetic etiology with perhaps hundreds of contributing risk loci, some of which are now known (eg. *SHANK2*, *SHANK3*, *NRXN1*, *PTCHD1*, 16p11.2). Recently, Next Generation Sequencing technologies have proven powerful tools for identifying mutations underlying Mendelian diseases and their application to more heterogeneous disorders may discover novel sequence variants, identifying new loci and verifying previously implicated genes. With the aim of discovering rare genetic variants associated with ASD, we are carrying out high throughput exome sequencing in a cohort of 1000 Canadian patients. All individuals have previously been genotyped using a high resolution microarray (more than one million probes) and thorough copy number variant (CNV) analysis conducted. We are currently focusing on individuals carrying potentially pathogenic large CNVs under the hypothesis that there may be additional sequence changes or other contributing loci. We are using the Agilent 50Mb whole exome SureSelect target enrichment protocol with Life Technologies' SOLiD platform for sequencing. We have developed an analysis pipeline using alignment tool SHRiMP2 and the Genome Analysis Toolkit (GATK) genotyper, followed by filtration through dbSNP and data from the 1000 genomes project with functional annotation using SIFT and PolyPhen. Variants of interest identified in this discovery stage are being validated and segregation patterns examined. From the first 100 sequenced exomes, we typically detect approximately 15,000 single nucleotide variants per individual in exonic regions, 3000-5000 of which are novel. We have identified numerous distinct, potentially pathogenic sequence changes in genes previously associated with ASD susceptibility. Inherited missense and nonsense variants have been discovered in genes such as *NRXN1*, *RPGRIP1L*, *EFHC1* and *CEP290* among others and appear to segregate with the phenotype. Further characterisation will be achieved by assessment of frequencies in independent case and control populations and through functional studies. We speculate that sequencing of these individuals will yield novel genes and variants influencing ASD susceptibility and shed light on the broader issues of allelic architecture, variable penetrance and phenotypic heterogeneity in complex disorders.

876T

Long Range Sequence Scaffolding of Large Genomes using the Argus™ Optical Mapping System. *N. Xiao[1], R. Ptashkin[1], T. Anantharaman[1], B. Zhu[1], D. Sweeney[1], X. Xun[2], J. Henkhaus[1].* 1) OpGen, Inc., Gaithersburg, MD; 2) Beijing Genome Insitute, Shenzhen, China.

Despite the continued improvements in DNA sequencing technologies, whole genome sequencing of large complex organisms remains a significant bioinformatics challenge, particularly when trying to discern the order and orientation of the hundreds or thousands of sequencing scaffolds typical in these projects. Optical Mapping is a single molecule technology that generates de novo, ordered, high-resolution restriction maps. Whole genome Optical Maps are assembled from collections of single molecule restriction maps, and are routinely used in comparative genomics, sequence assembly and sequence finishing of microorganisms. Recent improvements in data throughput and data quality suggest that the Argus™ Optical Mapping System can be practically applied to large complex genomes such as plants and animals, including the human genome. In order to understand the utility of Optical Mapping in sequencing large genomes, we conducted proof-of-concept studies on the human and goat genomes. With this approach, sequencing information and Optical mapping data were used in combination where Optical Mapping linked otherwise unordered scaffolds into much larger, super-scaffolds. For both the human and goat genomes, millions of single molecule restriction maps that typically ranged from 250Kb to 1 Mb in length were obtained from the Argus Optical Mapping System. In an iterative fashion, these molecules were then aligned to sequence scaffolds to extend the ends with de novo Optical Mapping data. Scaffolds that shared overlapping mapping information were then joined. For the human genome, a test data set was used where artificial gaps of known size (10 - 200 Kb) and position were incorporated into existing human genome sequence (NCBI Build 37). Optical Mapping was performed directly from human blood. The goat genome involved an active de novo sequencing project where whole-genome, shotgun sequence information was obtained using the Illumina platform. Optical Mapping was independently performed directly from frozen, epithelial cells. Results from the human test set successfully joined >90% of the gaps; the error rate (mis-join rate) was < 2%. For the goat genome, scaffold N50 was improved nearly 8-fold (from 2.2 Mb to 16.9 Mb). The N90 was similarly improved from 0.5 Mb to 2.8 Mb. These results indicate that the Argus Optical Mapping System can facilitate sequence finishing of large complex genomes, reduce the bioinformatics burden and improve the overall sequence quality.

877T

Whole genome sequencing of one 100-year old and one discordant MZ twin pair. *K. Ye[1], M. Beekman[1,2], E. Lameijer11, J. Houwing-Duistermaat[3], D. Kremer[1], K. Raine[4], B. Blackburne[5], S. Potluri[6], R. van der Breggen[1], R. Westendorp[7], P. t' Hoen[8], J. den Dunnen[8], G. van Ommen[8], G. Willemse[9], D. Cox[6], Z. Ning[4], D. Boomsma[9], E. Slagboom[1,2].* 1) Leiden University Medical Center, Leiden, Leiden, Netherlands; 2) Netherlands Consortium for Healthy Ageing; 3) Medical Statistics and Bioinformatics, Leiden University Medical Center, Leiden, the Netherlands; 4) The Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK; 5) Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester M13 9PT, UK; 6) Pfizer Inc., South San Francisco, US; 7) Gerontology and Geriatrics, Leiden University Medical Center, Leiden, the Netherlands; 8) Human Genetics, Leiden University Medical Center, Leiden, the Netherlands; 9) Department of Biological Psychology, VU University, Amsterdam, The Netherlands.

Monozygotic (MZ) twins develop from one zygote that splits and forms two embryos. Genomic studies and recent whole genome sequencing efforts all tend to conclude that MZ twins are genetically identical. However, very old MZ twins may accumulate somatic mutations and phenotypically discordant MZ twins may carry germline variants. Therefore, we investigated whether we could detect genetic differences within MZ twin pairs by applying next generation sequencing from two platforms. We identified the oldest living Dutch MZ twin pair of 100 years old. These twins were collected as part of the Leiden Longevity Study (LLS) and they are highly concordant for a range of metabolic phenotypes. In contrast, a 40-year old MZ twin pair from the Netherlands Twin Register (NTR) discordant for BMI from an early age onwards. To identify somatic and germline discordant variants, the whole genomes of both MZ twins were sequenced on Illumina GAII sequencers. We first compared the nucleotide base counts per location between the co-twins to generate a list of potentially discordant base substitutions. After filtering the data on the basis of the distance among these base substitutions, the distance to other identified variants (indels and structural variants) and the coverage, we prioritized a list of 1,349 potentially discordant base substitutions between the co-twins for both pairs (LLS 690 and NTR 659). We validated a first set of top 485 sites using Sequenom MassArray. Between the phenotypically discordant NTR co-twins we did not confirm any of the single base discordancies whereas in the centenarian LLS MZ twin pair we confirmed three single base discordances, which were further confirmed by Sanger sequencing. For the centenarian LLS twin pair, whole genome sequencing data were also generated using the Complete Genomics platform. We observed 19 potentially discordant base substations supported by the data from both Illumina and Complete Genomics sequencing platforms, 17 variants additional to the ones described above. We are investigating these additional potential variants by Sanger sequencing and sofar confirmed 7 of them. In summary, so far there was no evidence for discordant single base substitution in the BMI-discordant MZ twin pair. This could indicate that epigenetic variation may contribute to the discordancy in phenotype. In contrast, we confirmed a modest number of somatic single base substitutions in the very old MZ twins.

878T

Identification of novel loci for hereditary cataract in dogs. *S. Ahonen[1,2,3], SL. Ricketts[4], C. Mellersh[4], H. Lohi[1,2,3].* 1) Dept Med Gen, Univ Helsinki, Helsinki, Finland; 2) Dept. of Molecular Genetics, the Folkhälsan Institute of Genetics, Helsinki, Finland; 3) Dept. of Veterinary Biosciences, University of Helsinki, Finland; 4) Centre for Preventive Medicine, Animal Health Trust, Newmarket, Suffolk, UK.

Dogs suffer from many of the same hereditary eye diseases as humans including hereditary cataract (HC), which is the leading cause of blindness in both species. Cataract is defined simply as an opacity of the lens and there are many causes of cataract in both species, including accompanying ocular disorders, metabolic disease, senility, trauma and inherited defects. In humans about 10 % of congenital cataracts are caused by hereditary cataract and to date 25 genes have been associated with the development of cataract. However, there are many patients for whom a molecular diagnosis is unavailable, indicating that there are many additional genes involved with the development of cataract. In dogs it is estimated that around 100 breeds are affected with HC. Thus far only one gene has been associated with HC in dogs. Characteristics such as age of onset, rate of progression and position of the opacities vary between breeds, whereas inherited cataracts usually exhibit marked intra-breed specificity, indicating their genetic homogeneity within a breed. Due to the genetic homogeneity, dogs may be useful animal models to map novel cataract genes, which can then be screened in humans patients with similar phenotype. We have established a large sample cohort for posterior polar subcapsular cataract in the Siberian Husky, Alaskan Malamute and Samoyed breeds and for posterior polar subcapsular and posterior polar cortical cataract in the Karelian Beardog (KBD). We have performed a genome-wide association study (GWAS) with Illumina's 173K canineHD SNP array to attempt to map HC-associated loci in each breed. GWAS identified a shared HC-associated locus in the Siberian Husky and Samoyed on canine chromosome 18 (CFA18) (Praw=4.6 x 10^{-7}, Pgenome =0.01). A tentative association was identified in Karelian Beardogs on CFA11 (Praw=2.66 x 10^{-6}, Pgenome =0.18). No association was identified in the Alaskan Malamute possibly due to overall homozygosity. However, it is suspected that the Alaskan Malamute may share the same genetic background due to its close ancestral relationship with the Siberian Husky. Further studies will include re-sequencing of the associated loci in the Siberian Husky and replication study of the KBD locus in a larger sample cohort. This is likely to reveal novel canine cataract genes. Mapping new causative genes for canine HC will enable important new insights into the molecular pathogenesis of hereditary cataract in both dogs and humans.

879T

A genome-wide association meta-analysis reveals new childhood obesity loci. *J.P. Bradfield[1], S.F.A. Grant[1,2] on behalf of the Early Growth Genetics Consortium.* 1) Center for Applied Genomics, The Children's Hospital of Philadelphia Research Institute, Philadelphia, Pennsylvania 19104, USA; 2) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, USA.

A number of genetic determinants of adult obesity have already been established through large scale meta-analyses of genome wide association studies (GWAS), several of which were also confirmed in the context of childhood obesity. However, less progress has been made to establish genetic influences specific to childhood obesity though similar approaches. To identify novel genetic factors that influence early-onset obesity, we performed a meta-analysis of genome-wide genotyped datasets from 14 study sites consisting of 5,447 cases (≥ 95th percentile of BMI achieved any time from age 2 to 18 years old) and 8,185 controls (<50th percentile of BMI consistent throughout all measures during childhood) of European ancestry. Following the meta-analysis of ~2.54 million SNPs (directly genotyped or imputed), variation at seven loci yielded association at the level of genome wide significance ($P < 5x10^{-8}$). All these loci have been previously reported in the context of adult BMI GWAS (*FTO*, *TMEM18*, *POMC*, *MC4R*, *FAIM2*, *TNNI3K* and *SEC16B*). *FTO* yielded the strongest evidence for association while *TNNI3K* and *POMC*, which were only detected in adult studies when using hundreds of thousands of participants, were readily detected in our relatively small sample size. We elected to take forward all novel signals yielding association with $P < 5x10^{-6}$ (n = 8) in order to test for replication in independent datasets. We observed two loci that yielded a genome wide significant P-value when combined with the discovery cohort, namely near keratoconus gene 6 (*KC6*) on 18q12 (rs17697518; combined $P = 9.05x10^{-10}$) and near olfactomedin 4 (*OLFM4*) on 13q14 (rs9568856; combined $P = 2.03x10^{-8}$). By also exploring association with the inclusion of extreme childhood obesity, we further confirmed the locus near OLFM4 (rs9568856; combined $P = 1.00x10^{-8}$). In summary, as a consequence of extensive North American-Australian-European collaborative meta-analyses of genome-wide genotyped datasets on children, we have uncovered at least two novel obesity loci.

880T

The Axiom® Genomic Database and the Axiom® Design Center: Designing custom genotyping arrays made easy. *J. Burrill, J. Bleyhl, Y. Lu, D. Le, R. Shigeta, W. Short, A. Tracy, R. Wheeler, B. Wong, Y. Zhan.* Affymetrix, Inc, Santa Clara, CA.

The Axiom® Genomic Database (AGD) and the Axiom® Design Center (ADC) have been created to facilitate marker selection for inclusion on SNP genotyping arrays. The AGD is a collection of more than 11M markers, annotation information, and genotype information across three major populations (CEU, ASI [comprised of CHB and JPT], and YRI). Linkage disequilibrium (LD) was computed across the database, enabling LD-based selection methods in the ADC. The database contains two types of markers: Axiom-validated markers and "taggable" markers. Axiom-validated markers have been shown to genotype with high accuracy and call rate in the Axiom Assay, and the minor allele was seen in more than two individuals. Taggable markers can be reliably tagged in at least one population by a validated marker. The ADC is a web-based tool, accessible at www.affymetrix.com/designcenter, that enables users to query the AGD to create custom microarray designs. The selection of markers is divided into two steps: defining the target set and choosing the selection method. Researchers can utilize the AGD as a target set, or use lists of markers, regions, or genes to define their target set. Users can then filter the available markers by their relationship to transcripts (*e.g.,* cSNPs) or minor allele frequency in a population. Four methods are available to select markers for the design; a different method can be used for each set of target markers. The direct selection method includes all of the Axiom-validated markers in the target set. The best tag method first performs a direct selection, then selects the validated marker with the highest r^2 value for each taggable marker. The greedy tag method "greedily" selects validated markers that cover the most target set markers at a user-defined r^2 threshold. Finally, the physical density method uses a "gap-filling" strategy to obtain uniform physical coverage across the region. The ADC processes the marker group queries (target set and selection rule) and generates reports and BED files detailing the markers selected by each query and for the overall design.

The Axiom Genomic Database and the Axiom Design Center were successfully used internally to create several array plates, including the Axiom Genome-Wide CHB 1 and YRI Arrays. Together, the Axiom Genomic Database and Design Center provide an easy-to-use, web-based tool that enables the rapid selection of markers for custom array designs.

881T

A Genome-Wide Search for Non-UGT1A1 Markers Associated with Unconjugated Bilirubin Level Reveals Significant Association with a Polymorphic Marker in a Gene of the Nucleoporin Family. *S. Datta[1], A. Chowdhury[2], M. Ghosh[3], K. Das[2], P. Jha[4], R. Colah[5], M. Mukerji[4], P.P. Majumder[1,6].* 1) Human Genetics Unit, Indian Statistical Institute, Kolkata, West Bengal, India; 2) Department of Gastroenterology, S.S.K.M. Hospital, Kolkata, India; 3) Department of Haematology, N.R.S. Medical College & Hospital, Kolkata, India; 4) Institute of Genetics & Integrative Biology, New Delhi, India; 5) Institute of Immunohaematology, Mumbai, India; 6) National Institute of Biomedical Genomics, Kalyani, India.

Background & Aims.Variants in *UGT1A1* gene and its promoter are known to determine levels of unconjugated bilirubin (UCB), but do not explain all cases of unconjugated hyperbilirubinemia. We undertook a genome-wide association study with unconjugated bilirubin level to discover associations with variants in genes other than *UGT1A1*.

Methods.We recruited 200 participants to cover the entire range of quantitative variation in UCB level. Genome-wide scans were performed using 512349 polymorphic markers. Quantitative trait locus (QTL) association analyses were performed with these markers and UCB level adjusted for effects of age, gender and genotype at the dinucleotide (TA) insertion locus in the promoter of *UGT1A1* that is known to significantly modulate UCB level.

Results.Association analysis performed with age- and gender-adjusted UCB level, but without adjusting for the effect of the dinucleotide insertion in the *UGT1A1* promoter, revealed statistically significant associations with polymorphic markers in *UGT1A1*, *UGT1A6*, *UGT1A10*, *NUP153*, and *FAM155A*. When further adjustment for genotype effect of the dinucleotide insertion was done, the significant association of a polymorphic marker (rs2328136) in the *NUP153* gene (which produces a 153 kDanucleoporin) was sustained.The frequency of the variant allele (A) at the rs2328136 locus in our study population is 9.95%, similar to European populations. *NUP153* is associated with the transport of biliverdinreductase, which is important for bilirubin conjugation.

Conclusions.In addition to *UGT1A1*, the nucleoporin gene *NUP153*, whose product is a major regulatory factor in bidirectional transport of biomolecules across nucleus to cytosol, is important in the regulation of unconjugated bilirubin level.

882T

Genome-wide association analysis of lactose consumption measured longitudinally identifies a novel variant 500kb downstream of the LCT gene region. *K.M. Eny[1], S.B. Bull[2,3], A.J. Canty[4], L. Sun[3,5], A.P. Boright[6], S.M. Hosseini[1], P.A. Cleary[7], J. Lachin[7], A.D. Paterson[1,3], DCCT/EDIC Research Group.* 1) Genetics & Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Samuel Lunenfeld Research Institute of Mount Sinai Hospital, Prosserman Centre for Health Research, Toronto, Canada; 3) Dalla Lana School of Public Health, University of Toronto, Toronto, Canada; 4) Department of Mathematics and Statistics, McMaster University, Hamilton, Ontario, Canada; 5) Department of Statistics, University of Toronto, Canada; 6) Department of Medicine, University of Toronto, Toronto, Canada; 7) The Biostatistics Center, The George Washington University, Rockville, Maryland.

Lactase non-persistence occurs due to the decrease in lactase activity post-weaning, resulting in the inability to digest lactose in adulthood. rs4988235, 14 kb upstream of the lactase (*LCT* Chr 2q21) gene first discovered in a Finnish linkage study, showed complete co-segregation and association of the CC genotype with lactase non-persistence, and was later associated with lower lactose consumption in young Finnish adults. *In vitro* functional studies demonstrated differential enhancer activity of the *LCT* promoter by rs4988235, suggesting this SNP to be the causal variant in Europeans. However, given the long-range linkage disequilibrium (LD) known to occur in this region, it is conceivable a variant other than rs4988235 could be the causal polymorphism. To date, no genome-wide association study (GWAS) has been conducted on lactase non-persistence or lactose consumption. We therefore conducted a GWAS of lactose intake assessed using a diet history questionnaire in 1304 white participants with type 1 diabetes from the Diabetes Control and Complications Trial. Given that repeated measures may improve power, we used mean lactose intake measured an average of 3.6 (range: 1-6) times per subject over a mean of 6.5 years. To detect significant loci for lactose consumption, we applied linear regression examining the association of 841,342 SNPs with mean lactose intake transformed into a normal score. Several SNPs in the *LCT* region were associated in an additive manner with mean lactose intake. The top SNP identified, rs1561277, () ±SE =-0.23±0.04, p= 1.2e-8) is located in the *ZRANB3* gene, 517kb centromeric from rs4988235 () ±SE=-0.21±0.04, p=1.8e-7) with pairwise r^2=0.77. The lambda statistic for the full GWAS analysis was 1.06, reducing to 1.04 when a 2MB region around the LCT gene was removed. In analysis of rs1561277 with rs4988235, the association of rs4988235 was diminished (p=0.68), while the effect at rs1561277 was attenuated, but not completely diminished () ±SE =-0.20±0.09, p=0.02). This suggests that rs1561277 or another variant in linkage disequilibrium with it are alternative causal variants. In cross-sectional analysis, the effect at rs1561277 was mildly attenuated () ±SE = -0.21±0.04, p=2.2e-7), indicating that use of longitudinal measures of lactose intake may be more powerful than use of a single measure. Taken together, our GWAS identified a novel variant associated with lactose consumption and demonstrates the value of longitudinal trait measures.

883T

Identification of candidate genes of complex diseases through an integrated analysis of genome-wide association studies and expression quantitative trait loci. *C.K. Fuller, X. He, H. Li.* Integrative Program in Quantitative Biology, University of California, San Francisco, CA.

Genome-wide association studies (GWAS) have successfully identified many loci for a range of complex diseases, but there have been relatively few functional studies of these loci to date. Association studies of molecular traits, such as gene expression level, could greatly facilitate such functional studies by directly linking genetic variants to their putative functional consequences. In this work, we propose a general strategy for integrating association data of complex diseases and molecular traits. Our approach is based on the idea that if a molecular trait has a causal relation to a complex phenotype, then any genetic variation (whether in cis- or trans-) that affects this molecular trait will likely affect the phenotype as well. We translate this intuition into a novel Bayesian statistical method to identify genes and gene networks involved in the phenotype. We test our method on GWAS data of Crohn's disease, using gene expression as the molecular trait. Genes predicted by our method are highly enriched with putative causal genes of Crohn's disease, including those already confirmed by recent studies or those known to play an important role in regulating immune functions. Compared with earlier studies that utilize expression quantitative trait loci (eQTL) data for interpreting GWAS results, our method is capable of leveraging the information in all gene expression SNPs (not just those in cis-), can distinguish causality from coincidence, and is generalizable to any molecular trait (e.g. metabolite level). With the coming explosion of genomic sequencing and molecular phenotyping data, we believe integrative methods such as what we describe here are essential for determining the mechanistic basis of complex diseases.

884T

Implication of European-derived adiposity loci in African Americans. *J. Hester[1,2,3], J. Li[1,2], P. Hicks[1,2,4], C. Langefeld[5,6], B. Freedman[7], D. Bowden[1,2,4,7], M. Ng[1,2].* 1) Center for Diabetes Research, Wake Forest University School of Medicine, Winston-Salem, NC; 2) Center for Genomics and Personalized Medicine Research, Wake Forest University School of Medicine, Winston-Salem, NC; 3) Molecular Genetics and Genomics Program, Wake Forest University School of Medicine, Winston-Salem, NC; 4) Department of Biochemistry, Wake Forest University School of Medicine, Winston-Salem, NC; 5) Department of Biostatistical Sciences, Wake Forest University School of Medicine, Winston-Salem, NC; 6) Division of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC; 7) Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC.

Recent genome-wide association studies (GWAS) have identified multiple novel loci associated with adiposity in European-derived study populations. Limited study of these loci has been reported in African Americans. Here we examined the effects of these previously identified adiposity loci in African Americans. 74 representative SNPs in 47 loci that were previously reported in GWAS in Europeans (including *FTO* and *MC4R*) were genotyped in 4992 subjects from six African American cohorts. These SNPs were tested for association with BMI after adjustment for age, gender, disease status, and population structure in each cohort. Meta-analysis was conducted to combine the results. Meta-analysis of 4992 subjects revealed eight SNPs near five loci including *NEGR1*, *NISCH/STAB1*, *TMEM18*, *SH2B1/ATP2A1*, and *MC4R*, showing significant association at $0.005 < P < 0.05$ and had effect sizes between 0.04 and 0.06 SD units (or 0.30 to 0.44 kg/m^2) of BMI for each copy of the BMI-increasing allele. The most significantly associated SNPs (rs9424977, rs3101336, and rs2568958) are located in the *NEGR1* gene ($P = 0.005$, 0.020 and 0.019, respectively). We replicated association of variants at four loci in six African American cohorts that demonstrated a consistent direction of association with previous studies of adiposity in Europeans. These loci are all highly expressed in the brain, consistent with an important role for central nervous system processes in weight regulation. However, further comprehensive examination of these regions may be necessary to fine map and elucidate for possible genetic differences between these two populations.

885T

Toward a deep catalog of results from >1,000 genome-wide association studies. *A. Johnson, R. Leslie, C. O'Donnell.* Division of Intramural Research, NHLBI's Framingham Heart Study, Framingham, MA.

Purpose: We previously reported on a catalog and analysis of results from 118 genome-wide association studies (GWAS). We report the ongoing construction of a catalog of >1,000 GWAS. **Method:** Controlled search terms were used to construct a list of GWAS. QUOSA was used to automate article retrieval. All referenced supplemental files were additionally downloaded. Each significant SNP association ($P<0.05$) was extracted manually, in semi-automated or automated fashion. Custom programs were used to remove redundant associations. Phenotypic labels were assigned to each study to facilitate cross-study queries. Due to privacy concerns we conducted a temporal survey of all results publicly available from 11/1/02 to 7/1/10 to assess community standards for release of GWAS results. **Results:** At the time of submission 1,055 GWAS were identified. We conducted interim analysis of 650 GWAS; cataloguing of further studies is ongoing. This catalog differs from the NHGRI catalog in having more studies, multiple SNP-phenotype associations allowed per SNP per study, and including all results with $P<0.05$. Differences with the NHGRI catalog are due to search strategies and several criteria for inclusion. Major phenotypic divisions are: Neurological (n= 257), Cardiovascular disease (broadly-defined CVD [n=53], diabetes [n= 55]) and its risk factors (total n=225), Blood-related (n=148), Cancer-related (n=139), and Drug/treatment-response (n=77). In our prior survey of 118 GWAS we predicted regions of pleiotropic effect. In interim prospective analysis of 650 subsequently published GWAS we find further evidence of pleiotropic effects for predicted regions (e.g., OAS1 at 12q24.13 which mediates immune response to viral infections). **Discussion:** Our temporal survey shows historical shifts with increased results disclosure over time, but a parallel increase in protected access modes. To our knowledge this is the largest genetics results database constructed to date (currently >2.5 million SNP entries with $P<0.05$ from 768 GWAS). While many entries are expected to be false positives or correlated markers this database has features important for data mining including depth of results, SNP result redundancy (retaining results in linkage disequilibrium), standardized annotation and exact source references for each result. A deeply annotated results catalog will facilitate hypothesis generation and help genomic scientists identify cross-dataset opportunities for mining and collaboration.

886T

Fine Mapping and Biological Functional Study of 14q24.1 Breast Cancer Susceptibility Locus. *P.S. Lee[1], YP. Fu[1], J. Figueroa[2], L. Prokunina-Olsson[1], S. Chanock[1], The Cancer Genetic Markers of Susceptibility (CGEMS) Consortium.* 1) NCI-DCEG, Gaithersburg, MD; 2) NCI-DCEG, Rockville, MD.

A single nucleotide polymorphism (SNP) marker, rs999737, which maps to the last intron of the RAD51-like 1 (RAD51L1) gene coding region, has been associated with breast cancer risk in a genome-wide association study. Located within the 14q24.1 region, RAD51L1 coding region is composed of 13 exons and at least three mRNA splicing variants. The protein has been proposed to promote homologous recombination repair and to maintain genome integrity; another RAD51-paralog has also been associated with breast cancer risk. Our experiments further elucidate the biological relationship between rs999737, RAD51L1, and breast cancer risk. First, we fine mapped the 14q24.1 region to determine whether additional SNPs can pose as targets of additional interest for our biological validation work. We imputed a 3.93MB region flanking rs999737 for Stages 1 and 2 of the Cancer Genetic Markers of Susceptibility study (5,692 cases, 5,576 controls, and 11,268 SNP markers) using the combined reference panels of the HapMap 3 and the 1000 Genomes Project. Single-marker association testing and variable-sized sliding-window haplotype analysis were performed, and for both, the tagging SNP rs999737 exhibited the strongest association with breast cancer risk. Investigation of contiguous regions did not reveal evidence of an additional independent signal. We concluded that rs999737 was an optimal tagging SNP for common variants in the 14q24.1 region and should be the focus of further biological validation studies. We hypothesize that rs999737 alters the RAD51L1 mRNA splicing patter due to its location within the coding region. To examine this hypothesis, we performed Rapid Amplification of cDNA Ends (RACE) experiments to determine the 3' ends of RAD51L1 mRNAs in T47D and Hs578Bst cells, a breast tumor cell line heterozygous for the risk allele and a normal breast cell line homozygous for the risk allele, respectively. We cloned and then sequenced the results from the 3' RACE experiments and observed mRNA with a terminal site located within intron 12, but only in the T47D cell line. This preliminary finding suggests that either the RAD51L1 splice pattern varies among cancer and non-cancer samples, or, that the genomic element tagged by rs999737 is associated with variation in mRNA splicing in the 3' terminal of RAD51L1. As the preliminary RACE experiment data were encouraging, we plan to expand the RACE and sequencing experiments to additional tissue RNA samples.

887T

Genome-wide Association Study Identified 3q13 and 6p12 as Candidate Loci for Childhood Eczema. *T.F. Leung[1], H.Y. Sy[1], S.S. Wang[1], A.P.S. Kong[2], I.H.S. Chan[3], L.Y. Tse[1], W.C. Chan[1], G.W.K. Wong[1], J.C.N. Chan[2], K.L. Hon[1].* 1) Department of Pediatrics, The Chinese University of Hong Kong, Hong Kong, Hong Kong; 2) Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong, Hong Kong; 3) Department of Chemical Pathology, The Chinese University of Hong Kong, Hong Kong, Hong Kong.

Rationale: Eczema is the commonest chronic skin disease in children. It is caused by complex interactions between multiple predisposition genes and environmental factors. The only published genome-wide association study (GWAS) found single-nucleotide polymorphisms (SNPs) on 11q13.5 locus to be associated with eczema in German children (Esparza-Gordillo JA, et al. Nat Genet 2009), but such findings have not been replicated in other ethnic groups. We performed a multi-stage GWAS for eczema in Hong Kong Chinese children. **Methods:** Over 590K SNPs were genotyped in 264 patients (151 males; mean [SD] age: 10.7 [4.2] years) with early-onset, moderate-to-severe eczema using Illumina HumanHap 610-Quad BeadChips. Genotypic data was compared with those of 1000 random non-diseased Chinese controls by PLINK, adjusted for relatedness by dividing χ^2 statistic by the genomic inflation factor 1.594. Significant SNP signals were replicated in an independent population using TaqMan or Sequenom platforms, and results were analyzed by multivariate logistic regression. Interactions between SNPs for eczema were analyzed by generalized multifactor dimensionality reduction (GMDR). **Results:** 458K SNPs with genotyping call rate⁄ 99.9% satisfied quality control criteria. Sixty-two SNPs reached *P*-value threshold of 4.75×10^{-5}. Three clusters of SNPs could be identified: 15 on chromosome 3q13.32, three on 6p21.3 and nine on 6p12.1. Their respective odds ratios for eczema diagnosis were 1.67-1.80 ($P = 3.96 \times 10^{-5}$-8.48×10^{-6}), 1.95-2.17 ($P = 2.09 \times 10^{-5}$-2.22×10^{-6}) and 1.88-2.22 ($P = 1.91 \times 10^{-5}$-2.29×10^{-7}). No association was found between eczema and 11q13.5. Chromosome 6p21.3 being in the HLA locus was not replicated because of its known effects on allergies. Findings for 24 SNPs on 3q13.32 and 6p12.1 were replicated in another 992 patients (mean [SD] age: 11.7 [4.5] years) and 1022 controls, especially for the phenotypes of early-onset disease and moderate-to-severe eczema. No functional gene could be mapped within 1-megabase of the signals on 3q13.32, and bioinformatics analyses revealed possible epigenetic influences due to abundance of non-coding RNAs in this intergenic locus. The 6p12.1 locus contains two genes, namely *GFRAL* and *HMGCCL1*. GMDR did not reveal any interaction between SNPs on 3q13.32 and 6p12.1. **Conclusion:** Chromosomal regions 3q13.32 and 6p12.1 are putative susceptibility loci for eczema in Chinese children. **Funding:** RGC General Research Fund (469908), Hong Kong SAR.

888T

A Replication Study of Two Genetic Variants for Bone Mineral Density Variation in Caucasians. *J. Li[1], L.S. Zhang[2], H.G. Hu[2], Y.J. Liu[1], P. Yu[1], F. Zhang[3], T.L. Yang[3], Q. Tian[1], Y.P. Zheng[2], Y. Guo[3], H.W. Deng[1,2,4].* 1) Center for Bioinformatics and Genomics, Department of Biostatistics and Bioinformatics, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, 70112; 2) College of life sciences and bioengineering, School of Science, Beijing Jiaotong University, Beijing 100044, P R China; 3) The Key Laboratory of Biomedical Information Engineering, Ministry of Education and Institute of Molecular Genetics, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, P R China; 4) Center of System Biomedical Sciences, Shanghai University of Science and Technology, Shanghai, PR China.

A recent genome-wide association study in a Korean population suggested two SNPs, rs7776725 and rs1721400, were significantly associated with bone mineral density (BMD) variation at the radius, tibia and calcaneus. In this study, we aimed to test whether the association of these two genetic variants with BMD can be replicated in Caucasians and whether the association can be extended to BMDs at other skeletal sites that are more clinically relevant to osteoporotic fractures. We tested these two SNPs for association with BMD in two independent US Caucasian cohorts, including a cohort of 2,286 unrelated adults living in Kansas City area, and a cohort of 1,000 unrelated adults living in Omaha and its surrounding areas. We found significant association of rs7776725 with wrist BMD (p=1.42E-16) and suggestive association of rs1721400 with wrist BMD (p=0.017). In addition, rs7776725 was also associated with BMDs at hip, spine and whole body (p=0.013-1.26E-8). The SNP rs7776725 is located in the FAM3C gene. Interestingly, several additional FAM3C SNPs (e.g., rs4727922, rs1803389, and rs718766) also showed association with BMD variation, independent of the effect of rs7776725, which further highlights the potential importance of the FAM3C gene for BMD variation. Our results demonstrated that the SNPs rs7776725 and rs1721400 are associated with BMD variations at multiple skeletal sites in Caucasians. Further in-depth re-sequencing study and functional assays are necessary to elucidate the underlying mechanisms.

889T

Genome Wide Association Study of Hematological Traits among African American Children. *J. Li[1], P. Sleiman[1], C. Kim[1], C. Hou[1], J. Glessner[1], F. Mentch[1], H. Zhang[2], Y. Guo[1], S.L. Furth[3], H. Hakonarson[1].* 1) Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Office of Biostatistics and Epidemiology, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Rockville, MD; 3) Division of Nephrology, School of Medicine, University of Pennsylvania, Philadelphia, PA.

Hematological traits, such as the number and volume of red blood cells, white blood cells, and platelets, are important diagnostic factors for a variety of diseases. Several studies have been carried out to address the question of what genetic loci are associated with blood cell traits in Caucasians adults. However, little is known about the genetic determinants among African American children. We performed a genome-wide association study of eight hematological traits among 6208 African American children as the discovery cohort and additional 1796 African American children as our replication cohort. The hematological traits that we studied include hemoglobin concentration (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) , red blood cell count (RBC), white blood cell count (WBC) and Mean platelet volume (MPV). We also carried out a meta-analysis of the discovery and replication cohorts. Our results indicate a locus on chromosome 11p15.4 (HBE1) is significantly associated with Hct, Hgb and RBC. Two loci on chromosome 3p14.3 (ARHGEF3) and 10q21.3(JMJD1C) reached genome-wide significance for MPV and a locus on 16p13.3 (C16orf35, LUC7L) is significantly associated with MCV, MCH and MCHC. Thus multiple previously reported hematological loci among Caucasians were replicated among our cohorts of African American children. Interestingly, the locus on 16p13.3 shows a more significant association among African American children than it does among Caucasians adults.

890T

Multi-cohort genome-wide association study reveals a new signal of protection against HIV-1 acquisition. *S. Limou[1,2,3,4], O. Delaneau[2], D. van Manen[5], P. An[1], E. Sezgin[6], S. Le Clerc[2,3,4], C. Coulonges[2,4], J.L. Troyer[7], J.H. Veldink[8], L.H. van den Berg[8], J.L. Spadoni[2], L. Taing[2], T. Labib[2], M. Montes[2], J.F. Delfraissy[4], F. Schachter[2], S.J. O'Brien[6], S. Buchbinder[9], M.L. van Natta[10], D.A. Jabs[10,11], P. Froguel[12,13], H. Schuitemaker[5], J.F. Zagury[2,3,4], C.A. Winkler[1].* 1) Basic Science Program, SAIC, NCI-Frederick, Frederick, MD; 2) Chaire de Bioinformatique, Conservatoire National des Arts et Metiers, Paris 75003, France; 3) Université Paris 12, INSERM U955, Créteil 94010, France; 4) ANRS Genomic Group (French Agency for Research on AIDS and Hepatitis), Paris 75013, France; 5) Department of Experimental Immunology, Sanquin Research, Landsteiner Laboratory, Center for Infectious Diseases and Immunity Amsterdam (CINIMA) Academic Medical Center, University of Amsterdam, Amsterdam 1105 AZ, Netherlands; 6) Laboratory of Genomic Diversity, National Cancer Institute-Frederick, Frederick, MD 21702, USA; 7) Laboratory of Genomic Diversity, SAIC-Frederick, Inc., National Cancer Institute-Frederick, Frederick, MD 21702, USA; 8) Rudolf Magnus Institute of Neuroscience, Department of Neurology, University Medical Center Utrecht, Utrecht 3584 CX, The Netherlands; 9) San Francisco Department of Public Health, HIV Research Section, San Francisco, CA 94102, USA; 10) Department of Epidemiology, the Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD 21205, USA; 11) Departments of Ophthalmology and Medicine, the Mount Sinai School of Medicine, New York, NY 10029, USA; 12) UMR CNRS 8090, Institut Pasteur de Lille, Lille 59000, France; 13) Genomic Medicine, Hammersmith Hospital, Imperial College London, London SW7 2AZ, UK.

To date only mutations in *CCR5* have been shown to confer resistance to HIV-1 infection and these explain only a small fraction of the observed variability in HIV susceptibility. We therefore performed a meta-analysis between two independent European genome-wide association studies, each one comparing HIV-1 seropositive cases with normal population controls known to be HIV uninfected, to identify SNPs associated with the HIV-1 acquisition phenotype. A single highly significant association with the chromosome 8 SNP rs6996198 was revealed ($P = 8.91 \times 10^{-6}$) and this SNP was moved forward for a second stage analysis in two independent USA cohorts of European descent: rs6996198 was replicated in both stage 2 cohorts ($P = 1.77 \times 10^{-2}$ and $P = 2.80 \times 10^{-3}$). Among the four groups, the rs6996198-T allele was significantly associated with a reduced risk from HIV-1 infection, and this protective allele was correlated to a higher *CYP7B1* gene expression. Overall, the meta-analysis between the four independent groups reached the genome-wide significance: $P_{combined} = 7.76 \times 10^{-8}$. In conclusion, we provide strong evidence of association for a common chromosome 8 variant with HIV-1 acquisition in populations of European ancestry. This signal is the first association with protection against HIV-1 infection identified outside the CCR5 nexus. It provides a putative new molecular mechanism of resistance potentially involving the *CYP7B1* gene, encoding a member of the cytochrome P450 family which has been reported to impact inflammation, apoptosis, and regulation of IgA levels.

891T

Genome-wide Association Study in the GAIT Project Disclose Sex-specific Regulation of Mitochondrial DNA Levels. *S. Lopez[1], A. Buil[1], J.C. Souto[2], J. Casademont[3], J. Blangero[4], A. Martínez[1], L. Rib[1], J. Fontcuberta[2], L. Almasy[4], J.M. Soria[1].* 1) Genomics of Complex Diseases, IIB - Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; 2) Haemostasis and Thrombosis Unit, Department of Haematology, Hospital de la Santa Creu i Sant Pau, Universitat Autonoma de Barcelona, Barcelona, Spain; 3) Internal Medicine Department, Hospital de la Santa Creu i Sant Pau, Universitat Autonoma de Barcelona, Barcelona, Spain; 4) Department of Population Genetics, The Texas Biomedical Research Institute, Sant Antonio, TX, USA.

An increasing number of common diseases in humans, such as cancer, diabetes, cardiovascular diseases and neurological disorders, are associated with altered mitochondrial DNA levels. These altered mtDNA levels affect normal mitochondrial function. There is great interest in defining the factors that control mitochondrial biogenesis, especially the genes that regulate the mtDNA copy number. We searched for genetic variants that influence mtDNA levels using a genome-wide association (GWAS) method and families from the Genetic Analysis of Idiopathic Thrombophilia Project (GAIT). MtDNA levels were measured by quantitative real-time PCR in 386 individuals from 21 extended Spanish families. A GWAS using the Illumina infinitum 317 Beadchip was performed to detect single nucleotide polymorphisms (SNPs) associated with variation of mtDNA levels. The first GWAS was carried without regard for sex. In addition, association analyses were performed with individuals according to their sex. Also, we fine-mapped three linkage regions, which were sex-specific and that were reported previously in the same individuals. Twenty-two SNPs showed significant association with mtDNA levels (p value ≤ 1e-6). We grouped the SNPs according to sex-specificity, since 6 SNPs showed strong statistical significance in males only, 6 SNPs were strongly associated only in females and 10 SNPs showed significant associations with mtDNA levels independent of sex. These three mitochondrial-related genes *MGC25181*, *MRPL37* and *PARK2* harbour some of the SNPs with significant associations without regard for sex, in males only or in females only, respectively. Interestingly, fine-mapping within the male-specific linkage region that was reported previously (in GAIT) revealed the most significant SNP association with mtDNA levels, which was located in *MRPL37* when analyzed in males only. Our study demonstrates the potential to find genes using GWAS with families. This approach could help to identify genes that affect variation of mtDNA levels. Our objective was to dissect the sex-specific architecture of mtDNA and to describe the mechanisms involved in the control of this quantitative trait. However, our findings require replication and functional validation.

892T

Genome-wide association study of juvenile idiopathic arthritis identifies novel susceptibility loci associated with early disease onset. *M. Marion[1], P. Ramos[1], M. Sudman[2], M. Ryan[2], M. Tsoras[2], S. Kramer[2], M. Wagner[2], D. Glass[2], C. Langefeld[1], S. Thompson[2].* 1) Department of Biostatistical Sciences and Center for Public Health Genomics, Wake Forest School of Medicine, Winston-Salem, NC; 2) Division of Rheumatology, Children's Hospital Medical Center, Cincinnati, OH.

Juvenile idiopathic arthritis (JIA) is the most common chronic childhood rheumatic disease in the Western world. It is comprised of several clinically heterogeneous groups of arthritides with onset before the age of 16 years. Given its early disease onset, JIA may have a stronger genetic contribution than other adult onset disorders. Our goal was to identify genetic risk factors associated with early age of onset in JIA. We performed a genome-wide association study (GWAS) in 771 Caucasian children with oligoarticular and polyarticular rheumatoid factor negative forms of JIA, using the Affymetrix SNP Array 6.0. Tests for association were adjusted for potential confounding effects of population structure by including principal components derived from the GWAS as covariates in logistic regression models. The strongest associations were in the nitric oxide synthase 1 adaptor protein (NOS1AP; rs10753784, P=4.0E-06), the chemokine ligand 2 (CXCL2; rs11815919, P=5.6E-06), and between the dendritic cell-derived BTB/POZ zinc finger (ZBTB20) and the T cell immunoreceptor with Ig and ITIM domains (TIGIT) genes (rs9837270, P=5.8E-06). None of these immune-related genes herein associated with early JIA onset have been associated with the presence of JIA. Current efforts are focusing on replication of top results. These results may help explain the genetic basis of JIA's clinical heterogeneity, and provide tools to aid in a more specific genetic-based phenotypic classification of the disease.

893T

A three-stage genome-wide association study in progressive multiple sclerosis. *F. Martinelli Boneschi[1], P. Brambilla[1], F. Esposito[1], H. Abderrahim[2], M. Rodegher[1], B. Colombo[1], L. Moiola[1], V. Martinelli[1], J. Hillert[3], E. Lindstrom[3], H.F. Harbo[4], A.B. Oturai[5], J. Wojcik[2], D. Booth[6], T.J. Kilpatrick[8], G. Stewart[6], J. Stankovich[7], J. Rubio[8], G. Comi[1].* 1) Department of Neurology, Scientific Institute of San Raffaele, Milan, Italy; 2) Merck-Serono Genetics Research Center, Geneva, Switzerland; 3) Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden; 4) Department of Neurology, Hospital and University of Oslo, Oslo, Norway; 5) The Danish Multiple Sclerosis Center, Department of Neurology, Copenaghen University Hospital, Rigshospitalet, Copenaghen, Denmark; 6) Westmead Millennium Institute, University of Sydney, Australia; 7) Menzies Research Institute, University of Tasmania, Tasmania, Australia; 8) Centre for Neuroscience, University of Melbourne, Melbourne, Australia.

Objective: To identify genetic susceptibility loci associated with the risk of progressive multiple sclerosis (PrMS). Methods: We conducted a genome-wide association study (GWAS) in 197 patients affected with primary progressive (PP), progressive-relapsing (PR) and single attack progressive (SAP) multiple sclerosis (MS) and 234 age- and sex-matched controls of Italian origin. We tested 20 SNPs with suggestive evidence of association in the GWAS study ($p \leq 10^{-4}$) in two independent sets of PPMS cases and healthy controls. Results: We identified a risk-associated SNP in the HLA region in linkage-disequilibrium (LD) with the DRB1*1501 and DQB*0602 loci with evidence of genome-wide significance (rs3129934T, pcombined= 6.7×10^{-16}, OR=2.34, 95% CI=1.90-2.87), and a novel locus on chromosome 7q35 with suggestive evidence of association (rs996343G, pcombined=2.4×10^{-5}, OR=0.7, 95% CI=0.59-0.83) present in an intergenic region which resides within a human endogenous retroviral (HERV) element, belonging to the HERV16 family. The new locus did not appear to have a “cis” effect on RNA expression in PBMCs, but pathway analyses of “trans” effect point to a regulation of the expression levels of genes involved in neurodegeneration, including glutamate metabolism (p<0.02) and axonal guidance signalling (p<0.03) . Conclusions: We performed a three-stage GWAS study in PrMS cases. We confirmed that the association of the HLA-DRB/DQB loci locus with MS is independent of disease course, while the second strongest signal was found for rs996343 which is in an intergenic region on chromosome 7 and could play a regulatory role.

894T

Evaluating genomic copy number variations in a Brazilian population of adult sickle cell anemia patients with and without stroke complication. *F. Menaa[1,2], MA. Bezerra[3], A. Silva Araujo[3], G. Ananina[1], P. Rodrigues Souza da Cruz[1], G. Pereira Gil[1], F. Ferreira Costa[2], M. Barbosa de Melo[1,2].* 1) Center of Molecular Biology and Genetic Engineering (CBMEG), Laboratory of Human Molecular Genetics, State University of Campinas, Campinas-São Paulo, Brazil; 2) Department of Hematology and Hemotherapy - HEMOCENTRO, School of Medical Sciences, State University of Campinas, Campinas-São Paulo, Brazil; 3) Department of Hematology and Hemotherapy-HEMOPE, Recife-Pernambuco, Brasil.

Background - Sickle cell anemia (SCA), an autosomal recessive disorder caused by a missense mutation (Glu6Val) in the beta polypeptide chain of hemoglobin, displays distinct clinical complications that suggest a complex etiology. To the best of our knowledge, little is known about the submicroscopic genomic alterations (e.g., SNPs, CNVs) among Brazilian SCA patients (SS), especially those with stroke-associated complication. The main purpose of our study was to identify, classify and compare the copy number variations (CNVs) in a cohort-case of Brazilian SS patients, in order to determine possible genomic regions associated with the risk of this critical complication. Material and Methods - Two independent groups of unrelated adult Brazilian SS patients - one with stroke and one without stroke - were enrolled in this study. The patient stratification was performed in accordance to eligibility criteria based on patient's information as well as clinical, laboratorial and imaging data when available. Genomic DNA was isolated from 2 ml of peripheral blood collected from each informed consent patient. CNVs were screened using the Genome-Wide Human SNP Array 6.0 (Affymetrix Inc., CA, USA) according to the manufacturer's instructions. Our comparative CNV analysis first focused on large events (> 100 kbps). Results - Comparing SS patients with stroke versus SS patients without stroke defined as reference, we observed an average of 13.22 ± 3.72 events per patient. The number of gain events was about 2.7 times higher than the number of losses. The de novo CNVs represented an average of 1.56 ± 1.51 per patient. This number of de novo CNVs could change, depending on the size of genomic fragments analyzed. We further observed genomic regions of major differences (e.g., 4q13.2, 14q11.2 or 15q11.1) containing known coding proteins (e.g., olfactory receptor family member). Current investigation of those CNV regions against our Brazilian healthy group population as well as against available HapMap populations, is providing a better understanding about the meaning of such genomic variations. Conclusions - Genome wide-association studies (GWAS) are opening new frontiers in the comprehension of some diseases. This study in the Brazilian population is getting extended to a much larger number of Brazilian samples and will be validated using other techniques (e.g., qPCR) and population groups. Financial support - FAPESP.

895T

Translation of genomic medicine : Use of GWAS studies for drug repositioning. *V. Mooser[1], P. Agarwal[2], M. Barnes[2], L. Cardon[1], P. Sanseau[2].* 1) Genetics, GlaxoSmithKline, King of Prussia, PA; 2) Computational Biology, GlaxoSmithKl.ine, King of Prussia, PA.

The promises that GWAS studies would lead to novel therapeutics haven't materialized yet. This is due in part to the 10+year lag time between the time a new drug target is identified and the discovery and development of novel medicines modulating this target. One way to accelerate the translation from GWAS studies into clinical benefits is to use GWAS results to identify unsuspected indications for existing molecules. That is, some GWAS loci contain the target of existing drugs; if the conditions these loci are associated with differ from the diseases considered for these drugs, such mismatches may point to alternative indications for these drugs. To capture these opportunities, we first looked at the overlap between known drug targets and GWAS hits. The list of drug targets was derived from PharmaProjects; this database listed 3,613 marketed therapeutics and 10,093 drugs under development, which together target 1,191 human gene products. The list of GWAS hits was derived from the NHGRI database; on 02/03/11, this database reported 796 publications and 4,818 associations between a trait and a locus. We excluded associations with p > 10E-7, anthropometric traits and unreplicated associations, leaving 991 items. The overlap, consisting of GWAS hits containing genes for which there is a drug, consisted of 155 genes. For 49 genes (e.g. HMGCOaR), the drug (e.g. statins) indication (i.e. LDL-lowering) matched the GWAS trait (e.g. LDL-cholesterol). The remaining 106 associations were manually evaluated. We removed 57 GWAS traits which do not represent strong unmet medical needs (like platelet volumes) and GWAS hits containing several genes with ambiguous association with target genes. This left 49 potential repositioning opportunities. Thirty eight (78%) mismatches correspond to immunology target genes. An example is JAK2, an active drug target for Rheumatoid Arthritis, which is associated in several GWAS studies with Crohn's Disease. As such, these GWAS observation strongly support very recent trials designed to develop JAK2 inhibitors for Crohn's. In conclusion, GWAS studies have the potential to identify well-validated alternative indications for marketed medicines or molecules in development, especially for drugs designed to modulate immunology targets. These repositioning opportunities shall provide a much faster route to clinical utility for GWAS studies than the ab initio discovery and development of drugs targeting novel GWAS-derived loci.

896T

A genome-wide association study identifies the association of HLA-DP locus with chronic hepatitis B and viral clearance. *N. Nishida[1, 2], H. Sawai[1], Y. Mawatari[1, 2], M. Yamaoka[1], A. Koike[3], K. Matsuura[4], Y. Tanaka[4], M. Sugiyama[2], k. Ito[2], M. Mizokami[2], K. Tokunaga[1].* 1) Dept Human Genet, Univ Tokyo, Tokyo, Japan; 2) Research Center for Hepatitis and Immunology, NCGM, Ichikawa, Japan; 3) Central Research Laboratory, Hitachi Ltd., Kokubunji, Japan; 4) Department of Virology & Liver unit, Nagoya City University, Nagoya, Japan.

In 2009, a genome-wide association study identified a significant association of chronic hepatitis B with a region including HLA-DPA1 and HLA-DPB1 using 179 Japanese individuals with chronic hepatitis B and 934 control individuals (Kamatani et al. 2009). We conducted a genome-wide association study, using an independent set of 181 Japanese individuals with chronic hepatitis B (CHB), 184 controls and 185 individuals who were recovered from HBV (HBsAg-negatibe and anti-HBc-positive), to identify the host genetic factors related with protective effects for CHB and/or viral clearance. All samples were genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0 including more than 906,600 SNPs and more than 946,000 probes for the detection of CNVs (copy number variations). The genotype calls of a total of 906,600 SNPs were determined using the Birdseed algorism using the genotyping data with >95% QC call rate. The SNP filtering was performed using the following criteria: (i) SNP call rate≧95%, (ii) minor allele frequency (MAF)≧1% and (iii) deviation from Hardy-Weinberg equilibrium (HWE) P≧0.001 in control samples. We identified significant associations of chronic hepatitis B with 5 SNPs in a region including HLA-DPA1 and HLA-DPB1 in a GWAS using 181 individuals with chronic hepatitis B and 184 controls (rs3077; $P = 1.01 \times 10^{-7}$, OR=2.41 and rs3117229; $P = 3.25 \times 10^{-8}$, OR=2.41). We also found strong associations of chronic hepatitis B with SNPs in the same region in a GWAS using 181 individuals with CHB and 185 HBV-recovered individuals (rs3077; $P = 1.91 \times 10^{-6}$, OR=2.21 and rs3117229; $P = 2.88 \times 10^{-5}$, OR=1.97). In addition, we found several candidate genetic regions with $P<10^{-5}$ in the single-point association data based on allele frequency, which would be expected to be associated with viral clearance. Our findings confirmed the significant association of CHB with the HLA-DP locus using an independent set of Japanese individuals. In addition, the HLA-DP locus would be associated with clearance of hepatitis B virus with several other genetic factors. Further investigations are necessary to clear the molecular mechanism by which the HLA-DP locus and/or other genetic factors confer the risk of chronic hepatitis B and lead viral clearance. URLs. The results of the present GWAS will be registered at a public database: https://gwas.lifesciencedb.jp/cgi-bin/gwasdb/gwas_top.cgi.

897T

Genomewide association study identified new susceptibility loci for Kawasaki disease. *Y. Onouchi[1,6,19], K. Ozaki[1], J.C. Burns[2,3,20], C. Shimizu[2,3,20], M. Terai[4], H. Hamada[4], H. Suzuki[5], T. Suenaga[5], Y. Suzuki[6], K. Yasukawa[7], R. Ebata[7], T. Saji[8,19], Y. Kemmotsu[8], K. Ouchi[9], F. Kishi[10], T. Yoshikawa[11], T. Nagai[12], K. Hamamoto[13], Y. Sato[14], K. Sasago[6], A. Takahashi[15], N. Kamatani[15], M. Kubo[16], T. Tsunoda[17], A. Hata[6], Y. Nakamura[18], T. Tanaka[1].* 1) Lab. Cardiovascular Disease, Ctr. Genomic Med. RIKEN, Yokohama, Kanagawa, Japan; 2) Dept. Pediatrics, School of Medicine, Univ. of California San Diego, La Jolla CA; 3) Rady Children's Hospital San Diego, CA; 4) Dept. Pediatrics, Tokyo Women's Medical Univ., Yachiyo Medical Ctr., Yachiyo, Chiba, Japan; 5) Dept. Pediatrics, Wakayama Medical Univ., Wakayama, Wakayama, Japan; 6) Dept. Public Health, Chiba Univ. Graduate School of Medicine, Chiba, Chiba, Japan; 7) Dept. Pediatrics, Chiba Univ. Graduate School of Medicine, Chiba, Chiba, Japan; 8) Dept. Pediatrics, Toho Univ. School of Medicine, Tokyo, Japan; 9) Dept. Pediatrics, Kawasaki Medical School, Kurashiki, Okayama, Japan; 10) Dept. Molecular Genetics, Kawasaki Medical School, Kurashiki, Okayama, Japan; 11) Dept. Pediatrics, Fujita Health Univ., Toyoake, Aichi, Japan; 12) Dept. Pediatrics, Dokkyo Medical Univ. Koshigaya Hospital, Koshigaya, Saitama, Japan; 13) Dept. Speech and Hearing Sciences, International Univ. of health and welfare, Fukuoka, Fukuoka, Japan; 14) Dept. Pediatrics, Fuji Heavy Industry LTD. Health Insurance Society General Ohta Hospital, Ohta, Gunma, Japan; 15) Lab. Statistical Analysis, Ctr. for Genomic Med., RIKEN, Yokohama, Kanagawa, Japan; 16) Lab. Genotyping Development, Ctr. for Genomic Med., RIKEN, Yokohama, Kanagawa, Japan; 17) Lab. Medical Informatics, Ctr. for Genomic Med., RIKEN, Yokohama, Kanagawa, Japan; 18) Lab. Molecular Medicine, Human Genome Ctr., Institute of Medical Science, Univ. of Tokyo, Tokyo, Japan; 19) Japan Kawasaki Disease Genome Consortium; 20) U. S. Kawasaki Disease Genetics Consortium.

Kawasaki disease (KD; OMIM 611775) is an acute systemic vasculitis syndrome of infants and young children. Previously we performed a genome-wide linkage study of affected sib pairs of KD and mapped several candidate loci for susceptibility genes. From these loci, we have identified SNPs in *ITPKC* at 19q13.2 and *CASP3* at 4q35 which confer risk for KD in the Japanese and Caucasian children. To identify additional KD risk loci, we performed a GWAS. We genotyped 447 KD cases for 551,767 tag SNPs on Illumina Human Hap550v3 BeadChip. As a control population, we used 3397 individuals constituted by 906 healthy adults and 2,491 adults with various common diseases unrelated to KD and genotyped them for 620,480 tag SNPs on Illumina Human610-Quad DNA Analysis BeadChip. After quality control, we compared frequencies of 463,883 autosomal SNPs in 438 KD cases and 3397 controls by Cochrane-Armitage trend test. Association with genome-wide significance ($P < 1.0 \times 10^{-7}$) was observed at two SNPs; $P = 8.0 \times 10^{-12}$ for SNP 1 and $P = 3.3 \times 10^{-8}$ for SNP 2, respectively. For validation of these two SNPs and following up of other marginally associated SNPs, we prepared two case-control panels (panel 1; 470 KD cases and 378 controls, panel 2; 284 KD cases and 569 controls) independent from those used in GWAS and genotyped them for the top 100 SNPs showing P values smaller than 6.3×10^{-5}. In both panels, SNP 1 showed association with KD and the P value in a meta-analysis was 2.0×10^{-21}. Initial association of SNP 2 was not replicated in either of the panels. Association of genome wide significance level was additionally achieved at five loci including those of *ITPKC* and *CASP3* in combined data analyses of GWAS and follow up studies. Thus we identified four novel susceptibility loci for KD. We believe further investigation of these loci will foster better understanding of the pathogenesis and pathophysiology of the disease.

898T

GWAS in Neurofibromatosis Type 1 (NF1): progress update. *A. Pemov[1], H. Sung[2], A.F. Wilson[2], D.R. Stewart[1].* 1) NIH/NCI/DCEG, Bethesda, MD; 2) NIH/NHGRI/IDRB, Baltimore, MD.

Background. NF1 is known for phenotypic variable expressivity and limited correlation between genotype and phenotype. However, the cause of such variability is unknown. We hypothesize that this variable expressivity is genetically determined, in part, by elements other than variants in coding regions at the *NF1* locus. We tested this hypothesis with a genome-wide association study (GWAS) of quantitative NF1 phenotypes. **Methods.** A single observer quantified severity in multiple NF1 sub-phenotypes, including head circumference (OFC), and the number of café-au-lait macules (CALM), cutaneous neuorofibromas (cNF), Lisch nodules (LN) and cherry hemangiomas (CH). Germline DNA was hybridized on the Illumina Human OmniQuad-1M SNP genotyping array for 117 Caucasians (82 families, average family size = 1.4). Traditional test of association, ignoring family structure was performed on 605,630 SNPs (MAF >= 0.10) on the 22 autosomal chromosomes in an attempt to identify non-NF1 variants associated with sub-phenotypes. Each sub-phenotype was adjusted for selected covariates such as age, gender and eye color. **Results.** Several SNPs were identified for each studied NF1 clinical sub-phenotypes at a significance level of 10^{-5} reflecting the modest sample size. Significant SNPs for CH and LN sub-phenotypes resided in gene-free regions of the genome, while the SNPs for CALM, cNF and OFC were found inside *RPS6KA2*, *KCNJ6* and *FOXP2* genes, respectively. *RPS6KA2* encodes p90 ribosomal S6 kinase, which, like *NF1*, is a part of MAPK and mTOR pathways. *KCNJ6* encodes one of the subunits of inward rectifier potassium ion channels; it has been shown that inactivation of *NF1* in Schwann cells leads to increased levels of cAMP and as consequence to increased outward K+ current and cell proliferation. *FOXP2* is a transcription factor that controls expression of ~300 human genes and is highly expressed in developing brain; this gene is required for proper development of speech and language regions of the brain during embryogenesis **Conclusions.** We expect that GWAS of quantitative traits in NF1 to be a useful first step in understanding NF1 phenotypic diversity. Future analyses will include other NF1-related traits. Principal component analysis will be performed on these phenotypes to identify independent phenotypic components for analysis.

899T

Genome-wide association of structural MRI data identifies variants associated with amygdala volume. *P.M.A Sleiman[1], T. Satterthwaite[2], K. Ruparel[2], C. Kim[1], R. Chiavacci[1], J. Richard[4], M.E. Calkins[5], R.C Gur[3], J. Loughead[2], R.E Gur[3], H. Hakonarson[1].* 1) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Brain Behavior Laboratory Neuropsychiatry Section Department of Psychiatry University of Pennsylvania Philadelphia, PA 19104; 3) Department of Psychiatry University of Pennsylvania Philadelphia, PA 19104; 4) Director, Computerized Neurocognitive Testing/Research Data Manager Department of Psychiatry/ Neuropsychiatry Section University of Pennsylvania Medical Center 10th Floor Gates 3400 Spruce Street Philadelphia, PA 19104; 5) Schizophrenia Research Center and Brain Behavior Laboratory Department of Psychiatry, Neuropsychiatry Section University of Pennsylvania School of Medicine 9 Maloney, 3600 Spruce Street Philadelphia, PA 19104.

We carried out a genome-wide association study of cortical and subcortical brain volumes derived from the structural MRI data of 374 individuals, comprising 189 Caucasians and 185 African Americans, all were between the ages of 8 and 21. All samples were genotyped on either Illumina HH550 or HH610 arrays, SNPs common to both were carried forward after standard quality controls. Genetic ancestry was determined by principal component analysis. Volumetric data for whole brain, grey matter, white matter and individual subcortical regions was scaled to account for differences in intracranial volume and tested for association as quantitative traits in linear models. After multiple testing correction, variants at one locus on chromosome 11p15 remained significantly associated with amygdala volume ($P = 1.6x10^{-8}$). All associated variants mapped to a single gene, NAV2, that is highly expressed in the nervous system in both developing embryos and adults. Previous work suggests the gene is a cytoskeletal-interacting protein that functions in neurite outgrowth and axonal elongation. Interestingly, homozygous mouse mutants have previously been shown to exhibit reduced cerebellar volume, which resulted from a reduction in the number of cells undergoing proliferation, limiting the expansion of the cerebellar primordium during development. We will therefore report on the identification of the first genetic variants associated with amygdala volume in both Caucasians and African Americans using subcortical volumetric data derived from structural MRI. There is intense interest in the volume of the human amygdala and its asymmetry between the hemispheres in relation to a broad range of phenotypes including fearfulness, emotion recognition, neuropsychiatric conditions such as autism, schizophrenia and bipolar, normal ageing and neurodegenerative disorders such as Alzheimer's and even obesity and cardiovascular disease. The identification of common variants that influence normally developing human amygdala volume could therefore inform research across a broad range of phenotypes.

900T

A Genome-Wide Association Study Examining Obese Factors in an Arab Family with a History of Type 2 Diabetes. *G. Tay[1], H. alsafar[2], H. Cordell[3], S. Jamieson[4], K. Khazanehdari[5], R. Francis[4], J. Blackwell[4,6], alsafar.* 1) Centre for Forensic Science, The University of Wes, Perth, Australia; 2) Khalifa University of Science, Technology and Research; 3) Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, United Kingdom; 4) Telethon Institute for Child Health Research, Centre for Child Health Research, The University of Western Australia, Subiaco, Western Australia; 5) Molecular Biology and Genetics, Central Veterinary Research Laboratory, Dubai, United Arab Emirates; 6) Cambridge Institute for Medical Research and Department of Medicine, School of Clinical, Medicine University of Cambridge, Cambridge, United Kingdom.

ABSTRACT Overweight and obesity are major risk factors for a number of chronic diseases, including Type 2 Diabetes (T2D), cardiovascular disease and cancer. In the United Arab Emirates (UAE), it has been estimated that some twenty percent of adults suffer from obesity. The incidence of T2D in the UAE population is also among the highest in the world. To identify factors that result in obesity, and its association with T2D, we conducted a Genome-Wide Association Study (GWAS) and specifically assessed genetic associations with “Body Mass Index” (BMI) and “Waist Circumference” (WC). GWAS analysis of 178 individuals in an extended family of Arab descent revealed four loci that reached genome-wide significance, two of which were found in previous studies. The previously described association between the Single Nucleotide Polymorphism (SNP) at position rs2793823 within the ADAM30 locus (identified through meta-analysis of a GWAS study of subjects of Caucasian descent) was also shown to be associated with the disease in Arabs (p = 1.86E-8). Our study also confirmed the association between SNPs within the JAZF1 loci and BMI, WC and T2D as reported in other studies. Two novel associations were noted in our study: (1) a novel locus on chromosome 16 within the FBXO31 locus (rs9308437, p = 7.5E-7) was shown to be associated with the WC phenotype, and (2) the SNP (rs7120774) in GALNTL4 of chromosome 11 was found to be associated with BMI (p =1.82E-10). FBX031 is a candidate gene for breast cancer, whereas GALNTL4 plays a role in insulin stimulated glucose transport in muscle. Work continues to replicate the two latter findings in independent cohorts to confirm the involvement of FBXO31 and GALNTL4.

901T

Meta-analysis of genome-wide association studies identifies novel loci for lung function. *M.D. Tobin[1], M. Soler Artigas[1], D.W. Loth[2,3], L.V. Wain[1], S.A. Gharib[4,5], M. Obeidat[6], W. Tang[7], B.H. Stricker[2,3,8,9], P. Elliott[10,11], G.T. O'Connor[12,13], D.P. Strachan[14], S.J. London[15], I.P. Hall[6], V. Gudnason[16,17], the. SpiroMeta consortium[18], the. CHARGE consortium[19].* 1) Departments of Health Sciences and Genetics, University of Leicester, Leicester, UK; 2) Department of Epidemiology, Erasmus MC, Rotterdam, , The Netherlands; 3) Inspectorate of Healthcare, The Hague, The Netherlands; 4) Center for Lung Biology, University of Washington, Seattle, Washington, USA; 5) Department of Medicine, University of Washington, Seattle, Washington, USA; 6) Division of Therapeutics and Molecular Medicine, Nottingham Respiratory Biomedical Research Unit, University Hospital of Nottingham, Nottingham, UK; 7) Division of Nutritional Sciences, Cornell University Ithaca, New York, USA; 8) Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands; 9) Netherlands Genomics Initiative (NGI)-sponsored Netherlands Consortium for Healthy Aging (NCHA), The Netherlands; 10) MRC Health Protection Agency (HPA) Centre for Environment and Health, Imperial College London, London, UK; 11) Department of Epidemiology and Public Health, Imperial College London, St Marys Campus, London, UK; 12) Pulmonary Center, Boston University School of Medicine, Boston, Massachusetts, USA; 13) The NHLBI's Framingham Heart Study, Framingham, Massachusetts, USA; 14) Division of Community Health Sciences, St George's University of London, London, UK; 15) Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, North Carolina, USA; 16) Icelandic Heart Association, Kopavogur, Iceland; 17) University of Iceland, Reykjavik, Iceland; 18) SpiroMeta consortium contact Martin D.Tobin, Departments of Health Sciences and Genetics, University of Leicester, Leicester, UK; 19) CHARGE consortium contact Stephanie J. London, Institutes of Health, Department of Health and Human Services, Research Triangle Park, North Carolina, USA.

Lung function measures are heritable traits that predict population morbidity and mortality and are used in the diagnosis of chronic obstructive pulmonary disease. Recently, two large meta-analyses of genome-wide association studies (GWAS), each of them with sample sizes over 20,000 individuals of European ancestry, discovered novel loci associated with lung function. To increase the power to detect genetic variants of modest effect sizes we undertook a larger meta-analysis including a total of 23 lung function GWAS in Stage 1 (n=48,201) and followed-up potentially novel loci in 17 studies in Stage 2 (n=46,411). In each study, forced expiratory volume in 1 second (FEV1) and the ratio of FEV1 to forced vital capacity (FVC) were adjusted for age, sex, height and ancestry principal components, and inverse normal transformed. Additive genetic models stratified for ever/never-smoking status were used to test the association of approximately 2.5 million genotyped or imputed SNPs with the adjusted traits. Inverse variance weighting was used to meta-analyse the smoking strata within studies first and then to obtain the pooled estimates across studies in Stage 1 and 2. Ten previously discovered loci (*TNS1, FAM13A, GSTCD/NPNT, HHIP, HTR4, ADAM19, AGER, GPR126, PTCH1,* and *TSHD4*) showed genome-wide significance (P<5x10^{-8}) in our Stage 1 data. We also describe novel loci reaching genome-wide significance after meta-analysing Stage 1 and 2 and the relation of these loci to other complex traits. These new loci may provide insight into the molecular mechanisms regulating pulmonary function and into molecular targets for future therapy to alleviate reduced lung function.

902T

Genome-wide association analysis of rare variants with Type 2 Diabetes. *M.E. Travers[1], R. Magi[2], J. Chen[2], K. Gaulton[2], A.L Gloyn[1], M.I McCarthy[1,2], I. Prokopenko[1,2], A.P Morris[2].* 1) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, United Kingdom; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK.

Genome-wide association studies (GWAS) of common variants have been successful in identifying novel type 2 diabetes (T2D) susceptibility loci. However, the joint effects of these common variants account for no more than 15% of T2D heritability. The aim of this study was to assess the evidence for association of T2D with rare genetic variation, defined here to have minor allele frequency (MAF) less than 0.01 (1%), through imputation from existing GWAS genotyping data.

We performed imputation in 1,926 T2D cases and 2,942 controls of European descent genotyped using the Affymetrix 500K GeneChip. Imputation was undertaken using the European reference panel from the 1000 Genomes Project (August 2010 release). We tested for association of T2D with accumulations of minor alleles at rare variants within genes. The analysis was performed using GRANVIL, which models disease status as a function of the proportion of rare variants at which an individual carries a minor allele in a logistic regression framework.

The strongest signal of association was observed for *BMP2* (p=1.0x10-6, genome-wide significant correcting for 30,000 genes). Common variants within this gene are associated with height and body mass index. The gene contained 36 rare variants (mean MAF = 4.3x10-3), with odds ratios of 1.26 (1.15-1.38) per minor allele. Strong evidence of association (p<10-5) was also observed for *IGFL4* (p=2.4x10-6) and *CLK3* (p=6.4x10-6). *IGFL4* belongs to a family of signalling molecules that play crucial roles in cellular energy metabolism and in growth and development.

We have designed and validated assays to genotype nine of the most strongly associated variants, and our validations have confirmed presence of the expected rare variants in samples with the highest confidence imputation. Further genotyping will assess the overall accuracy of imputation using this reference panel, as well as the impact of replication genotyping upon disease association strength. Our analysis has demonstrated strong evidence of association of T2D with rare variation in three genes. Our results highlight the potential for the identification of rare variant associations using existing GWAS genotyping data, supplemented with imputation from high-density reference panels, without the need for costly re-sequencing experiments.

903T

Genome-wide Association (GWAS) Results in dbGaP. *Z. Wang, Y. Jin, M. Xu, Y. Shao, M. Kimura, R. Bagoutdinov, N. Popova, J. Paschall, N. Sharopova, S. Pretel, A. Sturcke, L. Hao, L. Ziyabari, L. Phan, S. Sherry, M. Feolo.* Information Engineering Branch, National Center for Biotechnology Information, NLM/NIH, Bethesda, MD 20892, United States.

A huge investment in genome-wide association studies (GWAS) in the past few years have produced billions of associations between genetic variants and phenotypic or exposure variables or biomarkers. In most cases, the volume of generated data is so large that only the associations with the lowest p-values are reported in manuscripts. Consequently, many genetic association results are not accessible to public even though they may account for a non-trivial fraction of variance contributing to an inherited trait. Since 2006, NCBI's dbGaP has been populated with over 3100 analysis datasets, including 2808 allelic association and/or linkage scans from approximately 1000 traits in the Framingham Cohort and ~300 other result sets spanning 44 different dbGaP studies. These submitter-produced results are curated, linked to existing NCBI resources, and included as controlled access components available to researchers authorized to download data for that study. Public users can visit the dbGaP Association Results Browser website: http://www.ncbi.nlm.nih.gov/projects/gapplus/sgap_plus.html and efficiently query for significant results by SNP identifier (rs#), chromosomal location, gene, or phenotype trait (MeSH term). Users can explore dbGaP association results using dbGaP interactive genome display, where testing statistics such as p-value, effect size (OR and Beta) and the coding allele (QTL), genotype quality (call rate and p-value from HWE test) are shown on a sequence view that contains recombination rates, genes, proteins and neighboring markers in that region. The locus positions, feature orientations and alleles are updated with successive Genome and dbSNP builds. The data presented in the browser for each analysis are downloadable from the public dbGaP ftp site. The original submission, including genotype counts and allele frequencies, are fully accessible through NCBI Controlled Access system. This presentation will describe the current association results, use of tools to explore and download the results, and future development of GWAS results data at dbGaP.

904T

A New Reference Dataset for Imputing Common and Uncommon SNPs. *Z. Wang[1,2], K.B. Jacobs[1,2], M. Yeager[1,2], A. Hutchinson[1,2], J. Sampson[1], M. Tucker[1], S.J. Chanock[1].* 1) Core Genotyping Facility, SAIC-F/NCI, Bethesda, MD; 2) Division of Cancer Epidemiology and Genetics, NCI, NIH, Bethesda, MD.

Statistical imputation of SNP genotype data is a commonly used technique in the analysis of genome-wide association studies. This approach is used to infer additional genetic variants to test for association with medically-relevant states, to remove missing data from dense datasets to add statistical power or to apply methods that are intolerant of missing data, and most importantly to allow the combined analysis of genotype data obtained from different SNP assays and assay versions. The accuracy of genotype imputation depends on the size, data quality and population representation of the imputation reference panels. We have developed a dataset that, in combination with data from HapMap and 1000 Genomes Project, is a novel set for performing genotype imputation of common and uncommon SNPs (MAF greater than 1%): (1) ~750 cancer-free individuals of European descent from three large prospectively sampled cohort studies genotyped on the Illumina HumanHap1M, HumanOmni1M, HumanOmni2.5 arrays; (2) 75 African American individuals and 75 Chinese individuals genotyped on the HumanOmni2.5 array; and (3) 441 HapMap samples genotyped on the HumanOmni2.5 array. The cleaned and harmonized dataset contains approximately 3.1 million SNPs and 1,300 individuals. Using this reference set, we explore different imputation scenarios starting from datasets genotyped using the Illumina HumanHap660W and HumanOmniExpress assays. We show a substantial improvement in imputation accuracy when using our reference panel to impute all 3.1 million SNPs versus the standardly used combination of 1000 Genomes and HapMap data for both scenarios using the IMPUTE2 and BEAGLE software. We also demonstrate several interesting results on the power of imputation analysis when the reference population does not precisely match that of the inference set and for hybrid genome-wide scans that combine data from the Illumina HumanHap660W and OmniExpress assays. This reference dataset will be shared with the scientific community through the NCBI dbGaP portal and be provided as conventional genotype data files, preformatted for use with the IMPUTE2, Beagle and MACH software. An upcoming version of the dataset will include Affymetrix 6.0 array data and additional data from non-European populations. Funded by NCI Contract N01-CO-12400.

905T

Genome-wide association study identifies novel susceptibility candidates for Kawasaki disease. *J. Wu[1], Y. Lee[1], F. Tsai[2], L. Chang[1], Y. Liu[1], Y. Chen[1], C. Chen[1], Y. Chen[1].* 1) Institue Biomedical Sci, Academia Sinica, Taipei, Taiwan; 2) Department of Medical Genetics, Medical Research and Pediatrics, China Medical University Hospital, Taichung, Taiwan.

Kawasaki disease (KD) is an acute systemic vasculitis syndrome that primarily affects infants and young children with epidemiological observations suggesting a substantial genetic contribution to disease susceptibility. Taiwan has the third-highest incidence of KD in the world, after Japan and Korea. To investigate novel mechanisms that might predispose individuals to KD, we conducted a genome-wide association study (GWAS) in 622 KD patients and 1107 controls in a Han Chinese population residing in Taiwan, and further validated our findings in an independent Han Chinese cohort of 261 cases and 577 controls. Three loci with most strongly associated single-nucleotide polymorphisms (SNPs) were detected, of which two novel loci were identified and a COPB2 locus previously identified in GWAS was confirmed. The first novel locus is associated with the tolerance mechanisms of B cells; the second locus is involved in stimulatory signal of immune response. Both regions were previously identified associated with several other autoimmune diseases. These findings have implications for understanding the immune activation in KD pathogenesis.

906T

In search of genetic attributors of handedness. *M. Xu[1], G. Pare[4], P. Kraft[1], E.B. Rimm[1,2], D.J. Hunter[1,2], D. Chasman[4], J. Han[1,2,3].* 1) Epidemiology, Harvard School of Public Health, Boston, MA; 2) Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston; 3) Clinical Research Program, Department of Dermatology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 4) Division of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

About 10% of humans are left-handed and it has previously been estimated from twin studies that the heritability of handedness is around 24.64%. Left-handedness has been shown to be associated with pathological conditions such as lower birth weights, higher incidence of schizophrenia. We conducted genome-wide association studies (GWAS) of genotyped and imputed 2.54 million SNPs (data set 1) on handedness with 9,428 subjects and another GWAS in an independent population with 18,994 subjects (data set 2) followed by a meta-analysis of these two GWASs. The rs16879276 SNP on chromosome 6 had the smallest p-value 2.8 x 10-7, which is 64kb upstream of ATXN1, a gene that is associated with schizophrenia and ataxia (inability to coordinate muscle movements). GWAS with data set 1 identified a SNP rs6719499 on chromosome 2 with p-value = 1.04 x 10-6 in the CTNNA2 gene region previously reported to be associated with handedness and schizophrenia, and the p-value of this SNP is 0.0013 in the meta-analysis. To investigate the role of common variants in handedness heritability, we used 151,154 SNPs genotyped in data set 2 and either genotyped or imputed with R-squared greater than 0.99 in dataset 1 to estimate additive genetic contribution to handedness and found these SNPs explain 14.7% (95% CI 0, 35.3%) variance. After adjusting for the top 4 eigenvectors, these SNPs explain 4.22% (95% CI 0, 26.9%) variance, suggesting the strong influence of population stratification on handedness for either genetic or cultural implications. Analyses on the eigenvectors found the Jewish groups have significantly higher incidence (10.8%) of left-handed people than other religious groups (7.0%). The low percentage of variance explained by commons SNPs suggests a large portion of handedness heritability may come from other genetic factors such as rare SNPs, imprinting, and copy number variation whose information is not contained in the common genetic variants.

907T

Bimodality filtering to prioritize genes for eQTL analysis of allelic imbalance. *K. Azad[1,2], B. Ge[4], T. Pastinen[4,5], C.M.T. Greenwood[1,2,3].* 1) Lady Davis Institute for Medical Research, Montreal, QC, Canada; 2) Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, QC; 3) Department of Oncology, McGill University, Montreal, QC; 4) McGill University and Genome Quebec Innovation Centre, Montreal QC; 5) Department of Human Genetics, McGill University, Montreal, QC.

Introduction: eQTL analysis usually involves many tests looking for associations between cis-SNPs and gene expression levels. However, severe corrections for multiple testing are necessary due to the number of tests performed. Here, we are investigating a two-stage process to prioritize genes for eQTL analysis of allelic imbalance, by first identifying genes showing evidence for bimodal distributions of allelic imbalance. Association tests at bimodal genes are then given higher priority. We expect this strategy to lead to increased specificity.

Methods: High-density SNP genotype data for 11,365,000 markers, after imputation, was extracted for the 56 YRI samples from HapMap. We use a Tukey-biweighted mean of allelic imbalance across all heterozygous SNPs within each gene to obtain a measure of differential allelic expression. We then use three indexes of bimodality, variance reduction score (VRS), bimodality index (BI), and the dip test (DIP) of unimodality, to identify a subset of genes whose distributions seem to support a two-component mixture model. In this subset, we look for cis associations between allelic imbalance and nearby SNPs in a window around each gene +/-50kb.

Results: Among 5005 full-length transcripts on chromosome 1, bimodality could be evaluated for all but 29. Intraclass-correlation (ICC) was high (0.7) for VRS and BI, but DIP agreed poorly with the other two (ICC of all three measures: -0.4). On chromosome 1, we selected the genes with top 2% evidence for bimodality by any of the 3 measures, obtaining 282 bimodal genes. For 48 of these bimodal genes, at least one nearby SNP was associated with allelic imbalance with a p-value less than 0.001.

Conclusions: It is interesting that a large proportion of these genes demonstrating evidence of bimodality are not associated with a nearby common SNP. We anticipate that additional power to detect cis-SNPs will be obtained by this 2-stage strategy combined with stratified false discovery rate estimation.

908T

A linear complexity HMM phasing method for unrelateds and nuclear families. *O. Delaneau[1,2], J. Marchini[2], J.F. Zagury[1].* 1) Chaire de Bioinformatique, Conservatoire National des Arts et Métiers, Paris, France; 2) Department of Statistics, University of Oxford.

Phase information in diploid sequences is of crucial interest in genetic studies of human disease and population genetics. However, experimental methods to determine this information are still in development, making statistical alternatives widely used in practice. With datasets ever increasing in the number of markers, samples and diversity of populations from which samples originate, as highlighted by the 1000 Genomes Project, it is important to develop accurate, fast and flexible statistical phasing methods. Here we describe a phasing method called SHAPEIT, that relies on the same Gibbs sampler scheme used by methods such as PHASE, IMPUTE or MACH, but differs in the way that each individual's haplotypes are updated conditional upon the other haplotypes in the sample. We have made two improvements to this core step. First, we build a graph representation of the full list of the conditioning haplotypes on which we can derive a compact hidden Markov model (CHMM) similar to the Li & Stephens model but with a limited number of states. Second, we use a separate graph per individual to represent the space of possible haplotypes consistent with each individual's genotypes. The transition probabilities between nodes of this graph are quickly computed using the CHMM and then pairs of haplotypes consistent with each genotype can be efficiently sampled. This new method has several notable features (1) it scales linearly in the number of haplotypes used in each iteration, which leads to improved accuracy and speed, (2) it can handle mixtures of unrelated samples and small nuclear families, (3) it can be run efficiently on whole chromosomes at once, and (4) phasing uncertainty is captured within the graph constructed for each individual. We illustrate the improved performance of the method compared to IMPUTE, BEAGLE, FASTPHASE and MACH on several real datasets with both European and African ancestries.

909T

RNA Polymorphisms: Impact on the Transcriptome and on Complex Traits. *E.R. Gamazon[1], D.S. Park[2], N.J. Cox[1].* 1) University of Chicago, Chicago, IL; 2) Columbia University, New York, NY.

RNA editing characterizes a wide variety of molecular phenomena in which nucleotide information in an RNA molecule is modified, causing a difference between the amino acid sequence of the encoded protein and the predicted sequence from the template genomic DNA. The mechanisms by which these differences between DNA template and RNA product arise remain to be elucidated, and their biological implications are little understood. Recent advances in assaying the genome and the transcriptome at single nucleotide resolution may facilitate studies of RNA editing and promise to expand our understanding of this relatively novel form of gene regulation. While studies of disease susceptibility and drug response have focused on DNA sequence polymorphisms or mRNA expression variability, RNA sequence polymorphisms are likely to contribute to the etiology of at least certain complex traits. In this study, we set out to clarify the biological impact of DNA and RNA sequence differences. Using RNASeq data from samples of European and African ancestry, we uncovered loci with high coverage where the DNA and RNA sequences show a consistent type of nucleotide difference in multiple individuals within each population and across populations. Through the use of an independent gene expression assay, we found that genes with RNA editing sites are significantly enriched for the most differentially expressed genes across a range of p value thresholds. We conducted a systematic study of the genes identified as harboring such RNA editing sites for their role in pharmacologic phenotypes. Our findings strongly support the hypothesis that identified and validated DNA-RNA differences have biological significance and their inclusion in studies of complex traits are likely to advance our understanding of etiology.

910T

TAG-PCR allows rapid high-throughput sequencing of targeted genomic regions in pooled barcode-labeled samples. *G. Haller, C. Cruchaga, A. Goate.* Department of Psychiatry Washington University 660 S. Euclid Ave. St. Louis, MO 63110.

We demonstrate the use of Tagged, Adapter-ligated Genomic DNA (TAG) fragments as template for the amplification and sequencing of targeted genomic regions in pooled samples using next-generation technology. Individual DNA samples were prepared for Illumina sequencing using single-end barcoded adapters. Samples were then pooled and used as the template for PCR amplification of targeted genomic regions using one primer complimentary to the adapter used for Illumina sequencing and one region-specific primer with a 5' overhang complementary to the second adapter and ending with a biotin molecule. The product of this reaction was then purified with streptavidin-coated magnetic beads and sequenced on an Illumina GAIIx. Using this method, we sequenced the entirety of the *TAS2R16* gene in 96 individuals. We obtained ~20 million reads from one lane of single-end 36bp Illumina sequencing. These reads were first separated based on barcode, allowing a total of ~19 million reads (95%) to be unambiguously assigned to one of the 96 sequenced individuals. Barcodes were specifically designed to differ from all other barcodes at a minimum of 2 sites, allowing even those reads containing one misincorporated base to be assigned. Reads for each individual were then aligned to the reference sequence using MAQ. Greater than 30% of individual assigned reads aligned to amplified regions. This figure will likely improve with changes to the PCR protocol as well as primer characteristics, however. Individual genotypes were then determined at all sites with at least 8-fold coverage. Variant calling was possible at an average of 95% of sequenced sites across the sequenced individuals. We then validated a subset of sites found to be polymorphic using previously obtained genotyping results. All chosen predicted polymorphic sites were confirmed. Dideoxy-sequencing of 10 sequenced individuals across the entirety of *TAS2R16* verified the absence of variants not identified by pooled sequencing. Additionally, TAG-PCR utilizes DNA samples prepared and pooled before amplification, reducing reagent costs substantially, allowing for rapid sequencing of future loci of interest within the same pooled sample and providing the flexibility to sequence hundreds of loci in a single individual or a single locus in hundreds of individuals.

911T

High-throughput single molecule DNA mapping in nano-channels. *E.T. Lam[1], A. Hastie[2], D.S. Ehrlich[1], D. Somas[2], M.D. Austin[2], P. Deshpande[2], C. Lin[1], H. Cao[2], M. Xiao[2], P.-Y. Kwok[1].* 1) Institute of Human Genetics, University of California, San Francisco, San Francisco, CA; 2) Bionanomatrix, Philadelphia, PA.

Many genomes from normal and diseased individuals have recently been sequenced by short-read technologies. The advancement in sequencing has led to better characterization of single-nucleotide variants. Despite the explosion of data, mapping of short reads relies on the reference genome sequence, and de novo genome assembly using short reads remains difficult. Also, structural variants and long-range haplotypes are inferred from short sequencing reads by computational means with great uncertainty. In-depth analysis of structural variants requires laborious methods such as PCR and fluorescence in situ hybridization. Here, we present an optical mapping approach that complements short-read data in sequence assembly and analysis of structural features. Silicon-based nano-channels are fabricated for uniform DNA linearization. Labeling of sequence-specific sites and subsequent high resolution imaging of DNA molecules enable analysis of individual DNA molecules of several hundred kilobases (kb) in a linear, single haplotype form. To illustrate the utility of our method in mapping, we have used previously mapped bacterial artificial chromosomes (BACs) to reconstruct the major histocompatibility complex (MHC) region, a gene-rich and highly polymorphic region important for immune functions and known to be structurally complex. 49 BACs from the PGF library and 46 BACs from the COX library were analyzed as single BACs and as mixtures. An automated pipeline was used for unsupervised clustering of molecules with different labeling patterns, deriving consensus optical maps, and assembly. Molecules from individual BACs were recovered, and labels of more than 2 kb apart could be clearly resolved. In parallel, the BACs were sequenced using the Illumina HiSeq 2000 for comparison of short-read sequencing and our method for assembly of the MHC region. Our approach will enable high-throughput assembly of structurally complex regions and characterization of structural variants.

912T

Genomic Analysis of 15q Duplication/Deletion Disorders Reveals Differential Regulation of Genes with FOXP1 and NF-Y Binding Sites. *L. Reiter[1,2,3], T.J. Bischell[4], W. Taylor[2,5], N. Urraca[1].* 1) Dept Neurology, UTHSC, Memphis, TN; 2) Dept. of Anatomy and Neurobiology, UTHSC, Memphis, TN; 3) Dept. of Pediatrics, UTHSC, Memphis, TN; 4) Vanderbilt Kennedy Center, Vanderbilt University, Nashville, TN; 5) Molecular Resource Center, UTHSC, Memphis, TN.

Angelman syndrome (AS) and interstitial duplication 15q autism (int dup(15)) are two reciprocal genomic disorders caused by maternal allele deletion or duplication of the 15q11.2-q13 region. Although AS is caused by maternal loss of the *UBE3A* gene most AS patients have a more complex phenotype, in part due to the deletion of additional genes at the 15q locus. Maternal duplications of this region cause an autism phenotype, while paternal duplications do not, clearly implicating the maternally expressed *UBE3A* gene in the autism phenotype. Although *UBE3A* is not imprinted in peripheral blood mononucleocytes (PBMC), UBE3A protein levels are elevated in blood. We investigated both chromatin and gene expression changes in blood from three int dup(15) Class II and three reciprocal AS deletion Class II subjects to identify global genomic and gene expression changes that may influence the AS and autism phenotypes. Using whole genome sequencing combined with formaldehyde-assisted isolation of regulatory elements (FAIRE) we identified 1104 regions of differential open chromatin in AS deletion and 2344 regions int dup(15) indicating global changes in chromatin could influence gene expression in these regions. Microarray analysis from PBMC revealed 1236 genes that were elevated in AS deletion PBMC vs int dup(15) and 981 genes that were elevated in int dup(15) vs AS deletion PBMC (pval<0.05). As expected, significant differences in expression were found for genes in the duplicated/deleted locus like *UBE3A*, *ATP10A* and *HERC2*. Among the 981 genes elevated in int dup(15) there was a significant enhancement for genes with binding sites for transcription factors NF-YA and FOXP1. Although we expected to find peaks indicative of open chromatin at loci for differential gene expression, we actually found a large set of genes with FAIRE peaks in AS deletion but increased transcription in Int dup(15) subjects. Among these were genes involved in chromatin remodeling, DNA repair and neurogenesis. This combined chromatin and microarray analysis provides the first insights into transcriptional changes which may be mediated by the transcriptional co-activation function of the UBE3A protein unveiling new sets of genes and pathways contributing to both AS and int dup(15) autism pathogenesis.

913T

Extensive characterization of NF-2B binding uncovers non-canonical motifs and advances the interpretation of genetic functional traits. *D. Wong[1], A. Teixeira[1], S. Oikonomopoulos[1], P. Humburg[1], I.N. Lone[3], D. Saliba[2], T. Siggers[5], M. Bulyk[5,6,7], D. Angelov[3], S. Dimitrov[4], I. Udalova[2], I. Ragoussis[1].* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, Oxfordshire, United Kingdom; 2) Kennedy Institute of Rheumatology, Imperial College, 65 Aspenlea Road, London W6 8LH, UK; 3) Université de Lyon, Laboratoire de Biologie Moléculaire de la Cellule, CNRS-UMR 5239/INRA 1237/IFR128 Biosciences, Ecole Normale Supérieure de Lyon, 46 Allée d'Italie, 69007 Lyon, France; 4) Université Joseph Fourier - Grenoble 1; INSERM Institut Albert Bonniot, U823, Site Santé-BP 170, 38042 Grenoble Cedex 9, France; 5) Division of Genetics, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA; 6) Harvard-MIT Division of Health Sciences and Technology (HST), Harvard Medical School, Boston, MA 02115, USA; 7) Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA.

Genetic studies have provided ample evidence of the influence of non-coding DNA polymorphisms on trait variance, particularly those occurring within transcription factor binding sites (TFBSs). Protein binding microarrays and other high-throughput platforms that map TFBS with great precision have enhanced our understanding of how a single nucleotide polymorphism (SNP) can alter binding potential allowing for a prediction of its effect on a TFBS. When used in conjunction with ChIP-Seq, these enable allele-specific analyses of expression and DNA-TF interactions on a genome-wide scale. We have used both arrays and Electrophoretic Mobility Shift Assay-Sequencing (EMSA-Seq), a deep sequencing based method we developed to analyze the DNA binding preferences of 9 distinct NF-2B TF-dimers. Despite this TF-family being one that has been extensively studied, our understanding of its DNA binding preferences is largely limited to the consensus motif, GGRRNNYYCC which was first described 25 years ago. We highlight differences in the binding preferences of NF-2B family members and also, put under the spotlight non-canonical motifs that are bound by NF-2B dimers and that may be found within genomic regions in which a canonical motif is not evident. When overlaid onto a dataset describing the binding of NF-2B RELA immunoprecipitated from lymphoblastoid cells across eight individuals (Kasowski *et al*., 2010), our binding affinity data enhances the interpretation of TF-binding across 1405 genomic regions laden with SNPs. The overall approach is equally applicable to other TFs and is a relevant resource for immunologists, geneticists and systems biologists and the wider scientific community.

914T

Characteristics of an Online Consumer Genetic Research Cohort. *J.Y. Tung, N. Eriksson, A.K. Kiefer, J.M. Macpherson, B.T. Naughton, A.B. Chowdry, C.B. Do, D.A. Hinds, A. Wojcicki, J.L. Mountain.* 23andMe, Inc, Mountain View, CA.

The increasing availability of internet access and the decreasing costs of generating genetic information have created a new group of individuals who are active internet users with access to their own genetic information. We hypothesized that these individuals would be interested in sharing their genotypic and phenotypic information for research if given the opportunity to do so by providing data online, thus removing any geographic restriction for participating in a study. All customers of 23andMe, a direct-to-consumer genetic testing company, were recruited for an ongoing research project entitled 23andWe. We evaluated consent rate, demographics (including sex, age, education, self-identified race, and genetic ancestry), and research questionnaire response rate in this cohort. Most customers consented to participate in 23andWe, and the majority of those further agreed to have their saliva sample banked and also potentially used for future research. Over half of participants answered at least one research question, with an average of 10 questionnaires completed, and 75 singular "Research Snippet" questions answered. Despite the fact that the majority of customers did not obtain the 23andMe service in order to participate in research, we have found that most are willing to contribute their data for research and that more than half actively contribute additional phenotypic data online, suggesting that tapping into consumer interest in genetics and research is a viable method for conducting large-scale studies.

915T

Simultaneous Evaluation of Small RNA, Whole Transcriptome, Whole Genome and Targeted Resequencing on Next Generation Sequencing Platforms. *C. Patel, N. Fong, J. Briggs, Q. Doan, M. Storm, Y. Sun, P. Kinney, R. Padilla, H. Shepherd, L. Joe, T. Burcham.* Life Technologies, Foster City, CA.

Researchers are using Next Generation Sequencing platforms to support more and more applications. It is critical for such platforms to be flexible enough to support many applications at the same time while maintaining low cost, high quality and high throughput. In this study we describe the development of an end to end solution from sample prep to analyzing the sequencing run. The test was to interrogate up to six independent samples simultaneously on a single run. In a single run we sequenced two lanes with single-plex human long mate pair sample, a multi-plex of universal human reference whole exome, ChIP-Seq sample, a kinome enriched sample, and a whole transcriptome sample. Each sample type used required a different sequencing run, yet with this new process they all were run together saving time, reagents, and freeing the researcher from having to wait to collect sample of the same type to run together.

916T

Enhancer mixture for amplifying genes with CpG islands in PCR. *F. DE LA CRUZ-RUIZ, M.C. MARTINEZ-LOPEZ, J.L. CORTES-PEÑALOZA, D. CADENA-SANDOVAL, E. ZAMARRON-LICONA.* LABORATORIO DE DIAGNOSTICO MOL, Universidad Juárez Autónoma de Tabasco (UJAT), VILLAHERMOSA, TABASCO, Mexico.

Summary Background: Several biological additives have been used to make sure polymerization in GC-rich DNA sequences Objective: To design a PCR enhancer mixture for amplifying genes with CpG islands. Methods: Fragments of IRS2 and HNF1(; genes were analyzed using EMBOSS CpG Report software, Primers were designed with the Primer3 software and were tested with e-PCR. Three additives were used: BSA (0,1 µg/µl), DMSO (5%) and Formamide (5%), in five assays of PCR, two using one additive, two combining two additives and one with all additives. DNA sequences were amplified with the enzymes: Taq native, recombinant and platinum. Amplicons quality was tested by sequencing. Results: HNF1(gene were amplified only with the three enzymes with no additives, however sequence was not pure. IRS2 gene sequences achieved pure amplification in all fragments with the whole enzymes package and DMSO-BSA-Formamide mixture addition. Conclusion: DMSO-BSA-Formamide mixture can be used to obtain GC-rich DNA amplicons with such a high quality that generates pure chromatograms during sequencing.

917T

Non-invasive specimen collection for molecular diagnostics and point-of-care testing. *C. Dobbin, M. Abdalla, M. Elmogy, W.-S. Kim, Y. Haj-Ahmad.* Norgen Biotek Corp, Thorold, Ontario, Canada.

Blood has traditionally been the specimen of choice for various molecular diagnostic analyses of disease-specific biomarkers. However, there are a number of drawbacks when using blood for diagnostics, including: 1) it is complicated to collect, requiring highly trained personnel; 2) it is infectious and poses a risk to technicians performing the collection; 3) it is invasive and painful for the patient; 4) collections must be performed into filling tubes with additives, such as anti-coagulants, which may affect downstream assays; 5) blood is a very complex media and contains inhibitory compounds known to interfere with molecular assays such as PCR and; 6) there is also a substantial risk to neonates or young children who risk becoming anemic by repeated blood collections. As a result Norgen Biotek is now focusing on the use of non-invasive specimens as a source of biomarkers for disease diagnostics. Much research has now accumulated which has unveiled a number of disease biomarkers in these biological fluids for the diagnosis of microbial and systemic disease. The advantages of using biological specimens such as saliva, urine or sputum for disease diagnostics include: 1) ease of access; 2) noninvasive sample collection; 3) increased acceptance by patients; 4) reduced risks of infectious disease transmission and; 5) lower costs. Whereas blood exists in the circulation for several days, passing through organs that can potentially alter biomarkers that are present, saliva is a "real-time" fluid since the salivary glands are exocrine glands that produce biomarker profiles indicative of the individual's status at the moment of collection. In addition, specimens such as urine are abundant and allow for repeated samples to be collected. As saliva, urine and sputum can be collected non-invasively, by untrained personnel they have also gained widespread interest as a diagnostic specimen for rapid point-of-care testing. The objective of this research was therefore to develop a universal diagnostic solution for a complete, ready-to-use system for the isolation and detection of disease from non-invasively collected samples. In particular, the rapid isolation of total nucleic acids (DNA & RNA) and proteins from various specimen types (urine, saliva or sputum) using Norgen's proprietary resin. Preservation and detection of the collected specimens for sensitive detection assays will also be discussed.

918T

Measuring the chronology of technology transfer of molecular genetic discoveries. *F. Rousseau[1, 2, 3], C. Lindsay[2, 3], Y. Giguère[1, 2, 3], J. Jbilou[2, 4], R. Landry[2, 5], N. Amara[2, 5].* 1) Biol Medicale, Univ Laval, Quebec, PQ, Canada; 2) APOGEE-Net/CanGèneTest Network on Genetic Services and Policy, International; 3) URGHM, Centre de recherche du CHUQ, Hôpital St-François-d'Assise, PQ, Canada; 4) Dept of Public administration, University of Moncton, NS, Canada; 5) Dept Management, University Laval, PQ, Canada.

Purpose:The process of technology validation and transfer of new molecular diagnostic tools towards the clinic faces challenges and needs to be improved. Albeit massive investments in genomic research, molecular genetic tests represents <0.05% of all diagnostic laboratory tests in many Western countries. There is no empirical measure of the process nor of the pace of technology transfer of molecular genetic discoveries. We studied the chronology and pace of technology transfer for 28 different molecular genetic test discoveries with the objectives to 1)provide estimates of the timeframe between discovery and complete clinical implementation, and 2)compare the trajectories between different new tests to identify common patterns and bottlenecks. Methods:We developed a list of 11 publicly available "timestamps" for the technology transfer process. 28 different well known molecular genetics tests (with different characteristics) were selected. For each test selected, public databases were searched in order to identify available timestamps and dates. Trajectories of individual tests, including chronology, were plotted and compared using standard statistics. Results:There is much variability in the chronology of transfer from test to test. The mean time between discovery and clinical test offer was 10 years (minimum 2). There was a mean time of 11 years between discovery and publication of the first health technology assessment report (minimum 2 years). A mean of 15 years spanned between test discovery and FDA approval (minimum 9), and it took a mean of 17 years between discovery and the availability of a certified reference material (minimum 13). Our analysis revealed variability between different molecular tests in terms of trajectory. Indeed the sequence of events is not always identical between tests, as well as the time between individual timestamps. Further, all steps are not present for all tests. Short time spans were typically observed for molecular tests with clear clinical utility, filling a need, or replacing a poorly performing test, amongst other factors. Conclusion:New molecular genetic tests seem to take significant time between discovery and clinical implementation. However, the trajectory and pace of transfer appears to be very variable and influenced by tests characteristics and performance. Further work is needed to pinpoint key factors, including policy and organization factors, that may allow for improving and streamlining this process.

919T

DUNES Environment: A Suite of Tools and Software to Support Data Harmonisation and Synthesis. *D. Doiron[1], V. Ferretti[2], F. L'Heureux[1], M. Naccache[1], I. Fortier[3].* 1) Public Population Project in Genomics (P3G), Montreal, Quebec, Canada; 2) Ontario Institute for Cancer Research, MaRS Centre, Toronto, Ontario, Canada; 3) Research Institute - McGill University Health Centre, Montreal Quebec, Canada.

Very few individual studies provide the statistical power needed to thoroughly investigate complex interactions between environmental and genetic factors in chronic disease etiology. It is therefore necessary and increasingly common to pool information collected from participants across several studies to increase the sample sizes available for statistical analyses. However, ensuring the quality of pooled information requires a formal and rigorous approach and specialized data harmonisation tools and software. Such an approach must not only ensure that pooled data is measured well but that the information each study conveys is effectively compatible. Impelled by a growing demand from epidemiology consortia and networks around the world, DUNES Environment has been launched to consolidate existing data harmonisation tools and methodologies developed under the umbrella of the Public Population Project in Genomics (P3G) and its partner projects. As a framework to support data harmonisation, DUNES Environment has established a five-step approach which is supported by a suite of web-based software. As a first step, the scientific objectives and scope of a cross-study harmonization initiative must be defined and its participating studies should be well documented. That is, study designs and sampling protocols, calendar times of exposures and outcomes, data access policies, as well as all relevant information describing data collected by participating studies (e.g. questionnaires, data dictionaries, SOPs) is properly catalogued. Second, target variables to be shared between studies are defined and annotated. Third, the harmonisation potential between the data collected by participating studies and the variables targeted by the harmonisation project is evaluated. Fourth, processing algorithms are developed and implemented to transform original study data into a common compatible format. Lastly, pooled data is disseminated to investigators and appropriate statistical analyses are conducted. For each step, open-source web-based software has been developed to streamline the entire process. Among these, the P3G Catalogues, DataSHaPER, Opal, and Mica software are being made freely accessible to the scientific community. DUNES Environment ultimately aims to facilitate collaborative research by offering a centralized web-based infrastructure that facilitates the harmonisation process and ensures the validity and compatibility of pooled data.

920T

Miniaturization and Automation of One-step Gene Expression using the Access™ Laboratory Workstation and the Echo® liquid handler. *R. Dyer, C. Glazer, S. Datwani, J. Barco, T. Allison.* Labcyte Inc., Sunnyvale, CA.

As gene expression workflows are pushed to the next step of miniaturization, sample preparation techniques must evolve. The Labcyte Echo liquid handler utilizes tipless, touchless, transfer of samples and reagents to enable the highest precision and accuracy at nanoliter volumes. With a seamless addition of automated plate handling, the Access workstation transforms the Echo platform into a walk-away solution tailored for gene expression. This poster illustrates the ability to prepare a one-step RT-qPCR reaction as low as 300 nl, directly from cell lysates, in both 384- and 1536-well plates. The automated workflow, along with the consistency and accuracy of these reactions are discussed. With the Access workstation, high throughput gene expression screens using more relevant cell models including primary cells can replace less sensitive cell-based reporter assays.

921T

Sequencing of expanded CGG repeats in the *FMR1* gene. *E. Loomis[1], J. Yen[2], J. Major[2], F. Tassone[3], P.J. Hagerman[1,3].* 1) Univ. of Calif. Davis School of Medicine, Biochemistry and Molecular Medicine, Davis, CA; 2) Pacific Biosciences of California Inc., Menlo Park, CA; 3) Univ. of Calif. Davis School of Medicine, MIND Institute, Sacramento, CA.

The 5' untranslated region (5' UTR) of the fragile X mental retardation 1 (*FMR1*) gene contains a CGG-repeat element that, when expanded beyond ~200 CGG repeats, generally undergoes methylation-coupled transcriptional silencing, giving rise to fragile X syndrome (FXS); smaller expansions (55-200 CGG repeats) result in elevated levels of *FMR1* mRNA, which is now thought to be responsible for the late-onset neurodegenerative disorder, fragile X-associated tremor/ataxia syndrome (FXTAS). Previous DNA sequencing methodologies have been unable to sequence cleanly through more than ~100 CGG repeats, thus limiting the ability to precisely characterize such disease-causing alleles. The recent development of single molecule, real-time sequencing (Pacific Biosciences) represents a novel approach to DNA sequencing that couples the intrinsic processivity of DNA polymerase with the ability to read polymerase activity on a single-molecule basis. Furthermore, the accuracy of the method is improved through the use of closed circular templates, such that each molecule can be read multiple times to produce a circular consensus sequence (CCS). We have succeeded in generating CCS reads representing multiple passes through both strands of large expanded CGG repeats (>400 CGGs) flanked by native *FMR1* sequence. This application will allow us to fully characterize the previously intractable CGG repeat sequence, leading to a better understanding of the distinct FXS and FXTAS molecular pathologies.

922T

Next-Gen Sequencing of HLA class I and class II exons with GS GType HLA Primer Sets on the GS Junior System. *C.S. Teiling[1], C. Holcomb[2], B. Hoglund[2], P. Moonsamy[2], B. Simen[3], E. Blake[3], K. McGowan[3], H.A. Erlich[2].* 1) Roche Applied Science, Indianapolis, IN; 2) Roche Molecular System, Pleasanton CA; 3) Roche/ 454 Sequencing, Branford CT.

Inherited DNA sequence variation in the HLA region on human chromosome 6 significantly influences the risk for autoimmune diseases and the host response to pathogenic infections. Collecting allelic sequence information at the HLA class I and class II genes is critical for studying hematopoietic stem cell transplantation for genetic association studies, but is complicated due to the high degree of polymorphism across the major histocompatibility (MHC) antigen region. Next-generation sequencing offers a cost-effective alternative to Sanger-based sequencing, which has been the standard for HLA typing, with the new GS GType HLA Primer Sets. The GS GType HLA Primer Sets together with the GS FLX Instrument or the benchtop GS Junior Instrument enable high- and medium-resolution genotyping of the class I and II loci of the HLA genes. The clonal, high-throughput, long reads generated by these 454 Sequencing systems provide a direct, unambiguous assignment of phase for linked polymorphisms within exons. This enables third-party genotyping software, such as the Conexio Genomics Assign ATF 454 software, to consolidate reads and, by comparing sequences to the IMGT HLA sequence database, assign alleles automatically. As shown by the data presented, in a typical run of 5 to 6 samples, the concordance of genotype assignments at 8 loci using the GS Junior Instrument and Conexio software was 100% and unambiguous allele assignment was 59%. 454, 454 SEQUENCING, GS FLX, GS JUNIOR, and GTYPE are trademarks of Roche. All other product names and trademarks are the property of their respective owners.

923T

Genetic modification of cancer cells using non-viral, episomal S/MAR vectors for in vivo tumour modelling. *S.P. Wong, O. Argyros, R. Harbottle.* Molecular Medicine, Imperial College London, London, Greater London, United Kingdom.

The development of genetically marked animal tumour xenografts is an area of ongoing research to enable easier and more reliable testing of cancer therapies. Genetically marked tumour models have a number of advantages over conventional tumour models, including the easy longitudinal monitoring of therapies and the reduced number of animals needed for trials. Several different methods have been used in previous studies to mark tumours genetically, however all have limitations, such as genotoxicity and other artefacts related to the use of integrating viral vectors. Recently we have generated an episomally maintained plasmid DNA (pDNA) expression system based on Scaffold/Matrix Attachment Region (S/MAR), which provides permanent genetic modification and persistent transgene expression in tumour cells. Here we describe the use of this pDNA vector to create stable genetically marked human hepatoma (Huh7), pancreatic carcinoma (MIA PaCa-2) and Birt-Hogg-Dube Syndrome folliculin knockout (UOK 257) cell lines. These modified cells were xenografted into NOD-SCID mice and monitored longitudinally over time. Each cell line exhibited the permanent episomal maintenance of the vector and sustained long-term transgene expression. Importantly, each formed tumours showing the pathological characteristics of hepatocellular carcinoma, pancreatic carcinoma and renal cancer respectively. This is the first demonstration that a non-viral, replicating, non-integrating pDNA vector can confer sustained transgene expression in various murine tumour models. We believe that this system provides great utility and advantages over currently used technologies and will be a useful resource to the field of cancer research.

924T

High throughput validation of next generation sequencing results from lung cancer samples using a novel custom genotyping platform. *R. Selzer[1], K. Lo[1], J. Patel[1], M. Watt[1], X. Zhang[1], T. Richmond[1], M. You[2], P. Liu[2], T. Albert[1].* 1) Roche NimbleGen, Inc., Madison, WI; 2) Medical College of Wisconsin, Milwaukee, WI.

Massively parallel sequencing technologies have revolutionized the way we conduct genomics research by providing an unbiased and comprehensive method of detecting novel single nucleotide polymorphisms (SNPs), insertions-deletions (Indels) and structural rearrangements within complex genomes. As sequencing cost declines, sequencing is fast becoming the method of choice for discovery. However, due to massively parallel nature of these novel technologies, even miniscule error rates can translate into hundreds or thousands of false discoveries. Thus, downstream validation is essential to ensure reporting accuracy. Today, Sanger sequencing remains the de facto gold standard for downstream validation but suffers from low throughput and high cost. Mass spectrometry based methods, such as Sequenom, improve upon throughput but are still limited to hundreds of putative SNPs. Genotyping arrays, such as Illumina iSelect HD, provide up to 1 million SNPs for validation but require steep up-front costs. The need for a high throughput and low cost validation method remains unmet. We have developed a novel SNP validation assay which utilizes both hybridization and on-array enzymatic reactions to provide enhanced specificity for SNP validation. The probe is specifically designed to provide discrimination between perfect match and mismatch by combining a long hybridization oligonucleotide with a hairpin. Stringent hybridization of genomic DNA to an array is followed by flap endonuclease (FEN) treatment, which provides the first layer of discrimination. The tripartite structure of the hairpin complexed with the hybridized genomic DNA is recognized by FEN, resulting in the cleavage of the 5' end overhang of the genomic DNA. Additional discrimination is provided by DNA ligase, which does not catalyze the ligation when a mismatch of the 5' end of the newly cleaved substrate is present. Covalent attachment of genomic DNA to the probe occurs when the probe sequence matches the cleaved genomic DNA sequence perfectly. In this study, we validated 13,500 SNPs from the DNA of 11 pairs of matched lung cancer and normal tissues that were previously exome-capture sequenced to an average depth of 86X using the Illumina paired end library prep protocol on an Illumina Genome Analyzer II sequencer. Overall, accuracy of 95.4% was achieved, demonstrating this method as an accurate and high-throughput solution to validating sequencing results.

925T

Single-Step Capture and Sequencing of Natural DNA for Detection of BRCA1 Mutations. *J. Thompson, J. Reifenberger, E. Giladi, K. Kerouac, J. Gill, E. Hansen, A. Kahvejian, P. Kapranov, T. Knope, D. Lipson, K. Steinmann, P. Milos.* Res & Development, Helicos Biosci, Cambridge, MA.

Genetic testing for disease risk is an increasingly important component of medical care. However, testing can be expensive which can lead to patients and physicians having limited access to the genetic information needed for medical decisions. To simplify DNA sample preparation and lower costs, we have developed a system in which any gene can be captured and sequenced directly from human genomic DNA without amplification, using no proteins or enzymes prior to sequencing. Extracted whole-genome DNA is acoustically sheared and loaded in a flow cell channel for single-molecule sequencing. Gene isolation, amplification, or ligation is not necessary. Accurate and low cost detection of DNA sequence variants is demonstrated for the BRCA1 gene. Disease-causing mutations as well as common variants from well-characterized samples are identified. Single-molecule sequencing generates very reproducible coverage patterns and these can be used to detect any size insertion or deletion directly, unlike PCR-based methods which require additional assays. Because no gene isolation or amplification is required for sequencing, the exceptionally low costs of sample preparation and analysis could make genetic tests more accessible to those who wish to know their own disease susceptibility. Additionally, this approach has applications for determining genomic sites of integration for gene therapy vectors, transposons, retroviruses, and other mobile DNA elements in a more facile manner than possible with other methods.

926T

Enhanced Multiplexing Capabilities Using the Agilent Technologies SureSelect*XTII* Target Enrichment System for Fast and Easy Pre-Capture Indexing. *B. Arezi[1], A. Giuffre[2], S. Joshi[2], H. Ravi[2], M. McCarthy[1], K. Chen[1], J. Ong[2], B. Novak[3], M. Visitacion[3], M. Hamady[3], C. Pabón-Peña[3], F. Useche[3], E. Lin[3], W. Liu[3], S. Hunt[3], D. Roberts[3], S. Happe[2], E. Leproust[3].* 1) Agilent Technologies, La Jolla, CA 92037; 2) Agilent Technologies, Cedar Creek, TX 78612; 3) Agilent Technologies, Santa Clara, CA 95051.

Massively parallel sequencing technologies have enabled scientists to discover rare mutations, structural variants, and novel transcripts at an unprecedented rate. Nevertheless, it remains cost-prohibitive to sequence entire genomes from large sample cohorts. To meet the demand for fast, inexpensive and accurate genome analysis method, Agilent Technologies has developed the SureSelect*XT* platform, an in- solution hybrid selection technology for systematic re-sequencing of candidate regions in the genome. To further reduce costs and take advantage of the increasing capacity of next-generation sequencers, we highlight the ability to pre-capture multiplex DNA samples in a single sequencing lane/slide while maintaining the coverage necessary to confidently make SNP calls. We demonstrate an automation-friendly, easy to use protocol where gDNA libraries are uniquely “tagged” and then combined pre-capture, to allow for maximum multiplexing (up to 96 indices). We also introduce the more streamlined SureSelect*XTII* kit that utilizes reagent master mixes for faster, easier, and more user-friendly preparation of samples for multiplex sequencing. We show high performance, as measured by capture efficiency, uniformity and reproducibility. The multiplexing capabilities of our kit extend to both small (<1Mb) and large (>50Mb) libraries. SureSelect captures maintain performance when sequenced alone in one lane/slide or multiplexed in a pool with other samples. When multiplexing HapMap samples, >98% concordance between SureSelect resequencing results and previously-determined genotype is observed. The unique 8bp 96 index show uniform performance across different bait sizes. Additionally, there are no dropouts and coverage and index representation is uniform. In summary, the SureSelect pre-capture indexing kit provides the ability to combine targeted enrichment with multiplexing, thus maximizing the number of samples that can be sequenced at one time, providing optimum time and cost savings without sacrificing performance.

927T

Automating high-throughput production of libraries for massively parallel sequencing platforms. *J. Bishop, M. Allen, W. Zhang, D. Mandelman, M. Landers, A. Harris, R. Bennett.* Life Technologies, Carlsbad, CA.

Advances in the capabilities of massively parallel sequencing platforms have outpaced methods for constructing sequencing libraries, which do not scale well to the tremendous speed and throughput capabilities of the sequencers. We describe here a set of protocols, robotics scripts, reagents and instrumentation developed to automate the simultaneous production of up to 96 high quality sequencing libraries. Methods for creating sequencing libraries are slow, tedious, prone to error and variation, and generally not optimized for automation. For robust, high throughput library creation, we describe here the development of robotic scripts for simultaneously producing 1-96 sequencing libraries on commonly used robotic fluidic-handling platforms. These automated protocols provide uniquely optimized adaptor concentrations and bead-based clean-up techniques to increase by several fold the yield of libraries from small amounts of input. The protocols accept 10-5000 ng of sheared DNA input, calculate and dilute barcode adaptors for each library, automate all intermediate processing and cleanup steps, and deliver purified libraries for amplification off-instrument. An additional script enabling post-PCR purification of the libraries is also provided. We further describe an alternative automation process for lower-throughput needs. Our analysis of sequencing data shows that libraries produced by all of the above procedures remove excess adaptors which interfere with quantitation and pooling, are unbiased, free from cross-contamination and of high complexity.

928T

Next Generation Sequencing High-throughput Solution for Illumina Fragment Library Sample Preparations. *M.E. Blair, K.L. Marshall, R. Zhang, A. Jackson.* Genomics, Beckman Coulter, Indianapolis, IN.

Illumina library sample preparation for Next Generation Sequencing (NGS) is a long and complicated process that can also require precise fragment size selection through agarose gel separations in order to obtain high quality sequencing results. Beckman Coulter has already released SPRIworks chemistry for low-throughput using the SPRI-TE Nucleic Acid Extractor and has now developed chemistry for high-throughput (HT) automation using Biomek® liquid handlers for Illumina compatible NGS library sample preparation. The HT solution can produce up to 96 libraries in a 96-well format with minimum hands on time and uses Solid Phase Reversible Immobilization (SPRI) paramagnetic beads to offer various fragment sizes selection (150-350 bp, 250-450 bp, 350-550 bp, and 150 bp up) with high accuracy as shown by bioanalyzer data. Downstream processes, including PCR reaction setup and cleanup, qPCR setup, and normalization, have been automated to create a suite of methods. This suite of automation methods has a single user interface (UI) to allow for easy selection of options.

929T

A Stream-lined Process for Amplicon Resequencing using Ion Torrent's Personal Genome Machine coupled with Fluidigm's Access Array. *J. Boland[1,2], D. Roberson[1,2], J. Bacior[1,2], V. Lonsberry[1,2], A. McCary[1,2], A. May[3], M. Pieperzyk[3], M. Yeager[1,2], K.B. Jacobs[1,2], A.A. Hutchinson[1,2], S.J. Chanock[1,2].* 1) Core Genotyping Facility, NCI Frederick, Bethesda, MD; 2) Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, Maryland USA; 3) Fluidigm Corporation, South San Francisco, California USA.

A multiplexed approach to sample processing is essential to maximize data return of a single run on a next generation sequencing platform. Using Fluidigm's Access Array and Ion Torrent's PGM, we have streamlined both our regional resequencing and follow-up validation for familial whole exome studies, with the ability to process hundreds of targets of interest across hundreds of samples in a single day. This presentation will focus on the sequencing of a region of chromosome 10 that was found to be associated with prostate cancer risk by genome-wide association studies (GWAS). This 97kb region, which includes *MSMB* and *NCOA4*, has also been resequenced using both the Illumina HiSeq 2000 and the 454 FLX platforms, providing ample comparative data. The ability to process hundreds of samples per day will undoubtedly lead to faster discovery of underlying genetic markers for many of today's current diseases. This project has been funded in whole or part with federal funds from the NCI, NIH under contract HHSN261200800001E.

930T

"Genetics, Meet Genomics": Using SNPs to Guide Variant Discovery in Sequencing Data. *R. Daber[1], S. Mulchandani[1], J. Ganesh[1], M. Mennuti[2], E.A. Tsai[1], H.C. Lin[1], B.A. Haber[1], P.F. Whitington[3], L.K. Conlin[1], H. Hakonarson[1], N.B. Spinner[1].* 1) Children's Hospital of Philadelphia, Philadelphia, Pa; 2) University of Pennsylvania, Philadelphia, Pa; 3) Children's Memorial Research Center, Northwestern University, Chicago, IL.

Next generation sequencing holds the promise of elucidating the cause of many genetic disorders. While various groups have had success in identifying new disease causing mutations associated with a variety of phenotypes, incorporation of next generation sequencing into routine variant discovery for genetic disorders remains challenging. Two major barriers that remain are the costs per individual genome and the difficulty in interpreting the meaning of the large number of variants that are found. To overcome these two challenges we have developed an analysis pipeline that utilizes traditional genetic approaches based on family history and genome-wide SNP arrays to reduce the number of samples that must be sequenced as well as the number of variants that need to be interpreted and subsequently validated. Depending upon the family history and availability of familial samples, genotype information is collected on SNP arrays and is used to determine allelic phase. Difference maps of allelic inheritance among affected and unaffected family members is then used to reduce the genome to chromosomal regions which are consistent with a model of inheritance displayed in the family history. In our first application, we compared genotypes of two affected siblings with their unaffected brother to define regions of shared inheritance following both a dominant and recessive model. This allowed us to sequence a single affected individual and instantly reduce the sequence data set to those reads found in the defined regions. When a dominant model of inheritance was assumed in this family, the number of genes analyzed for variants was reduced from 38,047 to fewer than 12,500. Under a recessive model the number of genes dropped even further to 6,173. This reduction of sequence search space by nearly 85% was completed well before the first base was even sequenced and reduces both the computational demands of analysis and the number of variants needing interpretation. In a second family where the disease was tracking through the maternal lineage, the genomes of two affected siblings were further filtered to only the regions of shared maternal inheritance. Thus, by incorporating information on the biological inheritance of chromosomal regions determined from SNP arrays, variant discovery by sequencing can be reduced to a single sample and variant interpretation is limited to only the regions that are biologically relevant.

931T

Real-time data quality feedback for next generation sequencing of a breast cancer whole transcriptome library. *R. Fish, S. Ngola, M.-Y. Shen, L. Jones, J. Ziegle, N. Fong, K. Perry, S. Chang, L. Lua, L. Liu, M. Mariano, A. Vadapalli, L. Xu, S. Yerramalli, E. Wang, C. Yang, L. Joe.* Life Technologies, Foster City, CA.

A large number of genes have been implicated in breast cancers when their expression is improperly or no longer regulated. With the advent of massively parallel sequencing in next generation sequencing systems, researchers have a powerful tool to study genome-wide changes in RNA expression. MCF-7 is a breast cancer cell line that has served as a model system for investigating the genome and transcriptome of a cancer cell. In order to study genetic variants and expression differences accurately, next generation sequencing samples and instrument runs have to be of high quality. Therefore, any feedback regarding sample and run quality is greatly beneficial to users. In order to address this need, the 5500 Series Genetic Analysis Systems incorporate sequencing control beads as a small percentage of the total sample population. As they represent less than one percent of the total beads, the overall impact on run throughput is negligible. The control template sequences contain a unique identifier that discriminates these beads from sample beads. The beads are comprised of a set of synthetic sequences that will support all run sample types from both DNA and RNA libraries, including mate pair and paired end sequencing. In this study, we show sequencing of a whole transcriptome library from MCF-7 in the presence of sequencing control beads. The same library was sequenced on multiple instruments, allowing an assessment of instrument to instrument and sample bead to sample bead differences by investigating the behavior of the sequencing control beads. As the run progresses the sequencing control provides real time comparison of the sequencing quality of the sample beads compared to the control beads. The sequencing control and associated software provide a powerful tool for monitoring and determining run quality and performance.

932T

Targeted Resequencing on Illumina's MiSeq: Methods for Rapid Validation and Cost Effective Extension Studies of Putative Genetic Variants. *S. Fisher, C. Russ, J. Flannick, N. Burtt, M. DePristo, W. Winckler, M. Carneiro, M. Parkin, J. Abreu, R. Hegarty, D. Perrin, T. DeSmet, M. Coole, J. Meldrim, C. Nusbaum, R. Nicol, S. Gabriel.* Broad Institute, Cambridge, MA.

Over the past several years whole exome or whole genome sequencing of significant number of samples from a wide variety of diseases has become common practice for identifying putative disease linked variants. However, follow up work to validate the large number of potential mutations identified in these studies and extending the study of specific subsets of targets across larger sample cohorts has been to date time consuming and costly. Here we present two approaches for targeted re-sequencing of small subsets of target regions using the Illumina MiSeq sequencer.

The first approach allows for rapid validation of target variants in less than 8 hours from PCR amplification to data analysis. In a proof of principle experiment, PCR amplicons approximately 600bp in length, for ~300 SNP containing targets, were designed, ordered and received within 48 hours. PCR was performed across eight samples and resulting amplicons were pooled by sample. Using Epicentre's Nextera transposon based library construction approach, amplicon pools were transformed into sequence ready libraries in under an hour. To enable sample multiplexing, a molecular barcode was added during the final library amplification step. Finally all eight sample libraries were pooled and sequenced on a single 36 base paired-end Illumina MiSeq run.

The second approach offers a cost effective method for extending studies to larger sample cohorts while examining small subsets of targets. Using Illumina's TruSeq Custom Amplicon kit, which enables highly multiplexed amplicon sequencing e.g. [700 amplicons of 250 bp in size], exonic regions totaling approximately 67Kb of sequence were selected and sequenced using 151 base paired-end reads on both Illumina's HiSeq2000 and MiSeq instruments. The resulting validation and extension data will be presented.

933T

Automation Method Suite for NGS Downstream Applications: PCR Cleanup, Real-Time qPCR Quantification, Normalization and Pooling Using Biomek FXp Laboratory Automation Workstation. *A. Jackson, R. Zhang, K. Marshall, M. Blair, J. Schlitz.* Application, Beckman Coulter, Inc., Indianapolis, IN.

Quality next generation sequencing (NGS) data depends on precise sample quantification. However, quantitation and normalization (Quant and Norm) are often the most time consuming and error prone processes in the laboratory. With the automation of library construction for higher throughput (HT), quant and norm has quickly become the bottleneck with the requirement for individual per-well volumes for sample and diluent. Once normalized, the NGS libraries are often pooled for sequencing, where uniform concentrations are critical. Using the Biomek FXP Laboratory workstation, PCR Clean-up using AMPure XP, and real-time qPCR Setup were automated in the 96-well plate format in addition to separate Norm and Pooling method. KAPA Biosystems Library Quantification Kits were used for real-time qPCR setup and provide easy to use standards. This suite of methods includes an intuitive user interface (UI) that enables simple entry of method defining information. There's flexibility to select specific samples to process for PCR clean-up, real-time qPCR setup, normalize and pooling samples. The Norm method utilizes Biomek Software's ability to interact with Microsoft Excel spreadsheets to allow the user to view the concentrations of the samples, select and note outliers and visually check sample volumes prior to normalization and pooling for better error handling and control of samples for downstream processing. Our preliminary real-time qPCR data has shown CVs <10% per plate, with +/-0.5 Ct from sample to sample variations. For sample tracking the UI assigns a plate ID that is tracked throughout the process with date and time processed. The user can also assign a unique ID manually through a barcode scan. All samples volumes of diluent transferred are displayed in the Quant/Norm spreadsheet and can be saved for note booking. This suite of methods is simple, fast, and convenient. It can be applied to NGS and other similar applications. Note: Beckman Coulter, the stylized logo, and Biomek are trademarks of Beckman Coulter, Inc., and are registered in the USPTO.

934T

Target enrichment for Human Kinome RNA and DNA sequencing. *E. Lin[1], A. Giuffre[2], B. Novak[1], M. Visitacion[1], M. Hamady[1], F. Useche[1], V. Kulkarni[1], W. Liu[1], T. Fjelsted[1], C. Pabón-Peña[1], S. Hunt[1], D. Roberts[1], S. Happe[2], E. Leproust[1].* 1) Genomics, Agilent Technologies, Santa Clara, CA; 2) Genomics, Agilent Technologies, Cedar Creek, TX.

Deep sequencing technology has revolutionized the discovery of rare polymorphisms, structural variation, and novel splice variants. To meet the demand for fast, cost-effective, and accurate genome and transcriptome analysis methods from small scale studies to large sample cohorts, Agilent Technologies has developed the SureSelect Target Enrichment System - a highly robust, customizable, and scalable system that focuses analyses on specific genomic loci or expressed transcripts by in-solution hybrid capture. Both RNA and DNA enrichment demonstrate excellent performance as measured by capture efficiency, uniformity, and reproducibility of sequence coverage. This enables the accurate detection of common and rare SNPs, indels, CNVs, allelic expression, and gene fusions. SureSelect for RNA-seq also retains relative transcript amounts after capture for accurate gene expression. In collaboration with the Netherlands Cancer Institute (NKI), 2 sets of probes were designed targeting over 500 kinases and a subset of clinically-relevant cancer genes (the “Kinome”) for use with DNA-seq and RNA-seq, respectively. We profiled a set of 6 cell lines and 2 tumor/normal pairs utilizing the each of the Kinome enrichment kits. The RNA-seq dataset will provide specific examples of allelic expression variance, differential splicing, and the capture of known disease mutations will be highlighted. The DNA-seq dataset from the same samples allows correlation of transcript, SNP, and indel information back to the genome, as well as identification of structural variants.

935T

Advanced Capture-Sequencing: New Designs and Multiplexing for Whole Exome and Regional Capture Sequencing Applications. *D. Muzny[1], I. Newsham[1], M. Wang[1], Y. Wu[1], C. Kovar[1], A. Sabo[1], R. Chen[1], J. Reid[1], M. Bainbridge[1], E. Boerwinkle[2], T. Albert[3], R. Gibbs[1].* 1) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX; 2) University of Texas Health Science Center at Houston, School of Public Health, Houston, TX 77030; 3) Roche NimbleGen, Inc., 1 Science Court, Madison, WI 53719.

Since the initial report of targeted-enrichment (Albert et al, 2007) we have been evolving the design and utility of capture reagents and methods, taking advantage of the parallel advances in sequencing platforms. New exome designs target a comprehensive set of coding exons from 6 different gene databases, as well as computationally predicted coding and non-coding elements: regulatory regions, and conserved UTRs. These reagents have shown that different regions of the exome have different capture qualities, and that variant densities from targeted exome regions outside CCDS, are 2X higher than the CCDS. Library automation, reduction of DNA input samples, capture hybridization multiplexing and application of faster read mapping tools such as BFAST and BWA, together allow a rate of >1500 libraries/captures per month, with >10,000 exome and regional capture libraries already completed. For regional capture designs (0.5-2 Mb target region), up to 48 barcoded samples have been pooled for capture hybridization with equal representation of samples and >90% of the target regions at 20X coverage with ~400Mb of raw sequence. Capture applications have also been developed for the Ion Torrent platform using a custom design (~1Mb), targeting all 167 known retinal disease genes. Excellent coverage was achieved with 85% of the target regions at 20X coverage from 200Mb of sequence and high concordance (99.1%) to known genotype calls was observed. Exome co-capture has now been implemented in production routinely achieving 90% coverage of targeted regions at 20X, from 10Gb of total sequence. Up to 12 samples have been pooled for exome capture hybridization, showing equal representation of samples. Future applications will include SNP validation and screening for genetic disorders and cancer mutations in clinical settings.

936T

Rapid Illumina Library Quality Assessment Using MiSeq. *D. Perrin[1], I. Rasolonjatovo[2], D. Dionne[1], T. Fennell[1], M. Costello[1], S. Humphray[2], T. DeSmet[1], J. Meldrim[1], S. Fisher[1], R. Nicol[1].* 1) Genome Sequencing Platform, Broad Institute, Cambridge, MA., USA; 2) Illumina, Inc.

Increased yields from the Illumina HiSeq 2000 have enabled large scale multiplexing of a variety of sample types. At the Broad Institute, we routinely pool sets of 96 barcoded libraries and run the pools across multiple HiSeq lanes. Uneven barcode representation or poor library quality can be costly and time consuming to fix. Here we demonstrate the utility of the Illumina MiSeq in an inexpensive, rapid, accurate, highly reproducible QC method for both libraries and pools of barcoded libraries that can be run prior to sequencing at high coverage on HiSeq. Running a library on a single MiSeq run enables the calculation of key performance indicators such as estimated library size, percent duplication, and insert size distribution, as well as GC bias and targeted sequencing metrics. In addition, running a pool of barcoded libraries on a single MiSeq run enables assessment of the evenness of representation of libraries within the pool, as well as the frequency of orphaned reads (reads for which a barcode cannot be confidently assigned). Finally, MiSeq cluster density can also be used to predict HiSeq cluster density, reducing rework and maximizing yields on HiSeq flowcells.

937T

Scaling with the flow: advantages of a MapReduce-based scalable and high-throughput sequencing workflow. *L. Pireddu[1], S. Leo[1], F. Reinier[1], R. Berutti[1,2], R. Atzeni[1], G. Zanetti[1].* 1) CRS4, Pula, CA, Italy; 2) Dept. of Biomedical Sciences, University of Sassari, Sassari, Italy.

The continuous increase in sequencing throughput imposes a new generation of tools for data processing. The alternative is to continue suffering scalability problems in processing workflows and IT infrastructure. We evaluate the advantages that the CRS4 Sequencing and Genotyping Platform (CSGP), equipped with 6 Illumina sequencers, gained by replacing its conventional workflow with a new one based on Seal (http://biodoop-seal.sf.net) and Hadoop. The former was a standard pipeline that demultiplexed samples, aligned reads with BWA, removed duplicates with Picard and recalibrated base qualities with GATK. It parallelized computation through concurrent jobs, using a centralized file system to share data. This implementation showed weaknesses as the workload increased: low parallelism; I/O bottleneck at central storage; failure of entire analyses due to node failures or transient cluster problems. The new workflow is a custom, distributed pipeline based on the open-source Seal suite, which provides a set of tools (including a distributed BWA aligner) that run on the Hadoop MapReduce framework, leveraging its functionality for genomic sequencing applications. By switching to a Seal-based workflow we have acquired computational scalability out-of-the-box. Therefore, we can now easily meet the demands imposed by the growing sequencing platform by adding more computing nodes. In addition, the much-increased parallelism has improved overall computational throughput by taking advantage of all available computing power. Notably, we drastically sped up alignment and duplicates removal by 5x without adding computation nodes; adding nodes would result in additional throughput. Moreover, the effort required by our operators to run the analyses has been reduced, since Hadoop transparently handles most hardware and transient network problems and provides a friendly web interface to monitor job progress and logs. Finally, we eliminated the need for our expensive shared parallel storage devices. Our tests reveal that Seal is efficient, achieving close to 70% of the theoretical maximum throughput per node (measured with a single-node version of the workflow on a small data set) and scales linearly at least up to 128 nodes. In summary, this case study suggests that the MapReduce programming model, Seal and Hadoop provide considerable benefits in the genomic sequencing domain. Seal now includes our new workflow as a downloadable sample application.

938T

Automated Pyrosequencing using Digital Microfluidics. *M.G. Pollack, D.J. Boles, J.L. Benton, G.J. Siew, M.H. Levy, M.A. Sandahl, J.L. Rouse, L.C. Perkins, V.K. Pamula, V. Srinivasan, A.E. Eckhardt.* Advanced Liquid Logic, Inc, Research Triangle Park, NC.

Most DNA sequencing technologies employ large instruments incapable of functioning outside of laboratory environments. Advanced Liquid Logic's (ALL) digital microfluidics technology represents a novel means of liquid handling, streamlining molecular workflows in a broad range of settings. By automating a series of assay steps, complex molecular biology procedures (or techniques) can be performed by personnel with minimal training. Meeting these criteria for the automation of DNA sequencing would provide a tool for a range of research, clinical and forensic applications including microbial identification and DNA fingerprinting. Pyrosequencing is a sequencing-by-synthesis method by which nucleotides are sequentially added to a template-of-interest. Incorporation of nucleotides into the template complement is monitored by the release of pyrophosphate. ALL has tested the feasibility of implementing pyrosequencing chemistry within droplets using electrowetting-based digital microfluidics. The digital microfluidic cartridge is composed of an array of electrodes patterned on a printed-circuit board, bonded to a plastic top-plate containing various reagents which are programmably dispensed based on software commands. The cartridge is inserted into an analyzer, which houses detection systems, heaters and magnets for various processes. Using this system, a three-enzyme protocol was developed in which individual droplets containing enzymes, dNTPs, and DNA templates were combined to perform the pyrosequencing reaction. DNA templates were anchored to magnetic beads, enabling thorough washing between nucleotide additions. Reagents and protocols were optimized to maximize signal over background, linearity of response, cycle efficiency, and wash efficiency. As an initial demonstration of feasibility, a 229 bp Candida parapsilosis template was sequenced using both a de novo protocol and a resequencing protocol. Up to 67 bp of sequence was obtained with 100% sequence accuracy based on raw pyrogram signals. Excellent linearity was observed for homopolymers (2, 3, or 4 nucleotides) contained in the C. parapsilosis sequence and control experiments demonstrated the possibility of extending the linear response to at least 8 nucleotides. With improvements in microfluidic design, it is expected that longer reads, higher throughput and improved process integration (i.e. “sample-to-sequence” capability) could be achieved using this droplet-based platform.

939T

High Accuracy Genome Sequencing on the Illumina HiSeq Platform. *S.R. Rawlings[1], S. Humphray[1], K. Maisinger[1], V.P. Smith[1], J. Gordon[2], S. Tanner[2], C. Tregidgo[1], G. Smith[1], K. Hall[1], D. Bentley[1].* 1) Illumina UK, Chesterford Research Park, Little Chesterford, Nr Saffron Walden, Essex, United Kingdom; 2) Illumina Inc, 9885 Towne Centre Drive San Diego, CA 92121 USA.

Illumina Sequencing platforms have been instrumental in transforming the use of sequence information in a wide range of genomic, genetic and biological studies. Applications include whole genome and targeted sequencing, de novo genome assembly, RNA sequencing, functional and epigenetic studies. Together the range of applications and launch of the complementary fast-turnaround MiSeq platform enable integrated studies in both the research and clinical setting. Common to all the sequencing platforms is a simple sample preparation workflow that enables preparation of DNA fragments to form high density random arrays of clusters that are then sequenced using reversible terminator chemistry. Single- or paired-read datasets (read length 35-150 bases) are generated either for de novo assembly or alignment to a previously known reference. Recent developments in chemistry, hardware and software have resulted in higher accuracy, improved genome coverage (low GC bias), longer reads and faster cycle times. Major improvements to image analysis algorithms, analysis software and base calling calibration tables have contributed to greater accuracy, density and hence yield, with paired 100-base runs regularly providing >600 Gb per run and >80% of base calls with quality values >Q30 (>99.9% raw read accuracy at each base) on all platforms. When the sequencing or detection accuracy of SBS is isolated from errors introduced during sample prep, it is found to be compatible with overall system base-quality scores of Q60 or greater. Whole genome sequencing (WGS) has been widely adopted during the past year for the analysis of germ line and somatic mutations in human disease, with a particular focus on cancer. A recent study of a time-series on chronic lymphocytic leukaemia (CLL) patients enabled characterisation of the changing profiles of somatic mutations during cycles of treatment, remission and relapse. Tumour heterogeneity and subclonal populations were defined using deep targeted sequencing of somatic mutations. The CLIA-certified, CAP-accredited WGS laboratory has expanded activity particularly in patient sequencing in response to an increased number of requests from physicians and we anticipate progress in the direct clinical application of whole genome and targeted sequencing in personalised medicine.

940T

Optimizing DNA Shearing Utilizing Bulk Lateral Ultrasonic™ Energy. *S. Sharma, K. Dev, v. Vivek, J. Shieh, B. Jamieson.* Microsonic Systems, San Jose, CA.

As Next-Gen sequencing throughput continues to accelerate, a serious sample preparation bottleneck is emerging, calling for a higher throughput DNA shearing technique. The solution must not compromise the quality of the sheared fragments and should not add to the existing high cost of sample prep kits. Microsonics has pioneered the use of a unique, new ultrasonic technology "Bulk Lateral Ultrasonic™ Energy" and implemented a core building block "The Microprocessor for Life Sciences" to commercialize this exciting new technology, making possible a multi-channel DNA shearing device that is scalable to any number of simultaneous shearing channels.

This poster will reveal the most critical parameters and explain how this new form of ultrasonic energy can be optimized for DNA shearing, including: 1.The effect of ultrasonic energy power level and shearing time, 2. The effect of the starting sample size and source of DNA, 3. The effect of starting sample volume, 4. DNA concentration independence and then provide a comparative chart to highlight the advantages of BLU over other technologies like probe sonication, pressure differential, nebulization, enzymatic and focused acoustics.

Careful optimization of these critical parameters has led to a successful implementation of Bulk Lateral Ultrasonic energy for high throughput DNA shearing, sufficient to meet the needs of current and emerging Next-Gen sequencing throughput demands.

941T

De novo Assembly and Haplotype Sequencing from Short Sequencing Reads. *F. Steemers[1], S. Amini[1], N. Pignatelli[1], C. Turk[1], I. Goryshin[2], T. Royce[1], M. Ronaghi[1], K. Gunderson[1].* 1) Advanced Research, Illumina, San Diego, CA; 2) Research, Illumina, Madison, WI.

Next-generation sequencing is transforming the field of genomics. With a growing list of assembled genomes, these technologies are enabling a wide range of applications including genomic re-sequencing, RNA-seq, and ChIP-seq. However, some applications are limited by the shorter read lengths involved in next-generation sequencing. De novo assembly can be facilitated by mate-pair libraries to enable scaffolding of assembled short read contigs, but generating these libraries can be time-consuming and can require large amounts of DNA. Limitations in read length can also create challenges for mapping reads to highly variable and repeat regions. Furthermore, some previous short read technologies were unable to reassemble molecular haplotypes — information that has significant medical importance. The completeness and accuracy of these assemblies are critical to the information extracted from genomes. We propose a revolutionary new sequencing approach that enables the rapid and accurate assembly of sequencing reads into long meta-reads by preserving order and contiguity information during fragmentation. During library preparation, each fragment is automatically tagged with information to identify neighboring sequences in the original DNA molecule. Simple look-up tables enable neighboring sequences to be reassembled bioinformatically, removing the necessity of a reference genome for assembly. This unique molecular tagging also enables haplotype reconstruction at the single-molecule level. Metagenomics can also benefit by simultaneously assembling reads from multiple unknown and diverse microorganisms. This new approach facilitates accurate de novo genome assembly, haplotype sequencing, sequencing of previously uncharacterized genomes, and metagenome sequencing.

942T

Automated Library Preparation for Next Generation Sequencing using Digital Microfluidics. *A.P. Sudarsan[1], J.L. Rouse[1], R.R. Dhopeshawrkar[1], A. Rival[1], A.E. Eckhardt[1], N. Mushero[2], M. Weiand[2], B. Minie[2], P. Cahill[2], M.G. Pollack[2].* 1) Advanced Liquid Logic, Inc., Research Triangle Park, NC; 2) Broad Institute of MIT and Harvard, 320 Charles St. Cambridge, MA.

The preparation of fragment libraries for next generation sequencing remains a cost and labor intensive bottleneck in most genomics laboratory workflows. Advanced Liquid Logic (ALL) and the Broad Institute have teamed to develop a cost-effective automation solution for library preparation, using ALL's proprietary "digital microfluidics" technology. ALL's system consists of an instrument and cartridge, the latter of which contains a network of liquid handling electrodes. Using the "electrowetting" effect as a motive force, fluid droplets are manipulated on the electrode network based on a predefined set of software commands. The instrument component of the system houses electronics, thermal management equipment and magnet arrays. To date, capabilities for sequential end-repair, A-tailing and adapter ligation have been demonstrated, with additional on-cartridge workflow components under development. Because specific protocols are defined in the system's software, new protocols and enhancements to existing protocols can be rapidly incorporated. The flexibility of the ALL system enabled the examination of both published and novel library preparation protocols for use with next generation sequencers. Data will be presented regarding the quality of the DNA libraries prepared, resulting sequencing data, as well as ease-of-use and cost-effectiveness metrics.

943T

Complete Solution for Next-Generation Sequencing Sample Preparation of Roche's GS FLX Titanium Series Kits. *R. Zhang.* Application, Beckman Coulter, Inc., Indianapolis, IN.

This poster presents a suite of high-throughput (HT) NGS sample preparation methods that automated the Roche 454* GS FLX Titanium* Series Kits in a 96-well plate format with samples starting from random sheared gDNA to the enriched emPCR beads that are ready-to-load onto the Roche FLX Genome Sequencer. It consists four major applications: (1) Preparation of up to 96 libraries using Roche GS FLX Titanium* Rapid Library Preparation Kit (< 3 hours). (2) NGS Library Quantification and Normalization using Rapid Library (RL) Standard and PicoGreen Assay Kit (Invitrogen) (< 1 hour). (3) emPCR Reaction Setup using Roche GS FLX Titanium* LV and SV emPCR Kits (1-1.5 hours). (4) Large Volume (LV), Medium Volume (MV) and Small Volume (SV) emPCR enrichment and sequencing primer annealing processes (~ 4 hours). Random sheared DNA (500 ng) samples from clonal isolates of Daphnia pulex provided by Indiana University were used to generate libraries for sequencing on the Roche/454 Life Science* platform. The data shows that compared to libraries prepared manually, libraries prepared using automation were proportionally equal across libraries but the yield was slightly higher, and the size distribution of DNA fragments prepared using automation fit within acceptable boundaries, but with a slightly shortened average length of the reads (361 bp automated vs. 391 bp manual). Note: Beckman Coulter, the stylized logo, and Biomek are trademarks of Beckman Coulter, Inc., and are registered in the USPTO.

944T

Molecular analysis of mutations in *COL4A3* gene, in Mexican patients with Alport syndrome. *B. Rodriguez-Espino[1], A. Sanchez-Boiso[2], R. Garcia-Vazquez[1], A.M. Hernandez-Sanchez[1], L.M. Ortiz-Vaquez[1], M.I.P. Garcia-Roca[1], B. Romero-Navarro[1], Y. Fuentes-Velasco[1], M. Medeiros-Domingo[1].* 1) Investigacion en Nefrologia, HIMFG, Mexico, D.F., Mexico; 2) Departamento de Genetica, , HIMFG, Mexico, D.F., Mexico.

Introduction. Alport syndrome is a genetic disorder characterized by glomerulo-nephritis, endstage kidney disease, and hearing loss. Alport syndrome is caused by mutations in *COL4A3*, *COL4A4*, and *COL4A5*, collagen biosynthesis genes. Mutations in any of these genes prevent the proper production or assembly of the type IV collagen network, which is an important structural component of basement membranes in the kidney, inner ear, and eye. In most cases with Alport syndrome, the condition is inherited in an X-linked pattern, due to mutations in the *COL4A5* gene. However, it is possible an autosomal recessive inherit, if both copies of the gene *COL4A3* and *COL4A4*, located on chromosome 2, have been mutated. There are many mutations reported in Alport syndrome, but most cases are unique to each family and there are no records in the Mexican population. Aims. To determine the type of mutations in the *COL4A3* gene in four positive Mexican index cases for Alport syndrome. Methods. Genomic DNA was isolated from patients and a control group of healthy individuals. The coding regions of *COL4A3* genes were analyzed by DHPLC and direct sequencing, and compared against international data bases. Results. In one patient was detect the mutation [*c*.1141-1142G/C], this produce a missense change of the amino acid G327P. In a second patient, was found [*c*.IVS17 + 35G/T] in the intronic sequence, which prevents adequate RNA splicing. In two different patients more, we observed the insertion of a nucleotide [*c*.1141_1142insN], this produces a framework shift in the polypeptide translation. Conclusions. It is the first molecular study of *COL4A3* gene in Mexican population, which reported four new mutations associated with Alport syndrome. These studies together with clinical data, can support the family genetic counseling of affected individuals and also to identify the molecular variables of the Alport syndrome in the Mexican population.

945T

Overlapping features of Mehes and Potocki-Lupski syndromes: Report of an adult patient with Mehes phenotype and 17p11.2 duplication. *P. Sarda[1], K. Hollody[2], M. Vincent[1], M. Girard[1], A. Schneider[1], G. Lefort[1], J. Puechberty[1], D. Genevieve[1].* 1) Dept Med Gen, Arnaud de Villeneuve Hosp, Montpellier, herault, France; 2) Department of Paediatrics, University of Pécs, Pécs, Hungary.

We describe a 24-year-old woman presenting with delayed speech development, mild developmental delay, facial dysmorphism with asymmetry, strabismus and transverse earlobe creases. All these features are reported in patients with Mehes syndrome. The patient has mild mental retardation associated with psychiatric troubles not defined as autistic syndrome. Adaptive functioning was uniformly low with a complete lack of social skills and autonomy for daily life activities and the inability to have any independent work activity at an adult age. Microarray analysis found a de novo duplication of chromosomal region 17p11.2 confirming diagnosis of Potocki Lupski syndrome. We looked for 17p11.2 duplication by interphase FISH in the four patients with Mehes syndrome published by K. Hollody (2005) and found no duplication. This new observation of a patient with “delayed speech development associated with facial asymmetry, strabismus and transverse earlobe creases” confirms the existence of this association, but this entity is certainly heterogeneous. Our observation shows an overlap between the phenotypes of Mehes and Potocki-Lupski syndromes. Consequently it seems important to look for a chromosome 17p11.2 duplication using a specific probe in patients with this phenotype. It also appears that the diagnosis of Potocki Lupski syndrome is difficult to evoke through the sole observation of dysmorphic traits. This diagnosis should be considered in patients with severe language delay associated with particular behavioural difficulties including some or all traits of the autism spectrum. In these patients, the non-specific and variable dysmorphic traits present in Potocki-Lupski syndrome will then suggest cytogenetic analysis to look for a 17p11.2 duplication.

946T

22q11 deletion syndrome at Siriraj Hospital, Bangkok, Thailand - 16 years review (1995-2011). *A. Sathienkijkanchai, N. Vatanavicharn, P. Wasant.* Division of Medical Genetics, Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Background: 22q11 deletion syndrome is the most common microdeletion syndrome in humans, affecting 1 in 4,000 live births. It has variable phenotypes, including congenital heart disease (CHD), facial dysmorphism, palatal anomalies, immune deficiency, hypocalcemia, and learning difficulties. This syndrome is diagnosed in individuals with a submicroscopic deletion of chromosome 22 detected by Fluorescence *in situ* Hybridization (FISH).

Methods: Retrospective study included all children clinically diagnosed with 22q11 deletion syndrome, DiGeorge syndrome, or velocardiofacial syndrome who were seen in the Genetics clinic, Department of Pediatrics, Siriraj hospital between1995-2011. Children who had no FISH analysis done were excluded from the study.

Results: A total of 20 new cases with the deletion of chromosome 22q11 confirmed by FISH analysis were seen in the clinic. Eight cases (40%) were referred by pediatric cardiologists due to CHD with facial dysmorphism. Four cases (20%) presented with seizures resulting from hypocalcemia. The rest of them were referred because of developmental delay, or facial dysmorphism. 75% of patients (15/20) had CHD, e.g. ventricular septal defect (5/20), MAPCA/VSD/PDA (major aortopulmonary collaterals) (4/20), tetralogy of Fallot (3/20), interrupted aortic arch typeB (1/20). Two patients died from CHD at very young age. Hypocalcemia was found in 8 patients (40%), and 5 of them had seizures due to hypocalcemia. Eight patients had laboratory analysis of immune function performed which revealed that 2 of them had T-cell defect. 45% of patients (9/20) had palatal anomalies. More than half of patients (11/20) had failure to thrive.

Conclusion: Most of the clinical findings in this study reflect previous report. However, we found a higher incidence of MAPCA/VSD/PDA in our patients. This is a hospital-based study, so further studies on a national scale are warranted to better elucidate clinical features of 22q11 deletion syndrome in Thai population.

947T

Molecular confirmation of Costello syndrome in siblings and use of allelic specific amplification to determine parent of origin in a cohort of patient with *HRAS* germline mutations. *K. Sol-Church.* Dept Biomedical Res, Alfred I duPont Hosp Children, Wilmington, DE.

Here we report on the identification of an HRAS mutation c.34G>A, predicting a p.G12S amino acid substitution, in the surviving brother of a previously reported sibling pair, and documentation of the same change in autopsy material from his deceased sister. This represents, to our knowledge, the first molecularly confirmed Costello syndrome in siblings. Using single nucleotide polymorphic markers and allele specific amplification, we clearly identified the mutation in the surviving sibling to be of maternal origin. While we cannot exclude two independently occurring de novo mutations, the complete sharing of polymorphic markers around the mutation site in both siblings supports maternal germ cell mosaicism. We also report of parental origin using Allelic speaific amplification in additional families with costello syndrome.

948T

Analysis of deletions in 22 Brazilian cases of Williams-Beuren syndrome. *D.H. Souza[1], M. Yoshimoto[2], L.R. Martelli[3], J.A. Squire[2], D. Moretti-Ferreira[1].* 1) Genetic, São Paulo State University - UNESP, Botucatu, São Paulo State, Brazil; 2) Queen's University, Kingston, Ontario, Canada; 3) Dept. Genetics, University of São Paulo, Ribeirao Preto, SP, Brazil.

Williams-Beuren syndrome (WBS) is a developmental genomic disorder with an incidence of 1/7,500 [Strømme et al., 2002]. WBS has multisystemic manifestations, mainly characterized by vascular stenosis (predominantly supravalvular aortic stenosis), distinctive craniofacial features, mental retardation with a characteristic neurocognitive profile, short stature and some endocrine and connective tissue abnormalities. The WBS syndrome is caused by a heterozygous deletion of contiguous genes at chromosomal region 7q11.23. The common deletions in WBS patients span a genomic region of ~1.5 (94%) or ~1.8 (5%) megabase pairs (Mb), encompassing approximately 28 genes (Frohnauer et al., 2010). Deletions occur due to nonallelic homologous recombination (NAHR) in a region of chromosome 7 containing blocks of unstable low copy repeat, with high sequence homology, that predispose to rearrangements during meiosis. There is considerable heterogeneity in deletion locations within this cytoband, as NAHR can occur within any of the blocks of low copy repeats that cluster to the WBS region. Furthermore, a genotype-phenotype correlative study of patients with WBS requires that the complexity of the pattern of genomic imbalance within this region be considered In this study an integrative clinical, cytogenetic and high-resolution array Comparative Genomic Hybridization (CGH) map of 7q11.23 deletions in 22 Brazilian patients with WBS has been performed, to compare the pattern of genomic imbalance with variations in the WBS phenotype. In all patients normal karyotype (550 band level) was observed, but analysis by FISH (probe LPU 011 - Cytocell) showed that all 22 cases, had a deletion of the WBS region. To analyze and precisely map the position of each deletion in this region array CGH (60K x 8 platform Agillent oligonucleotides) was performed. In 18 cases of the deletions had a minimal region of loss of 1.5 Mb (Type I deletions) and in 4 cases genomic losses were more extensive, approaching 1.8 Mb (Type II deletions). Direct correlations of phenotypical characteristics with the genomic mapping data failed to detect a significant differences between these two deletion classes of WBS patients. This is the first comprehensive high resolution genomic deletion analysis of WSB in Brazilian patients. The use of array-CGH technology allowed better delineation of the deletion maps that may be helpful for the patient prognosis.

949T

Refinement of the Jacobsen syndrome critical region for thrombocytopathy by aCGH. *D.J. Stavropoulos[1], Y. Diab[2], W.H. Kahr[2], R. Babul-Hirji[3], D. Chitayat[3].* 1) Department of Paediatric Laboratory Medicine; 2) Division of Haematology/Oncology; 3) Division of Clinical and Metabolic Genetics, Department of Paediatrics, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada.

Jacobsen syndrome (JS) is a rare contiguous gene disorder characterized by a deletion within distal 11q ranging in size from 7 to 20 Mb. The clinical symptoms include dysmorphic features, growth and psychomotor delays, and congenital anomalies involving the brain, heart, kidneys and gastrointestinal systems. Some of the cases have transient neonatal thrombocytopenia and occasional pancytopenia that resolves over time, and persistent thrombocytopathy. The phenotype varies depending on the deleted segment. Recent studies of cases with partial deletions of 11q have implicated the FLI1 gene as a strong candidate for the haematological manifestation of this disorder. We present a 4-year-old girl with clinical symptoms of JS who was found to have a 2.899 Mb deletion at 11q24.2-11q24.3, with haploinsufficiency of the OMIM Morbid Map genes KIRREL3 (OMIM 607761), FLI1 (133450) and KCNJ1 (OMIM 600359), as well as MIR3167, ETS1, KCNJ5, C11orf45, TP53AIP1, ARHGAP32, and BARX2 genes. She had normal platelet counts but her platelet ATP release was significantly decreased and platelet electron microscopy revealed markedly decreased numbers of dense granules and the presence of giant alpha granules, likely caused by the haploinsufficiency of FLI1, as supported by previous studies. The BARX2 gene has been suggested as a candidate for the dysmorphic features and craniosynistosis due to its expression pattern, and her small deletion is supportive of that hypothesis. This deletion further refines the critical region associated with JS.

950T

A novel FOXG1 mutation causing congenital Rett syndrome presenting with lactic acidosis. *M. Strenk[1], A. Atherton[1], C. Saunders[2], B. Heese[1].* 1) Department of Pediatrics, Children's Mercy Hospitals and Clinics, Kansas City, MO; 2) Department of Pathology, Children's Mercy Hospitals and Clinics, Kansas City, MO.

Introduction: In the last few years, several variants of Rett syndrome (RTT) have been identified, both due to mutations in MECP2, as well as CDKL5 (variant RTT) and FOXG1 (congenital RTT). Features associated with RTT caused by FOXG1 mutations include hypotonia, acquired microcephaly, impaired motor development, and hypoplasia of the corpus callosum. At this time, 26 patients (19 females, 7 males) with FOXG1 mutations have been described in the literature. To our knowledge, none have presented with elevated lactic acid levels. Case History: We present a 21 month old Caucasian male with lactic acidosis, developmental delay and regression of skills following frequent illness. Other than feeding difficulties shortly after birth, his development was apparently normal until about four months, at which time he experienced frequent respiratory illness associated with loss of skills. He had laryngomalacia, GERD, abnormal sleep study, neutropenia, and seizures. Feeding difficulties and poor growth eventually necessitated placement of a G-tube. At 5 months of age, an initial metabolic work up included plasma amino acids that revealed an elevated alanine level of 636 umol/L (ref <439). Lactic acid was found to be elevated (8.4 mmol/L and 6.1 mmol/L (ref <2.1)) on two occasions. Comprehensive metabolic and genetic testing, including chromosomes, microarray, and methylation studies for Angelman syndrome, was otherwise negative. Prior to a planned biopsy to proceed with evaluation of pyruvate metabolism, the patient was found to have a de novo c.506delG mutation in FOXG1. Conclusion: Our patient has a novel FOXG1 mutation associated with a congenital RTT phenotype. His clinical picture is interesting given his elevated lactate, which lead to initial concerns for mitochondrial dysfunction. There are several reports in the literature of classical RTT (due to mutations in MECP2) associated with mitochondrial dysfunction. It has also been suggested that FOXG1 may share molecular pathways with MECP2 during brain development. This raises the possibility that FOXG1 mutations may also be associated with mitochondrial dysfunction. We recommend consideration of congenital Rett syndrome in patients who present with developmental delay/regression and lactic acidosis or clinical findings suggestive of mitochondrial dysfunction.

951T

Müllerian anomalies should be considered a component of the clinical phenotype of 16p11.2 deletion syndrome. *R.D. Susman[1], R.M. Kimble[2].* 1) Genetic Health Queensland, Royal Brisbane and Women's Hospital, Brisbane, QLD, Australia; 2) Department of Obstetrics and Gynaecology, Royal Brisbane and Women's Hospital, Brisbane, QLD, Australia.

With the increasing use of array comparative genomic hybridisation (aCGH), 16p11.2 deletion syndrome is becoming increasingly recognised. The recurrent ~550kb deletion (from 29.5-30.1Mb) is associated with variable clinical phenotypes including delayed language development, intellectual disability, autism, and congenital anomalies. Some people with this deletion have a normal phenotype. We report a 16 year old girl with multiple problems including intellectual disability, speech and language impairment, epilepsy, and Müllerian anomalies who has a 16p11.2 deletion. We will present further details of her phenotype and review the literature.

952T

A Novel Frame-Shift Mutation in the NHS Gene Associated with Significant Dental and Behavioural Phenotypes in Females in a Family with Nance Horan syndrome. *M. Szybowska[1], A. Levine[2], A. Toutain[3], C. Li[1].* 1) Clinical Genetics Program, McMaster University Medical Centre, Hamilton, ON, Canada; 2) Department of Ophthalmology, The Children's Hospital of Philadelphia, Philadelphia, PA, USA; 3) Service de Genetique, Hopital Bretonneau, Tours Cedex, FRANCE.

Nance Horan syndrome (NHS) is a rare X-linked recessive condition typically characterized by bilateral congenital cataracts and abnormal dentition in males with milder lens opacities and dental issues in females. Mental retardation, behavioural issues and dysmorphic features have also been documented. NHS is caused by mutations in the NHS gene on Xp22.13 whose protein product is thought to be involved in the development of the eye, teeth, brain, face, and skull. The overwhelming majority of reported mutations in NHS are nonsense, although microdeletions and missense mutations have also been observed. We report on a family with two sons clinically diagnosed NHS and their mother and two sisters. A novel frameshift mutation in NHS [c.2393dupC; p.Gly799TrpfsX34] that causes a premature stop codon was found in this family. Features of NHS in our family include not only congenital bilateral cataracts and dental anomalies in the two affected males but also very significant dental anomalies in the three affected females including: diastema, positional anomalies, persistence of deciduous teeth, tapering incisor crown, “screwdriver shaped teeth”, incisal notching, enamel hypoplasia, caries, and pulp anomalies. Indeed the dental phenotypes observed in the females are more severe in certain aspects than in the males in this family. One of the females is confirmed to have sutural cataracts and ophthalmology assessments are pending for the other two women. Additionally, the male and female siblings have difficulty with attention span but do not have mental retardation. Our report draws attention to a new frameshift mutation as well as the fact that significant dental and behavioural issues can manifest in females to the same degree as in males with NHS.

953T

Palate abnormalities in Chilean patients with chromosome 22q11 microdeletion syndrome. *M. Vasquez[1], M. Palomares[2,3], M.L. Guzman[1], G. Lay-Son[1,3,4], G.M. Repetto[1,4].* 1) Genetics, Clinica Alemana-Universidad del Desarrollo, Santiago, RM, Chile; 2) Fundación Gantz, Santiago, Chile; 3) Hospital Calvo Mackenna, Santiago, Chile; 4) Hospital Padre Hurtado, Santiago, Chile.

Chromosome 22q11 microdeletion syndrome (del22q11) has complex clinical features involving several organs and systems. The findings include structural anomalies such as cleft palate and cardiac malformations among others, as well as developmental and neuropsychiatric manifestations. It is the most frequent microdeletion syndrome in humans, with an estimated incidence of 1/4000 and is a common identifiable cause of cleft palate. We characterized the palatal abnormalities in a large cohort of Chilean patients with del22q11. Two hundred and one patients with the deletion were evaluated by geneticists and speech pathologists, including nasopharyngoscopy when indicated. The average age at the time of the study was 12.2 years, ranging from 1.4 to 49 years. Males represented 48% of the group. Nine cases (4.5%) were inherited from an affected parent. Palate abnormalities were diagnosed in 141 patients (70%). The most frequent findings were submucous cleft palate and velopharyngeal insufficiency, seen in 112 patients (59% of the total group). Overt cleft palate was seen in 21 patients (11%) and cleft lip and palate in 5 (2.6%). Patients with or without palate anomalies had similar gender distribution. Patients with palate anomalies were older at the time of diagnosis of the deletion compared to those without palate abnormlaties (average 5.9 vs 4.4 years, respectively). Congenital heart disease was seen in 48% of patients with palate abnormalities and in 70% of patients with normal palates (p 0.002, Fisher's exact test). Our data show a high frequency of palate abnormalities, similar to other published series, but no evidence of association was found with gender, in contrast with other reports. Heart disease was more frequent in patients with normal palates. The most common types of palate defects seen in this series are usually not evident on physical examination and thus require a high index of suspicion and active evaluation through nasopharyngoscopy. Funded by Fondecyt-Chile grant #1100131.

954T

The Pitt-Hopkins syndrome: report of 20 new patients and proposal of a clinical score for the molecular test. *M. Zollino, S. Ricciardi, D. Orteschi, S. Lattante, M. Murdolo, G. Marangi.* Institute of Medical Genetics, Università Cattolica del Sacro Cuore, Roma, Italy.

Pitt-Hopkins syndrome (PTHS) is an emerging condition characterized by severe mental retardation, typical facial gestalt and additional features, such as breathing anomalies. Following the discovery of the causative haploinsufficiency of TCF4, about 60 cases have been reported. Because of the overlapping phenotype of severe mental retardation with absent speech, epilepsy, microcephaly, large mouth and constipation, differential diagnosis of PTHS with respect to Angelman (AS), Rett (RTTS) and Mowat-Wilson (MWS) syndromes represents a relevant clinical issue, and many patients are currently undergoing genetic tests for different conditions that are assumed to fall within the PTHS clinical spectrum. We performed a search for TCF4 mutations in 72 patients with a suspected PTHS by means of both quantitative genome analyses and/or direct gene sequencing. Haploinsufficiency of TCF4 was identified in 17 patients, as a consequence of complete gene deletions (2 patients), gene mutations (14 patients) and a t(14q;18q) balanced translocation disrupting TCF4 (one patient). By evaluating clinical features of these patients, we noticed that, in addition to the typical facial gestalt, the PTHS phenotype results from the various combination of the following characteristics: mental retardation with severe speech impairment, normal growth parameters at birth, postnatal microcephaly, breathing anomalies, motor incoordination, ocular anomalies, constipation, seizures, typical behaviour and subtle brain abnormalities. We assigned specific score to each signs, and a clinical checklist driving a first choice molecular test for Pitt-Hopkins syndrome is proposed and discussed. We found that patients with a proven TCF4 mutation reached scores significantly different from those of 16 patients with a proven mutation causing AS and of 13 patients with MECP2 mutation associated with RTTS. This checklist allowed the enrolment into the genetic test, with positive results, of three patients lacking the typical PTHS facial gestalt. On the same basis, a diagnosis of PTHS was made clinically in three patients who had normal TCF4. Although it was considered that PTHS evolves as a differential diagnosis to several encephalopathies with severe mental retardation and microcephaly, including Angelman and Rett syndromes, we observed that a molecular test of choice for PTHS is possible on a standardized clinical evaluation.

955T

***JARID2* isolated deletion is associated with velo-palatal insufficiency, hypotonia and developmental delay.** *T. Zvi, Y. Yaron, A. Orr-Urtreger, S. Ben-Shachar.* The Genetic Institute Tel-Aviv Sourasky Medical Center.

Palatal anomalies are amongst the most common major congenital anomalies. They include an array of defects such as cleft palate and velo-palatal insufficiency. The etiology of cleft palate is usually complex and believed to be associated with both genetic and environmental factors. However, many palatal anomalies are associated with chromosomal aberrations or monogenic disorders. Velo-palatal insufficiency without clefting has been reported in some genetic conditions as well. We report a 4-year-old male with speech delay, hypotonia, and a nasopharyngeal abnormality leading to velo-palatal insufficiency. Array based comperative genomic hybridization (aCGH) analysis, that was performed in Signature Genomics (Spokane, WA), revealed a ~220Kb deletion in chromosome 6p22-23, containing a complete heterozygous deletion of a single gene, *JARID2*, with no other annotated genes located in the deleted region. The deletion was confirmed by fluorescence *in situ* hybridization (FISH). Parental FISH analyses did not reveal the deletion. Neither deletions/duplications nor disease causing mutations have previously been described in *JARID2* gene. However, genome-wide-association studies have previously shown that *JARID2* mutations are associated with isolated cleft palate. We suggest, therefore that *JARID2* heterozygous isolated loss-of-function is a cause of congenital palatal abnormalities as well as intellectual disability. Furthermore, it is possible that *JARID2* intragenic mutations may be associated with cleft palate and/or intellectual disability as well.

956T

Mosiac trisomy 22 and normal developmental outcome: Report of two cases and review of the literature. *D. Abdelgadir[1], C. Li[2], M.J.M. Nowaczyk[2, 3].* 1) Department of Pediatrics, Pediatric Residency Program, McMaster Univesity Hamilton, Ontario, Canada; 2) Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada; 3) Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada.

Mosiac trisomy 22 is known to be compatible with prolonged survival. However, there are fewer than 20 reports in the literature of live born children with minimal information about their neurodevelopmental outcome. We report 2 girls with mosaic trisomy 22 with dysmorphic features, but documented normal development at ages 3 and 5 years, respectively. Case 1 was diagnosed at 30 weeks gestation because of IUGR and cardiac abnormalities. Amniocentesis at that time showed mos 47,46,XX,+22[6]/46,XX,[11]. Analysis of skin fibroblasts showed mos 47,XX,+22[16]/46,XX,[5]. ASD and pulmonary stenosis were repaired in the newborn period, she also had LV non-compaction, but her cardiac function was normal. At age 5 years she presented with growth parameters <3rd centile, left-sided hemihyperplasia associated with hypomelanosis of Ito, and normal developmental milestones except mild gross motor delays. Case 2 presented antenatally at 18 weeks gestation with IUGR and tetralogy of Fallot. At birth, peripheral karyotype was normal, 46,XX, but cytogenetic testing of umbilical cord fibroblasts showed mos 47,XX,+22[4]/46,XX[4]; mosaic karyotype was also found in cardiomyocytes. At age 5 years she presented with normal social, language and fine motor skills. She had left-sided hemihyperplasia, but all her growth parameters were <3rd centile. Both girls had characteristic dysmorphic features including flat nasal bridge, preauricular pits, epicanthic folds and 5th finger clinodactyly. Approximately 50% of reported mosaic trisomy 22 cases had normal development. Prenatal and postnatal growth failure were the most common complications. Skeletal abnormalities including body asymmetry and 5th finger clinodactyly were also common. It is possible that children with trisomy 22 mosacisim, minimal physical findings and normal development are not diagnosed. Missing these mildly involved patients would lead to skewing of the prognosis for the final development outcome in this population. Appropriate information regarding developmental outcome is necessary for genetic counseling, especially in prenatal situations.

957T

A complex de novo 4 cell line mosaic with gains of 4p15.32pter and 12p13.31pter in a patient with intellectual disability, scoliosis and foot abnormalities. *S. Ahmed, K. Reddy.* Genetics department, Kaiser Permanente Southern CA.

A child with a complex mosaic karyotype had additional material on chromosomes 4 or 12, a dicentric 4, 12 and a normal cell line. A decade and a half later FISH characterized each of the abnormal cell lines to have duplications of 4p15.32pter and 12p13.31pter. The size of these gains and genes involved were defined using aCGH. After confirming this mosaicism in the buccal epithelial cells by iFISH we were able to proceed with confidence to draw a genotype to phenotype correlation.

958T

A de novo 4.7 Mb deletion 6p21.2p21.31 and an insertion of chromosome 6p23p21.31 into chromosome 3q26.32 with a pericentric inversion 3p25q26.32. *D. Broome, K. Reddy.* Dept Gen, Kaiser Permanente, Anaheim, CA.

A well adjusted, independent teenager who had attained menarche also had mild facial dysmorphism, developmental delay and mild intellectual impairment. Her childhood karyotype showed an apparent translocation between chromosomes 3 and 6. On revaluation at high resolution banding the chromosome 3 involved a pericentric inversion of 3p25q26.32 and an inverted insertion of 6p23p21.31 into 3q26.32. By array CGH a subtle de novo loss of 4.7 Mb from chromosome 6 short arm, arr 6p21.31p21.2 (35,597,487-40,283,968)x1 was identified and confirmed using FISH. A complete understanding of the 5-break, complex abnormality was possible because of combining the multiple techniques, which in turn provided an accurate recurrence risk. Because of the high risk, Genetic counseling included possible PGD diagnosis for any future pregnancy. The phenotype associated with a proximal 6p deletion, del(6)(p21.2p21.31) is described for the first time.

959T

Characterization of the derivative X chromosome resulting from Xq;Yq translocation in a female with recurrent abortion. *E.Y. Choi[1], D.E. Lee[1], B.Y. Lee[1], J.Y. Park[1], I.S. Kang[2], H.M. Ryu[2], S.Y. Park[1].* 1) Laboratory of Medical Genetics, Cheil Medical Research Institute, Cheil General Hospital and Women's Healthcare Center, Kwandong University College of Medicine, Seoul, Korea; 2) Department of Obsterics and Gynecology, Cheil General Hospital and Women's Healthcare Center, Kwandong University College of Medicine, Seoul, Korea.

We present a cytogenetic and molecular cytogenetic study of a phenotypically normal female with an X;Y translocation. In this particular case, the breakpoints were located at the distal portions of the long arms on the X and Y chromosomes. Translocations between the Xq and Yq chromosomes occur rarely in humans. Chromosome analysis by G-banding showed a slight difference between the long arms of the two X chromosomes in a 28 year-old female with recurrent spontaneous abortion. Result of the fluorescent in situ hybridization revealed that there was Y chromosome material on the Xq. X inactivation study by R banding showed that the derivative X chromosome was late replicated in all 100 cells. Although the patient has a regular menstrual cycle to date, the Xq deletions have been known as a cause of premature ovarian failure. We have tried to localize the breakpoint positions of the X and Y chromosomes through methylation specific Southern blot analysis for FMR1 gene, multiplex PCR of Y-specific sequence tagged sites and array CGH.

960T

Mosaicism for deletion 8p22-p21.2, duplication 8p21.2-p12, and loss of the Y chromosome in an adult male with de novo robertsonian translocation 13;14. *D. Copenheaver[1], J. Meck[2], S. Aradhya[2], P. Tanpaiboon[1].* 1) Children's National Medical Center, Division of Genetics and Metabolism Washington, DC 20010; 2) GeneDx, Gaithersburg, MD 20877.

A cognitively impaired 28-year old male with a reported balanced robertsonian translocation between chromosomes 13 and 14, diagnosed at the time of amniocentesis, was referred to Genetics for re-evaluation. A whole genome oligonucleotide array CGH was requested to determine whether the translocation was truly balanced. The results instead revealed an 11.5 Mb mosaic interstitial deletion of 8p22-p21.2, an immediately adjacent 10.4 Mb mosaic duplication of 8p21.2-p12, and mosaic loss of the Y chromosome. There were no copy number abnormalities involving chromosomes 13 or 14. Both parents were phenotypically and karyotypically normal and neither had the copy number aberrations found in their son. The patient now presents with cognitive impairment, impulsive talking and bilateral hearing impairment requiring hearing aids, with the latter perhaps due to a lifelong history of recurrent ear infections. The patient has progressive bilateral keratoconus that may require bilateral corneal transplantation. At the age of 25, he started experiencing rapid hair loss. Physical examination revealed no dysmorphism except for simple, low set ears. This constellation of findings has not been previously reported in conjunction with duplication or deletion of 8p and/or mosaic 45,X/46,XY. Patients with full, non-mosaic 8p21.2-p22 deletion typically present with dysplastic ears, cognitive impairment, and microcephaly. Features of full, non-mosaic duplication of 8p similar to that found in our patient are markedly variable and do not constitute a distinct phenotype. The only consistent finding is mental retardation. Clinical findings in individuals with postnatally diagnosed mosaic 45,X/46,XY range from Turner syndrome to mixed gonadal dysgenesis, male pseudohermaphroditism, or a normal male phenotype. This proband had an unexpected number of chromosome abnormalities including a de novo balanced robertsonian translocation, a mosaic deletion/duplication abnormality of 8p, and mosaic loss of the Y chromosome. The mosaic nature of the unbalanced aberrations likely explain this unusual phenotypic presentation. Given the patient's de novo robertsonian translocation, investigation for possible uniparental disomy 14 should also be performed since this could contribute to the phenotype. Furthermore, the findings may represent the natural history of an individual with imbalance of genetic material of chromosome 8 and/or Y, since most reports describe phenotypes in children.

961T

Unexpectedly Variable Phenotype of a Combined Chromosomal Disorder of 18p Monosomy and 18q Partial Trisomy: Autopsy Findings. *M. Doi[1], H. Kuniba[1], S. Miura[2], M. Nakashima[3], T. Hayashi[4], A. Yamashita[1], A. Yanai[1], M. Obatake[5], K. Mochizuki[5], H. Moriuchi[1].* 1) Dept Pediatrics, Nagasaki University Hospital, Nagasaki, Japan; 2) Tissue and Histopathology Section, Division of Scientific Data Registry, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; 3) Dept Tumor and Diagnostic Pathology, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; 4) Dep Pathology, Nagasaki University Hospital; 5) Dept Pediatric Surgery, Nagasaki University Hospital, Nagasaki, Japan.

Introduction 18p deletion syndrome is characterized by mild to moderate mental retardation, speech delay, short stature, and characteristic face. While patients with holoprosencephaly-type defect usually have a poor prognosis, patients without severe brain malformations are believed to have unimpaired life expectancy. We herein report a fatal case of 18p depletion syndrome with unexpectedly variable clinicopathological manifestations and a unique combination of chromosome 18 abnormalities. **Case report** A 40-year-old woman (gravida 4, para 3, spontaneous abortion 1) underwent ultrasound examinations at 12 weeks of gestation, which showed fetal hygroma. Chromosomal analysis by amniocentesis at 16 weeks of gestation revealed 18p monosomy. The female baby was born at 39 weeks of gestation, weighing 2064 g. Soon after birth, she developed respiratory failure requiring mechanical ventilation. Her face looked “coarse”with a protruding forehead, low-set malformed ears and a low nasal bridge. She also suffered from heart failure, hyperbilirubinemia, panhypopituitalism, normocytic anemia, increased susceptibility to infection and feeding difficulty with continuous abdominal distention. Although she was intensively treated with mechanical ventilation, various medications, and enteral nutrition with duodenal tube for 3 months, she died of shock associated with sudden deterioration of paralytic ileus, adrenal insufficiency and severe anemia. Autopsy revealed that aplasia of the pituitary gland (empty sella), lung saccular hypoplasia characterized by failure to terminally differentiate alveolar sacs, non-compaction of the ventricular myocardium, hypoganglionosis of the ileum and cecum, myelodysplastic dysplasia, liver hemochromatosis, thymic hypoplasia, and hypoplasia of pancreatic) -cell within the islets. Array-comparative genomic hybridization (CGH) with Agilent 1M platform detected 18q partial duplication (18q22.3 - q23, 5.0-Mb) in addition to 18p deletion (18p11.32 - p11.21, 14.8-Mb). **Discussion** Our patient showed a variety of clinicopathological manifestations apparently different from those known for 18p monosomy. Little has been known about clinicopathological characteristics in patients with a combined chromosomal disorder of 18p monosomy and 18q partial trisomy. The correlation between those unique features proven by autopsy and the karyotype identified with array-CGH in details will shed light on the functional mapping of chromosome 18.

962T

Duplication of 10q associated with multiple congenital anomalies, dysmorphism, cognitive impairment and autism. *H. El-Shanti[1], Y. Al-Sarraj[1], R. Taha[1], H. Khair[2], Z. Hamed[2], I. Shafeeullah[2], B. Saleh[2], J. Alami[1].* 1) Shafallah Medical Genetics Center, Doha, Qatar; 2) Shafallah Center for Children with Special Needs, Doha, Qatar.

Characterizing chromosomal rearrangements at the molecular level is an often successful approach to identify monogenic disorder genes, as well as susceptibility loci for complex disorders. Autism spectrum disorders (ASD) are characterized by language impairment, social deficits, and repetitive behaviors and are associated with cognitive impairment in a fraction of affected individuals. Genetic factors play a significant role in the etiology of cognitive impairment, as well as ASD. Several studies have shown that DNA copy number variants (CNV) are associated with cognitive impairment, as well as ASD. We describe a 20 year old young man with multiple congenital anomalies including cleft palate and microphthalmia, dysmorphism including marfanoid habitus, prominent lips and prognathism, severe cognitive impairment and ASD. He has severe cognitive impairment with poor verbal and non-verbal communication skills. He talked at the age of 7 year but never acquired phrase speech. His ASD evaluation denotes a Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS). Cytogenetic analysis of his chromosomes revealed an unbalanced chromosomal abnormality with an additional chromosomal material attached to the short arm of chromosome 14. The chromosomal analysis of the parents was normal. The techniques employed for the molecular analysis included multiplex ligase-dependent probe amplification (MLPA), real-time PCR (RT-PCR) and SNP genotyping on an Illumina platform. The MLPA provided evidence that there are three copies of chromosome 10q subtelomeric sequences, which was confirmed by RT-PCR on two genes, *TUBGCP2* and *VENTX*, which are centromeric to 10q subtelomeric sequences. The Illumina SNP genotyping showed duplication of the telomeric 30 Mb of the long arm of chromosome 10. The RT-PCR refined the breakpoint to show that it does not disrupt any gene sequences but is present between *NT5C2* (2 copies) and *HCG* (3 copies). The duplicated region contains hundreds of genes. In conclusion, we describe a young adult with multiple congenital anomalies, dysmorphism and cognitive impairment and ASD, who carries a *de novo* duplication of the telomeric 30 Mb of the long arm of chromosome 10.

963T

Prenatal Somatic Overgrowth and Postnatal Growth Deceleration in Pallister Killian syndrome: Phenotypic and Mechanistic Overlap with Beckwith-Wiedemann syndrome. *K. Izumi[1], M. Kaur[1], L. Conlin[2], N. Spinner[2], A. Wilkens[1], Z. Zhang[3], I. Krantz[1].* 1) Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Pathology, Children's Hospital of Philadelphia, Philadelphia, PA; 3) Department of Bioinformatics, Children's Hospital of Philadelphia, Philadelphia, PA.

Pallister Killian syndrome (PKS) is a multi-system developmental disorder typically caused by tetrasomy 12p mosaicism. Clinical manifestations in PKS includes craniofacial dysmorphia, clefts, ophthalmologic, audiologic, cardiac, musculoskeletal, diaphragmatic, gastrointestinal, gentiourinary, cutaneous anomalies, intellectual disability and seizures. Growth parameters are often normal to elevated at birth with deceleration of growth postnatally. The purpose of this presentation is 1) to further delineate the growth pattern of PKS, 2) to suggest PKS as an important differential diagnosis for Beckwith-Wiedemann syndrome (BWS), and 3) to discuss the potential mechanism of overgrowth in PKS. We conducted a retrospective chart review of 60 probands with PKS enrolled in the PKS study cohort at the Children's Hospital of Philadelphia. Birth weight was available on 50 probands and was greater than 90th centile in 18 probands (36%). Birth weight was greater than 50th centile in 42 probands (84%). None of the probands had birth weight of less than 10th percentile. Birth length was available on 45 probands and was greater than 90th centile in 17 (37%). Birth head circumference was available on 22 probands and was greater than 90th percentile in 11 (50%). Postnatal growth pattern was analyzed in 15 probands with birth weights greater than 90th centile. Weight and height usually drops within first year of life, with the exception of a single proband who maintained a weight greater than 97th percentile at 6 years. In addition to macrosomia, additional findings overlapping with BWS in this cohort included umbilical hernia and ear pits, and 2 PKS probands were clinically misdiagnosed with BWS, one of whom exhibited limb asymmetry. Our data demonstrates a unique growth pattern for PKS which consists of macrosomia at birth and subsequent growth deceleration after birth. Genome-wide expression array analysis revealed dysregulated expression of insulin-like growth factor binding proteins (IGFBPs). Upregulation of IGF2 activity is thought to play a pivotal role in the pathogenesis of BWS, suggesting a mechanistic connection between these two diagnoses.

964T

VCP mutations associated with varied phenotypes in the dominant and homozygous state. *V. Kimonis[1], A. Nalbandian[1], E. Dec[1], S. Donkervoort[1], H. Yin[2], G.D. Watts[3], B. Martin[4], C. Smith[4], V. Caiozzo[5], A. Wang[2], T. Mozaffar[2], J. Weiss[2].* 1) Division of Genetics, Department of Pediatrics, University of California Irvine, CA; 2) Department of Neurology, University of California, irvine, CA; 3) School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, Norfolk, UK; 4) Department of Neurology, University of Kentucky Medical School, Lexington, KY; 5) Department of Orthopedic Surgery, University of California, Irvine, CA.

Hereditary inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia is an increasingly recognized disorder caused by VCP mutations and is now identified in 2% of familial amyotrophic lateral sclerosis. VCP is at the crossroads of many cellular functions including ubiquitin proteasome-mediated degradation, and p62-associated autophagy. Pathology includes ubiquitin and TDP-43 positive inclusions also seen in ALS and other proteinopathies. Genotype-phenotype correlations reveal marked intrafamilial variations and varied phenotypes including cardiomyopathy, ALS, Parkinson's, myotonia, cataracts and anal incompetence. Our studies in this fascinating disorder in patients, myoblasts and the knock-in VCP R155H mouse model adds some insight in the disrupted pathways in VCP disease. Our R155H VCP knock-in heterozygous mice demonstrate progressive muscle, bone, and brain pathology. Spinal cords show typical ALS pathology which increases with the age of the mice. Preliminary studies with our homozygous mice produce fewer than the 25% homozygous pups expected. The mice are small and die by 14-21 days of age. There is a decrease in muscle mass, wide variation in muscle fiber size and angulated myofibers, increase in nuclei and lymphocytes. The heart depicts marked bilateral hypertrophy and dilated vascular channels. Electron microscopy identifies abnormal mitochondrial structure in the homozygote tissue and immunohistochemistry studies implicate abnormal autophagy. Understanding the pathogenesis of VCP disease has implications for potential therapeutic targets in more common related progressive muscle, bone and brain diseases.

965T

19p13.3 pure duplication. *K. Kurosawa[1], A. Ishikawa[1], K. Enomoto[1], M. Tominaga[1], N. Furuya[1], M. Masuno[2].* 1) Kanagawa Children's Med Ctr, Yokohama, Japan; 2) Kawasaki University of Medical Welfare, Kurashiki, Japan.

19p13.3 aberration has been rarely described, and published reports are so limited. With the advent of array CGH, the newly recognized microdeletion/duplication syndromes have been emerging. We described a girl with 19p13.3 pure duplication caused by unbalanced translocation der(10)t(10;1-9)(qter;p13.3). She was born to non-consanguineous healthy parents. At the 28th weeks of pregnancy, severe IUGR was diagnosed. Due to fetal distress, a cesarean section was performed at 35 weeks of gestation. Her birth weight was 1216g, length 36.5cm, and OFC 28cm. APGAR were 4/9. She was referred at age 3 years for evaluation of a severe developmental and growth delay, dysmorphic facial appearance, and cardiac failure caused by pulmonary hypertension and mitral valve abnormalities associated with endocardial cushion defect. The detailed examination revealed left renal agenesis, scoliosis, strabismus, and dislocation of hip joints. Standard GTG is normal. Subtelomere screening by FISH with subtel probes showed cryptic translocation of 19pter to 10qter, but the 10qter signal was retained. Both parents had normal karyotype. Array CGH (Agilent, SurePrint G3, 60K) identified 5.7Mb duplication of 19p13.3 (arr 19p13.3(327,273-6,106,229)x3dn). FISH with BAC clones also confirmed the range of duplication. Only 3 cases with pure duplication 19p13.3 were reported, but the range of duplication varied. The common clinical features are developmental and growth delay. Chromosome 19 has the highest gene density among all chromosomes. Further analyses are required for genotype-phenotype correlation in the 19p13.3 duplication syndrome.

966T

Clinical Characteristics of Chromosome 18 Deletion Anomalies. *J. Lin[1], S. Wang[1], F. Lo[2].* 1) Division of Medical Genetics, Department of Pediatrics, Chang-Gung Memorial Hospital, Chung Gung University College of Medicine, Taoyuan, Taiwan; 2) Division of Pediatric Endocrinology, Department of Pediatrics, Chang Gung Memorial Hospital, Chung Gung University College of Medicine, Taoyuan, Taiwan.

Chromosome 18 abnormalities, including deletion of 18q, deletion of 18p, and ring chromosome 18, are among the most frequent autosomal anomalies, together occurring in approximately 1/40,000 live births. We retrospectively reviewed the chromosome 18-deletion patients in our hospital and compared them with previous reports. There were two patients with 18p deletion, four with 18q deletion and 6 with ring chromosome 18. Our patients had a higher proportion of ring 18 than 18q deletion. Mitotic instability for ring 18 was also noted. This might contribute to the clinical manifestation changes in future life. Malignancies, behavioral problems and thyroid functions should be carefully assessed when ring 18 patients were followed up.

967T

Duplication of GPC3 in the boy with growth retardation and developmental delay. *N. Nakashima, T. Yamagata, M. Saito, Y. Nozaki, M. Y. Momoi.* Dept Pediatrics, Jichi Medical Univ, Tochigi, Japan.

GPC3 mutations and deletion were reported to be responsible for Simpson-Golabi-Behmel syndrome (SGBS) that is one of the over growth syndrome such as Beckwith-Wiedemann syndrome (BWS). A boy with severe growth retardation and multiple anomalies had duplication on Xq26 including clusters of *glypican (GPC) 3* and *GPC4*. (Case report) The patient was a four-year-old boy. He was born at 35 weeks of gestation, and his height and weight at birth were 36cm and 1,440g, respectively. At four years of age, his height and weight were 75.8cm (-5.7SD) and 7.558g (-4.1SD), respectively. He also had severe mental retardation, double-outlet right ventricle, narrow palpebral fissures, micrognathia, cleft palate, hearing difficulty, low set ear, overlapping fingers, micropenis, etc. And he often showed fasting hypoglycemia. The secretion of thyroid hormone, growth hormone, ACTH and cortisol were all retained. No patient with *GPC3* duplication was reported, thus far. (Results) On his array CGH analysis using Agilent Human genome CGH 244K, about 4.6MB duplication on Xq25-q26 was detected. Main genes involved in this duplicated region were *ARHGAP36*, *IGSF1*, *MST4*, *RAP2C*, *HS6ST2*, *TFDP3*, *GPC4*, *GPC3*, *PHF6*, etc. and microRNAs. (Discussion) Among these genes, loss of function of *GPC3* causes SGBS which shows overgrowth with high stature, obesity and large head. Addition to that SGBS shows mild to moderate mental retardation, cardiac anomalies such as VSD, PA, TGA, etc, down slanting palpable, large mouth and gnathia, cleft palate, hearing difficulties, syndactyly skeletal abnormalities, etc. And *GPC4* may contribute to SGBS. Members of the glypican family, including *GPC3*, are heparan sulfate proteoglycans that bind to the exocytoplasmic surface of the plasma membrane and regulate the signaling of WNTs, Hedgehogs, fibroblast growth factors, and bone morphogenetic proteins. It was considered that over expression of *GPC3* causes growth retardation opposite to over growth induced by disruption of *GPC3*, like *IGF2* where over expression of *IGF2* induced BWS and suppression of *IGF2* induced intrauterine growth retardation. The fact that many anomalies in this patient and SGB were common organs and tissues also suggested that *GPC3* contributed to this patient.

968T

Interstitial duplication of 1p13.3-p22.3: Report of a patient and review of the literature. *I. Ohashi[1], T. Sasaki[1], T. Kusaka[2], Y. Shimanouchi[1], M. Masuno[3], S. Itoh[2].* 1) Pediatrics, Mitoyo General Hospital, Kanonji, Kagawa, Japan; 2) Pediatrics, Kagawa university, Miki-cho Kita-gun, Kagawa, Japan; 3) Graduate School of Health and Welfare, Kawasaki University of Medical Welfare , Kurashiki, Okayama, Japan.

Chromosome duplications of 1p are rare, and less than 20 such patients have been reported. We describe a three-year-old girl with an interstitial duplication of the short arm of chromosome 1 [46,XX,dup(1)(p13.3p22.3)]. The patient presented with some clinical findings, such as postnatal growth retardation, developmental delay, mild sensorial hearing impairment, dolichocephaly, epicanthus, telecanthus, sparse eyebrows, downturned corners of the mouth, soft cleft palate, and clinodactyly of the fifth fingers. She was born at 36 weeks of gestation by normal vaginal delivery with an Apgar score of 10 at 5 min. Her parents were nonconsanguineous. During gestation, her mother did not receive medication or radiation. There had not been intrauterine growth retardation; birth weight was 2,680 g and length was 46 cm. The family history was unremarkable. During the neonatal period, she demonstrated feeding problems and marked growth retardation. At the age of one year old, she also demonstrated developmental delay, especially that of language due to cleft palate and mild hearing impairment. She began to walk independently at 18 months. At the age of three years, chromosome study on peripheral blood was performed by GTG banding and showed a 1p duplication. Her karyotype was 46,XX,dup(1)(p13.3p22.3). At the age of three and half years, her height was 81.8cm (-2.6 S.D.) and weight was 9.0kg (-2.6 S.D.). Her mental development was equivalent to about two years of age. There were no cardiac or urogenital complications. Only 3 cases demonstrating a similar duplication of 1p have been reported, but the range of duplication varied: dup(1)(p13.1p22.1) [Lo et al., 1998], dup(1)(p13.3p22.1) [Utkus et al., 1999], dup(1)(p13.3p21.2) [Piccione et al., 2010]. Clinical manifestations are quite variable among these 4 patients including the present patient. A patient with dup(1)(p13.3p22.1) demonstrated Kabuki make-up syndrome [Lo et al., 1998]. However, our patient did not show any of the typical features of Kabuki syndrome. In another paper, the microduplication of 1p21.2-p13.3 was detected using array CGH technique and the patient was diagnosed as having autism spectral disorder [Piccione et al., 2010]. In this case, we should monitor her mental development, especially for signs of autism spectrum. Further analyses using array CGH are required for genotype-phenotype correlations in 1p13-p22 duplication patients.

969T

MLPA and Cytogenetic Microarray: Utility in Evaluation of Mental Retardation. *S. Phadke[1], V. Boggula[1], R. Kumar[2].* 1) Dept Med Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India; 2) Department of Pediatrics, Chhatrapati ShahujI Maharaj Medical University, Lucknow.

Identification of etiology of developmental delay / mental retardation (DD / MR) continues to remain a challenge for clinicians as well as scientists. With new molecular cytogenetic techniques there is improved diagnostic yield. We applied these techniques to study the cases of DD /MR without identified etiology after evaluation by a clinical geneticist and appropriate investigations like traditional karyotype, neuroimaging, fragile X study and other investigations like Fluorescent in situ hybridization, evaluation for genetic metabolic disorders as per clinical situation. One hundred and seventy four cases of DD /MR with or without malformations and dysmorphism were studied by Multiplex Ligation Probe Amplification (MLPA) using P245 kit by MRC, Holland for common microdeletion syndromes. One hundred and seventy four cases were studied by MLPA using kits for subtelomeres (P70 and P36 - MRC, Holland). Sixty males with DD /MR were studied by kit for X linked genes for DD /MR. Cytogenetic Microarray (CMA) analysis was carried out in 49 cases of idiopathic DD /MR and 2 cases of unbalanced karyotypes detected by MLPA and traditional karyotype. Out of 49 cases 7 genomic imbalances were detected and in 2 other cases CMA provided additional information. Two of these deletions could have been detected by MLPA as well. MLPA for common microdeletions identified imbalances in 13 out of 174 cases (7.4%) and kit for subtelomeres identified abnormalities in 4 out of 174 cases (2.3%). No abnormality was detected using the MLPA kit for X linked genes for MR/ DD. The molecular cytogenetic techniques improve the diagnostic yield of cases with DD/ MR and help greatly in genetic counseling. The choice of first test at present depends on the availability of the test and relative cost.

970T

Identification of deletions in patients with cleft palate and/or velopharyngeal insufficiency without a definitive diagnosis. *L.A. Ribeiro-Bicudo[1], R.M.C. Sandri-Souza[2], N.C. Lorenco[1], R.M. Zechi-Ceide[1].* 1) Genetics, Hospital for Rehabilitation of Craniofacial Anomal, Bauru, Sao Paulo, Brazil; 2) Center for Human Genome Studies - Institute of Biosciences/USP, Sao Paulo, Brazil.

Aberrations of the 22q11 region are amongst the most common constitutional chromosomal abnormalities, with an incidence of around 1 per 4000. The resulting varied and complex phenotypes make these disorders a significant health problem. Congenital conotruncal cardiac defects, immune deficiency, palatal abnormalities, characteristic facial features, learning disabilities, and psychiatric symptoms present as the most common features. Although most individuals with 22q11 deletion syndrome (22q11DS) are considered to have a “common 3MB deletion” that contains more than 45 known genes, 8% show a 1.5Mb deletion, and a minority has smaller overlapping and non-overlapping 22q11.2 deletions. Thus, a molecular test that could identify deletion variants is relevant not only for increasing diagnostic rates, but also for identifying specific genes that could be involved in different 22q11DS phenotype. MLPA is a technology based on simultaneous hybridization of 40 DNA sequence-specific probes enabling the detection and delineation of deletions and duplications in the 22q11 region. MLPA kit is a cost-effective, rapid, and sensitive method effective for not only detecting, but also sizing the recurrent deletions and duplications in proximal 22q11. In the present work we evaluated a sample of 35 individuals presenting cleft palate and/or velopharyngeal insufficiency features without definite diagnosis by the MLPA technology. The results, confirmed by the use of positive and normal controls identified that 13 individuals had deletions in the region under study. The MLPA technique has been implemented successfully demonstrating that their inclusion in the diagnosis of chromosomal abnormalities in certain clinical conditions, such as the 22q11 microdeletion, can significantly increase the detection rate and therefore a more appropriate genetic counseling. We believe that a definitive diagnosis of 22q11DS helps the team predict the future needs of the patient and aids early intervention and allocation of the appropriate resources.

971T

Analphoid supernumerary marker chromosome characterized by high resolution array: a *de novo* 3q26.32-q29 duplication in a child with pigmentary mosaicism of Ito. *C.E. Steiner[1], K.S. Cunha[1], M. Simioni[1], T.A.P. Vieira[1], V.L. Gil-da-Silva-Lopes[1], M.B. Puzzi[2].* 1) Department of Medical Genetics, State University of Campinas Medical School, Campinas, Sao Paulo, Brazil; 2) Skin Cell Culture Laboratory, State University of Campinas Medical School, Sao Paulo, Brazil.

Pigmentary mosaicism of Ito is a skin abnormality often characterized by hypopigmentation of the skin following, in most cases, the Blaschko lines, usually associated with extracutaneous abnormalities, especially of the central nervous system. Here we report a sporadic case of a four year old girl presenting with developmental delay, speech abnormalities, congenital cardiac defects, umbilical hernia, high arched palate, cubitus valgus, short metacarpals and metatarsus, intermamilar increased distance, hirsutism, hypertrichosis, and low set ears. She also presented with hypopigmented streaks and whorls along the Blaschko's lines on face, trunk, legs, and arms. Chromosomal analysis on lymphocytes and cultured skin fibroblasts revealed a de novo supernumerary marker chromosome in mosaic (47,XX,+mar[38]/46,XX[12]). The karyotype of the parents was normal. Considering this, additional studies were undertaken to enhance the laboratory investigation. The marker chromosome was characterized as an analphoid with C-banding and subsequent study with a high resolution array (Genome-Wide Human SNPArray 6.0 Affymetrix®) was performed. SNP array analysis showed an amplification of 18045 kb in region q26.32q28 of chromosome 3, confirming the origin and breakpoints of the marker chromosome. These results are being validated with fluorescence in situ hybridization (FISH) studies. Hypopigmentation and hyperpigmentation following the Blaschko's lines are relatively common in individuals with chromosomal mosaicism. In cases where there is presence of marker chromosomes, the phenotype depending on the chromosomal region involved, the euchromatic content present and degree of mosaicism. Thus, it is important to characterize this chromosome markers to define the genes involved. Duplication of a specific autosomal segment tends to result in comparable phenotypes, thus the identification of more and more similar cases of analphoid duplication 3q marker will help in establishing a better genotype-phenotype correlation. The combined approach of karyotype and array analysis allowed the correct characterization of the cytogenetics abnormality on this case and also contributed to provide an accurate genetic diagnosis and better counseling to the family.

972T

Clinical features in a 4 year old male with trisomy 8p23.3p11.1. *D.L. Sternen[1], J. Conta[1], S.E. Wallace[1, 2].* 1) Division of Genetic Medicine, Seattle Children's Hospital, Seattle, WA; 2) Division of Genetic Medicine, University of Washington, Seattle, WA.

We report the clinical features of a 4 year old male with trisomy 8p23.3p11.1. There is a limited amount of literature on children with this specific duplication. Oligonucleotide array identified a gain at 8p23.3p11.1 that at minimum is 43.2 Mb (position 313560-43519004 based on hg18). G-banded karyotype showed an unbalanced translocation between chromosomes 8p11.1 and 14p11.1. The deleted 14p region was not covered by the array. A paternal balanced translocation between 8p and 14p was detected. Our patient has remained at the upper end of the growth chart, with current height 75%; weight 90%; head is relatively macrocephalic. He has minimal dysmorphic features including two posterior hair whorls, a short philtrum and wide-spaced teeth. He has mild joint laxity at the elbows and digits, a right transverse proximal palmar crease, and hypoplastic 5th toenails. His hair is coarse and skin shows persistent cutis marmorata. He has severe progressive neuromuscular scoliosis that required VEPTR placement. He has osteopenia, attributed to decreased weightbearing, and suffered two fractures of the right tibia. Frequent infections include chronic otitis media and sinus infections. Quantitative immunoglobulins showed low IgG and IgA; additional work-up for immune deficiency was negative. An echocardiogram, EKG, and renal ultrasound were normal. Head MRI showed absent corpus callosum. Spine MRI was normal. A brainstem auditory evoked response was normal. He has a history of early hypotonia that improved with time. He has gross and fine motor delays with no regression. At 4 years of age he is able to scoot and walks with a gait trainer. He uses approximately 10 signs, 20 words, and a few 2-word combinations. G-tube feeding was initiated due to poor and inconsistent oral intake presumed secondary to severe reflux and oral aversion. We wish to identify additional cases of older children with trisomy 8p23.3p11.1 to expand the phenotype and better characterize the natural history of this disorder.

973T

A long-term survivor with double aneuploidy of trisomy 18 and Klinefelter syndrome (48,XXY,+18): a successful shift from neonatal intensive care to home care. *Y. Ushiroda, M. Doi, H. Motomura, A. Yamashita, A. Yanai, H. Kuniba, H. Moriuchi.* Pediatrics, Nagasaki University Hospital, Nagasaki, Japan.

Introduction Double aneuploidy of trisomy 18 and Klinefelter syndrome is very rare, the natural history of which is hardly known. We herein report a patient with the double aneuploidy whom we have been following for 20 months since his birth until successful home care. Case report The male infant was born at 38 weeks of gestation from a 43-year-old mother. Birth weight was 2082 g. He was hypotonic, cried weakly, and had features characteristic of trisomy 18, such as hypoplastic and low-set ears, micrognathia, overlapping fingers, and rocker-bottom feet. He also had interruption of aortic arch and double outlet right ventricle. Chromosomal analysis was done after obtaining parents' informed consent, revealing a karyotype 48,XXY,+18. The patient's parents desired that his cardiac defects be repaired surgically; however, his underlying condition did not allow him to undergo a major operation. He has been treated with prostaglandins, initially by continuous intravenous infusion and later perorally. His other clinical problems included apnea attack, gastroesophageal reflux, and deafness. Respiratory condition has been well managed with oxygen administration and noninvasive positive pressure ventilation (NPPV), and appropriate nutrition has been maintained by enteral feeding through a duodenal tube. He was discharged home after 222-day stay at the neonatal intensive care unit. The aforementioned supportive care and medical treatment have been maintained with the family's dedicated involvement, enabling him to have a substantial time with his family. He became 20 months old, weighing 3500 g as of June 2011. Discussion Only less than 10 cases of double aneuploidy of trisomy 18 and Kleinfelter syndrome have been reported in the literature, and a 15-month-old infant lived the longest life. In most cases as well as the present one, the main manifestations were those of trisomy 18, including a number of malformations, cardiovascular defects, and respiratory distress. In this case, it was fortunate that his respiratory and circulatory conditions were relatively stable, so that he can survive any of those previously reported and live at home. Conclusion Double aneuploidy of trisomy 18 and Klinefelter syndrome is a rare chromosomal aberration with poor prognosis. However, coping appropriately with each clinical problem may allow patients longer survival and better management in a home care setting.

974T

Challenges in the clinical interpretation of *de novo* single gene deletions detected by high resolution genomic microarray screening. *N. Van der Aa[1,2], G. vandeweyer[2], F. Kooy[2].* 1) University Hospital Antwerp, Antwerp, Antwerp, Belgium; 2) University of Antwerp, Antwerp, Belgium.

The introduction of high resolution arrays allows the detection of very small genomic abnormalities. We screen our patients with intellectual disability routinely on an Illumina iScan system using the HumanCytoSNP-12 BeadChip for the possible presence of chromosomal abnormalities. The data are analyzed using our integrated platform CNV Webstore (BMC Bioinformatics, 2011, 12:4). This allows the detection of abnormalities of 10 consecutive probes at the routine level and even higher for cases of special interest. As a consequence, we have detected a relatively high number of deletions at the single candidate gene level. Examples of de novo single gene deletions in our patient cohort include NLGN4, CLIP2, CMIP and NETO1. The NLGN4 deletion was detected in a male patient with severe autism, and can therefore be considered causative. The deletion in CLIP2 was detected in a patient with autism that also had a 16p11.2 duplication. The phenotype of the index patient was due to the latter duplication, but the deletion in CLIP2, one of the 3 key genes that has been postulated to play a role in the typical cognitive profile observed in the Williams-Beuren syndrome, was present in the apparently unaffected mother and uncle of the patient and the effect of this mutation is under study. The de novo deletion of CMIP (deleted along with GAN that has no clinical consequences in this patient), was identified in a patient with delayed speech development and an autism spectrum disorder. CMIP was recently implicated in the etiology of specific language impairment by genome wide association analysis, suggesting a causative relationship between the CMIP haploinsufficiency and the clinical manifestation of the patient. A de novo deletion of NETO1 was identified in a patient with severe rolandic epilepsy. NETO1 was recently identified as an NMDA-receptor interacting protein required for synaptic plasticity and learning. The detection of these often unique abnormalities poses specific challenges for clinical counselling. We have obtained detailed clinical information for the patients described above, and were therefore able to make the diagnoses. At the same time we learned more on the role of individual genes in human disease. These cases stress the importance of the Decipher database, as more cases with the same abnormalities are needed for optimal interpretation of the data.

975T

Choreoathetosis, congenital hypothyroidism and neonatal respiratory distress syndrome caused by 14q13.3 deletion not encompassing *NKX2-1*: First reported case. *C.P. Barnett[1], S.M Kirwin[2], K.M.B Vinette[2], W. Waters[3], J. Mencel[1].* 1) SA Clinical Genetics, Women's and Children's Hospital/SA Pathology, North Adelaide, South Australia, Australia; 2) Nemours/Alfred I. duPont Hospital for Children, Wilmington, DE; 3) Cytogenetics, Women's and Children's Hospital/SA Pathology, North Adelaide, South Australia, Australia.

Background: Mutations in the NK2 homeobox 1 gene (*NKX2-1*) cause the rare but well defined syndrome known as choreoathetosis, congenital hypothyroidism and neonatal respiratory distress syndrome (OMIM 610978). Here we present the first reported patient with this condition caused by a 14q13.3 deletion which is adjacent to but does not interrupt *NKX2-1*, and review the literature on this condition. Case report: A 23 month old infant presented with a history of developmental delay, hyperkinesia, recurrent respiratory infections and hypothyroidism. Past history included unexplained neonatal respiratory distress requiring oxygen for 24 hours. Choreiform movements and delayed motor milestones were first noted at 6-8 months of age and extensive investigation for known causes of choreoathetosis were normal. At 23 months, examination revealed choreoathetosis, hypotonia and mildly dysmorphic facial features. TSH levels had been consistently elevated (2-4 x normal) from 8 months of age. Brain MRI revealed a hypoplastic pituitary gland but was otherwise normal. The clinical presentation was suggestive of choreoathetosis, congenital hypothyroidism and neonatal respiratory distress syndrome. Sequencing of all exons and splice site junctions of *NKX2-1* was performed but was normal. Array CGH was then performed and a 3.29 Mb interstitial deletion at 14q13.1-q13.3 was detected. The distal region of loss of the deletion disrupted the surfactant associated 3 gene (*SFTA3*) and was adjacent to, but did disrupt, *NKX2-1*. A multiplex PCR assay using fluorescently labelled primers amplifying a region of all exons of *NKX2-1* indicated the *NKX2-1* copy number was normal, ruling out the possibility of small deletions or insertions involving *NKX2-1*. Discussion: *NKX2-1*, located at 14q13.3, encodes important transcriptional factors involved in the developmental pathways for thyroid, lung and brain. The 14q13 deletion in our patient, adjacent to but not involving *NKX2-1*, emphasizes the role that the promoter region of *NKX2-1* or other genes in the region may play in the causation of this condition, including other known neurodevelopmental pathway genes (*RALGAPA1 and NPAS3*) and the surfactant pathway gene *SFTA3*. Conclusion: Deletions at 14q13.3 adjacent to but not involving *NKX2-1* can cause choreoathetosis, congenital hypothyroidism and neonatal respiratory distress syndrome. Array CGH should be done when *NKX2-1* mutations are not identified by sequencing or copy number analysis.

976T

Reproductive fitness in adults with 22q11.2 deletion syndrome. *G. Costain[1,2], E.W.C. Chow[1,3], C.K. Silversides[4,5], A.S. Bassett[1,2,3,5].* 1) Clinical Genetics Research Program, Centre for Addiction and Mental Health, Toronto, Ontario, Canada; 2) Institute of Medical Science, University of Toronto, Toronto, Ontario, Canada; 3) Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada; 4) Toronto Congenital Cardiac Centre for Adults, Toronto General Hospital, University Health Network, Toronto, Ontario, Canada; 5) Division of Cardiology, Department of Medicine, University Health Network, Toronto, Ontario, Canada.

22q11.2 deletion syndrome (22q11.2DS) is the most common microdeletion syndrome in humans. Intellectual disability, schizophrenia (SZ), and serious congenital heart disease (CHD) are frequently associated features. These conditions can negatively affect reproductive fitness. As for other genomic disorders, fitness in individuals with 22q11.2DS is unknown.

The proportion with at least one child (PC) and the overall rate of liveborn offspring (LB) in 142 carefully phenotyped Canadian adults (>17 years) with 22q11.2DS [47.9% male; mean (SD) age 34.2 (11.6) years] were compared with those of their 207 unaffected siblings [48.3% male; mean age 36.0 (12.1) years]. Similarly, we also compared these fitness parameters in the subsets of adults with 22q11.2DS with SZ (n=54) and with serious CHD (n= 60) to age-, sex-, birth cohort-, and severity-matched disease comparison groups without 22q11.2 deletions. We identified phenotypic features in 22q11.2DS associated with childlessness using a multivariate logistic regression model.

Twenty (14.1%) adults with 22q11.2DS had in total 40 children; half inherited their parent's 22q11.2 deletion. Eighty-three (40.1%) siblings had 168 children (22q11.2DS/sibling relative PC=0.35, p<0.0001; relative LB=0.36, p<0.0001). Three (5.6%) individuals with SZ and 22q11.2DS had in total 10 children, while 13 (24.1%) individuals with SZ without 22q11.2DS had 25 children (22q11.2DS-SZ/SZ-comparison relative PC=0.23, p=0.01; relative LB=0.40, p=0.12). In contrast, there were no significant fitness differences between those individuals with CHD with and without 22q11.2DS. In addition to current age and male sex, mild to severe mental retardation (OR=5.96, p=0.02) and SZ (OR=22.54, p<0.01) were significant predictors of childlessness in 22q11.2DS. Notably, CHD was not a significant predictor of childlessness in 22q11.2DS.

Individuals with 22q11.2DS have significantly reduced reproductive fitness. This appears primarily related to the severity of the neuropsychiatric phenotype. Nevertheless, these data and the high rate of *de novo* mutations predict an increasing number of individuals with 22q11.2DS in the general population.

977T

A 2.42 Mb interstitial deletion at 12p13.1p13.2 in a female infant with neural tube defect and distinct dysmorphic features. *J. Fahrner[1], E. Wohler[2], C. Dinsmore[1], L. Henderson[1], D. Batista[2], T. Wang[1].* 1) Johns Hopkins University School of Medicine, Baltimore, MD; 2) Kennedy Krieger Institute, Baltimore, MD.

Neural tube defects (NTDs) are a group of common congenital malformations with significant morbidity and mortality. NTDs are known to occur sporadically and result from combined gene-environment interactions. Genes involved in folic acid metabolism have long been recognized and extensively studied as contributing risk factors to developing NTDs. However, the genetic landscape and molecular mechanisms that underlie the risks and severity of NTDs remain poorly understood. Here we report a 2 month-old female with spina bifida, myelomeningocele, hydrocephalus, Chiari II malformation, agenesis of the corpus callosum, developmental delay, and distinct facial and skeletal features. One maternal first cousin of the proband was noted to have spina bifida and hydrocephalus. SNP array was performed using the HumanQuad610 Beadchip and the Illumina BeadArray reader, and the data were analyzed using CNV partition v2.4.4.0 (Illumina, Inc., USA). A 2.42 Mb interstitial deletion at 12p13.1p13.2 (12,170,628-14,586,144; NCBI build 36.1) was identified in the proband but was not found in her unaffected mother and in over 2000 controls. This deletion involves 24 known genes, including low density lipoprotein receptor-related protein 6, or LRP6. Consistent with the predicted LRP6 deletion from the SNP array, the transcript levels of LRP6 in lymphoblasts were found to be lower in the proband than in her mother. LRP6 acts as a co-receptor for the canonical WNT signaling pathway, and a mutation in the gene has been associated with an increased risk of coronary artery disease in humans. However, mutations in LRP6 have not been linked with NTDs in humans. Interestingly, the lack of Lrp6 in mice results in neonatal lethality and multiple congenital anomalies, including spina bifida. Two naturally occurring NTD mouse models were found to have either homozygous hypomorphic or hypermorphic mutations in Lrp6, suggesting a gene dosage effect on the severity of the NTD phenotype. Our report provides suggestive evidence that abnormal LRP6 dosage and/or function may be involved in the pathogenesis of NTDs in humans.

978T

Trisomy X and 7p15.2-p21.1 deletion in a patient with cleft lip and hand-foot-genital syndrome. *A.N. Filose, G.E. Tiller.* Department of Genetics, Kaiser Permanente, Los Angeles, CA.

Hand-foot-genital syndrome is a rare autosomal dominant disorder due to mutations or deletions of the HOXA13 gene on chromosome 7p15.2. We report a three year old female with trisomy X and an interstitial deletion of chromosome 7p15.2-p21.1. She was a former 2000gm term infant born to a 41 year old G9P5sAb1elAb2 Sephardic Jewish mother. Pregnancy was complicated by advanced maternal age and gestational diabetes. Amniocentesis revealed 47,XXX karyotype, and fetal anatomic survey revealed left-sided cleft lip; the couple elected to continue the pregnancy. At birth, a left-sided cleft lip and alveolar defect was evident, with simple low-set ears, short thumbs and great toes, and camptodactyly; the placenta was small with multiple infarcts and calcifications. The infant underwent placement of a gastrostomy tube in the first month of life for poor feeding. At three years of age, she has global developmental and growth delay (weight and head circumference 50% for 14 months). Array CGH revealed a 7.7Mb deletion from 7p15.2-p21.1. The deletion includes the entire HOXA gene cluster, but spares the TWIST locus distally. At least five other patients have been reported with deletion of the HOXA gene cluster and hand-foot-genital syndrome, but none have had cleft lip +/- palate. A patient reported by Hoover-Fong et al. (Am J Med Genet 117A:47, 2003) with deletion from 7p15.1-p21.1 had bilateral cleft lip and unilateral cleft palate, as well as other craniofacial anomalies. This suggests that a locus for facial clefting may lie between TWIST (distal) and the HOXA gene cluster (proximal) on chromosome 7p.

979T

Preliminary analyses of de novo CNVs and sequence variation in the DDD project. *M. Hurles, Deciphering Developmental Disorders Project.* Wellcome Trust Sanger Inst, Cambridge, United Kingdom.

The Deciphering Developmental Disorders (DDD) project is a translational research project that is a collaboration between the Wellcome Trust Sanger Institute and the 23 NHS regional genetics services. The primary objective of the project is to investigate the utility of new genomic technologies for providing genetic diagnoses, by profiling ~12,000 families with children with severe undiagnosed developmental disorders using array-CGH, SNP genotyping and exome sequencing. I will outline the design of custom array-CGH, SNP genotyping and exome sequencing reagents to maximise the ability to identify causative variants, and the development of new, improved analytical approaches to discovering variation, determining de novo status, and interpreting genetic causation. I will summarise the application of these approaches to ~1,000 control samples, and estimation of the background rate of de novo CNVs. I will also review the preliminary analyses of 100+ families and identification of de novo CNVs and sequence variants in these families.

980T

The patterns of deletion at chromosome 15q11q13 defined with array comparative genomic hybridization in Korean Prader-Willi/Angelman syndrome patients. *H.W. Kim[1], S.Y. Shin[1,2,3], E.J. Seo[1,2,3], M. Hong[3], G.H Kim[2,3], J.Y Lee[2], H.W. Yoo[2,3,4].* 1) Dept. of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea; 2) Genome Research Center for Birth defects and Genetic disorders, Asan Medical Center, Seoul, Korea; 3) Medical Genetic Center, Asan Medical Center, Seoul, Korea; 4) Dept. of Pediatrics, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea.

Introduction : Prader-Willi/Angelman syndromes(PWS/AS) are imprinting disorders caused by deletion or nonfunctioning of imprinted genes at chromosome 15q11q13. Deletion at the critical regions of Prader-Willi/Angelman syndrome accounts for about 70% of both PWA and AS. According to the location and size of the deletion, a number of other loci rather than the crucial deleted regions may be related to present diverse phenotypes. In the present study, we performed chromosomal microarray study to characterize the pattern of deletions in Korean PWS/AS patients. **Materials and methods :** Among 58 PWS and 21 AS patients who were confirmed by methylation-specific PCR, 40 PWS and 17 AS patients with deletion by FISH study were subjected. Array comparative genome hybridization was performed using 60k oligonucleotide microarray (Agilent Technologies, Santa Clara, CA). Commercial pooled male DNA was used for reference (Promega, Madison, WI). Electronic medical records were retrospectively reviewed. **Results :** The size of deletion ranged from 4.0 Mb to 8.7 Mb. We could classify the patients into three groups according to patterns of deletion; type I (BP1 to BP3), type II (BP2 to BP3), and atypical type (BP2 to BP5). Type II deletion was found in 23 (57.5%) of 40 PWS and 9 (52.9%) of 17 AS. Approximately 1.9 Mb loss of known CNV region proximal to BP1 was more frequent in type I than type II deletion; 45.8% vs 21.9%. A distinct large deletion of 8.7 Mb from BP2 to BP5 was observed in a AS patient presenting seizure, which contains CHRNA7 gene reported as a candidate for the seizure phenotype in 15q13.3 microdeletion syndrome. **Conclusions :** Statistically significant differences were not found in the distribution of deletion patterns between PWS and AS. Common breakpoints were observed determining the consistent deletion patterns in majority. The distinct AS case with large deletion encompassing BP2 to BP5 may underline the contribution of the CHRNA7 gene for seizure phenotype.

981T

The smallest region of deletion on 3p25 in a patient with 3p deletion syndrome. *I. Peltekova[1], A. MacDonald[1], C. Armour[2].* 1) Pediatrics, Kingston General Hospital, Kingston, Ontario, Canada; 2) Clinical Genetics, Kingston General Hospital, Kingston, Ontario, Canada.

The rare 3p deletion syndrome presents with a spectrum of anomalies caused by deletions within the distal short arm of chromosome 3. Some of the phenotypic features of this syndrome include facial dysmorphisms, cognitive impairment, growth retardation, congenital heart defects, and renal and intestinal anomalies. The clinical severity of the 3p deletion syndrome is variable because different deletion sizes and locations within the short arm of chromosome 3 have been shown to cause the disorder. Recently, the smallest causative deletion, consisting of 1.6Mb within 3p25.3-p26.1, was identified in a patient with 3p deletion syndrome. Here we describe an even smaller heterozygous interstitial deletion of 643kb in a girl who displayed typical features of the syndrome. We have performed a comparative analysis of the phenotypes and the deleted regions shared between our patient and the one recently described with the 1.6Mb deletion. The two patients have several typical features of the 3p deletion syndrome, such as mental retardation, seizures and congenital heart defects. In addition, they share a 518kb region of overlap that contains 12 candidate genes: *THUMPD3*, *SETD5*, *LHFPL4*, *MTMR14, CPNE9, BRPF1, OGG1, CAMK1, TADA3, ARPC4, TTLL3,* and *RPUSD3*. The putative functions of several genes as reported in the literature are discussed with respect to their involvement in the 3p deletion syndrome phenotype. We focus on *CRELD1* and its relation to congenital heart defects and organogenesis. We evaluate the implication of *CAMK1* and *SRGAP3* in abnormal cognitive development and mental retardation. We also examine the involvement of *TADA3* in the deregulation of physiologic processes that may account for the multiple anomalies seen in the 3p deletion syndrome. In summary, this report further refines the 3p deletion region and reviews some of the candidate genes implicated in this syndrome.

982T

A new case of interstitial 6p22.3 chromosome deletion : confirmation of the minimal critical region. *L. PINSON[1], A. SCHNEIDER[1,2], M. TOURNAIRE[1,2], M. GIRARD[1,2], P. BLANCHET[1], C. COUBES[1], D. GENEVIEVE[1], P. SARDA[1], G. LEFORT[1,2], F. PELLESTOR[1,2], S. TAVIAUX[1,2], E. HAQUET[1], J. PUECHBERTY[1,2].* 1) Medical genetics, Hôpital Arnaud de Villeneuve, CHRU, MONTPELLIER, Languedoc, France; 2) Chromosomal genetics, Hôpital Arnaud de Villeneuve, CHRU, MONTPELLIER, Languedoc, France.

Deletions of the short arm of chromosome 6 are rare events, mostly discovered by standard cytogenetics. Using molecular cytogenetics, two distinct 6p deletion syndromes were described, corresponding to terminal (6p24-pter) and interstitial (6p22-p24) regions. The introduction of array CGH in clinical diagnostics has led to improved detection and characterization of chromosomal microrearrangement syndromes. Seven cases with an interstitial deletion of the short arm of chromosome 6 involving the 6p22.3 region have been described so far in the literature. From these cases, Bremer et al., (2009) defined a 2.2-Mb minimal critical region (MCR) for the 6p22 deletions, containing 12 genes. The main features reported are developmental delay, hypotonia, brain, heart, and kidney defects, craniofacial anomalies (abnormal skull shape, structural eye and ears abnormalities, short neck) and clinodactyly or syndactyly. We report a de novo interstitial 6p22.3 deletion in an 5.5-year-old girl. The patient was referred to our Department of Medical Genetics because of developmental delay of undefined origin. She presented with psychomotor delay, moderate lower limb spasticity and moderate postnatal microcephaly. The patient has always had friendly and amiable behavior. Clinical examination revealed a triangular face, deeply set eyes, hypotelorism, thin upper lip, posteriorly rotated ears, short neck, clinodactyly of the fifth digit and big hallux. Magnetic resonance imaging of the brain was normal. Conventional cytogenetic studies showed a normal female karyotype (46,XX). Microarray analysis (SNP6.0, Affymetrix) showed a 2.25Mb interstitial deletion of the 6p22.3 region encompassing 11 genes. Interestingly, this deletion corresponds to the MCR defined by Bremer et al., except for the most proximal gene which is not deleted in our patient. Also, it is interesting to note that our patient did not present with heart or kidney defects. We compare the phenotype observed in our patient and the patients with overlapping deletions and discuss candidate genes linked to the features described in the patients with 6p22.3 deletion. Further observations of a larger number of patients with 6p22.3 chromosomal deletions may lead to the recognition of a specific phenotype. To achieve this, it seems important that microarray analyses revealing 6p22.3 deletions be accompanised by detailed clinical data as well as the recording of all the cases in international genetic databases.

983T

Phenotypic variability in individuals with chromosome 15q11.2 microdeletion. *J. Ranells, P. Newkirk, T. Ferlita.* Univ South Florida Col Med, Tampa, FL.

Recent reports describe a broad range of developmental, neurologic and behavioral abnormalities in individuals with microdeletion between breakpoints 1 and 2 proximal to the Prader-Willi/Angelman critical region. This _500 kb region contains four highly conserved non-imprinted genes — *NIPA1*, *NIPA2*, *CYFIP1* and *TUBGCP5*, three of which have a role in central nervous system function. The microdeletion is often inherited from a phenotypically normal parent. We present clinical features of 6 individuals (4 probands and 2 parents) with this microdeletion evaluated in a recent 6 month period. SNP microarray analysis was performed at LabCorp on each proband using the Affymetrix v.6.0 platform. Parental follow-up studies were performed by FISH analysis. Patient 1 is an 8 month old female with left auditory neuropathy, mild developmental delay, hypotelorism and a 627 kb deletion. Father, a college graduate, carries the deletion. Family history is negative for behavioral, neurologic and psychiatric difficulties. Patient 2 is a 4 year old girl with mild developmental delay, hypotonia, language regression at 18 months, autistic characteristics and a 638 kb deletion. Parental studies were normal. Family history is significant for: sister with mild learning difficulties, odd behavior and immaturity; mother with mild anxiety; maternal aunt with seizures; paternal aunt with learning disability, schizophrenia and bipolar disorder. Patient 3 is an 8 year old boy with ADHD, mild intellectual disability, autistic characteristics, minimal ptosis, inferior epicanthal folds, prominent fetal finger pads and 581 kb deletion of 15q11.2, 202 kb deletion of 9p23 and 236 kb deletion of 12q23.1. Mother shares the 15q and 12q deletions and father has the 9p deletion. Twin sister is a slow learner and in speech therapy. Mother had speech therapy. Father and his parents are “loners”. Patient 4 is a 9 year old boy with moderate intellectual disability, severe developmental regression at age 15 months, hypertonia, scaphocephaly, high narrow palate and preauricular pit. He is non-verbal, has oral aversion, GERD and is gastrostomy dependent. He has a 2.193 Mb deletion including the BP1-BP2 region and extending proximally. Mother has schizophrenia. Parents are unavailable for testing. These cases illustrate the difficulty in defining the spectrum of clinical features associated with this deletion and the need for further studies to identify factors that contribute to the variable phenotype.

984T

An 81kb deletion detected by aCGH in a patient with Rubinstein-Taybi syndrome. *E.C. Tan[1], A.H.M. Lai[2], E.C.P. Lim[1], J.S.H. Ng[1], M.S. Brett[1].* 1) KK Research Center, KK Women's & Children's Hospital, Singapore; 2) Genetics Service, KK Women's & Children's Hospital, Singapore.

Rubinstein-Taybi syndrome is a multiple congenital anomaly syndrome characterized by mental retardation, postnatal growth deficiency, microcephaly, broad thumbs and halluces, and dysmorphic facial features. The pattern of inheritance is autosomal dominant although most cases are sporadic. The disorder is usually caused by de novo mutations in the gene encoding transcriptional coactivator CREB-binding protein (*CREBBP*) on chromosome 16p13.3 or the 300KD E1A-binding protein (*EP300*) on chromosome 22q13.

We report a patient who is the second offspring of biologically unrelated parents who are phenotypically normal. They have two other healthy children but the mother had a cousin who had absent speech at age 15. The patient was born at full-term with normal birthweight. At the initial assessment at the Genetics Clinic at 2 years of age, her height was within normal range but her head circumference was on the 10th centile and her weight was on the 90-97th centile for age. At 7 years of age, her height was on the 25th centile but her weight was on the 97th centile for age. Clinical diagnosis of Rubinstein-Taybi syndrome was made based on her physical features of downslanting palpebral fissures, a prominent nose with the nasal septum extending below the alae nasi, broad thumbs and big toes and postaxial polydactyly on the right foot. Renal ultrasound scan revealed bilateral hydronephrosis. She had global developmental delay since infancy. Other than persistent constipation since infancy, her general health is good. She does not speak but can communicate with gestures and understands instructions.

Peripheral blood karyotype analysis was reported as normal (46,XX). Results from Agilent 400K Human CGH array showed copy number loss for chromosome 16p13.3. Amplification of the region across the breakpoint and subsequent sequencing results showed the deletion to be approximately 81 kb. It starts from the 5' untranslated region of the deoxyribonuclease I gene (*DNAse I*), includes the whole of the TNF receptor-associated protein 1 gene (*TRAP1*), and extends into the CREB-binding protein gene (*CREBBP*) which has the last three exons deleted. The molecular results confirm the clinical diagnosis of the patient whose phenotypic presentations are almost certainly due to the identified 16p13.3 microdeletion.

985T

A Rubinstein-Taybi syndrome patient with partial deletion of the CREB binding protein (CREBBP) gene. *E. Wohler[1], A. Bytyci[2], L. Henderson[2], E. Germain-Lee[3], J. Hoover-Fong[2,4], DAS. Batista[1,2,5].* 1) Cytogenetics Laboratory, Kennedy Krieger Institute, Baltimore, MD; 2) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 3) Hugo W. Moser Research Institute and Albright Clinic at Kennedy Krieger Institute, Baltimore, MD; 4) Greenberg Center for Skeletal Dysplasias, Johns Hopkins University, Baltimore, MD; 5) Pathology Department, Johns Hopkins University, Baltimore, MD.

Rubinstein-Taybi syndrome (RTS) is a rare autosomal dominant genetic disorder affecting approximately 1 in 125,000 individuals. Most patients with RTS have a de novo defect in the CREB binding protein (CREBBP) gene. Features of RTS include broad thumbs with radial deviation and broad great toes, seizures, moderate to severe intellectual disability, constipation, short stature and characteristic facies with downslanting palpebral fissures, arched brows, bitemporal narrowing and columella lower than alae. Our patient is a 5-month-old former term male with bilateral thumb deformities, crumpled auricles, hypertrichosis of the forehead with confluence of anterior hairline to lateral brows, mild truncal hypotonia, chronic constipation, and slow motor skill acquisition. Birth weight and length were 7 lbs, 14 oz. (25-50th percentile) and 21 inches (90th percentile), respectively. By 5 months he had symmetric growth failure with height and length below the 3rd percentile and head circumference at the 5th percentile, an abnormal growth pattern that is typical for RTS. The clinical diagnosis of RTS syndrome was suspected in this case. A single nucleotide polymorphism (SNP) array (Illumina 610K BeadChip) revealed a 41 kb deletion on chromosome 16 (3,699,394-3,740,546 bp (NCBI Build 36/hg18)) involving 11 consecutive markers overlapping the distal 3' end of CREBBP. Further gene-specific testing was performed in another laboratory using the oligonucleotide aCGHExonArrayDx platform (GeneDx). This established a heterozygous deletion of exons 17-31 of CREBBP. 5-10% of patients with RTS have a defect in one or more exons of CREBBP. Only one case with RTS has been reported with a partial deletion of CREBBP spanning the same exons 17-31 as in our patient. The clinical indication of RTS in this patient was instrumental for the detection of the abnormality by the SNP array since the number of markers involved and the size of the deletion were below our reporting range.

986T

Severe Aortic Stenosis in a Child with Joubert Syndrome and Related Disorders (JSRD) - a Case Report and Review of Congenital Heart Defects Reported in the Human Ciliopathies. *S.C. Bowdin[1], N. Karp[1], L. Grosse-Wortmann[3], S. Blaser[2].* 1) Clinical & Metabolic Gen, Hosp Sick Children, Toronto, ON, Canada; 2) Department of Radiology,Hosp Sick Children, Toronto, ON, Canada; 3) Department of Cardiology, Hosp Sick Children, Toronto, ON, Canada.

We report a 2 year-old boy with classical features of JSRD including oculomotor apraxia, postaxial polydactyly, episodes of rapid breathing and developmental delay. MRI demonstrated the “molar tooth sign”. The child was also diagnosed with severe congenital aortic stenosis. Sequencing of the AHI, TMEM67, CEP 290 and NPHP1 was negative for mutations. We await results for CC2D2A, INPP5E, RPGRIPIL, ARL13B and TMEM216. JSRD is one of a group of conditions known as `ciliopathies', whose multi-organ involvement results from primary cilia dysfunction. There have not been other reported cases of aortic stenosis associated with JSRD. Cardiac screening is not currently recommended in the management guidelines for JSRD. We speculate that while the presence of congenital aortic stenosis in this child could be caused by an unrelated genetic mechanism, it could also represent a phenotypic overlap with another ciliopathy, the Bardet Biedl syndrome, in which aortic stenosis is present in about 38% of cases. Primary cilia coordinate cellular signal transduction pathways during embryonic development, including Hedgehog. In mouse models of early cardiogenesis, cardiomyocyte differentiation is coordinated by the primary cilium. This implies a role for cardiac primary cilia, as well as nodal cilia, in the pathogenesis of cardiac malformations in human ciliopathies.

987T

Characteristics of kidney and liver disease in 38 patients with Joubert syndrome and related disorders (JSRD). *J. De Dios[1], T. Vilboux[1], K. Daryanani[2], I.B. Turkbey[3], P. Choyke[3], D. Doherty[4], I. Glass[4], M. Parisi[5], J. Bryant[1], M. Huizing[1], T. Heller[1], W.A. Gahl[1], M. Gunay-Aygun[1].* 1) NHGRI, NIH, Bethesda, MD, MD; 2) NIH Clinical Center, Bethesda, MD; 3) Molecular Imaging Program, NCI, NIH, Bethesda, MD; 4) University of Washington, Seattle, WA; 5) NICHD, NIH, Bethesda, MD, MD.

Joubert syndrome and related disorders (JSRD) are a genetically and phenotypically heterogeneous group of ciliopathies defined based on the unique combination of midbrain and hindbrain abnormalities resulting in the pathognomonic “molar tooth sign” on axial brain imaging. JSRD patients can have mutations in a variety of genes that are also associated with other ciliopathies. Similar to other ciliopathies, a subset of patients with JSRD develop hepatorenal fibrocystic disease. Under the NIH clinical protocol, “Clinical Investigations into the Kidney and Liver Disease in Autosomal Recessive Polycystic Kidney Disease (ARPKD)/Congenital Hepatic Fibrosis and other Ciliopathies” (ClinicalTrials.gov: NCT00068224), we evaluated 38 JSRD patients. All patients had the typical “molar tooth sign”. Age at NIH evaluation ranged from 0.9 to 36.2 years (8.6 + 7.3). Four patients from 4 families carried the diagnosis of Senior-Loken syndrome (SLS) and 12 from 10 families were classified as COACH syndrome based on presence of liver involvement. There was history of oligohydramnios in 4 and polyhydramnios in 2 patients. In 5 patients, prenatal ultrasound showed enlarged hyperechoic kidneys, indistinguishable from ARPKD. Six individuals including 3 SLS and 3 COACH syndrome patients received kidney transplantation, one after the NIH visit. Age at kidney transplantation ranged from 4 to 13 years (7.5 + 3). High resolution ultrasound (HR-USG) of the kidneys was normal in 20 of the 33 patients with native kidneys (ages 0.9 to 36.2 years, mean 7.0 + 8.1). The most common ultrasound finding was diffusely increased echogenicity with loss of corticomedullary distinction with or without discrete cysts; kidney size was enlarged in 2, small in 1 and normal in others. Two patients had unilateral multicystic dysplastic kidney. Glomerular filtration rate (GFR) was less than 80 ml/min/1.73 m2 in 12 patients, including 2 who had normal ultrasounds and GFRs of 52 and 65 at ages 9 and 3.5 years, respectively. While all COACH patients (11.6 + 8.8 years) had elevated liver enzymes, review of records showed normal liver enzymes in the first years of life. Nine patients including 7 with COACH had splenomegaly suggesting portal hypertension. No patients showed liver cysts. JSRD patients should be monitored for kidney and liver disease. Next generation DNA sequencing is underway. Genotype phenotype correlations might enable prediction of hepatorenal disease.

988T

New syndrome? situs inversus totalis and infantile spasm and generalized hypotonia with severe global developmental delay. *B. Hashemi[1], K. Siriwardena[1], S. Jain[1], M. Moharir[2], O. Bar-yousef[2], A. Ali[3], D. Chitayat[1,4].* 1) Department of Pediatrics, Divisions of CIINICAL and METABOLIC GENETICS, Hospital for sick children, Toronto, Canada; 2) Department of Pediatrics, Divisions of NEUROLOGY,Hospital for sick children, Toronto, Canada; 3) Department of Pediatrics, Divisions of OPHTALMOLOGY,Hospital for sick children, Toronto, Canada; 4) The Prenatal Diagnosis and Medical Genetics Program, Department of Obstetrics and Gynaecology, Mount Sinai Hospital; UoT, Toronto, ON, Canada.

Situs inversus totalis (SIT) is in most cases a rare sporadic and asymptomatic condition but about 3-5% of these patients have heart defect. CNS abnormalities associated with this condition is rare. We report a patient with SIT and idiopathic infantile spasm. Since to our best knowledge this has not been reported previously it may be a new syndrome caused by a gene associated with laterality determination and brain function. This female patient is 18 month old, born to non-consanguineous parents of Indian origin. The pregnancy was uncomplicated and delivery was at term by C/S due to decrease fetal movement and breech presentation. Postnatal investigation showed situs inversus totalis and hypotonia with roving eyes were noted. At 2 months of age she presented with infantile spasm and Hypsarrythmia on EEG with no response to anti-seizure medications. Ophthalmologic exam showed recently bilateral papilledema and hyperpigmented fovea with cortical blindness. On physical examination all of the growth parameters are WNR, her facial features showed mild frontal bossing, curly hair (not familial), high arched palate and small teeth. Brain MRI showed mild generalized decrease in volume and hypomyelination. Detailed metabolic investigations showed no abnormalities and microarray analysis was normal. Muscle biopsy for mitochondrial cytopathy and hair EM were also normal. SIT is known to be associated with ciliary abnormalities but little is known about cilia function in brain morphogenesis and function. In the mouse mutant for the ciliopathy gene Ftm, olfactory bulbs are present in an ectopic location in the telencephalon. Recent publication showed that a mutation in the in KIF7, a gene associated with human primary ciliary function, causes hydrolethalus and acrocallosal syndromes. Further studies are being done to identify the etiology of the ciliopathy causing the SIT and brain dysfunction in our patient.

989T

Confirmation of the Chromosomal Microarray as a first-tier Clinical Diagnostic Test for Individuals with DD/ID and MCA. *A. Battaglia[1,2], A. Novelli[3], L. Bernardini[3], T. Filippi[1], V. Doccini[1], JC. Carey[2].* 1) Dev Neurosciences, Stella Maris nst/Univ Pisa, Pisa, Italy; 2) Division of Medical Genetics, Dept. of Pediatrics, University of Utah Health Sciences Center, SLC, USA; 3) IRCCS Hospital S. Giovanni Rotondo & CSS Mendel Institute, Roma, Italy.

Submicroscopic structural chromosomal rearrangements are the most common identifiable causes of Developmental Delay/Intellectual Disability (DD/ID) associated with multiple congenital anomalies (MCA). Chromosomal microarray (CMA), able to detect rearrangements as small as 3 Mb or less and to identify position, size and presence of known genes, is increasingly utilized for genetic testing of such individuals. In this study, we analyzed 343 karyotyped patients affected by DD/ID/MCA, observed at the Stella Maris Institute between May 2004 and May 2011. There were 219 (63.8%) males and 124 (36.2%) females, aged 5 months to 19 years. Blood samples, collected from patients and their parents, were analyzed, at the Mendel institute, with CMA at a resolution ranging from 1 Mb to 75 Kb. We identified pathogenic CNVs in 22% (75/343) of our sample (53 males vs 22 females). 41.1% were inherited and 41.7% de novo. 48 of the detected CNVs were deletions and 35 duplications. Most CNVs were detected by CMA at 200 Kb resolution. All CNVs were confirmed using qPCR or FISH. Our study provides further evidence of the high diagnostic yield of CMA for genetic testing of individuals with unexplained DD/ID and MCA, confirming its first-tier use in such disorders.

990T

1p36.2 CNVs Cause Setleis Syndrome, A Focal Facial Dermal Dysplasia. *R.J. Desnick[1], A. Yang[1], L. Edelmann[1], L. Liang[1], I. Nazarenko[1], A. Ma'ayan[2], B.E. Blessing[3], V.K. Proud[3], J.E. Ming[7], J.A. Rosenfled[4], C. Cadilla[5], A.R. Norby[6], D.D. Weaver[6].* 1) Dept Gen/Genomic Sci, Box 1498, Mount Sinai Sch Med, New York, NY; 2) Dept Pharmacology and Systems Therapeutics, Mount Sinai Sch Med, New York, NY; 3) Children's Hospital of the King's Daughters', Norfolk, Virginia 23507; 4) Signature Genomic Laboratories, Spokane, Washington 99207; 5) Department of Biochemistry, University of Puerto Rico School of Medicine, San Juan, PR 00936; 6) Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, Indiana 46202; 7) Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104.

Setleis syndrome is a rare focal facial dermal dysplasia (FFDD; Type III) characterized by bitemporal scar-like lesions similar to forceps marks as well as other facial abnormalities. Recently, we reported that homozygous nonsense mutations in the transcription factor, *TWIST2*, caused the disease in patients from two unrelated consanguineous families, but not in five other unrelated Setleis syndrome patients (Tukel et al., Am J Hum Genet. 88:289-296, 2010). Array CGH studies of unrelated patients with normal *TWIST2* alleles who had facial dysmorphic features, and developmental delay, mental retardation, autism, attention deficit disorder and/or learning disability, revealed overlapping de novo duplications and a triplication within a 1.2 Mb region on 1p36.22 to p36.21, which contains ~26 genes. Compared to the facial phenotype in *TWIST2*-mutant Setleis syndrome patients, the two patients with the duplication had more subtle dysmorphic facial findings, while the patient with the triplication had more typical facial findings seen in the patients with *TWIST2* mutations, evidencing a dosage effect of a gene(s) in the 1p36.2 region. Since the TWIST2 160 amino acid protein is generally an inhibitory transcription factor in mammalian development, it is likely that one or more of the genes in the 1p36.2 smallest region of overlap (SRO) is normally inhibited during facial development by TWIST2. In this model, increased dosage of a gene(s) on 1p36.2, caused by duplications and triplications in the presence of normal *TWIST2* alleles, would mimic the loss of function *TWIST2* mutations and lead to the Setleis syndrome phenotype. Efforts are underway to identify the specific gene(s) that are normally inhibited by TWIST2 in facial development by systems biology approaches and direct sequencing. Thus, TWIST2 loss-of-function mutations and CNVs that increase the expression of TWIST2 regulated genes can cause Setleis syndrome. Similar etiologic CNV alterations may be responsible for the heterogeneity in other transcription factor diseases and may facilitate identification of the specific genes that they regulate.

991T

Phenotypic heterogeneity in a family segregating two different *NRXN1* deletions. *L.B. Henderson[1], C.D. Applegate[1], R.L. McClellan[2], H.T. Bjornsson[1], S.A. Morsey[3], D.A.S. Batista[3,4], S. Naidu[5], A. Hamosh[1].* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Division of Metabolism, Kennedy Krieger Institute, Baltimore, MD; 3) Cytogenetics Laboratory, Kennedy Krieger Institute, Baltimore, MD; 4) Department of Pathology, Johns Hopkins University, Baltimore, MD; 5) Department of Neurogenetics, Kennedy Krieger Institute, Baltimore, MD.

Neurexins are presynaptic cell adhesion molecules that function in neurotransmitter release. Exonic deletions in the neurexin 1 (*NRXN1*) gene are associated with a variety of neuropsychiatric phenotypes including autism, developmental delay (DD), intellectual disability, and schizophrenia. Here we report a family segregating two different *NRXN1* deletions and a spectrum of developmental disorders. The proband was referred to genetics at 23 months of age with a history of failure to thrive, hypotonia, Chiari malformation, and global DD with regression of skills. He also had mild aortic root dilatation and a normal brain MRI. Illumina 610k SNP array was performed and two deletions were identified in band 2p16.3: a 267 kb deletion encompassing the first four exons of the *NRXN1*-(isoform and a 138 kb deletion spanning the last two exons of the *NRXN1*-(/) isoforms. Neither deletion was reported in 2,026 healthy controls. To determine whether these deletions were in *cis* or *trans*, parental arrays were performed and revealed that the larger 5' deletion was maternally inherited and the smaller 3' deletion was paternally inherited. The mother had no history of delayed development, but the father had a history of ADD, scoliosis, and was in a special education setting until age 10. Three of their four other children also had neuropsychiatric features, though less severe than in the proband. A 9 year old girl had ADHD, paranoia, texture avoidance, and learning difficulties; an 8 year old boy had DD, behavioral problems, and a diagnosis of autism; a 3 year old girl had DD and anti-social behavior; and a 2 year old boy was neurotypical. SNP array detected the paternal 3' *NRXN1* deletion in all but the neurotypical 2 year old boy. Mild dysmorphic features in the family included epicanthal folds, down-slanting palpebral fissures, 5th finger clinodactyly, 2,3-toe syndactyly, and inverted nipples; however, these features did not segregate with the *NRXN1* deletions. Furthermore, *PTEN* sequencing in the proband revealed a paternally inherited promoter variant of unknown significance. This variant was not present in the neurotypical child. We are aware of only one other case with homozygous disruption of *NRXN1*, in which a girl presented with Pitt-Hopkins-like syndrome. The family described here exemplifies the incomplete penetrance and variable expression of heterozygous *NRXN1* alterations, which may be attributed to modifier genes such as *PTEN*.

992T

Clinical Dilemma in Interpretation of Microaaray Results: Report on Series of Inherited Copy Number Gain and Loss with Variable Expression. *J. Hiemenga, K. Withrow, C. Forgeng, V. Kirkland, M. Jaworski, J. Bodurtha, A. Pandya.* Human and Molecular Genetics, Virginia Commonwealth University, Richmond, VA.

CGH chromosomal microarray studies have opened new diagnostic frontiers; they are often the first tier test in evaluation of children with developmental delay, multiple congenital anomalies, and autism spectrum disorder. Although efforts to create national datasets with phenotypic information on the identified del/dup variants are underway, a paucity of reported phenotypes for novel copy number variants (CNV) makes interpretation of results a challenge. In addition, the notion that CNVs are also noted commonly in the general population further complicates the interpretation of an inherited CNV. De novo alterations in a proband are most often considered causative; however, inheritance of a variant from an unaffected parent raises questions and poses challenges in attributing the phenotype to a given result. We report on the parentally inherited micro deletions /duplications diagnosed through CGH microarray evaluation of symptomatic infants and children from 2009 through 2011 at our center. We have identified 18 children with an inherited CNV. Seventy per cent of affected probands are male. Mothers were the transmitting parent in 2/3 of our series. Five of the transmitting parents were noted to have some phenotypic changes; however the offspring of the transmitting parents always demonstrated a more severe phenotype. One phenotypically normal father transmitted the CNV to two of his affected sons, and one woman with short stature and subtle distinctive features was diagnosed with the CNV after the affected stillborn conceptus was positive for a CNV by microarray. The variable phenotypic expression for our transmitting parents and their affected children will be presented. Possible mechanisms for the variable phenotypic presentation in these cases include variable expressivity, effects of a second “hit” to genomic integrity, unmasking of heterozygous recessive conditions and epigenetic or environmental interactions. A careful medical history and physical evaluation of the parent is warranted to assess for subtle phenotypic expression and when possible, a complete microarray study in the parents may be helpful in delineating the differences. Caution needs to be exercised in counseling these families about the potential relevance of a CNV to the phenotype, especially in prenatal cases.

993T

Alström Syndrome in Taiwan: Familial Variable Expression of Metabolic Abnormalities and Revisit of Syndromic Obesity. *J. Hou.* Pediatrics, Cathay General Hospital, Taipei, Taiwan.

Background: Alström syndrome (AS) is a rare autosomal recessive disorder characterized by infancy-onset obesity, retinal degeneration, sensorineural hearing loss, type 2 diabetes mellitus and chronic nephropathy, caused by *ALMS1* mutations.The natural history of the insulin resistance and development of obesity is unknown. **Aims**: Our objectives were to characterize metabolic parameters, to establish *ALMS1* mutations of Taiwanese AS patients, and to determine whether a genotype-phenotype correlation exists. **Design and Patients**: Six Taiwanese patients (M:F=3:3, age at diagnosis: 10-15y) with cardinal features of AS from different 5 families were evaluated both clinically and biochemically. The 23 exons (locus: 2p13) and intron-exon boundaries of ALMS1 were directly sequenced. **Results**: Specific features vary from patient to patient. All of them have eye and hearing problems, and overweight since childhood, early onset of liver dysfunction with hepatic steatosis, acanthosis nigricans, hyperlipidemia, and insulin-resistance. Other features include early onset lung fibrosis(n=3), overt Type 2 DM (n=3), cerebral atrophy(n=3), gynecomastia (n=3), renal insufficiency(n=1), and pancreatitis associated with hypertriglyceridemia (n=1). DNA studies on the candidate gene *ALMS1* showed homozygous 19-bp deletion: nt(del)11116_11135 in 2 patients, homozygote nt(del)11116_11135 in 1 patient, and compound heterozygous nt(del)11116_11135 with a secondary heterozygous 2-bp deletion (AG) at nt c.10831_10832 in 3 patients, all leading to a frameshift mutation at exon 16. Mutations of *ALMS1* are clustered in exon 16 among Taiwanese patients, with a possible founder effect. **Discussion**: The clinical features, time of onset, and severity vary greatly among and within families. No genotype-phenotype correlation exists. Two novel mutations in the *ALMS1* gene causative for AS have been reported here, thereby increasing the number of reported mutations and providing a wider basis for mutational screening among affected individuals.

994T

Array comparative genomic hybridization (aCGH) in diagnosing Mendelian disorders: how far do we go and when do we stop?! *A. Tsai, D. Klepacka, C. Walton.* Sect Clinical Gen & Metabolism, Childrens Hosp, Denver, B300, Denver, CO.

Array comparative genomic hybridization (aCGH) is a powerful tool for the molecular elucidation and diagnosis of disorders resulting from genomic copy-number variation (CNV). Intra-genic deletions or duplications have become available through increasing probes and improving genomic resolution. However, benign CNV can confound clinical interpretation. We reported the aCGH results on 423 patients who were ascertained in a pediatric genetic clinic during 2009-2011 performed by a single referenced lab using 180K oligoarray. 112 patients were initially found to be abnormal; further parental studies showed 42 were confirmed to have a chromosomal anomaly, 28 have presumed anomalies, 5 have iatrogenic deletion involving a AD gene. 37 have one or more inherited/de novo CNVs yet not sufficient to explain the problems. We report two unique cases in this group to highlight the diagnostic dilemma and ethical concerns. Case 1 is a-11 year-old girl with developmental delays and dysmorphic features, a 180K microarray identified a 0.853 Mb-1.736 Mb duplication at 8p22 which is not found on her mother and a 2.462 Mb duplication at 22q11 that is inherited. Her mother has similar learning concerns and required special education. Because of her facial features are suggestive of Rubinstein-Taybi syndrome, analysis for the EP300 gene was done which revealed a novel variation p.H1744R:c.5231A>G, predicted to be disease-causing by Mutation Taster. Mother does not carry the same variation. Paternal studies were not available. Case 2 is a 5 year-old boy with hypotonia, hypospadias, microcephaly, big teeth, autism and dysmorphism. A 180 K aCGH revealed a 50 kb loss at 8q22.2, involving COH1 gene (Cohen syndrome). Sequencing for COH1 gene revelaed a heterozygous Nonsense mutation: W1328X. Subsequent studies confirmed the neutropenia and eye problems. Here we raise a few cpncerns. In a child with a CMA anomaly, how far do we go for testing, and when do we stop?! Most people argue aqainst further testing as the cost is high. Should this be the case, Case 1 would be missed and future prenatal diagnosis would be misled. In Case 2, the aCGH reveals a clue which makes testing COH1 an exploratory instead of confirmatory testing. This case demonstrated the favorable outcome of exploratory testing as a Nonsense mutation were identified; however, should this a missense mutation, interpreting SNP of unknown significance would be challenging.

995T

Using a combination of MLPA kits to detect microdeletion and subtelomeric rearrangement in Iranian patients with mental retardation. *S. Zeinali[1], M.S. Falah[2], T. Pourmostafaei[3], A. Bidmeshki-pour[4], H. Bagherian[2], Z. Sharifi[2], N. Khazaei[2], M. Mohammadi[5], F. Fardanesh[5], F. Mohammadi[6], S. Amini[2], P. Foroghi[2], S. Malvandi[2], M. Masoudifard[2], S. Kianfar[2], A. Sarhaddi[2].* 1) Department of Molecular Medicine, Biotech Research Center, Pasteur Institute of Iran, Tehran, Iran; 2) Zeinali's Medical Genetics Lab (ZMGL), Kawsar Human Genetics Research Center (KHGRC), Tehran, Iran; 3) Student of MSc in Molecular biology, Razi University, Kermanshah, Iran; 4) Asistant Prof of Molecular Genetics, Razi University, Kermanshah, Iran; 5) National Genetic Counselling Center, State Welfare Organization, Tehran, Iran; 6) Genetic Counselling Center, State Welfare Organization, Kermanshah, Iran.

Introduction: Mental retardation (MR) is a major health issue and it has been estimated that 1 to 3% of the population suffer from it. In syndromic-MR, mental retardation is not the only presentation of the disease and other physical and/or behavioral abnormalities is present. Submicroscopic chromosomal del/dup and sub-telomeric rearrangements are one of the most common causes of MR. Such variations are not routinely detected by conventional chromosomal analysis. These cases need to be investigated with technique like MLPA. The aim of this study was to investigate syndromic-MR using of MLPA method. Methods: Thirty unrelated cases with mental retardation were selected. Blood sample were taken from probands and their parents after obtaining consent. Karyotype analysis was carried out for probands. Those cases that remained normal were investigated for microdeletion syndromes and sub-telomeric rearrangement using MLPA technique. Results: 1st step screening revealed trisomy in chromosome 21 in one cases. In further MLPA study in other cases, we found an abnormality in 3 cases. A deletion in 7q11.23 region (Williams's syndrome), a deletion in 17p11.2 region (Smith-Magenis syndrome) and a duplication in 4p16.3 region were detected in 3 unrelated cases. Deletion in 17p11.2 region was confirmed with by another MLPA kit (P374) with more probes in the region. Conclusions: Micro-deletion syndromes and sub-telomeric rearrangements as two main categories of genetic causes of MR could be investigated easily by MLPA method. It is an cost-benefit and easy method that is recommend as first step screening in patients with syndromic MR and normal karyotype.

996T

Predictors of adaptive functioning in adults with 22q11.2 deletion syndrome. *A.S. Bassett[1, 2], N. Butcher[2], A. Ho[2], G. Costain[2], D. Young[2], E.W.C. Chow[1, 2].* 1) Department of Psychiatry, University of Toronto, Toronto, ON, Canada; 2) Clinical Genetics Research Program, Centre for Addiction and Mental Health, Toronto, ON, Canada.

22q11.2 deletion syndrome (22q11.2DS) is a common genomic disorder with congenital and later onset features including congenital heart disease (CHD), intellectual disability and schizophrenia that may affect long term functioning. Data on adaptive functioning, that is, the relative ability of a person to effectively interact with society and care for one's self, in adults with 22q11.2DS are limited. The Vineland Adaptive Behaviour Scale (VABS) was administered to the primary caregivers of 115 well phenotyped Canadian adults (>17 years) with 22q11.2DS. After excluding eight with moderate to severe mental retardation, the sample comprised 52 men and 55 women with mean age 28.7 (SD 10.2) years. There were 39 subjects (36.5%) with serious CHD and 49 (45.8%) with schizophrenia. Mean full scale IQ, assessed using the Wechsler Adult Intelligence Scale (WAIS-III), was 71.6 (SD 9.3). We used a multivariate linear regression model to identify major phenotypic features associated with functioning. A minority had ever had a spouse or equivalent partner (14%). About one in four had a driver's license. The mean overall VABS score for the sample was 64.7 (SD 18.4), significantly lower than the normal mean of 100 (SD 15) for the VABS scale ($p<0.001$). The proportion of subjects with scores in the functional deficit range was significantly different across the three main VABS domains, with Daily Living Skills less affected than Communication or Socialization ($p<0.001$). IQ and a diagnosis of schizophrenia were significant predictors of overall adaptive functioning ($p<0.001$). Results were similar for the three VABS domains examined. Notably, sex, age and a diagnosis of serious CHD or anxiety/depression were not significant predictors of functioning in 22q11.2DS. There is considerable functional impairment in 22q11.2DS in early to mid-adulthood. This appears to be primarily mediated by the severity of the neuropsychiatric phenotype. Relative strengths in domestic and community interaction skills have important implications for vocational and other service planning.

997T

Unique Phenotype Associated with a Novel Partial Chromosome 5p Deletion/Duplication. *L. Brick[1], J.M. Meck[2], S. Aradhya[2], C. Li[1].* 1) Genetics Department, McMaster Children's Hospital, Hamilton, ON, Canada; 2) GeneDx, Gaithersburg, MD.

Both deletions and duplications of the 5p region have been noted in the literature. The finding of a terminal deletion in tandem with a 5p duplication, however, is rarely reported for chromosome 5p. We report on a 3-year old boy with a complex chromosome 5 abnormality, who presented with a high-pitched cat-like cry, unilateral cryptorchidism, bilateral esotropia, dysmorphic features, short stature (height <5th%), macrocephaly (head circumference >98th%) and developmental delay. The patient was born at 36 weeks gestation and initially required intubation. Medical history is also significant for gastroesophageal reflux, constipation, mild unilateral ankle talipes, a small ventricular septal defect and asthma. Initial karyotype analysis revealed an abnormal male karyotype with extra chromosomal material of unknown origin, attached to 5p15.3. Further characterization of the area using whole-genome oligonucleotide array CGH revealed a 6.0 Mb terminal deletion of chromosome bands 5p15.3 to 5p15.33, as well as an adjacent 24Mb duplication of chromosome bands 5p13.3 to 5p15.31. The co-occurrence of these two genomic imbalances suggests a U-type exchange mechanism. Parental karyotype yielded normal results. The terminal deletion in our patient involves a partial deletion of the cri-du-chat syndrome (CdCS) region (5p13-5p15), and encompasses only the specific critical region for the high-pitched cat-like cry (5p15.3). Our patient exhibits none of the other clinical features of CdCS, a condition that manifests with microcephaly, dysmorphic features, hypotonia, severe mental retardation, and the characteristic cat-like cry. Large duplications in the 5p region lead to a variable phenotype. A review of the literature suggests that duplications distal to 5p13.3 result in significantly fewer physical anomalies than duplications proximal to that region. Our case is novel in that the patient has several significant congenital, medical and developmental anomalies, despite having a duplication that is limited to the region distal to 5p13.3. Since our clinical findings cannot be explained solely by the 5p terminal deletion, we propose that the duplication distal to 5p13.3 can lead to a significant clinical phenotype.

998T

Case report: of a newborn with ambiguous genitalia and mosaicism for the SRY locus. *C. Bupp, J.H. Hersch, J.J. Wetherbee-Landis, C.M. Rajakaruna, K.M. Goodin.* University of Louisville, Louisville, KY.

Ambiguous genitalia in a newborn is a medically and psychosocially difficult finding with a broad differential diagnosis. Initial examination and laboratory results guide further investigations in order to find a diagnosis. We present a patient born with ambiguous genitalia including nonvisible vaginal opening, nonpalpable gonads, hypertrophic clitoris versus micropenis, posterior labial scrotal fusion, and patent anus. Initial pelvic ultrasound identified a uterus but could not identify gonads. Karyotype was 46,XX. Congenital adrenal hyperplasia (CAH) is the most common cause of ambiguous genitalia with normal female karyotype but our patient's 17-hydroxyprogesterone level was not markedly elevated. Less common causes of CAH were ruled out by additional laboratory tests and negative ACTH stimulation test. Antimullerian hormone (AMH) was found to be in the male range. Comparative genomic hybridization (CGH) and FISH for SRY found mosaicism of the SRY locus (3.8-26% positive). Diagnostic laparoscopy demonstrated right ovary and fallopian tube, left testicle with epididymis and nonconnecting vas deferens, and unilateral uterine remnant without functional uterus. A multidisciplinary team care conference was held with the family where it was decided to remove the testicle due to concerns about future malignancy. Pathologic evaluation demonstrated an ovotestis with 90% testicular tissue and 10% ovarian tissue. The patient is being raised as a female and monitored by the multidisciplinary team for further complications and management issues associated with disorders of sexual development (DSD).

999T

Mosaic dup/del 6q22.1q22.2 including COL10A1 in a patient with syndromic developmental delay and symptoms of osteogenesis imperfecta. *I. Filges[1], B. Roethlisberger[2], P. Dill[3], P. Weber[3], A.R. Huber[2], F. Wenzel[1], P. Miny[1].* 1) Division of Medical Genetics, University Children's Hospital and Department of Biomedicine, Basel, Switzerland; 2) Division of Medical Genetics, Center of Laboratory Medicine, Cantonal Hospital Aarau, Switzerland; 3) Division of Neuropediatrics and Developmental Medicine, University Children's Hospital, Basel, Switzerland.

Segmental aneuploidy plays a significant role in the origin of syndromic and non-syndromic developmental delay. Complex mosaic structural aberrations, however, are very rare. We report on a unique mosaic interstitial aberration in 6q22.1q22.2 including a duplication in one and a deletion in a second cell line, initially identified by a dosage aberration in array genomic hybridization (AGH) in a patient with dysmorphisms, mild developmental delay (DD) and an osteogenesis imperfecta (OI) phenotype. This 15 year-old patient with global DD shows turricephaly, sparse hair, dysplastic ears, long face, blue sclerae, crowded teeth, kyphosis, joint hyperlaxity, myopia, conductive hearing loss, frequent bone fractures in early childhood and a proximal fibular osteochondroma. High resolution AGH of DNA from blood lymphocates identified an increased copy number state (2.2) of 4.2 Mb in region 6q22.1q22.2 suggesting mosaicism. FISH studies with locus-specific probes revealed an interstitial duplication in about 2/3 of cells examined, and a deletion of the same region in about 1/3 of cells well explaining array dosage results. Parental array and FISH analyses were normal. The aberrant region contains about 25 genes including COL10A1 which is expressed by hypertrophic chondrocytes of the growth plate. Mutations in COL10A1 lead to Schmid type metaphyseal chondrodysplasia (SMCD). Nonsense mediated decay and dominant negative mechanisms have been proposed. Although AGH has shown its potential in the detection of low-level mosaicism, FISH studies are necessary to further elucidate its nature. The rare interstitial duplication-deletion mosaicism in our patient may result from a single postzygotic event by unequal sister chromatid exchange producing three cell lines, the normal one not detected or present in blood lymphocytes. Studies on cultured fibroblasts are ongoing. Genotype-phenotype correlations of the affected region using existing databases and publications reveal a few overlapping deletions including COL10A1 of patients with DD but no symptoms reminiscent of OI, SMCD or connective tissue disease. We could not identify patients with a duplication of the affected region for comparison. The deletion in our patient may explain the DD phenotype.We hypothesize that the cell line with the duplicated region contributes to the OI phenotype possibly by increased dosage effects of COL10A1 and/or impaired gene regulation. Further functional studies are needed.

1000T

Renal Tubular Dysgenesis in Two Siblings with Novel ACE Mutations. *A. Alfares[1], N. Braverman[1], C. Antignac[2], K.B. Brosnihan[3], V. Morinière[2], G. Gubler[2], R. Vanneste[1], R. Gosselin[1], C. Bernard[1], M. Bitzan[1].* 1) Medical Genetics, McGill University, Montreal, Quebec, Canada; 2) INSERM U574, Hôpital Necker Enfants-Malades, Paris, France; 3) Wake Forest University, The Hypertension and Vascular Research Center, Winston-Salem, NC, USA.

Renal tubular dysgenesis (RTD, OMIM #267430) is a rare, autosomal recessive developmental nephron abnormality characterized clinically by fetal anuria and oligohydramnios, and severe postnatal hypotension. It is causally linked to homozygous or compound heterozygous mutations in genes encoding components of the renin-angiotensin system (angiotensinogen [AGT], renin [REN], angiotensin-converting enzyme [ACE], and angiotensin II receptor type 1 [AGTR1]). Here, we report two siblings with novel mutations in ACE. Case 1: The first child of healthy, non-consanguineous parents was delivered by C-section at 33 weeks due to IUGR, severe oligohydramnios and decreased fetal movement. Growth parameters were all < 3rd %ile. She had persistent anuria and systemic, treatment-resistant hypotension. She died of necrotizing enterocolitis at age 14 days, with functioning dialysis. Post-mortem histology showed immature, overcrowded glomeruli, slightly distended microcystic cortical tubules and absence of definite proximal tubules, consistent with RTD. The colon demonstrated small calibre in its entire length. Serum ACE activity was undetectable (<3.8 nmol/mL/min; normal 18-55 nmol/mL/min), angiotensin I (Ang I) greatly increased (1765.6 pg/mL; N 40-80 pg/mL). Case 2: Prenatal ultrasound imaging of a subsequent child of the same parents at 12 and 24 weeks gestation was unremarkable, but showed severe oligohydramnios and IUGR at 28 weeks. Pre-term labour and fetal decelerations at 35 wks prompted C-section. There was profound hypoxia related to pulmonary hypoplasia and pulmonary hypertension that failed to respond to intensive treatment. She remained anuric until her death at 14h post partum. Post-mortem histology was similar to the first case. A novel, maternally inherited heterozygous nonsense mutation in the ACE gene (c.793C>T) was detected, predicting a premature stop codon (p.Arg265X). Exon array analysis with multiple oligonucleotide probes within the ACE gene identified a paternally inherited, novel heterozygous deletion of exons 14 & 15. The biochemical findings of immeasurable ACE activity and excessive Ang I concentration point to deleterious ACE gene mutations. The observed microcolon is a novel finding not previously described in patients with RTD. This report further demonstrates the utility of exon array technology for an autosomal recessive disorder where only a heterozygous mutation is identified by conventional sequencing.

1001T

Central choroidal areolar dystrophy type 2: genotype-phenotype correlation. *L. Gabriel[1, 2, 3], M. Avila[1, 3].* 1) Ophthalmology, UFG, Goiania, Goias, Brazil; 2) Dcode Laboratory, Goiania, Goias, Brazil; 3) CBCO, Goiania, Goias, Brazil.

Purpose: Herein we present a case of a 35-year-old brazilian patient with type 2 central choroidal areolar dystrophy caused by the previously described p.G208D mutation in exon 2 of the peripherin 2 (*PRPH2*) gene with a never before reported symptom of progressive intermitent diplopia for the last three years, besides refering central scotomas on both eyes. **Methods**: The patient was submitted to the ocular genetics clinics in Goiania and has undergone bidirectional DNA sequencing of all three coding exons and splice sites of *PRPH2* gene. **Summary of results**: The p.G208D mutation causes a missense alteration in a highly conserved region of the PRPH2 gene affecting an important domain (ROM1) of the peripherin 2 protein that, in this particular case, showed a new symptom to the natural history of type 2 central choroidal areolar dystrophy.

1002T

Novel de novo AVPR2 gene mutation causing CNDI in Swedish patient. *S.S. Joshi[1], J.H. Christensen[1, 2], P. Brandstrom[3], J. Knudsen[1], N. Gregersen[4], S. Rittig[1].* 1) Pediatric Research Lab-A, Aarhus University Hospital, Skejby, 8200, Aarhus N, Denmark; 2) Institute of Human Genetics, Aarhus University, Aarhus, Denmark; 3) Children's Clinic, Ryhov County Hospital, Jönköping, Sweden; 4) Research Unit for Molecular Medicine, Aarhus University Hospital, Skejby, Aarhus, Denmark.

Congenital Nephrogenic Diabetes Insipidus (CNDI) is a rare inherited disorder characterized by reduced ability to reabsorb water in response to the antidiuretic effect of vasopressin on renal collecting duct cells. Patients exhibit polyuria, nocturia, hypernatremic dehydration, failure to thrive, irritability, and fever. CNDI is genetically heterogeneous and may be inherited in an X-linked or autosomal recessive manner. The proband (7.5 years Swedish male), was identified to have CNDI at 1 month of age and diagnosed at 6 months of age during an episode of gastroenteritis. There was no family history of diabetes insipidus. We sequenced coding regions of arginine-vasopressin receptor-2 gene - AVPR2 gene in patient and 4 unaffected family members. We identified a novel de novo mutation (80bp duplication) in exon 2 of AVPR2 gene in the patient. Duplication begins from nucleotide 1312 (gDNA sequence) and consists of 80bp that are identical to nucleotide sequence from 1232-1311. This duplication leads to a frame shift and a stop codon- 4 codons downstream (AAWD 294-297>PRLX). There were no mutations in coding regions of AVPR2 gene, in other family members. In this case, clinical data including the water deprivation test and duplication mutation will aid in understanding the pathophysiology of CNDI. Identification of such mutations will facilitate in early diagnosis of CNDI, genetic counseling and early intervention aimed at reducing morbidity with reference to diagnostic testing.

1003T

Wide phenotypic variability of Kabuki syndrome with MLL2 mutations. *K. Kosaki[1], R. Tanaka[2], R. Kosaki[3], K. Uchida[4], C. Torii[2], T. Ishi[2], T. Sato[5], H. Yoshihashi[6].* 1) Center of Clinical Genetics, Keio University School of Medicine, Tokyo Japan; 2) Department of Pediatrics, Keio University School of Medicine, Tokyo Japan; 3) Department of Clinical Genetics and Molecular Medicine, National Children's Medical Center, Tokyo, Japan; 4) Department of Health and Nutrition, Tokyo Kasei Gakuin University & Junior College, Tokyo, Japan; 5) Department of Endocrinology and Metabolism, Kanagawa Children's Medical Center, Yokohama, Japan; 6) Departments of Clinical Genetics, Tokyo Metropolitan Children's Medical Center, Tokyo, Japan.

Kabuki syndrome is characterized by long palpebral fissures with eversion of the lateral aspect of the lower eyelids and developmental delay. Recently, MLL2 at chromosome 12q12-q14 has been identified as a causative gene of KS. To date, a broad range of complications has been reported in patients with KS. Yet, when “atypical complication” was present, the clinical diagnosis of KS may become less assuring prior to the identification of the causative gene. Here, we demonstrate a wide phenotypic variability among patients with Kabuki syndrome who have MLL2 mutations. The subjects were recruited from a group of patients with KS who were regularly followed at the authors' institutions at the time of study. A diagnosis of KS was made if they had: long palpebral fissures with eversion of the lateral portion of the lower eyelids, large, prominent or cupped ears; and developmental delay. The entire MLL2 coding region was screened for mutations by using the dideoxy sequencing method. Heterozyogous MLL2 mutations were identified in all of the patients: 6 frameshift mutations (4833 del G fs1721X, 6613-6614 insG fs2242X, 6620 del C fsX2263, 12201-12202 insC fs4106X, 12876-112885 del 10bp fs4380X, and 15529-15530 insCACA fs5199X), 3 nonsense mutations (E970X, Q2445X, and 5282X), and 2 missense mutations (G5189R and R5432W). No apparent genotype-phenotype correlations were noted among the 11 patients. All the patients demonstrate variable degrees of developmental delays including a 29 year-old male without speech ability and a 6 year-old boy with arithmetic ability. Two patients (R5432W and R5282X) had staphyloma of the eye with bulged anterior segments and structurally normal posterior segments. Appearance of the complication in two unrelated patients with different mutations in MLL2 suggests that Kabuki syndrome needs to be considered even in the presence of severe developmental defect of the eyes, such as staphyloma. One patient had a combination of rare complications: obstructive jaundice and severe persistent hypoglycemia due to hypopituitarism. In conclusion, we documented wide phenotypic variability among MLL mutation positive cases including staphyloma of the eyes, a previsouly undescribed servere ophthalmic complication. Such variability should be taken into consideration in the clinical evaluation of suspected Kabuki syndrome cases.

1004T

Trichohepatoenteric syndrome - phenotypic variability and molecular characterisation. *U.H. KOTECHA, R.D. PURI, S. MOVVA, I.C. VERMA.* CENTER OF MEDICAL GENETICS, SIR GANGARAM HOSPITAL, , NEW DELHI, INDIA.

We report two patients of Asian Indian origin, born to non consanguineous parents with clinical features of Trichohepatoenteric syndrome (THES). Both patients had onset of intractable diarrhea from 5-6 months of age, recurrent respiratory infections, growth retardation and developmental delay. Dysmorphic features in both cases were broad forehead, prominent cheeks with trichorrhexis nodosa.The first patient also had Café au lait spots distributed along the lower abdomen and thighs. Ventricular septal defect, atrial septal defect and patent foramen ovale as well as multiple hemangiomas in the liver which gradually regressed were present in the second case. Hepatic hemangiomas have not been observed in the earlier reported cases. Liver histology in case 2 showed chronic hepatitis with cirrhosis and copper accumulation. Other causes of chronic diarrhea were excluded in both patients. Duodenal biopsy showed a villous atrophy of the intestinal mucosa in both cases. Though both patients had a history of recurrent infections suggesting immunodeficiency the serum immunoglobulin levels were elevated in the first case and normal in the second as opposed to low concentrations found in all the previously described cases. The TTC37 gene on chromosome 5 is a large gene with 40 coding exons. In the publication where the molecular basis of THES was identified, four patients of South Asian origin (2 Indian and 2 Pakistani) were reported to harbor a homozygous mutation (c.2802G>A) in Exon 28.With this background we first analyzed our patients for this mutation by restriction enzyme analysis and sequencing of Exon 28 and identified the same mutation in both. Trichohepatoenteric syndrome is an autosomal recessive condition characterized by chronic diarrhea, immunodeficiency, facial dysmorphism, trichorrhexis nodosa and variable degrees of hepatic involvement. The two consistent features observed amongst all previously reported cases include chronic diarrhea and immunodeficiency characterized by low serum immunoglobulin levels. Our patients had normal and elevated levels of serum immunoglobulins as well as hepatic hemangiomas further defining the phenotypic heterogeneity in THES. The possibility of a founder mutation in South Asian population was suggested by Hartley et al, and is further reaffirmed by our findings.

1005T

Evaluation of three automated mutation detection software programs for clinical diagnostic sequencing. *J. Machado[1], N. Persaud[1], L. Han[1], M. Eliou[1], P. Ray[1,2], T. Stockley[1,3].* 1) Department of Paediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 3) Department of Laboratory Medicine and Pathobiology, Toronto, Ontario, Canada.

The Molecular Genetics laboratory at The Hospital for Sick Children analyzes more than 2000 specimens per year using genomic DNA sequence analysis. The increasing demand for genetic testing by DNA sequencing requires rapid, accurate detection of mutations. We evaluated three commercially available automated mutation detection software programs for performance in the commonly clinically sequenced cystic fibrosis conductance regulator (*CFTR*) gene.

Fifty specimens were sequenced for all 24 coding exons and intronic flanking regions of the *CFTR* gene. DNA sequence for each specimen was generated using fluorescent cycle sequencing reactions analyzed on an ABI™ 3730xl capillary sequencer. Each of the fifty specimens was analyzed by three mutation detection software programs: SeqScape™ v2.5, Mutation Surveyor™ v3.24 and SeqPilot™ v3.4.2. The sensitivity and specificity of the software programs were compared to 28 samples tested using the Luminex™ xTAG Cystic Fibrosis Assay.

SeqPilot™ allowed for easy sequence assembly, produced easy to read reports with proper nomenclature and was the most user friendly. SeqScape™ allowed the ability to lock the template, relatively easy sequence assembly, but did not provide variant information. Mutation Surveyor™ easily created templates, listed proper nomenclature, but didn't provide variant information and sequence assembly was difficult.

The SeqPilot™ software detected the least amount of false positives and no false negatives across the *CFTR* gene. Results from the Luminex recurrent mutation assay showed that Mutation Surveyor™ and SeqPilot™ correctly identified and excluded all mutations. SeqScape™ specificity was high, but the sensitivity was low.

Each of the three mutation detection software programs exhibited useful tools for the analysis of DNA sequence data. However SeqPilot™ contained the most favorable features for the specific requirements of mutation detection in a clinical laboratory setting, whereas SeqScape™ and Mutation Surveyor™ would be more appropriate for simple sequence detection analyses.

1006T

Cornelia de Lange syndrome: A five year follow-up and renal lithiasis. *M.A. Aceves-Aceves[1], I.M. Salazar-Dávalos[1], D. García-Cruz[2], A. Moreno-Andrade[3], M.G. González-Mercado[2,4], N.O. Dávalos[2], J.A. Cruz-Ramos[2,4], I.P. Dávalos[2,4].* 1) Facultad de Medicina, CUCS, Universidad de Guadalajara; 2) Instituto de Genética Humana, Doctorado en Genética Humana, CUCS, Universidad de Guadalajara; 3) Universidad Autónoma de Guadalajara; 4) División de Genética, CIBO, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México.

Introduction: Cornelia de Lange Syndrome (CDLS) is characterized by typical facies, including low anterior hairline, bushy eyebrows, synophrys, long eyelashes, low-set ears, anteverted nares, depressed nasal bridge, long philtrum, thin lips, cleft palate, mental retardation, upper limb anomalies and hypertrichosis. CDLS presents prenatal and postnatal growth retardation and shows clinical variability. CDLS1 can be caused by mutation in the NIPBL gene (608667) on chromosome 5p13.1, CDLS2, X-linked is caused by mutation in the SMC1A gene (300040) on chromosome X11.2-p11.21, CDLS3 syndrome is caused by mutation in the SMC3 gene (606062) on chromosome 10q25. Case report: The propositus, male, aged 5 years 1 month old,was the product of the 2nd pregnancy, from non-consanguineous and healthy parents, with 35 years (he) and 36 years (she) at this time, no relatives were similarly affected. He was obtained by caesarian section at 36 weeks due to pre-eclampsia. At birth his weight was of 2.100 kg (<pct3) and height of 47 cm (<pct3). Physical examination at two months persists with somatometric parameters <pct 3. Clinically: microcephaly, generalized hypertrichosis, synophrys, anteverted nares, single transverse palmar crease, clinodactyly of 5th finger, syndactyly of 2nd-3rd toes and bilateral cryptorchidism. Karyotype 46,XY. At one year old was detected left renal lithiasis and mild ectasia on the right one by renal ultrasound. The lithiasis was resolved spontaneously. A renal function as well as a cardiological evaluation were normal. At 3 years old, weight, height and OFC continue below the 3rd pct and psychomotor delay was evident (non language, non walking). At this time, renal tubular acidosis was excluded and orchidopexy was done, hypotrophy of the right testicle was reported. At 5 years old, weight of 10.6 k (<pct 3), height of 87 cm (<pct 3) and OFC of 43.5 cm (<pct 3). Radiological evaluation showed craniofacial disproportion, prominent occipital fossa, elongated sella turcica, plumbed anterior cranium, ovoid vertebral bodies, mild dorsal kyphosis, lumbar hyperlordosis, decrease interpedicular distance, slightly horizontal acetabular roofs, bilateral coxa valga and slightly closed sciatic notches. Renal ultrasound reported bilateral nephrocalcinosis. Conclusion: The aim of this work is to present the five year follow-up of a CDLS patient, with antecedent of renal lithiasis and nephrocalcinosis diagnosed by renal ultrasound.

1007T

Neu-Laxova syndrome (NLS) and congenital anomalies of the spectrum of this syndrome in a highly inbred family. Could these anomalies represent clinical manifestations in heterozygotes? *D. Cavalcanti[1], M.T. Sakata[1], F. Poletta[2], J. Lopez-Camelo[3,4], C. Rosenberg[5], E.E. Castilla[3].* 1) Medical Genetics, UNICAMP (FCM), Campinas, São Paulo, Brazil; 2) INAGEMP (Instituto Nacional de Genética Médica Populacional), Brazil; 3) ECLAMC (Estudo Colaborativo Latino-Americano de Malformações Congênitas), Lab. Epidemiologia Genética, Centro de Educação Médica e Investigações Clínicas, Buenos Aires, Argentina; 4) ECLAMC (Estudo Colaborativo Latino-Americano de Malformações Congênitas), Instituto Multidisciplinar de Biologia Celular, CEMIC, La Plata, Argentina; 5) Centro de Estudos do Genoma Humano, IB, USP.

The Neu-Laxova syndrome (NLS) is a lethal rare genetic disease described in 66 probands, with an excess of females (23♂ : 37♀). Of the 52 families with at least one affected, 23 referred parental consanguinity and recurrence occurred in 20, strongly suggesting recessive autosomal inheritance. From the clinical point of view this syndrome presents a great heterogeneity and is characterized by IUGR, microcephaly, ocular proptosis or ablepharon, structural anomalies of hands and feet, oedema and ichthyosis. The aim of this report is to present a large and highly inbred family living in a Brazilian rural region with around 2,000 inhabitants that seems a population isolate. In this family we found one proband with an unusual phenotype and several other individuals affected by apparently isolated congenital anomalies from the spectrum of NLS. The proband's parents are once removed cousins with the following obstetric history: GVIIPIVAIII, being GII a male infant that presented, hydrocephalus, congenital heart defect, club feet and neonatal death; GV was the proband; GVI and GVII normal children; GI, GIII e GIV were spontaneous abortions. The proband presented generalized oedema, ichthyosiform skin, craniorrachischisis and anomalies of hands and feet. Karyotype (46,XX) and CGH-a were both normal. In other family individuals we found four hydrocephalus, five spine bifid and six ichthyosis. All, except one case of spine bifid, had consanguineous parents. The only individual alive with spine bifid has also palpebral ptosis and ichthyosis. The full genealogy shows 809 individuals in seven generations with a mean inbreeding coefficient F=0.008. The simple segregation analysis included the AR inheritance model (p=16.9 IC=7.9-25.9). In conclusion, these data suggest the possibility of clinical manifestation in heterozygotes for this syndrome. Future studies could confirm this hypothesis and hopefully will lead to identify the molecular basis of the NLS. (Supported by: INAGEMP - Instituto Nacional de Genética Médica Populacional).

1008T

Genome-wide DNA Methylation Profiling of CpG Islands in Hypospadias. *S. Choudhry[1], K. Beckman[2], L. Qiao[1], H. Bengtsson[3], M. Segal[3], L. Baskin[1].* 1) Dept Urology, Univ California, San Francisco, CA; 2) Biomedical Genomics Center, Univ Minnesota, Minneapolis, MN; 3) Dept Epidemiology and Biostatistics, Univ California, San Francisco, CA.

Purpose: The etiology of hypospadias remains largely unknown despite intensive investigations. Since steroid hormones are involved in regulating fetal genitourinary development, a leading hypothesis has been to explore the role of endocrine disrupting chemicals (EDCs). EDCs found in the environment such as phthalates, bisphenol A, diethylstilbesterol and some pesticides have both estrogenic and anti-androgenic actions and can interfere with male genital development by competing with natural androgens for the ligand-binding domain of the androgen receptor. Recent studies have suggested that molecular mechanisms that underlie the effects of EDCs on the fetus may involve disruption of epigenetic programming of gene expression during development. We hypothesize that alteration in DNA methylation may contribute to development of hypospadias. **Methods:** To test our hypothesis we assessed whether epigenetic modification of DNA methylation is associated with hypospadias in a case-control study of 31 subjects. We performed genome-wide DNA methylation profiling in bisulphite converted genomic DNA obtained from prepuce tissue samples of 13 severe hypospadias and 19 control subjects using Illumina Infinium HumanMethylation27 BeadChip. Illumina methylation BeadChip enables the direct investigation of methylation status of 27,578 individual CpG sites throughout the genome, which are focused on the promoter regions of 14,495 genes. The methylation level at each CpG site was compared between cases and controls using t-test and linear regression after adjusting for potential confounders including age at the time of surgery and race/ethnicity. **Results:** We identified 10 CpG sites that were associated with hypospadias with a p-value <0.001. These CpG sites were either in or near SLC2A11, C3orf32, MPO, ITPKA, CXorf6, THY1, OSBPL5, MAGEB2, ABCD1 and COX11 genes. Notably, the CXorf6 also known as MAMLD1 gene, which is expressed in the testes during the critical period for sex development, has been previously shown to be mutated in patients with isolated hypospadias. Only one CpG site in the SLC2A11 gene remained statistically significant after correction for multiple testing (p = 7.04x10-06, pcorrected = 0.036). **Conclusion:** This is the first study to investigate hypospadias using a unique and novel epigenetic approach. Our findings suggest utility for the DNA methylation patterns in identifying new genes such as SLC2A11 that may be involved in the etiology of hypospadias.

1009T

A patient with marked hypoplasia of the thorax, split-feet, polysyndactyly, renal defect and malrotation of intestine. *K. Haraguchi[1,2], M. Doi[1], H. Kuniba[1], M. Sasaki[1], T. Kosaka[3], K. Mochizuki[3], T. Tokunaga[3], M. Obatake[3], A. Yamashita[1], A. Yanai[1], H. Motomura[1], H. Moriuchi[1].* 1) Department of Pediatrics, Nagasaki University Hospital, Nagasaki, Japan; 2) Department of Pediatrics, Sasebo Municipal General Hospital., Nagasaki, Japan; 3) Division of Pediatric Surgery, Department of Surgery, Nagasaki University Hospital, Nagasaki, Japan.

We report on a female infant presenting a previously undescribed combination of marked hypoplasia of the thorax, split-feet, polysyndactyly, renal defect, malrotation of the intestine, and segmentation defects of the lumbar and sacral vertebrae. A unique feature in the proband was severe deformity of the thorax. To the best of our knowledge, the combination of those defects has never been reported. She was born at 35 weeks of gestation by cesarean section, weighing 2128 g. Her mother had histories of syndactyly, bicornuate uterus and duplication of the intestine. Chest computed tomography with 3D reconstruction demonstrated marked thoracic deformity without significant anomalies of the respiratory tract. Intestinal malrotation and renal defects with hypoplasia on the right and ectopia on the left were also found. Her facial appearance was normal. She survived for five months by intensive care such as high frequency ventilation or high frequency oscillation, but died from sudden deterioration of respiratory condition. Differential diagnosis includes spondylothoracic dysostosis (Lavy-Moseley syndrome) (MIM 608681), acrorenal syndrome (MIM 102520), split-foot deformity with mandibulofacial dysostosis (MIM 183700), split-hand with obstructive uropathy, spina bifida and diaphragmatic defects (MIM 183802), spondylocostal dysostosis 5 or costovertebral segmentation anomalies (MIM 122600), acrorenal-mandibular syndrome (MIM 200980) and short-rib polydactyly syndrome 2 (MIM 263520). All of them are differentiated from the present case, based on the constellation of abnormalities. Chromosomal analysis showed normal karyotype: 46,XX. Molecular karyotyping was performed with array CGH of Agilent 1M platform to the proband-parents trio. We found a 24-kb deletion in 9q21.33 containing C9orf153 and a 123-kb duplication in 10q25.3 containing C10orf118, TDRD1 and VWA2 in the proband and the mother, which have not been registered as copy number variations. Combined with the previously undescribed combination of multiple anomalies, those findings indicate a new clinical entity. Interestingly, a de novo 28-kb microdeletion of 17p13.2, encompassing GPR172B, was also suspected using Agilent Genomic Workbench software. Although function of GPR172B (G protein-coupled receptor 172B) has not been well identified in mammals, our finding may suggest its role in thoracic formation.

1010T

16p13.11p12.3 Duplication in a Girl with Multiple Congenital Anomalies and Developmental Delay. *E. Leon, S. Lewin.* Medical Genetics, University of Utah, Salt Lake City, UT.

Chromosome 16 is rich in intrachromosomal segmental duplications or low copy repeats (LCRs) that mediate recurrent genomic rearrangements. Recurrent deletions and reciprocal duplications in 16p13.11 have been previously reported. Duplications have been implicated in autism spectrum disorders, intellectual disability, and schizophrenia. However, the phenotypes associated with CNVs of 16p13.11 are not consistent, and both deletions and duplications of the region have been observed in “phenotypically normal” individuals. We describe a 1-year-old girl with a 2.5Mb duplication in the 16p13.11p12.3 region detected by 2.7 Affimetrix SNP array confirmed by FISH. The proposita was born at term, by emergent C-Section due to placenta abruption, to nonconsanguineous Caucasian young parents. Her prenatal history was remarkable for preeclampsia. Family history is unremarkable. Her birth weight and length were in the 50th centile. She had feeding difficulties and GI reflux during infancy, as well as recurrent URIs and episodes of otitis media. At 11 months of age she had a 1st toe tendon release surgery. At her initial evaluation (1 year) her weight was in the 5th centile, length 50th centile, OFC 75th centile, and foot length < 3rd centile. She has left eyelid ptosis, epichantal folds, bulbous nasal tip, low set and posteriorly rotated ears, high arched/narrow palate, tapered fingers with dysplastic fingernails, severe bilateral 5th finger clindodactyly, and short thumbs. She also has wide sandal gaps, medially deviated and short first toes, hypoplastic toenails, right 2/3 toe syndactyly, left 2nd toe clinodactyly with 3/2 overlapping. She has hypotonia, speech and motor delays. Feet X-rays showed shortening of the 2nd and 3rd metatarsals. Echocardiogram and renal ultrasound were normal. FISH for the 16p13.11 duplication was negative on mother's patient. Paternal FISH analysis has not been performed yet. Reported patients with duplication of 16p13.11 have variable presentations including skeletal features with craniosynostosis, polydactyly, and joint hypermobility; cardiac malformations; cognitive impairment; and behavioral abnormalities. As far as we know, the striking hand and feet anomalies of this patient have not been previously reported in the literature. Description of this case will further the genotype-phenotype information available for 16p13.11 duplication.

1011T

Sirenomelia: A Tale of Four South African Mermaids. *S. Moosa[1,2], A. Krause[1,2].* 1) Division of Human Genetics, National Health Laboratory Service, Braamfontein, Johannesburg, Gauteng, South Africa; 2) School of Pathology, University of the Witwatersrand, Johannesburg, South Africa.

Background: Sirenomelia is a rare, serious congenital anomaly characterized by variable degrees of fusion of the lower limbs. The reported incidence is between 1.1 and 4.2 per 100 000 births, with a male to female ratio of 3:1. It is also known as the mermaid syndrome/malformation, derived from the physical similarity to the sirens of Greek and Roman mythology. Sirenomelia rarely occurs as an isolated anomaly and affected individuals exhibit a range of severe associated malformations, mainly involving the lower vertebral, pelvic and genito-urinary systems. Objective: To describe the largest series of African patients with sirenomelia and to highlight rarer associated anomalies. Design/Methods: Four patients with sirenomelia were seen at the Division of Human Genetics, National Health Laboratory Service and the University of the Witwatersrand, since 2005. Data collected from patient files included: maternal histories, antenatal findings, birth histories, clinical examinations, clinical photographs, X-rays and other test results. We present a detailed comparison of these patients based on clinical and X-ray findings and the results of genetic testing and post-mortem investigation, where applicable. Results: 4/4 patients were born to young, Black South African women. None of the mothers reported a history of maternal diabetes or any significant teratogen exposure. None of our patients survived beyond 2 days of age, and all had different associated abnormalities. 4/4 had a single umbilical artery. Of the 3/4 patients who had upper limb involvement, one patient presented with severe left-sided phocomelia, oligodactyly, radial and ulna ray defects and a rudimentary humerus. 4/4 had features of VACTERL association and 3/4 had X-ray features suggestive of the caudal dysgenesis syndrome (CD). Conclusions: No two patients with sirenomelia are alike, highlighting the variability of the condition. Although 4/4 displayed the characteristic lower limb fusion, they displayed different associated malformations of the upper limb, gastro-intestinal and skeletal systems. The phenotypic overlap between sirenomelia, CD and VACTERL, as seen in our patients, supports the hypothesis that these three entities are possibly part of the same pathogenic spectrum.

1012T

Natural history study of Arthrogryposis Multiplex Congenita, Amyoplasia type. *T. Nichols[1], S.S. Hashmi[1], P. Mancias[1], M. Raia[1], G. Gogola[2], J.T. Hecht[1].* 1) The University of Texas Health Science Center, Houston, TX; 2) Shriners Hospitals for Children, Houston, TX.

Arthrogryposis or Arthrogryposis Multiplex Congenita (AMC) are terms used to describe the clinical finding of multiple congenital contractures. There are more than 300 distinct disorders associated with arthrogryposis. Amyoplasia is the most common type of arthrogryposis and is often referred to as the “classic” type. There is no known cause of amyoplasia and no risk factors have been identified. Moreover, there is no established diagnostic criterion, which has led to inconsistency and confusion in the medical literature. The purpose of this study was to describe the natural history of amyoplasia, to determine if there are any identifiable risk factors and develop a list of diagnostic criteria. A retrospective chart review of 59 children with multiple contractures, ascertained at the Shriners Hospitals for Children in Houston, Texas, was performed. In addition, information about prenatal, birth and family histories was obtained from a questionnaire completed in the clinic by a parent/guardian. Analysis was performed using STATA v10.0 to generate frequencies, means and medians. Of the 59 children, 44 were found to have amyoplasia by the diagnostic criteria described below; 15 children had multiple congenital contractures (MCC) only and were used as a comparison group. For the amyoplasia group, with the exception of abnormal amniotic fluid levels during pregnancy, there were no significant demographic or prenatal risk factors identified. However, other common features that discriminate amyoplasia from other types of contracture syndromes include: internally rotated shoulders, extended elbows, and flexed wrists without joint pterygium, round face with one or more midline facial hemangiomas, and normal mouth. These features have been incorporated into an amyoplasia checklist that can be used as diagnostic criteria for discriminating amyoplasia from other isolated and multiple contracture conditions.

1013T

A novel mutation in GPC3 gene in a Patient with Simpson-Golabi-Behmel Syndrome (SGBS). *E. Nishi[1], S. Mizuno[1], T. Yamamoto[2].* 1) Dept Pediatrics, Central Hosp, Aichi Human Service Ctr, Kasugai, Aichi, Japan; 2) Tokyo Women's Medical University Institute for Integrated Medical Sciences, Tokyo, Japan.

Simpson-Golabi-Behmel Syndrome (SGBS MIM;312870) is es syndrome characterized by prenatal and postnatal over growth, distinctive craniofacies, and mental retardation. The craniofacial features are macrocephaly, hypertelorism, macrosomia, macroglossia, and palatal abnormalities. The mental retardation is commonly mild to severe, with or without brain anomalies. The diagnosis of SGBS is based on clinical findings, family history consistent with X-linked inheritance, and molecular biological testing of glypican-3 (GPC3;300037), which maps to chromosome Xq26. We report a novel mutation of the GPC3 gene in a boy who was born as an extremely-low birth weight baby at 26 gestational weeks and showed prenatal and postnatal overgrowth, hypothyroidism, pervasive developmental disorder. He was seven-year-old Japanese boy with nonconsanguineous parents. He was born at 26 weeks and 2 days by emergency Caesarean section because of fetal distress. His birth weight was 976g (>90th centile), length 37cm(75th-90th centile), head circumference 24.5cm(50th centile), and the Apgar scores were 1 and 3 at 1 and 5 min, respectively. He was large for his gestational age and presented a neonatal hypotonia. He needed tracheal intubation for 8 days and phototherapy for 7days. Also He had difficulties of sucking, so he needed tube feeding. He had a global developmental retardation. At the age of 1 year, because of macrosomia and macroglossia, he had been once diagnosed beckwith-wiedermann syndrome clinically. He had hypothyroidism , febrile convulsions, ventricular septal defect , sleep apnea syndrome by enlarged tonsil needed removing, repeated otitis media with effusion , pervasive developmental disorder diagnosed at the age of 4 year and 9 months old. At the age of five, his height was 114cm (>90th centile), his weight was 23kg (>90th centile), and his head ciecumference was 52.5cm(50th-75th centile), SGBS was suspected because of the assosiation of prenatal and postnatal overgrowth, severe mental retardation, the typical dysmorphic features of face. The moleculae analysis of the GPC3 gene detected a non-reported 2-bp insertion (c.91_92ins GC) in exon 1. He was diagnosed SGBS . This mutation is de novo because it was not revealed in the molecular analysis of the mother.

1014T

Particular clinical, psychologic and behavioral features identified in Prader Willi syndrome. *C. Rusu[1, 2], E. Braha[1, 2], M. Volosciuc[2], I. Ivanov[3], M. Gramescu[3], V. Gorduza[1, 3], M. Puiu[4], D. Dan[5].* 1) “Gr T Popa” University of Medicine and Pharmacy, Medical Genetics Department, Iasi, Romania; 2) “Sfanta Maria” Children's Hospital, Medical Genetics Center, Iasi, Romania; 3) Immunology and Genetics Laboratory, Specialty Outpatient Unit of “Sfantul Spiridon”Hospital, Iasi, Romania; 4) “Victor Babes” University of Medicine and Pharmacy, Medical Genetics Department, Timisoara, Romania; 5) Romanian Prader Willi Syndrome Association.

Prader-Willi syndrome (PWS) is a relatively common disorder due to abnormalities in the 15q11.2-q13 region. Major manifestations include hypotonia with poor suck and poor weight gain in infancy, early childhood-onset hyperphagia and obesity, characteristic appearance, hypogonadism, growth hormone insufficiency causing short stature, developmental delay and characteristic behavior. We present 8 cases with PWS recorded in the files of Iasi Medical Genetics Center in order to illustrate some special features and to discuss the diagnosis and management strategy. Clinical diagnosis was based on actualized diagnostic criteria and confirmed by genetic tests (karyotype, MLPA, FISH, methylation test). Frequency of characteristic clinical features was analyzed (globally and separately for different types of genetic defects, compared to literature data), as well as particularities recorded. The most common particular features were: multiple and severe allergies, skeletal abnormalities (tall stature, short metacarpals, marked genu var), seizures and brain MRI abnormalities, as well as behavior particularities (no food seeking with relatively normal weight, preference for computer games instead of puzzle etc). Most of the cases have been followed for a long time and the changing of the phenotype in time is presented. In conclusion, we present particular cases of PWS aiming to illustrate special features and to discuss diagnosis and management strategy.

1015T

Unusual multiple congenital anomalies in a newborn boy of complex etiology: Mosaic 45,XY/45,X, duplication Xp22.33 involving *SHOX*, duplication 15q11.2 and 18q22.2-22.3, and history of maternal liver failure due to paracetamol, illicit drugs and alcoholism abuse. *S. Sampath[1], J.J. Gershanik[2], Y. Lacassie[3].* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Neonatal Medical Group, New Orleans, LA; 3) Department of Pediatrics Louisiana State University Health Sciences Center and Children's Hospital of New Orleans, LA.

We present a NB boy with multiple congenital anomalies (MCA), born to a 37 yo AA father and a 35 yo G4P3 Caucasian mother with history of alcoholism since she was a teenager. The mother had two normal girls from different unions, and a normal 2-year-old boy with the father of the proband. Early in pregnancy mom was admitted to ICU for liver failure secondary to paracetamol abuse. She also reported alcohol and drugs abuse. US detected abnormal spinal cord ending in the lumbar area with only half the coccyx/pelvis being visible, abnormal leg crossing extending in opposite directions and two vessels cord. Amniocentesis showed a mosaic 46,XY[95%]/45,X[5%]. Because of IUGR and breech presentation, the proband was delivered at 38 weeks via C/S. BW was 2,500 g., Apgar score 9-9. Main findings included: very short neck, humeri and femurs, opisthotonic positioning, high-pitched cry, some facial dysmorphism, ASD and VSD, a large ventral abdominal hernia, 11 ribs and hypoplastic defective vertebrae with low spinal tethered cord, sacral agenesis, CALS over right hip, large penis and undescended testes with rugous scrotum, hip flexed and immobile with hip subluxation, and delayed tibial ossification. Brain anomalies included agenesis corpus callosum and wider intrahemispheric sulci with possible leptomeningeal heterotopia, and cerebellar atropy. Very large gallbladder and large renal calyces were also observed. Since MCA were not explained by the sex chromosomal mosaicism, aCGH on chord blood was performed. aCGH revealed duplication at three loci: 1) Xp22.33, spanning 86 kb including the entire SHOX gene, 2) 15q11.2, spanning 1 Mb that includes 9 RefSeq genes, and 3) 18q22.2-22.3, spanning 1.9 Mb containing one hypothetical transcript. As mutations in *SHOX* including copy number changes have been reported in idiopathic short-stature and Léri-Weill Dyschondrosteosis and *SHOX* is a transcription factor involved during early embryonic development to control the formation of many body structures, especially important in the development of the skeleton and particularly in the growth and maturation of bones in arms and legs, we suggest the skeletal anomalies are probably due to the Xp22.33 duplication. It is not clear however the origin of other anomalies, but a role for duplication involving many genes at 15q11.2 and 18q22.2-22.3 or their interaction effect cannot be discounted. Further studies in the proband and both parents are currently in progress.

1016T

“Holoprosencephaly-polydactyly” (pseudotrisomy 13) syndrome: a new case report. *C. Sergi[1], J. Gekas[2], D. Kamnasaran[3].* 1) Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Canada; 2) Human Genetics Research Unit, Centre de recherche de l'hôpital Saint-François d'Assise, Québec, Canada; 3) Department of Pediatrics, Laval University, Québec, Canada.

Background: The etiology of disorders during the development of the prosencephalon is still vastly unknown, although five genes have already been identified, including *SHH*, *SIX3*, *TGIF*, *ZIC2* and *GLI3*, with mutations in subjects with holoprosencephaly or polydactyly. Holoprosencephaly has been described in several genetic syndromes and it has been suggested that “Holoprosencephaly-Polydactyly” or pseudotrisomy 13 may represent a particular subset of prosencephalic developmental disorders. In this study, we report a new case of a fetus with holoprosencephaly-polydactyly syndrome. **Methods and Results:** The proband was the fourth child born from non-consanguineous parents. During the pregnancy, the mother smoked, but was otherwise healthy with no infections and sickness or even medication/drugs used. The family histories were unremarkable. Transvaginal ultrasonography was performed at 20.4 gestational weeks, which led to the prenatal diagnosis of an abnormal fetus, and the decision to terminate the pregnancy at 23 gestational weeks. Autopsy confirmed the diagnosis of a female fetus having alobar holoprosencephaly with an absence of midline structures of the brain and a dilated single ventricle, absent olfactory bulbs and hypoplasia of the optic chiasm. The proband also showed classic facial features of hypotelorism, fused palpebral fissures and cebocephaly. No other extra-cranial findings were noted except for unilateral hexapolydactyly of preaxial type (right thumb) and septal agenesis of the left lung. The parent's and proband's karyotypes were normal. Molecular genetic testing were undertaken by sequencing candidate holoprosencephalic genes (*SHH, SIX3, TGIF, ZIC2*), and a candidate for polydactyly (*GLI3*). No apparent mutations were identified. **Conclusion:** We propose that the abnormalities of our fetus support the demarcation of this syndrome as an autonomous phenotype. Specific diagnostic criteria for holoprosencephaly-polydactyly syndrome need to be complemented by the absence of mutations in the major holoprosencephaly genes.

1017T

Inherited subtelomeric 14qdel10pdup and 14qdup10pdel in mentally retarded family members with different phenotypes, revealed reciprocal 14q10p translocation in their fathers. *V. Adir, E. Shahak, J. Levitatz, N. Ekhilevitch, H. Bar-El, Z.U. Borochowitz.* Simon Winter Institute for Human Genetics, Bnai-Zion Medical Center, Technion Institute of Technology, Rappaport Faculty of Medicine, Haifa, Israel P.O. Box 4940, Haifa, 31048, Israel.

Subtelomeric aberrations are responsible for 5% to 10% of unexplained cases of mental retardation. Subtelomeric regions are rich in genes and any change from the normal amount of the genes can cause a pathological phenotype. Each chromosomal site that is unbalanced can cause a specific phenotype, and deletion of genes will cause a different phenotype than duplication of the same genes. In most reported cases of subtelomeric imbalances, the exact cause and effect have not been well described. Here we describe a large family with two distinct mental retardation and malformation phenotypes, which correlate with their two genotype groups. These two genotypes were the outcome of the same genetic founder event, translocation of 14qter with 10pter. Screening the effected children in this family for subtelomeric microdeletions with MLPA P070 and P036 sets of probes revealed two groups of genotypes , group A had a deletion in 14qter and duplication in 10pter, while group B had a duplication in 14qter and deletion in 10pter. Testing their parents and healthy siblings with MLPA showed normal results. However FISH testing revealed balanced translocation involved 10pter and 14qter in one of the fathers. The patients in group A have more severe phenotype with distinct features of malformation, while group B have a milder pathology.

1018T

High frequency of Copy Number Variants (CNVs) in the chromosome 11p15.5 region in patients with Beckwith-Wiedemann syndrome. *B. Baskin[1], S. Choufani[2], C. Shuman[2,3], N. Parkinson[4], E. Lemyre[5], P.N. Ray[4], R. Weksberg[2,3].* 1) Clinical Genetics, Uppsala University Hospital, Uppsala, Sweden; 2) Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Canada; 3) Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, Canada; 4) Division of Molecular Genetics, The Hospital for Sick Children, Toronto, Canada; 5) Medical Genetics Service, Department of Pediatrics, CHU Sainte-Justine, Montreal, Canada.

Beckwith-Wiedemann syndrome (BWS) is an etiologically heterogeneous overgrowth disorder that results from dysregulation of imprinted growth regulatory genes on chromosome 11p15.5. This condition is characterized by macrosomia, exomphalmos, and macroglossia as well as hemihyperplasia and embryonal tumours. The majority of BWS cases (85%) are sporadic and result from loss of methylation at imprinting center 2 (IC2), gain of methylation at imprinting center 1 (IC1) or paternal uniparental disomy (UPD) involving both imprinting centers. Inherited forms of BWS are mainly due to mutations in CDKN1C but there are an increasing number of reports of patients with CNVs in the 11p15.5 region, some presenting with a positive family history. In order to better define the frequency of such CNVs, we analyzed the data for 435 unrelated patients clinically suspected to have BWS and referred to the molecular diagnostic laboratory for clinical testing by MS-MLPA. In total, molecular alterations were detected in 168 (39%) patients of which 60% showed loss of methylation at IC2, 9% showed gain of methylation at IC1, 22% showed paternal UPD and 9% had CNVs of the chromosome 11p15.5 region. Five out of 14 index cases with CNVs were linked to maternally transmitted deletions and?all were found to have affected siblings. Our results demonstrate that CNVs of chromosome 11p15.5 occur at a frequency of 9% in BWS individuals with positive molecular findings and represent an important contribution to the familial recurrence risk associated with this disorder. These data emphasize the importance of performing deletion/duplication testing in addition to methylation analysis for BWS given the potential impact on genetic counseling for these families.

1019T

Analysis of CNVs of the BP1-BP2 region (15q11.2) suggests mild pathogenicity in autism families. *N. Kommu[1], S. Sanders[2], E. Kaminsky[3], P. Stankiewicz[1], C. Martin[3], A. Patel[4], J. Wiszniewska[4], A. Beaudet[1].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, BCM MS:225, Houston, TX 77030; 2) Department of Genetics, Center for Human Genetics and Genomics and Program on Neurogenetics, Yale University School of Medicine, New Haven, Connecticut 06510, USA; 3) Emory University School of Medicine Department of Human Genetics Whitehead Building Room 315 615 Michael Street Atlanta GA 30322; 4) Medical Genetics Laboratories Baylor College of Medicine One Baylor Plaza, MS NAB 2015 Houston, TX, 77030, U.S.A.

Reports on the involvement of CNVs of the BP1-BP2 region in schizophrenia, bipolar and epilepsy disorders are conflicting. Further, there is no adequate information about its role in autism families.We studied copy number of the BP1-BP2 region in 1184 Simons Simplex Collection (SSC) families, 1911 NIMH controls and 28,473 cases, mostly with developmental delay referred to the Molecular Genetics Laboratories (MGL). These data were compared to CNV data from ~7700 controls recently reported by other groups.Among the SSC families, there were seven deletions of which five were in the transmitted chromosomes, one in the non-transmitted chromosomes and one de novo event. These families also have 15 duplications: one de novo, six in transmitted chromosomes and eight in the non-transmitted chromosomes. Nine deletions and four duplications were found in 1911 NIMH controls. Among the clinical samples referred to MGL, 153 were deletions (1 in 186) and 90 duplications (1 in 309). About half of these cases have neurological symptoms. Among the 7700 previously reported controls, there were 16 deletions (1 in 481) and 25 duplications (1 in 308).No significant differences in the frequencies of CNVs were observed between non-transmitted and transmitted chromosomes of the SSC parents. Nor there are differences among the autism patients, their unaffected siblings and the controls. The frequency of deletions among samples referred for clinical diagnosis is significantly higher than the controls studied here. Taken together, our results suggest that BP1-BP2 deletions may confer risk in developing a group conditions that include developmental delays and different types of neurological disorders but have low penetrance. Funded by SFARI.

1020T

Copy Number Variants in Cerebral Palsy. *G. McMichael[1], A. Moreno-De-Luca[2], A. MacLennan[1], J. Gecz[3], S. Girirajan[4], E. Eichler[4], C. Martin[2].* 1) Obstetrics & Gynaecology, Universtiy of Adelaide, Adelaide, South Australia, Australia; 2) Human Genetics, Emory University School of Medicine, Atlanta, Georgia, USA; 3) Genetics and Molecular Pathology, SA Pathology at the Women's and Children's Hospital, Adelaide, South Australia, Australia; 4) Genome Sciences, University of Washington School of Medicine, Seattle, Washington, USA.

Cerebral palsy (CP) describes a group of permanent disorders of the development of movement and posture that are attributed to non-progressive disturbances occurring in the developing fetal or infant brain. We hypothesise that CP is caused by many diverse and individually rare genetic abnormalities, including copy number variants (CNVs). To explore our hypothesis that CNVs contribute to the aetiology of CP, we initially tested 50 DNA samples from individuals with CP on a custom-designed 180K chromosomal microarray with targeted plus whole genome coverage. The targeted coverage includes known clinically relevant regions such as microdeletion/duplication syndromes, telomeres and centromeres at a resolution of ~20-50 kb plus exon-level coverage of >1200 genes involved in neurodevelopmental disorders. The whole-genome backbone results in a resolution in unique DNA of ~225kb. Three cases were identified with a CNV that included interesting candidate genes for the CP phenotype; *CTNND2* (446 kb duplication including the first exon), *MCPH1* (219 kb duplication including exons 1-8) and *COPS3* (4 kb deletion including exons 6-8). All three CNVs were shown to be inherited from an unaffected parent. These CNVs may show variable expressivity, reduced penetrance, possible imprinting or the presence of a point mutation on the other allele in affected children, not identified by the CNV profiling. Several other CNV regions were identified including 22q11.22, 15q11.2 (BP1-BP2) and 12p12.1p12.2, as well as single-gene CNVs across *CNTNAP3*, *MC2R*, and *FCSB* and intragenic CNVs in *DLGAP2*, *PARK2, NBEA*, *PAK2*, *MACROD2*, *CNTN1*, *MPV17L* and *NF1*. These same samples were also separately assessed on a 135K custom designed array with targeted coverage of ~50kb in all genomic hotspots and backbone coverage of 350kb. Results confirmed CNV regions for *CTNND2*, *FCSB* and 12p12.1-p12.2. CNV regions were also identified involving Histone cluster genes, Olfactory receptors and 7p21 region as well as single-gene CNVs for *PTCHD3*, *NPHP1*and *TARP* and intragenic CNVs in *NCOR2*, *SH3GL3* and *C16orf62*. Parental samples need to be tested to determine if these imbalances are *de novo* or inherited, which will aid in establishing their significance. The pathogenicity of these rare CNVs is not currently resolved but these preliminary studies into the contribution of CNVs to CP justify further evaluation in a larger cohort which is currently underway.

1021T

Microdeletion 22q11.2 in a patient with Goldenhar Syndrome. *S.F. Oliveira[1], P.A.C. Santos[1], E.L. Freitras[2], H.P.N. Safatle[2], C. Rosenberg[2], I. Ferrari[1], J.F. Mazzeu[1].* 1) Genetica e Morfologia, Universidade de Brasilia, Brasilia, Brasilia (Distrito Federal, Brazil; 2) Depto de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo.

Goldenhar syndrome, also known as oculo-auriculo-vertebral syndrome, is a congenital defect from anomalous development of first and second branchial arches. It is heterogenous and its etiology includes unkwown genetic factors, different chromosome aberrations and environmental factors, like maternal vasoactive medication, thalidomide and maternal diabetes. We report a patient with clinical signs of Goldenhar syndrome and Di-George syndrome. We identified a 2,493,883 bp deletion at genomic position 17,276,772-19,770,655 (hg18) in the 22q11.21 region by array-CGH. Deletions of 22q11 have been described in patients with Di-George syndrome and Goldenhar syndrome, however the deletion here described is more proximal and do not overlap with the previously reported deletions associated with Goldenhar syndrome. Several genes mapped to this segment are involved in the embryogenesis of the branchial arches, namely TBX1, required for auricular development, and GSC2 that is related to GSC previously suggested as a candidate gene for Goldenhar syndrome. Therefore TBX1 and GSC2 are also candidates for Goldenhar syndrome.

1022T

Suspected gonadal mosaicism as a cause of Gorlin syndrome. *E. Stattin, P. Lundberg, I. Golovleva.* Clinical Genetics, Umeå University Hospital, SE 901 85, Umeå, Sweden.

Background: Autosomal dominant basal cell nevus syndrome (BCNS; Gorlin syndrome, MIM 109400) is caused by mutations in the human homolog of the Drosophila patched gene-1 (PTCH1) at 9q22.3. The mutation spectra are represented by point mutations (missence, nonsense and those affecting splice sites) and interstitial deletions at 9q22.3. At the same time interstitial 9q22.3 deletion is proposed as a newly recognised overgrowth syndrome. Tumors such as medulloblastoma, rhabdomyomas cardiac and ovarian fibromas, lymphoma and meningioma have been associated with GS. The risk of other malignant tumors is not increased. Delayed development is described but not mental retardation. Objectives: We describe siblings diagnosed with Gorlin syndrome (GS) clinically, verified molecularly and further analysed by SNP array using Illumina's 610-Quad BeadChips. The two boys were children of healthy non-consanguineous parents. Both of the boys fulfilled diagnostic criterion for the diagnosis GS with multiple jaw keratocysts and basal cell carcinomas. But both of them also have mental retardation. The phenotype could not only be explained by GS and therefore further analysis was performed. Methods and results: Conventional cytogenetic analysis by GTG banding on peripheral blood lymphocytes revealed normal karyotypes in both patients however deletion of entire PTCH1 gene was demonstrated by MLPA. To establish if the boys have a larger deletion, we performed SNP array which demonstrated presence of interstitial deletion at 9q22.3. The region covered by the deletion was 7.92Mb (CRCh37/hg19 97700731-105685265) and contained 73 genes, including the PTCH1 and TGFBR1 genes. The deletion was verified by specific probes RP11-435O5 (9q22.32) and RP11-75J9, RP11-185E13, RP11-438P9 (9q31.1) by FISH analysis. FISH with the same probes on parent's blood lymphocytes excluded presence of balanced translocation of 9q22.3 locus and array results did not reveal any aberrations at 9q22.3. Conclusions: This is the first report on possible gonadal mosaicism causing Gorlin syndrome in two siblings.

1023T

Angelman syndrome case report with hypothyroidism. *C.E. Monterrubio Ledezma[1], L. Bobadilla Morales[1,2], L.J. Rodríguez Casillas[1], H.J. Pimentel Gutiérrez[1], J.R. Corona Rivera[1,2], A. Corona Rivera[1,2].* 1) Biología Molecular y Genómica, Lab. Citogenética Genotoxicidad y Biomonitoreo “Dr. ECR”. CUCS, UdeG., Guadalajara, Jalisco , Mexico; 2) Unidad de citogenética, Servicio de Hematología Oncología Pediátrica, División de Pediatría, Hospital Civil “Dr Juan I Menchaca”.

Introduction. Angelman syndrome (AS) is a neurogenetic disorder with UB3A gene alterations in maternal imprinting (15q11-13). It is characterized by mental retardation, epilepsy, language deficit, facial dysmorphism and characteristic behavioral phenotype. It has an estimated frequency of 1:10,000 to 1:40,000. Objective. To report a new AS case associated with hypothyroidism without goiter. Clinical report. Female nine and a half years old, product of the second pregnancy, threatened abortion presented, delivery was uncomplicated. At birth showed hypertonia, natal teeth and gums cysts, psychomotor development and language retardation; febrile seizures and smiles to any stimulus. Congenital hypothyroidism was discarded. Hypothyroidism is diagnosed from 3 years old (T4 1.03 μg/dL, TSH 7.68 mUI/dL) and medicated whiht levothyronine. At physical examination she showed, weight 33 kg (PC 75), height 1.3 m (Pc 50), head circumference 50 cm (Pc 25); brachycephaly, narrow forehead, round face, almoud eyes, epicanthus, anteverted nostrils, thin lips, short neck, scoliosis, clinodactyly, blue and brown spot, ataxic gait and mirror movements. Paraclinical: electroencephalogram with hypervoltage slow waves; computerized axial tomography, cortico-subcortical atrophy; renal sonogram, ectopic and hypoplastic right kidney. Karyotype 46,XX; FISH probe to test Prader-Willi/Angelman LSI was compatible with deletion 15q11->13. Conclusions. Currently it is not stated that hypothyroidism belong to the spectrum of manifestations of AS. However there have been reported 3 patients with AS and hypothyroidism. Based on the findings of the case and the literature review, it is suggested the directed search of endocrinological alterations such as hypothyroidism in patients with AS.

1024T

Health-related quality of life in patients with cardio-facio-cutaneous syndrome. *S. Sottile[1], A. Kwan[2], P. Magoulas[3], K. Rauen[4].* 1) Department of Genetics, Stanford University, Stanford, CA; 2) Department of Pediatrics, Division of Medical Genetics, Stanford University, Stanford, CA; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 4) Department of Pediatrics, Division of Medical Genetics, University of California San Francisco, San Francisco, CA.

Cardio-facio-cutaneous (CFC) syndrome is a rare genetic disorder caused by germline mutations in genes in the Ras-induced mitogen activated protein kinase (Ras/MAPK) pathway, a signal transduction pathway with important roles in both human development and oncogenesis. The past ten years have seen incredible advancements for conditions of the Ras/MAPK pathway, most notably the promise of treating CFC and related conditions at the molecular level by utilizing cancer therapeutics that target the pathway. Future clinical trials for CFC will require measurement of certain endpoints, including health-related quality of life (HRQL), in order to determine the effects of treatment on the individual and caregiver's quality of life. We aimed to study HRQL in a large cohort of patients with CFC. This is the first study to assess HRQL in this population and will serve as a guideline for future studies. HRQL is a multidimensional construct representing a patient's subjective perception of the impact of disease. We assessed HRQL using the Pediatric Quality of Life Inventory™ and designed a survey tailored to the features of CFC by using a generic quality of life survey along with disease-specific modules. Due to age and/or intellectual disability a parent-proxy measure was used. Participants were recruited through CFC International, a support organization for families of individuals with CFC. The study was advertised to approximately 227 members of CFC International; 90 families requested surveys, and 56 surveys were returned (62% return rate). Our results indicated that individuals with CFC may have lower physical, psychosocial and total quality of life as compared to their healthy peers. Difficulty with physical functioning, such as ambulating, self-grooming, and participating in chores may greatly impact quality of life in patients with CFC. Additionally, psychosocial HRQL may be lower for older patients with CFC as compared to younger patients. In contrast, parent HRQL and the impact of CFC on a family may be better for older patients with CFC as compared to younger patients. Finally, HRQL is similar among individuals with different molecular causes of CFC. This is the first study to assess the extent to which CFC may impact an individual and/or caregiver's quality of life and our results serve as an important baseline measurement of HRQL. This survey instrument may be appropriate for measuring changes in HRQL in future clinical trials for CFC syndrome.

1025T

A new locus for autosomal dominant renal insufficiency associated with anemia and growth retardation. *B. Loeys[1], J. Huyghe[1], G. Van Camp[1], E. Matthys[2], E. Sys[3], M. Renard[4], A. Raes[5], J. Vandewalle[5], C. Van Hemelrijk[5], G. Mortier[1], L. Van Laer[1].* 1) Center for Medical Genetics, Antwerp University Hospital, Antwerp, Belgium; 2) Department of Nephrology, AZ-Sint Jan, Brugge, Belgium; 3) Department of Nephrology, AZ-Sint Lucas, Brugge, Belgium; 4) Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; 5) Department of Pediatrics, Ghent University Hospital, Ghent, Belgium.

In 2009, Zivna et al. reported a novel clinical syndrome characterized by autosomal dominant early-onset anemia, hyperuricemia and progressive kidney failure. Causal missense mutations in the renin gene lead to chronic endoplasmatic reticulum stress and unfolded protein response with subsequent site-specific attenuation of renin biosynthesis in the juxtaglomerular apparatus. Here we ascertained a three-generation family with autosomal dominant progressive renal insufficiency associated with congenital anemia, intrauterine and postnatal growth retardation. At term, birth weights ranged from 1800 gram to 2400 gram. Ultrasound exams showed small, dysplastic kidneys without cysts. Pertinent laboratory findings included normochromic, normocytic anemia, increased creatinemia, mild hyperuricemia and absence of proteinuria. Kidney biopsy revealed glomerular sclerosis and tubular atrophy and hypertrophy. Based on the similarities to the phenotype caused by renin mutations, we first excluded renin as the culprit gene based on Sanger sequencing of all exons and flanking intronic regions. In addition, whole genome micro-array analysis did not reveal any chromosomal deletions or duplications. Subsequently, we collected DNA from twelve family members, including seven affected subjects, and performed genomewide linkage analysis using the Illumina Human CytoSNP12. A subset of 10,521 Single Nucleotide Polymorphisms with an interval of circa 250kb were analyzed with Merlin. We identified a candidate region with a maximum LOD-score of 2.71 on chromosome 3q. The region was confirmed and finemapped with microsatellite markers. The linked region measures 32 Mb and contains a total of 357 genes. Based on the phenotype, we prioritized AGTR1 and SUCNR-1 as candidate genes. Both genes are involved in renin-related pathways. Sanger sequencing of both genes failed to reveal any causal mutation. We will apply whole exome sequencing to identify the responsible gene. Zivna et al, AJHG, 2009, 85, 204-213.

1026T

MEF2C mutations are a frequent cause of Rett- or Angelman syndrome like neurodevelopmental disorders. *A. Rauch[1], D. Bartholdi[1], C.M. Rueegger[1], M. Zweier[2], C. Zweier[2], E.K. Bijlsma[3], A. van Haeringen[3], W. Reardon[4], M. Zollino[5], A. Baumer[1].* 1) Institute of Medizinische Genetik, University of Zurich, Schwerzenbach-Zurich, Switzerland; 2) Institute of Human Genetics, Erlangen, Germany; 3) Department of Clinical Genetics, Leiden, The Netherlands; 4) Regional Hospital, Dooradoyle, Limerick, Ireland; 5) Institute of Medical Genetics A Gemelli Catholic University of Rome, Rome, Italy.

The transcription factor MEF2C was recently identified as the phenocritical candidate gene for the 5q14.3q15 microdeletion characterized by profound muscular hypotonia, severe intellectual disability (ID) and variable neurological and minor anomalies. Phenotypic overlap with Rett syndrome was explained by transcriptional interaction of MEF2C and MECP2. Point mutations in MEF2C have been shown to represent a relatively frequent autosomal dominant cause of moderate-severe ID accounting for as much as 1.1% of patients. Nevertheless, to date only 5 patients with MEF2C point mutations have been reported. Due to the phenotypic overlap we screened a cohort of 54 patients previously tested negative for Rett and/or Angelman syndrome for MEF2C mutations or deletions and detected two patients with novel MEF2C mutations (one frameshift and one missense mutation within the MADS domain) within this cohort. In addition we identified one further patient with Rett syndrome like phenotype caused by a MEF2C deletion detected by array-CGH screening after negative MECP2 testing. Two further patients with MEF2C mutation and intragenic deletion were identified subsequently due to suspicion of a MEF2C related disorder. Of note, our patient with the novel missense mutation is the first reported to be able to speak several words at the age of 4 years functioning at the mild to moderate intellectual disability level (IQ55). In summary we present 5 novel patients with MEF2C defects increasing the total number of reported cases to 10 and further delineating the phenotype which resembles both Rett and Angelman syndromes. Our results indicate that MEF2C mutations represent a common differential diagnosis to Rett- and Angelman syndrome accounting for approximately 4 % of such cases.

1027T

3p13p14.1 deletion: a new microdeletional syndrome associated with syndromic distal arthrogryposis. *J. Thevenon[1], K. Dieterich[2], P. Callier[3], M. Francoise[4], M. Splitt[5], S. Kjaergaard[6], K. Neas[7], J. Dixon[7], T.L. Dahm[8], F. Huet[9], C. Ragon[3], A.L. Boidron-Mosca[3], L. Duplomb[10], M.H. Aubriot-Lorton[11], F. Mugneret[3], N. Monnier[12], J. Lunardi[12], L. Faivre[1,10], P.S. Jouk[2], C. Thauvin-Robinet[1,10].* 1) Centre de Génétique et Centre de Référence (Anomalies du Développement et Syndromes Malformatifs) du Grand Est, Hôpital d'Enfants, CHU Dijon, Dijon, France; 2) Centre de Génétique et Centre de Référence (Anomalies du Développement et Syndromes Malformatifs) du Centre Est, CHU de Grenoble, Grenoble, France; 3) Laboratoire de Cytogénétique, Plateau Technique de Biologie, CHU Dijon, Dijon, France; 4) Service de Pédiatrie, Centre Hospitalier Wiliam Morey, Chalon-Sur-Saône, France; 5) Department of Clinical Genetics, Guy's Hospital, London, United Kingdom; 6) Department of Clinical Genetic, University Hospital Rigshospitalet, Copenhagens, Denmark; 7) Central and Southern Regional Genetic Services, Wellington Hospital Private, Wellington South, New Zealand; 8) Department of Pediatrics, Hillerød Hospital, Denmark; 9) Service de Pédiatrie 1, CHU de Dijon, Dijon, France; 10) Equipe émergente GAD (Génétique et Anomalies du Développement), IFR Santé STIC, Université de Bourgogne, Dijon, France; 11) Laboratoire d'Anatomopathologie, Plateau Technique de Biologie, CHU de Dijon, Dijon, France; 12) Laboratoire de Génétique Moléculaire, CHU de Grenoble, Grenoble, France.

Distal arthrogryposis is a heterogeneous clinical and genetic condition. Mutations in six different genes encoding the skeletal muscle contractile apparatus have been implicated in distal arthrogryposis and are responsible for 20 to 25% of molecular diagnoses. We report on four patients from 4 different countries with syndromic distal arthrogryposis presenting with a de novo 3p13p14.1 microdeletion gathered through the Decipher network. Several case reports of patients diagnosed by classical cytogenetic techniques with 3p13p14.1 deletion all presented with congenital distal multiple contractures anomalies associated with other phenotypic manifestations. The homogeneity of the clinical phenotype associated with the 3p13p14.1 deletion seems to suggest a microdeletional 3p13p14.1 syndrome. Patients presented with distal abnormalities with congenital multiple contractures (4/4 cases), developmental delay/mental retardation (4/4 cases) and feeding difficulties with low food intake (3/4 cases). Facial dysmorphism was constant (4/4 cases) with low-set and backward rotated ears (3/4 cases), hypertelorism (2/4 cases) and microstomy (2/4). Reviewing two previously reported cases with precisely mapped deletions, we limited the critical region for this condition to a 250kb region containing only 1 gene, EIF4E3 and the first two exons of the FOXP1 gene. The EIF4E3 gene was considered as a good candidate gene since it is a member of Eukaryotic initiation factor 4E family (eIF4E) a cap-binding protein, expressed only in brain and muscles. 5' cap-binding is necessary for translation initiation. The EIF complex participates in stabilisation, storage and degradation prevention of mRNAs before adressing to the ribosome for translation. Direct sequencing of the EIF4E3 exons and intron-exon boundaries and locus quantification by dedicated quantitative PCR in a cohort of 13 French patients affected by isolated or syndromic distal arthrogryposis appeared normal. To conclude, we report on a new candidate locus for distal arthrogryposis in 3p13p14.1 containing the EIF4E3 gene and the two first exons of the FOXP1 gene. Investigating this locus in a larger cohort is needed to determine whether the genes in this interval, FOXP1 or EIF4E3, play a role in other forms of distal arthrogryposis.

1028T

An Unusual Case of Developmental Delay with Plantar Lipomatosis. *L. Diefendorf[1], J. Humberson[2].* 1) Department of Internal Medicine and Pediatrics, Brody School of Medicine at East Carolina University, Pitt County Memorial Hospital , Greenville, NC; 2) Medical Geneticist and Clinical Assistant Professor, Department of Pediatrics, Brody School of Medicine of East Carolina University, Greenville NC.

Case Report: A full term 16-month old female with unremarkable prenatal history presented to genetics clinic with severe global developmental delay, onset at 2 months, without regression. She was unable to roll over or sit independently. Mom reported that she says “ma” and “da” but no other words. She was unable to reach for or pick up objects. Mother reports that she does not focus on people's faces or spontaneously smile. She had several physical exam findings including: midfacial hypoplasia, broad philtrum, high arched palate, elongated pinnae, wide tented upper lip with smaller lower “pouting” lip, deep palmar creases with pillowing, and fat pads (plantar lipomatosis) antero-medial to the heels of the feet with a “pillowing” effect on the soles of the feet. Head circumference was fifth percentile and height and weight were twenty-fifth to fiftieth percentile for age. Vision and hearing were normal. The rest of the physical exam was normal including normal tone and full range of motion of joints. Cytogenetic studies revealed a normal karotype 46, XX and significantly increased (8%) homozygosity on array. MRI was unremarkable. There were no reported seizures. **Discussion:** Fat pads antero-medial to the heels are a relatively rare finding. This can be an isolated congenital anomaly. A literature search on OMIM (NCBI, On-line Mendelian Inheritance in Man) revealed four entries including Cowden syndrome, Proteus syndrome, Multiple Lipomatosis, and Pierpont syndrome. The first three conditions are unlikely in this patient, however her presentation is suggestive of Pierpont syndrome. Pierpont described two children with developmental delay, planter lipomatoisis, and unusual facies. The phenotypical appearance included midfacial hypoplasia, large upper lip with small lower lip, broad philtrum, high anterior hair-line, palatal ridge, antero-medial fat pads on bilateral heel, and global developmental delay. One case had seizures. Another case report by Oudesluijs reported a male patient with developmental delay and similar dysmorphic features including the plantar lipomatosis. All the published case reports to date have been male. There are currently no confirmatory genetic tests available as the cause is unknown. There has been debate over the mechanism of inheritance. If this female patient with 8% homozygosity has Pierpont syndrome then it lends evidence to the autosomal recessive theory.

1029T

Deletion of *TWIST1* in a patient with Duane-radial ray syndrome. *S. Jougheh Doust, J. So.* Clinical and Metabolic Genetic, The Hospital for Sick Children, Toronto, On, Canada.

Mutations in the TWIST1 gene, which is located on chromosome 7p21.1, cause Saethre-Chotzen syndrome (SCS; MIM 101400), an autosomal dominant (AD) syndromic form of craniosynostosis. TWIST1 mutations are detectable in 46-80% of individuals with SCS. Clinical manifestations in SCS show inter- and intra-familial variability. Clinical features can include craniosynostosis, low frontal hairline, ptosis, strabismus, facial asymmetry (secondary to unilateral coronal synostosis), small ears, and limb abnormalities such as 2-3 finger syndactyly, radio-ulnar synostosis and hallux deformities. Craniosynostosis is not an obligate feature of SCS syndrome. Most patients have no intellectual disabilities (ID); however, ID has been reported in patients with large deletions of the chromosome 7p21.1 region. Duane-radial ray syndrome (DRRS; MIM 607323) is characterized by uni- or bilateral Duane anomaly and radial ray malformation. DRRS is inherited in an AD manner and SALL4 gene mutations are responsible for 40-50% of DRRS cases. We report a patient with right-sided Duane anomaly, mild developmental delay with more significant speech delay, obsessive-compulsive disorder, dysmorphic features that include bilateral ptosis, small ears, narrow palate, and radial ray anomalies including thenar and hypothenar hypoplasia with proximally inserted thumbs. No craniosynostosis was appreciated in this patient. High-resolution karyotype and metabolic screening, as well as SALL4 mutation analysis, were normal. Further studies with oligo array detected a 4.9 Mb deletion at chromosome region 7p21.2-7p21.1, which included a complete deletion of TWIST1 gene. Our patient carries a microdeletion at 7p21.1 affecting the TWIST1 gene with clinical manifestations suggestive of DRRS but overlapping with SCS. Mutations in the TWIST1 gene associated with severe craniosynostosis and radial ray hypoplasia have been reported by Grip et al (1999) and Seto et al (2001). Duane anomaly has never been reported in association with a TWIST mutation. This is the first report of a TWIST1 gene deletion in an individual presenting with DRRS. The highly variable phenotype of SCS, together with the genetic heterogeneity in DRRS, as demonstrated by the majority of cases not having SALL4 mutations, make the establishment of a clean-cut diagnosis challenging in this case. Given the microdeletion finding in this patient, we suggest that a DRRS-like phenotype might be part of the phenotypic spectrum of SCS.

1030T

Familial cosegregation of Coffin-Lowry syndrome inherited from the mother and Waardenburg type IV syndrome due to deletion of *EDNRB* inherited from the father: A diagnostic odyssey. *Y. Lacassie[1], S. Sampath[2].* 1) Dept Ped/Div Clin Gen, LSU Hlth Sci Ctr, New Orleans and Children's Hospital New Orleans, LA; 2) Dept. Gen, LSU Hlth Sci Ctr, New Orleans. Current address Postdoc Fellow, McKusick-Nathans Inst of Genet Med, JHU, Baltimore, MD.

The presence of two or more conditions in a patient has been recognized for a long time as a rare diagnostic problem due to the patient. We report an AA family that was identified after the proposita was referred for diagnostic evaluation at 4½ months with a history of Hirschsprung that was surgically repaired and few dysmorphic features. These included hypertelorism and telecanthus, and a peculiar light blue iris. We evaluated the whole family with a suspected diagnosis of Waardenburg syndrome (WS). The father had similar dysmorphic features supporting the clinical diagnosis of WS; however, examination of the mother revealed characteristic facial and digital features of Coffin-Lowry syndrome (CLS). The clinical evaluation of the five older siblings of the proposita revealed that three were affected with one or the other condition, one with apparently both disorders, and one possibly normal. The youngest sibling born in 2010 however was the most difficult to diagnose clinically. Molecular testing of the mother identified a novel 2 bp deletion (*c.865_866delCA*) in codon 289 of *RPS6KA3* leading to a frameshift and premature termination of translation 5 codons downstream (*NM_004586.2:p.Gln289ValfsX5*). This deletion also was identified in the proposita and her three sisters with a clinical suspicion of CLS, all of whom, as heterozygotes had very subtle manifestations. The molecular confirmation of WS type 4 (Shah-Waardenburg; WS4) was an odyssey. To evaluate WS types I-IV multiple sequential molecular testing was requested, including Sanger sequencing of all exons, and deletion/duplication analysis using MLPA for *PAX3*, *MITF*, *SOX10*, *EDN3* and *EDNRB*. Sequencing did not identify any disease causing variants, but MLPA identified a heterozygous deletion of the entire *EDNRB* in the father. This deletion was also found in the proposita. The oldest brother and a sister (who is also heterozygote for CLS) have characteristic clinical features of WS4; therefore, molecular testing was considered unnecessary. The youngest brother of the proposita was negative for both mutations. Since the heterozygous deletion was the only change identified in *EDNRB*, this family represents one of the few cases with an autosomal dominant inheritance of WS4. This kinship is an example of the coincidence of two conditions cosegregating in one family, with variable phenotypes requiring molecular testing to confirm the clinical diagnoses.

1031T

Genetics of Kabuki Syndrome: MLL2 mutational spectrum in 100 KS patients. *A.D.C. Paulussen[1,3], M.J. Blok[1,3], C.E. van Roozendaal[1,3], D. Tserpelis[1], C. Posma-Velter[1], R. Vijzelaar[4], J. Schrander[2], Y. Detisch[1,3], C.T.R.M. Schrander-Stumpel[1,3], A.P.A. Stegmann[1,3], H.J. Smeets[1,3].* 1) Dept Clinical Genomics, Maastricht Univ Med Ctr, Maastricht, Netherlands; 2) Department of Paediatrics, Maastricht UMC+,Maastricht, the Netherlands; 3) School for Oncology & Developmental Biology (GROW), Maastricht UMC+,Maastricht, the Netherlands; 4) MRC-Holland, Amsterdam, The Netherlands.

Kabuki syndrome (KS, MIM: 147920) is a clinically recognizable syndrome of multiple congenital anomalies and mental retardation affecting approximately 1:30,000 live births. Key features are a characteristic face, growth retardation, developmental delay and additional features such as hypodontia and persistent foetal fingertip pads. Recently, a gene causing KS was identified through exome sequencing, reporting de novo mutations in the histone methyl transferase (HMT) gene MLL2 in 66% of 53 patients with Kabuki syndrome (Ng et al., Nat Genet.42(9):790-3, 2010). We confirmed the pathogenic significance of this gene in KS in our first published series, demonstrating 76% MLL2 mutations in 45 KS cases (Paulussen et al, Hum Mutat. 32(2), 2011). In a larger follow-up study we screened 100 patients for MLL2 mutations and included MLPA analysis to study exonic deletions and duplications in the MLL2 mutation negative patients. Using Long-Range PCR we further examined the complete MLL2 gene, including introns and 5'/3' UTR in two typically clinical KS patients without a mutation in the coding region to search for exon deletions/duplications not covered by the MLPA kit and/or deep-intronic de novo mutations. The effect of predicted splice-site mutations was investigated at the RNA level. Finally, we determined the mutational origin (paternal/maternal) of several mutations with allele specific PCR. Our data yields a more complete picture of the MLL2 mutational spectrum and further insight in the pathogenic mechanisms leading to KS.

1032T

Noonan syndrome and other genetically related syndromes diagnosed by a custom multiplex mutation panel (CGC Mutation Panel). *P. Tavares[1], A. Lopes[2], L. Lameiras[2], L. Dias[1], J. Sá[2,3], P. Rendeiro[2], A. Palmeiro[2].* 1) CGC Genetics, Newark, NJ 07103, NJ. (www.cgcgenetics.com); 2) CGC Genetics, Porto, Portugal (www.cgcgenetics.com); 3) Hospital Pediátrico de Coimbra, Coimbra, Portugal.

Introduction: Noonan syndrome is a congenital genetic disease that affects both males and females equally (1:1.000-2.500 newborns). Great clinical variability has been identified: heart disease, valvular pulmonary stenosis or hypertrophic cardiomyopathy, short stature of postnatal onset, psychomotor development delay. Often this syndrome is not diagnosed, but it is related to many complex problems such as coagulation defects and lymphatic dysplasias. Differential diagnosis includes major diseases in the same metabolic pathway - Costello, Cardiofaciocutaneous and LEOPARD Syndrome. Method: We developed a custom multiplex mutation panel (CGC Mutation Panel - Pat. Pending) that contains a total of 80 point mutations, 52 point mutations in the 4 main genes involved in Noonan (PTPN11, RAF1, SOS1 and KRAS) and 30 point mutations (on HRAS, BRAF, MAP2K1 and MAP2K2) involved on LEOPARD, Costello and Cardiofaciocutaneous syndromes (genetically related) to identify the most frequent and severe forms. With this panel we analysed 6 cases of Noonan syndrome that had been also characterized by sequencing of the PTPN11 gene and 33 samples (10 prenatal and 23 postnatal) with suspicion of Noonan or related syndromes. Results: All the 6 previously known mutations were also detected with the panel. From the 33 samples (10 prenatal and 23 postnatal), in 1 prenatal sample we detected a Thr3Ile change on PTPN11 gene allowing the diagnosis of Noonan and in 2 of the postnatal samples we diagnosed a Met269Thr change on SOS1 gene and a Glu257Arg on BRAF gene allowing the diagnosis of Noonan and Cardiofaciocutaneous syndromes respectively. Conclusion: This approach is a valuable diagnostics tool, since it detects the most common mutations associated with Noonan Syndrome, not only on PTPN11 gene but also in 7 other genes, improving the capacity for diagnosis. The genes and mutations included in this panel allow a broader clinical spectrum and a faster diagnosis, independently of the sample type, allowing an earlier decision-making process in patient management, being particularly relevant in prenatal diagnosis (cases with increased nuchal translucency and normal karyotype). This panel represents a step forward allowing the molecular diagnosis of Noonan syndrome together with the differential diagnosis for LEOPARD, Costello and Cardiofaciocutaneous syndromes.

1033T

Clinical and Molecular Analysis of Patients with Neurocardiofaciocutaneous Syndromes From Turkey. *P.O. Şimşek Kiper[1], Y. Alanay[1], B. Gülhan[1], C. Lissewski[2], D. Türkyilmaz[3], D. Alehan[4], M. çetin[5], G.E. Utine[1], M. Zenker[2], K. Boduroğlu[1].* 1) Hacettepe University Faculty of Medicine Department of Pediatrics, Pediatric Genetics Unit, Ankara, Turkey; 2) University Hospital Magdeburg Institute of Human Genetics Magdeburg, Germany; 3) Hacettepe University Faculty of Medicine Department of Ear-Nose-Throat, Audiology Unit, Ankara, Turkey; 4) Hacettepe University Faculty of Medicine Department of Pediatrics, Pediatric Cardiology Unit, Ankara, Turkey; 5) Hacettepe University Faculty of Medicine Department of Pediatrics, Pediatric Hematology Unit, Ankara, Turkey.

ABSTRACT The neuro-cardio-facio-cutaneous (NCFC) syndromes are a group of disorders which share many clinical features and in which the verification of the diagnosis may require molecular analysis. These syndromes result from germline mutations in genes encoding proteins and kinases of RAS-MAPKinase signal transduction pathway. In our study, we have performed molecular analyses in 31 patients with a clinical diagnosis of NCFC syndromes (26 patients with Noonan, 3 patients with Costello and 2 patients with LEOPARD syndromes) to elucidate the underlying genetic etiology and to establish a genotype-phenotype correlation. Mutation screening of several genes including PTPN11, SOS1, KRAS, RAF1, SHOC2, HRAS, BRAF and RAF1 were performed. Twenty patients out of 31 (64.5%) were found to be mutation positive. Mutations in PTPN11, SOS1 and SHOC2 genes were detected in 15 patients (58%) with Noonan syndrome (NS). PTPN11 mutations were found in two patients with LEOPARD syndrome. HRAS mutations were positive in two of three patients with Costello syndrome (CS), one of which was HRAS:p.E63_D69dup. The other CS patient was found to have a BRAF mutation which led to a change in diagnosis as cardio-facio-cutaneous syndrome. Of 15 NS patients with a mutation, seven (26.9%) showed a mutation on PTPN11, five (19.2%) on SOS1, and three (11.5%) on SHOC2. Pulmonary stenosis was the most common (62%) cardiac anomaly observed. Thrombocyte segregation abnormality was detected in three patients each with one of the mutations of SOS1, PTPN11 and SHOC2 and in two other patients without a mutation. Of 26 patients with NS, 17 (65.3%) had a delay in motor development whereas 14 (53.8%) had cognitive delay. Hearing and speech perception thresholds levels were found within normal limits in all patients tested. In conclusion, the common genetic basis underlying NCFC might cause difficulties in the differential diagnosis and renders molecular analysis essential for confirmation of the clinical diagnosis and patient follow-up.

1034T

A Novel Point Mutation in the STS Gene in a Family with X-Linked Recessive Ichthyosis. *R. Badilla-Porras, L. Dupuis, R. Mendoza-Londono.* Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, Ontario, Canada.

Recessive X-linked ichthyosis is a keratinization disorder due to an inborn error of metabolism that affects roughly 1:2000 to 1:6000 males. The clinical manifestations present early in life and involve generalized dryness and scaling of the skin with polygonal, regular dark scales. The trunk, limbs, neck, ears and scalp are often involved while palms and soles are spared. Extracutaneous signs are described involving corneal opacities, testicular cancer, cryptorchidism and a family history of delayed progression of parturition. The disease is caused by partial or complete deletions or inactivating point mutations in the steroid sulfatase (STS) gene located on Xp22.3 and leading to deficient STS activity. Up to 90% of the described mutations of this gene are complete deletions, which is one of the highest percentages of chromosomal deletions among all genetic disorders. We present the clinical characterization of a family with a clear X-linked pattern of inheritance, an identified mutation and clinical findings in three generations. Our index case presented at 2 months of age with dryness and scaling of the scalp and lower limbs, and later he developed generalized skin lesions. He did not have any extracutaneous findings.He had normal development, non dysmorphic features and no other abnormalities.His mother presented with mild dry skin and pregnancy history of delayed progression of delivery in all her children. Her first delivery was particularly difficult and that child developed perinatal asphyxia.The maternal grandfather presented with classical dermatological manifestations during childhood.Initial molecular analysis by Fluorescent in-situ Hybridization (FISH) and Multiple Linked PCR Analysis (MLPA) showed no deletions in the STS gene. Direct sequencing was subsequently performed for the STS gene and revealed a novel mutation In exon 9 (c.1381 G>T, p.R454L). Two other missense mutations p.R454H and p.454C at the same position have been described to cause X-linked ichthyosis.“Polyphen” predicted the consequence of the variant p.R454L for the protein to be “probably damaging” supporting the suspicion of the c.1381G>T mutation being pathogenic. The cases presented herein highlight the clinical variability in the phenotype of patients with X-linked Ichthyosis and allow a better genotype-phenotype correlation. Mutations at p.R454L present with classical phenotype in males. Females present with mild skin dryness and delayed progress of parturition.

1035T

Clinical course of Noonan-like syndrome with loose anagen hair (NS/LAH, MIM 607721) in individuals carrying mutations of *SHOC2*. *D. Bartholdi[1], M. Lang-Muritano[2], E.J. Schoenle[2], A. Schinzel[1], M. Zenker[3], A. Rauch[1].* 1) Institute of Medical Genetics, University of Zürich, Schwerzenbach, Switzerland; 2) Division of Endocrinology and Diabetology, University Children's Hospital, Zürich, Switzerland; 3) Institute of Human Genetics, University Hospital Magdeburg, Otto-von-Guericke University, Magdeburg, Germany.

Noonan syndrome comprises a group of autosomal dominant inherited disorders with high clinical variability and genetic heterogeneity caused by dysregulation of the RAS-MAPK signaling pathway. The main clinical characteristics are postnatal growth retardation, cardiac defects, lymphatic dysplasia, facial dysmorphism, ectodermal abnormalities, cognitive disability of variable degree, and susceptibility to certain malignancies. Recently, it was demonstrated that mutations in *SHOC2*, coding for a leucine-rich repeat-containing protein which positively modulates the RAS-MAPK signal cascade, cause a clinically distinctive condition previously termed Noonan-like syndrome with loose anagen hair (NS/LAH, MIM 607721). All patients with NS/LAH reported so far share the same 4A>G missense change in *SHOC2* that predicts the substitution of Ser2 by Gly. This amino acid change introduces an N-myristoylation site, resulting in constitutive membrane targeting of SHOC2 that leads to increased MAPK activity. Until now only a limited number of patients carrying *SHOC2* mutations have been reported and information about the clinical course and long term perspective is sparse. We report on the phenotypes and course of 4 patients carrying the 4A>G missense mutations, ranging from 3 to 17 years in age. All 4 patients presented with a highly recognizable phenotype characterized by relative macrocephaly at birth, severe feeding difficulties with growth failure, and ectodermal abnormalities (dry and darkly pigmented skin with ichthyosis or eczema). Additional ectodermal features include thin and slowly growing hair which can be easily plucked, and long and twisted eyelashes. One patient displayed proven growth hormone deficiency. Hyperactive behavior was observed in all individuals as was a hypernasal speech. Cognitive development was within the low-normal range in the two older boys (aged 11 and 17 years). Overall, the patients displayed a highly recognizable phenotype which enabled targeted search of the 4A>G missense change in *SHOC2* and rapid diagnosis.

1036T

Computer assisted estimation of the prevalence of dysmorphic features in the general population. *L. Basel-Vanagaite[1 2], L. Karlinsky[2], L. Wolf[2 3], M. Shohat[1 2].* 1) Dept Medical Genetics, Rabin Medical Ctr, Petah Tikva, Israel; 2) FDNA Ltd., Herzlyia, Israel; 3) The Blavatnik School of Computer Science, Tel-Aviv University, Tel-Aviv, Israel.

Over the past few years, we have witnessed a significant improvement in the capacity of genomic data generation and the quality of genetic data analysis, driven mainly by development of high throughput technologies, such as comparative array hybridization and whole exome sequencing. However, these technologies alone are insufficient for revealing genetic aetiology of novel human genetic syndromes, due to the large amount of genomic variation among normal individuals. Therefore, clinical evaluation of children with developmental problems and dysmorphic features continues to present a major challenge to clinicians world-wide. With the ever-growing amount of data generated by these recent technologies, computerized approaches for analyzing facial traits are urgently needed to aid the genetic evaluation and diagnostic process. Currently, the scope and availability of 2D and 3D computer-based analysis of facial traits is limited and does not provide a suitable solution to the clinical requirements. Whereas genetic syndromes frequently involve distinct craniofacial malformations, we have explored the automatic recognition of these traits from 2D facial images, using advanced facial recognition techniques. For each trait out of a short list of subjective clinically relevant anatomical facial features, including the philtrum, the periorbital region and several other characteristics of the entire face, we have constructed a computerized model that describes it in mathematical terms and can detect such features in images automatically and reliably. These models were constructed by applying machine learning techniques to a set of hand labeled dysmorphic faces and incorporate relative measurements as well as variations in appearance. By employing these models to a large image dataset of normally developed school children, we have estimated the prevalence of each feature in the general population. For example, we were able to estimate that the prevalence in the general population of long philtrum is 11.6%; Widely spaced eyes 5.3%; Upslanted palpebral fissure 8.9% and downslanted palpebral fissues 1.8%. This enabling technology could also provide computer aided diagnoses, as well as phenotypic comparison of patients with dysmorphic features. This approach, in combination with whole exome sequencing, can aid in identification of novel genes causing genetic syndromes in unrelated dysmorphic patients having the same syndrome.

1037T

A Simpson-Golabi-Behmel patient with severe neonatal liver involvment. *B. DEMEER[1], D. DJEDDI[2], F. LACAILLE[3], G. MORIN[1], G. JEDRASZAK[1], J. MICHELI[4], R. RHAZLANE[5], S. LANTA[6], F. AMRAM[1], MP. MOIZART[7], M. MATHIEU[1].* 1) Genetics department, Amiens university hospital, France; 2) Pediatrical gastroenterology unit, Amiens university hospital, France; 3) Pediatrical gastroenterology department, Necker Enfants Malades hospital, Paris, France; 4) Pediatrical oncology and haematology unit, Amiens university hospital, France; 5) Paediatrics department, Amiens university hospital, France; 6) Gynaecology and Obstetrics department, Amiens University hospital, France; 7) Molecular genetics department, Tours University Hospital, France,.

The patient is the third child of an unrelated couple, with no known remarkable history. Pregnancy was marked from 22 gestation weeks, by hydramnios and fetal macrosomia, and from 27 GW, by bilateral nephromegaly. The patient was delivered by ceasarean section at 36 GW, and birth parameters were : weight : 4kg105 (P97), height 52cm (P97) and OFC 37cm (P97). On examination, he had hypotonia, feeding difficulties and was icteric. He also presented facial dysmorphism with coarse features, hypertelorism, short nose with anteverted nares and macrostomia. Three abdominal supernumerary nipples and a abdominal diastasis recti were noted. Extremities were normal. No neonatal hypoglycemia was noted. Abdominal ultrasounds confirmed the bilateral nephromegaly, and showed at 2 weeks of age, an hepatomegaly. Biology showed a cholestasis and jaundice. The study of the GPC3 gene showed a single base deletion in the exon 7, confirming the diagnosis of Simpson-Golabi-Behmel syndrome (SGBS). At the age of 6 weeks, because of worseness of cholestastis, persistent jaundice and difficulties displaying the biliary ducts on CT scan, a liver biopsy was performed. It showed no biliary atresia, but found a significant proliferation, portal fibrosis and cholestasis. Apart from the visceromegaly, neonatal liver involvment is rarely associated to the SGBS. We will precisely described the hepatic results and compare this case to the literature.

1038T

Genetic diagnosis of Duchenne and Becker muscular dystrophy using next-generation sequencing technology: comprehensive mutational search in a single platform. *B. Lim[1], J. Chae[1], K. Kim[1], S. Lee[2,3], J. Kim[2,3,4], J. Seo[2,3,4].* 1) Seoul Natinoal University Hospital, Seoul, Korea; 2) Genomic Medicine Institute (GMI), Medical Research Center, Seoul National University; 3) Department of Biomedical Sciences, Seoul National University Graduate School; 4) Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine.

Background: Duchenne and Becker muscular dystrophy (DMD/BMD) might be a suitable candidate disease for application of next-generation sequencing (NGS) in the genetic diagnosis because the complex mutational spectrum and large size of the DMD require two or more analytical methods and have a high cost. We tested whether large deletions/duplications or small mutations, such as point mutations or short insertions/deletions of DMD, could be predicted accurately in a single platform using NGS technology. Methods: A custom solution-based target enrichment kit was designed to capture whole genomic regions of DMD and other muscular dystrophy-related genes. A multiplexing strategy was applied in which four differently bar-coded samples were captured and sequenced together in a single lane of the Illumina Genome Analyzer. The study subjects were 25 patients: 16 with deficient dystrophin expression without a large deletion/duplication and nine with a known large deletion/duplication. Results: Nearly 100% of the DMD exonic region was covered by at least eight reads with a mean read depth of 107. Pathogenic small mutations were identified in 15 of the 16 patients without a large deletion/duplication. Using these 16 patients as negative controls, our method accurately predicted the deleted or duplicated exons in the nine patients with known mutations. Inclusion of non-coding regions and paired-end sequence analysis enabled accurate identification by increasing the read depth and providing information about the breakpoint junction. Conclusions: The current method has an advantage in the genetic diagnosis of DMD/BMD in that a comprehensive mutational search may be feasible in a single platform.

1039T

Dynamics of genetic polymorphisms linked to Hemophilia A gene (*F8*) in the Brazilian population. *J.D. Massaro[1], C.E.V. Wiezel[1], C.T. Mendes-Junior[2], M.R. Luizon[3], A.L. Simões[1].* 1) Department of Genetics, School of Medicine of Ribeirão Preto, University of São Paulo; 2) Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo; 3) Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, SP.

Hemophilia A is a bleeding disorder conditioned by a gene located on the X chromosome and caused by partial or total deficiency of the Factor VIII activity, a plasma glycoprotein necessary for normal blood coagulation. Due to difficulties faced on direct recognition of the *F8* gene mutation, carrier diagnosis is done indirectly by linkage analysis with polymorphic markers located within or near to the gene. These markers may determine the haplotype and the mutation co-segregation within the studied family, and thus detect the carrier status and possibly assist in prenatal diagnosis. This study aimed to evaluate the power of some of these markers in population differentiation and determine their degree of informativeness for diagnosis and genetic counseling of affected families, as well as verifying the possible forensic use of such markers. We determined the allele and haplotype frequencies, genetic diversity, population differentiation, linkage disequilibrium and ancestral composition in Brazilian urban (healthy not related individuals from São Paulo, Rio Grande do Sul and Pernambuco), Quilombo remnant (*Mimbó*, *Sítio Velho* and *Gaucinha* in the State of Piauí) and Amerindian (*Tikúna*, *Baníwa* and *Kashináwa*) population samples by the analysis of four intragenic microsatellites (*F8*Int1, *F8*Int13, *F8*Int22, *F8*Int25.3), located in *F8* introns, and one extragenic (IKBKG). When appropriated, the analysis included a group of hemophilic patients. DNA polymorphisms were detected by PCR, PAGE and silver nitrate staining. Statistical analysis was implemented by GENEPOP, Arlequin, Fstat, MVSP, GDA, ADMIX®3 and PHILIP. Diversity parameters showed differences among the populational samples. Such regional differences in allele frequencies must be taken into account when conducting indirect diagnosis of Hemophilia A. Except for IKBKG, all other microsatellites showed high rates of heterozygosity. Using these markers, the diagnosis was possible in 10 of the 11 families analyzed. The microsatellites *F8*Int22, *F8*Int1, *F8*Int13, *F8*Int25.3 and IKBKG were informative in 63.6% (7/11), 54.5% (6/11), 54.5% (6/11), 45.5% (5/11) and 18.2% (2/11) of the cases, respectively, demonstrating the effectiveness of using these microsatellites in prenatal diagnosis and carrier identification in the Brazilian population. Financial support: Capes, FAEPA.

1040T

Molecular analysis by an individual diagnosed with autosomal recessive polycystic kidney disease (ARPKD). *M. Nagel, S. Nagorka, M. Brzeska.* Molec Gen, Ctr Nephrology, Weisswasser, Germany.

Autosomal rezessive polycystic kidney disease (ARPKD) is a genetic disease inherited in a recessive manner. It is less common then autosomal dominant polycystic kidney disease . The incidence is 1 in 6000 bis 1 in 55000 in the US population, but the higher incidence was noticed in the population of Finish and African ancestry (1 in 1000 in Finland and 1 in 20000 in Africa). Mutation in the PKHD1 gene, chromosomal locus 6p12.2 causes ARPKD. In this study, we performed direct sequencing of the entire coding region of the PKHD1 gene in 44 unrelated individuals referred to our laboratory with a clinical diagnosis of autosomal rezessive polycystic kidney disease (ARPKD). The fetal DNA was analysed in 12 cases. There were also 13 patients between the age of 1 and 5 months and 6 patients between the ages of 1 and 2 years. The 5 patients of the deceased newborn were tested for carriership. Sixteen novel mutations were identified. We found eight novel missense mutations: W477S, T3626I, R559Q, V1232E, G894V, S2867C, D1944N, A1987P; two novel splice mutations, IVS35+3A>G, IVS21+1G>A; and six deletions/insertions: c.122delT, c.7132delA, c.1393delA, c.10775del, c.6393insC, and c.10447insT. Six novel mutations, W477S,T3626I,R559Q, D1944N, A1987P and c.10775del were also found in the family members. In conclusion, the new mutations found in these patients almost proved a clinical diagnosis of autosomal rezessive polycystic kidney disease and certainly had pathogenic relevance.

1041T

Hemophilia A diagnose with *F8*Int18B*cl*I SNP and DXS 1108 markers in Brazilian population. *F.A. Saiki[1,2], J.D. Massaro[1], A.L. Simões[1].* 1) Department of Genetics, FMRP, University of São Paulo, USP, Ribeirão Preto, SP, Brazil; 2) São Paulo State University, UNESP - FCAV, Jaboticabal, SP, Brazil.

Hemophilia A is an X linked recessive bleeding disorder caused by a deficiency in blood coagulation Factor VIII. High incidence and morbidity stress the importance of genetic counseling. The great number and heterogeneous nature of human mutations in *F8* gene make direct analysis a hard task. Therefore indirect analysis is necessary to determine linkage between polymorphic markers located close or within *F8* gene to map mutations in affected families by inherited forms of Hemophilia A (HA). Although the ideal is the direct detection and analysis, starting with the analysis of the inversions in Introns 1 and 22, PCR is a reliable diagnostic method and high-cost benefit. The *F8*Int18B*cl*I SNP and DXS1108 were used to check viability in genetic counseling and characterize allelic frequencies, Hardy Weinberg Equilibrium, linkage disequilibrium and heterozygosity in health and not related urban population samples of São Paulo state (men and women), and patients with Hemophilia A and his mothers. Genotyping was performed by PCR/RFLP and PCR, non-denaturing 8% and 11% PAGE and silver staining to *F8*Int18B*cl*I and DXS1108, respectively. Half of the alleles found in the present study of DXS1108 marker were cloned and sequenced. The *F8*Int18B*cl*I distribution in men and women were different for Urban population of São Paulo and HA. The presence of allele for B*cl*I restriction were higher in HA (+, 59% for men and 68% for women) than SP (+, men 43%, 48% women). Despite the high presence of allele restriction, it was lesser than found in Japan, North India and Hungary. The only sample that had a higher prevalence of restriction site absence(-) was Asian Indian. The most frequent allele for DXS1108 in women (SP and HA) and HA patients were *DXS1108*23*, followed by *DXS1108*19*. The sample of SP men presented *DXS1108*23* and *DXS1108*24* alleles as the most frequent. The sample in the present study does not share any allele with Spanish sample. The analysis of linkage disequilibrium revealed highly significant *P*-values. Total heterozygosity (0.6-0.61) considering both markers was higher than identified in other populations as Japanese, North Indian, Asian Indian, Spanish and Chinese. The most frequent haplotype in both markers is -/23 in men and women of SP sample and +/23 for HA sample. The results show that these markers are informative in genetic counseling and diagnose. Financial Support: Capes, FMRP/USP,CNPq.

1042T

A comparative study about death anxiety with Familial Amyloidotic Polyneuropathy familiars (caregivers). *P.Isabel. Santos.* Psychology, Universidade Fernando Pessoa, Porto, Porto, Portugal.

Familial Amyloid Polyneuropathy (FAP) is a neurodegenerative disease caused by a mutant gene in chromosome pair 18 in which methionine is substitute for valine at position 30 (TTR-Met30) characterized by a progressive peripheral and autonomic neuropathy with neural and systemic amyloid deposits. It is an incurable and hereditary disease with autosomal dominant transmission; the children of an affected mother/father have 50% of possibility to inherit the mutation. The late onset (about 30 yrs) and a fatal prognosis (14 years after the onset) are expected. Is a very insidious disease that causes great dependence on a caregivers usually direct relatives (wives, husbands and children). Even the family free from the risk of inheriting the disease (or because they already performed the test and was negative, or because they have no inbreeding), live the disease nearby, and live the pain of a dependent mother/father, husband or wife. So we intent to explore if familiars of FAP patients namely the caregivers have different levels of death anxiety comparatively with a control group. We administered Death Anxiety Scale (DAS, Temper, 1970) in a group familiars of FAP patients (FAM) (N = 91) and a control group, (sociodemographic identical sample, but with no bound to FAP disease) (N = 121). FAP patients score significantly higher in DAS (M= 52.18, DP= 8.32 vs 49.56, SD = 9.12) than a control group. We conclude that being a caregiver for FAP is a source of increasing Death Anxiety. Since Death Anxiety correlates with depression, anxiety and psychopathology (Templer, 1976; Templer; Lavoie; Chalgujian & Thomas-Dobson, 1990). Psychological support should be considered to the familiars of FAP patients.

1043T

A Comparison of Enrichment Techniques for Clinical Next-Generation Sequencing of Intellectual Disability, Early Infantile Epilepsy and Congenital Brain Malformations. *S. Topper, V. Nelakuditi, M.A. Dempsey, S. Das.* Human Genetics, University of Chicago, Chicago, IL.

The genetic diagnosis of non-syndromic neurological disease presents unique challenges to clinicians and diagnostic labs: recognizable phenotypes are genetically heterogeneous with many dozens of genes causally implicated, mutations in each gene only explain a negligible percentage of patient cases, and a complex and rapidly developing literature requires constant re-evaluation of the diagnostic strategy. We are pursuing a highly multiplexed approach to the diagnosis of non-syndromic intellectual disability, developmental delay and brain malformations and are evaluating three different enrichment approaches: multiplexed target amplification using the Raindance system, targeted capture using Agilent SureSelect biotinylated RNA baits, and Agilent exome capture. Our region of interest includes 1627 exons from 98 genes, spanning 500,000 bases of coding sequence. For each of 12 samples enriched using the Raindance system, we generated ~16 million 76bp paired-end reads on an Illumina GAIIx. On average, 92% of reads aligned to the human genome, with 25-45% deriving from the target region. With an average depth of 150x, more than 94% was covered at 30x leaving 260 exons (out of 1627) with some small regions insufficiently covered. On average, we identified 250 variants per sample within this region that included 25 missense variants (two to five of which were novel), and 0 or 1 small coding deletions per patient. We successfully identified a causative single base deletion in the AGTR2 gene in a patient with intellectual disability and early infantile seizures in whom extensive single-gene testing had previously been performed with no diagnostic success. We have also identified a number of potentially deleterious mutations in the remaining patients, and validation is currently underway. A subset of these same samples have been enriched for our genes of interest using a custom Agilent SureSelect design and by using Agilent Exome Capture, and comparative data analysis will be presented. We propose that reliable integration of next-generation sequencing into the clinical arena requires sufficient data generation to yield 100% coverage of the target region defined as a minimum coverage of 30x at each base to minimize false positive and false negative rates; and that the target region needs to be supplemented by Sanger-sequencing where it fails to meet those requirements.

1044T

A c.1372C→T mutation found in TSC2 gene in a TSC-affected family revealed that it is less likely to account for mental retardation. *T. Tsai[1], S. Lin[2], D. Chu[3].* 1) Graduate Institute of Biomedical Science, Ghang Gung University, Tao-Yuan, Taiwan; 2) Department of Pediatrics, Mackay Memorial Hospital, Taipei, Taiwan; 3) Department of Medical Biotechnology and Laboratory Science, Chang Gung University, Tao-Yuan, Taiwan.

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder characterized by hamartomas in multiple organs. Seizures and mental retardation (MR) are important problems in TSC patients. Mutations in TSC1 and TSC2 genes are major causes of this disorder. In order to depict the correlation between TSC gene mutation and mental retardation, genomic DNA from TSC patients were analyzed for mutation screening. In this report, an inherited pathogenic mutation and an inherited single nucleotide polymorphism (SNP) were identified from a TSC family. In this family, the proband is a TSC patient without MR, but her daughter is a TSC patient with mild MR and her son is a TSC patient with severe MR. Using high-resolution melting analysis followed by bidirectional DNA sequencing, TSC1 c.2829C→T (p.A943A) was identified from the proband and her son. On the other hand, TSC2 c.1372C→T (p.R458X) was identified from the proband, her daughter and son by using whole-gene DNA sequencing. TSC2 c.1372C→T is a pathogenic mutation because the nucleotide substitution creates a new stop codon, leading to production of premature or truncated protein in translation. This pathogenic mutation exists in the proband and her children, but the proband does not suffer from MR. The inconsistency between genotype and phenotype suggests that TSC2 c.1372C→T is less likely responsible for mental retardation in this TSC-affected family.

1045T

Ion Torrent as a potential platform for molecular diagnosis. *X. WANG[1], H. WANG[1,2], D. Muzny[2], I. Newsham[2], Y. Wu[2], C. Buhay[2], H. Dinh[2], R. Gibbs[1,2], R. CHEN[1,2].* 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Human Genome Sequencing Center,Baylor College of Medicine, Houston, TX.

As a third generation sequencing platform, Ion Torrent machine is cheaper, much smaller in size, and has faster run time compared to the current existing technology. By combing with the capture enrichment technology, it might sever as an ideal platform for molecular diagnosis. To test this idea, we have designed a custom gene capture panel that includes all currently known 167 retinal disease genes (http://www.sph.uth.tmc.edu/Retnet/). As highly heterogeneous diseases with each type of disease accounting for a small fraction of all patients, accurate diagnoses are essential for genetic counseling and matching specific treatments for each disease. To test the performance, we have enriched and sequenced a HapMap sample using Ion Torrent. A total of 3.5 million reads were generated in a model experiment. Of these, 3.2 million reads (92%) mapped to the human genome while 1.4 million reads (39%) mapped to the targeted region. Excellent coverage of the targeted region was achieved, with 93.7% of targeted bases at >10x sequence coverage. SNP genotypes for the same DNA sample were determined using the Affymetrix 6.0 SNP array. There were 1,379 variant positions within the targeted region that were genotyped by the SNP array (1205 homozygous, 174 heterozygous). Excellent genotype concordance was observed: Specifically, 1194 (99.1%) of homozygous bases and 173 (99.4%) of heterozygous bases were shared. Thus, the combination of Ion Torrent platform and disease specific panel has the potential as an ideal tool for clinic diagnosis in the near future. We are currently in the process of applying this approach to several patients with retinal diseases. Further evaluation of this approach will be reported.

1046T

Genetic Counseling: An analysis of awareness, opportunities and accessibility in India at the undergraduate level. *A. NEOGI.* BITS PILANI, India.

Introduction:As a profession,Genetic Counseling is quite new in India,with only one individual having a specialised degree in the same. This study aims to determine the prevalence of genetic counseling-in terms of awareness and opportunities,for potential graduate students from India. **Methods**:There are two parts to this study. **Part 1**: A 20 MCQ survey based on Genetic Counseling(GC)was conducted across 2 universities.75 senior students with life science related majors formed the test population.75 seniors with non-science majors formed the control. **Part 2** (request for training)was conducted in three stages.Stage 1:contacting centres which listed GC services on their website,Stage 2:contacting CGCs through the NSGC for advice and guidance and Stage 3:contacting individuals/centres referred to by the CGCs and other sources. **Results**: **Part 1**: 20% of the test population were aware of the existence of GC as a profession.This was higher than the 10% in the control group.Data excludes percentage of students who `guessed' the meaning of GC without prior knowledge. **Part 2**: Stage 1-Only 2 out of 8 listed organisations responded to a training request. Stage 2-a total of 15 CGCs were approached.2 (13.33%) of the contacted individuals replied,with referrals. Stage 3-contact was made with the 3 referrals.There was a positive response from 2 of them. **Conclusion:**On analysis,I found that the centres which provide genetic counseling and related services can be divided into 3 categories. Small or newly established centres(first category)acknowledge the need to raise awareness and are willing to offer GC related internships. Genetic testing facilities such as hospitals(second category)do not offer exact GC experience but provide exposure to cytogenetic labs to introduce techniques like FISH and diagnostic karyotyping. Well-established genetic counseling centres(third category)are very busy and difficult to reach.They are unable to provide training.Also,at the undergraduate level, no relation exists between genetics modules studied and knowledge of GC as a profession.The low level of awareness amongst students in India can be attributed to the lack of educational programs related to GC and limited access to training.These issues can be addressed if the established centres engage more in public outreach programs and include training in their services.

1047T

R143Q MUTATION OF THE POU1F1 GENE, UNDERLYING COMBINED PITUTIARY HORMON DEFICIENCY. *A. AYKUT[1], S. OZEN[2], D. GOKSEN[2], H. ONAY[1], O. COGULU[1], S. DARCAN[2], F. OZKINAY[1].* 1) Department of Medical Genetics,Medical Genetics, Ege University Faculty of Medicine , IZMIR, bornova izmir, Turkey; 2) Department of Pediatrics, Ege University Faculty of Medicine,Izmir,Turkey.

Congenital Hypotyroidism is one of the most common endocrinological problems which lead to irreversible neurological findings and growth retardation. Newborn screening is important for early diagnosis and treatment. The congenital onset of hypotyroidism may result from severely impaired or loss of function of the POU1F1 gene. Patients with POU1F1 mutations show a combined pituitary deficiency with low or absent levels of Growth Hormone (GH), prolactin and Thyroid-stimulating hormone (TSH). We present a 7 month year old girl with a combined pituitary hormone deficiency caused by a defect in POU1F1 gene. The patient, the second child of consanguineous parents was born after an unremarkable pregnancy at the 44th week of gestation by cesarean section. Her birth weight was 3200 gr. The patient was found to be positive for congenital hypothyroidism. She showed a dysmorphic appearance, including anteverted nostrils, downturned mouth, macroglossia, protruding tongue. She was hypotonic, had hypertrichosis and cutaneous hemangiomas. In addition to congenital hypothyroidism, GH and prolactin deficiencies were present. A homozygous mutation at codon 143, arginine to glutamine in patient's POU1F1 gene was detected. The parents were heterozygous for the mutation.

1048T

Glucose Transporter Type I Deficiency Syndrome epilepsy phenotypes and alternative therapies: implications for genetic testing, counseling, and treatment from the world's largest cohort. *A.W. Pong[1], K. Engelstad[1], B. Geary[1, 2], A. Natarajan[1], H. Yang[1], D.C. De Vivo[1].* 1) Neurology, Columbia University, New York, NY; 2) University of Notre Dame, Notre Dame, IN.

Background. Glut 1 deficiency syndrome or DeVivo Disease (Glut 1 DS, SLC2A1 gene, chromosome 1p34.2, MIM 606777) is defined by hypoglychorrachia with normoglycemia, acquired microcephaly, episodic movements, and epilepsy refractory to standard anti-epileptic drugs (AEDs). Gold standard treatment is the ketogenic diet (KD), which provides ketones for cerebral metabolism, thereby treating the effects of neuroglycopenia. Aims. (1)To describe epilepsy phenotypes in the world's largest Glut 1 DS cohort, to facilitate diagnosis and earlier institution of KD, thereby preventing disease progression. (2)To describe cases in which non-KD agents achieved seizure freedom (SF), to highlight potential alternative treatments. Methods. Retrospective chart review of 86 consecutive Glut 1 DS research patients (48 males:38 females, aged <1mo to 35yrs, average age of Glut 1 diagnosis at 6yrs) at the Giblin Laboratory, Columbia University, Aug 1989 to Dec 2010. Findings. 78/86 (90.5%) patients had epilepsy, with average onset at 7 months. Seizure types included: absence, simple/complex partial, generalized tonic-clonic, myoclonic, tonic, drop seizures, infantile and epileptic spasms. We describe the first 2 cases of infantile spasms at 6-9 months, and epileptic spasms at 23 months. 68% (53/78) had >1 seizure type, with generalized tonic-clonic (41/77) and absence (39/77) seizures most commonly. Electrophysiological abnormalities were highly variable over time and only 13/75 (17%) had exclusively normal findings. KD was used in 82% (64/78); 67% (41/61) were seizure free, 2/3 resolved within a week (68 %, 28/41) and ¾ within a month 76% (31/41). SF without KD occurred in 7 patients treated with VPA(1), LTG(1), ZNS and LTG(1), TPM(2), and modified KD(1). Conclusions. Glut 1 DS is a genetic metabolic encephalopathy with multiple neurological symptoms. Variable seizure types and EEG findings, including focal or multifocal findings, may be seen. Infants with seizures or paroxysmal events should be tested for Glut 1 DS. Glut 1 DS may manifest with infantile and epileptic spasms, and it is not clear how often the diagnosis may be missed. Evidence is insufficient to recommend specific AEDs as alternatives to KD. Early diagnosis and initiation of KD and prevention of unnecessary, potentially deleterious, AED trials in Glut1 DS are important goals for the medical community treating children with epilepsy. Will & C.Giblin Fdns, 5RO1NS37949, Milestones for Children.

1049T

Developing a Next-Generation Sequencing Gene Panel for Molecular Diagnosis of Genetic Epilepsy. *F. Xia, P. Stankiewicz, M. Vatta, C.M. Eng, Y. Yang.* Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Epilepsy is a common condition affecting more than three million Americans, many of whom have an underlying genetic cause. The list of possible genes for epilepsy has grown significantly during past decades, demonstrating extensive locus and phenotypic heterogeneity for the disorder. Testing a large number of genes in a patient by Sanger sequencing is often necessary but cost-prohibitive, labor-intensive and therefore impractical in clinical laboratories. In recent years, next generation sequencing (NGS) has emerged as a possible revolutionary force to change the paradigm of conventional genetic testing by offering high throughput and inexpensive DNA sequencing. Here we report the NGS analyses of a panel of 32 genes mostly associated with genetic epilepsy. The targeted region of 116 kb includes all the coding exons of the 32 genes as well as 15 bp intronic regions upstream and downstream of the coding exons. RainDance mega-droplet multiplex PCR and two-step tailed primer enrichment systems were utilized for target enrichment and addition of sequencing adaptors, bypassing the library construction steps. More than 1600 unique PCR products of 200 bp or shorter were amplified from the enrichment step and sequenced on the Illumina Hiseq 2000 platform. Approximately 13 Gb of sequencing data from a multiplex of seven samples were obtained from a single Hiseq lane. Aligned sequence data showed that about 77% of the reads are on-target and the average coverage across the panel is greater than 5000X with more than 97% of the reads covered 100x or more. Three control samples previously deep sequenced by the 1000 Genome Project were included in the development. By comparing the variant calls from our data with those from 1000 Genome Project, we observed a sensitivity of greater than 99% for our assay. False positives were observed , which are most likely caused by uneven distribution- of the data quality and coverage across the PCR amplicons and are expected to be reduced by using better data analysis algorithm and improved Illumina Sequencing-By-Synthesis chemistry.

In summary, the development of the NGS epilepsy gene panel will be a novel and improved clinical tool for identifying the genetic basis of the condition and contributing to our understanding of the phenotypic range of these disorders for counseling, education, and possibly the development of new treatments.

1050T

Parental attitudes toward the disclosure of individual genotype results for children enrolled in a gene discovery protocol. *J.C. Sapp[1], D.A. Dong[1,2], B.B. Biesecker[3], L.G. Biesecker[1].* 1) Genetic Diseases Research Branch, National Human Genome Research Institute, Bethesda, MD; 2) Genzyme Corporation, Cambridge, MA; 3) Social and Behavioral Research Branch, National Human Genome Research Institute, Bethesda, MD.

The number of rare disorders whose molecular etiologies have been determined using whole genome sequencing (WGS) continues to expand. Although significant and largely theoretical debate exists with respect to the return of individual genotype results to participants enrolled in research protocols employing WGS, little is known about participants' own attitudes towards and preferences regarding the return of results. Understanding and characterizing the personal meaning participants ascribe to receiving individual genotype results is a primary aim of our protocol, which seeks to identify the causative genetic variants of rare and ultra-rare phenotypes using massively parallel (primarily exome) sequencing of individuals and/or families and employs a qualified disclosure policy. Because most probands with rare disorders are children, parents of minor children enrolled in our protocol are asked to participate in qualitative, semi-structured interviews designed to ascertain and understand their attitudes toward WGS, the way they approach decision-making about different categories of results, and their preferences regarding which (if any) results they would wish to receive for their children. Preliminary analysis of the first dozen interviews suggest that parents consenting to WGS to determine the genetic cause of their child's condition approach this decision with attitudes ranging from deep ambivalence to hopeful excitement. They understand the complexity and scope of WGS and frame their preferences about learning many kinds of individual variant results in terms of a perceived possible benefit to their children. Although they express reservations about some categories of results, such as those that increase disease predisposition, most view the idea of choosing to not learn any available information researchers are willing to share with them as much less desirable and would prefer to learn any and all results generated. Interviews will be conducted until data saturation and we will describe our ongoing analysis and the potential implications of our results for the evolving debate about disclosure policies for WGS protocols.

1051T

Fragile X AGG genotyping reclassifies risk for expansion in intermediate and small premutation carriers: Results of a multicenter study of 469 mother-child transmissions. *S.L. Nolin[1], E. Allen[2], A. Glicksman[1], S.L. Sherman[2], E. Berry-Kravis[3], F. Tassone[4], C. Yrigollen[4], A. Cronister[5], M. Jodah[5], N. Ersalesi[1], W.T. Brown[1], R. Shroff[6], S. Sah[6], G.J. Latham[6], A.G. Hadd[6].* 1) New York State Institute for Basic Research in Developmental Disabilities, Dept of Human Genetics, Staten Island, NY; 2) Emory University, Dept of Human Genetics, Atlanta, GA; 3) Rush University Medical Center, Chicago, IL; 4) UC Davis and MIND Institute, Davis, CA; 5) Esoterix Genetic laboratories LLC, Westborough, MA; 6) Asuragen, Inc., Austin, TX.

Screening pregnant women to determine their fragile X status has become increasingly common in recent years resulting in the identification of intermediate and small premutation alleles whose stability is unknown. Earlier studies have suggested that the presence of AGGs interspersed within the repeat and the length of uninterrupted CGGs at the 3' end of the repeat may influence repeat expansion. Until now however, the presence of the second X chromosome in females has prevented analysis of the AGG pattern in the expanded allele. An elegant new PCR assay has allowed us to examine the effect of the FMR1 CGG repeat structure on repeat instability. Our study investigated whether the AGG patterns and the length of uninterrupted CGGs at the 3' end of the repeat predicts instability in newly identified intermediate and small premutation alleles with 45 to 69 repeats. Using this new PCR technology, the FMR1 repeat structure was determined for 469 mother-child transmissions from 4 centers. We found that the number of AGG interruptions and the length of 3' continuous CGG repeats were correlated with repeat instability on transmission. The shortest unstable 3'-length was 25 uninterrupted CGGs (in a 45 repeat allele); all alleles with 61 or more 3' uninterrupted CGGs expanded. The expansion risks for alleles with 50-54 repeats was 100% for those without AGGs, but only 28% for alleles with 1 AGG, and 11% with 2 AGGs. Moreover, the magnitude of repeat expansion was larger for alleles that lacked an AGG interruption. The smallest number of uninterrupted CGG repeats that expanded to a full mutation in a single transmission was 51 (of 61 total repeats). These results indicate that AGG genotyping will allow risk estimates to be established for repeat instability in newly identified intermediate and small premutation alleles.

1052T

Screening for LHON mutations in Brazilian patients. *E.L. Sartorato[1], P.M.A.D. Miranda[1], M.S.A. Fernandes[2], A.T. Maciel-Guerra[3].* 1) CBMEG, UNICAMP, Campinas - SP, Campinas, Brazil; 2) Departamento de Oftalmologia - FCM, UNICAMP, Campinas - SP, Campinas, Brazil; 3) Departamento de Genética Médica - FCM, UNICAMP, Campinas - SP, Campinas, Brazil.

Leber Hereditary Optic Neuropathy (LHON) is a mitochondrial disease characterized by sudden loss of vision in both eyes, due to optic nerve degeneration. Currently, 17 main LHON associated mutations were published, three of which account for 95% of the cases (primary mutations) and fourteen different mutations account for only 5% of the total (secondary mutations). There is no official data about the frequency of LHON mutations in Brazilian patients. Screening of LHON mutations is important for confirmation of clinical diagnosis, provide prognostic information and more appropriate genetic counseling. Therefore, the aim of this study was to define the LHON mutations frequency in Brazilian patients. We evaluated 55 patients with LHON diagnosis or acquired optic neuropathy of unknown origin. Primary mutations (G11778A, T14484C and G3460A) and secondary mutations were screened. Primary mutations were screened by the method of enzyme restriction and secondary mutations by direct sequencing. Primary mutations were found in 19 patients. The frequencies found were 67% for the G11778A mutation (13 cases), 28% for the T14484C mutation (5 cases) and 5% for the G3460A mutation (only 1 case). The G11778A mutation was more frequent, as well as in others parts of the world. However, the frequency of T14484C mutation was higher while the G3460A mutation frequency was lower. No secondary mutation was found. The absence of these mutations can be attributed to the presence of mutations in regions not analyzed in this study. Analyze molecular allowed us to confirm LHON diagnosis in 38% patients studied.

1053T

How do young adults with Leber congential amaurosis perceive gene therapy? *M.P. Napier[1], J.E. Sutherland[2], M.A. Day[2], R.Z. Hayeems[3], D. Chitayat[1,4,5,6,7], C. Shuman[1,7], E. Héon[2,8].* 1) Department of Molecular Genetics, The University of Toronto, Toronto, Canada; 2) Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, Toronto, Canada; 3) Department of Health Policy, Management and Evaluation, The University of Toronto, Toronto, Canada; 4) Department of Paediatrics, The University of Toronto, Toronto, Canada; 5) Department of Obstetrics and Gynecology, The University of Toronto, Toronto, Canada; 6) Department of Laboratory Medicine and Pathobiology, The University of Toronto, Toronto, Canada; 7) Department of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, Canada; 8) Department of Ophthalmology and Vision Sciences, The University of Toronto, Toronto, Canada.

Introduction: Successful gene replacement therapy for Leber congenital amaurosis (LCA) has been demonstrated in a canine model. The impressive findings in both safety and efficacy from these studies have led to ongoing human clinical trials for a subset of patients with LCA. To effectively integrate such advances into clinical care, it is important to evaluate potential candidates' expectations, decision-making processes and concerns regarding gene therapy. To this end, this study utilized a qualitative exploratory approach to elicit perceptions regarding gene therapy among individuals who might consider this type of therapy.

Methods: Young adults with a clinical diagnosis of LCA were recruited through the Ocular Genetics Programme at the Hospital for Sick Children. Semi-structured telephone interviews were conducted with 10 patients. These interviews were audio-taped and analyzed in accordance with the principles of qualitative description.

Results: Study participants were aware of ongoing gene therapy research trials and actively sought information regarding advances in ophthalmology and vision restoration. However, differences in opinion were expressed regarding the value of enrolling in clinical trials; two patients are currently enrolled in a gene-replacement therapy trial, five indicated that they would participate if given the opportunity, two were ambivalent, and one would not participate if given the opportunity. Participants' expectations regarding visual improvement varied and influenced their willingness to take part in research trials. However, intrinsic factors also influence decision-making including coping strategies and adaptation to vision loss, and personal resilience.

Conclusions: This is the first qualitative study to explore patients' perspectives on gene replacement therapy clinical trials. The results of this study highlight the complex factors that are involved in gene-therapy related decision-making and provide a framework for health care providers to use when counselling individuals considering gene therapy.

1054T

Unprogrammed presentation number

1055T

Using Next Generation Sequencing as a clinical diagnostic tool for autism. *T. Brandt[1], O. Jabado[1], S. Yoon[2], V. Makarov[2], Z. Peralta[1], R. Kornreich[1], J. Buxbaum[2], L. Edelmann[1].* 1) Gen & Genomic Sci, Mount Sinai, New York, NY; 2) Psychiatry, Mount Sinai, New York, NY.

The underlying genetic causes of autism are diverse and, consequently, many patients undergo a battery of diagnostic tests. Recently, a large set of individual autism genes has been identified and a prevailing theory is that many different rare mutations underlie the genetic etiology of autism. However, sequencing for many of these newly discovered genes is not yet clinically available. Next generation sequencing (NGS) technologies have made the interrogation of a large number of genes in a single experiment possible, and have made comprehensive sequencing of autism genes clinically feasible. A panel of cytogenetic and molecular genetic tests is being established as a clinical algorithm to offer the highest possible diagnostic yield while minimizing findings of uncertain significance. NGS, though widely used for research, is a relatively new technology for clinical laboratories. As such, the focus of this project is the validation of this tool for diagnostic use with special attention to the false negative and false positive rates as well as the cost effectiveness of sample pooling for this larger scale sequencing project. We are using a pooled targeted sequence enrichment strategy followed by multiplexed Illumina sequencing to interrogate the 33 autism genes with the strongest support in the literature (~200 kb). In addition, we are validating NextGENe commercial NGS analysis software (by Softgenetics, State College, PA) for clinical use by multiple approaches: comparison with an in-house analysis pipeline centered on BWA, SAMTools, and GATK, Sanger sequencing of all calls, and comparison with known calls in HapMap samples and samples from The 1,000 Genome Project. Our approach, clinical considerations, and progress will be outlined.

1056T

Novel Mutations in Iranian Rett syndrome patients. *M. Dehghan Manshadi[1], S. Dadgar[1], O. Ariyani[1], P. Karimzadeh[2], SH. Salehpour[2], H. Tonkaboni[2], M. Houshmand[1, 3].* 1) Genetic Department of Special Medical Center, Tehran, Iran; 2) Shahid Beheshti University of Medical Science, Tehran, Iran; 3) National Institute of Genetic Engineering & Biotechnology, Tehran, Iran.

MECP2-related disorders include classic Rett syndrome, variant or atypical Rett syndrome, and mild learning disabilities in females and neonatal encephalopathy and mental retardation syndromes in males. Classic Rett syndrome is a progressive neurologic disorder in girls characterized by normal birth and apparently normal psychomotor development during the first six to 18 months of life. The girls then enter a short period of developmental stagnation followed by rapid regression in language and motor skills. Seizures occur in up to 90% of affected females; generalized tonic-clonic seizures and partial complex seizures are the most common. Females with classic Rett syndrome typically survive into adulthood, but the incidence of sudden, unexplained death is significantly higher than in controls of similar age. Atypical Rett syndrome is increasingly observed as MECP2 mutations have been identified in individuals previously diagnosed with autism, mild learning disability, clinically suspected but molecularly unconfirmed Angelman syndrome, or mental retardation with spasticity or tremor. Rett syndrome is inherited in an X-linked dominant manner. Approximately 99.5% of cases are single occurrences in a family, resulting either from a de novo mutation in the child with Rett syndrome or from inheritance of the disease-causing mutation from one parent who has somatic or germ line mosaicism. PCR amplification and Sequencing of the three axons of MECP2 gene coding region performed in our research.

1057T

Creating community dialogues; Exploring public opinions about genetics research in Newfoundland Labrador. *E. Dicks, H. Etchegary, J. Green, D. Pullman, C. Street, P. Parfrey.* Clinical Epidemiology Unit, Health Sciences Ctr, St John's, NF, Canada.

Background: Continued advances in genetics have prompted calls for greater public debate and involvement in decision-making about genetics research. Newfoundland Labrador, an island province in Canada is well known as a site of genetics research; however, relatively little work has explored public opinion and knowledge about genetics. We undertook community consultations to better understand how the Newfoundland public perceives various aspects of genetic research. Objectives: To explore public knowledge and values in order to foster greater public involvement in decision-making about genetics research and genetics health services To better understand how the public perceives genetics research so that practitioners can improve existing genetics services Methods: Community groups were identified in St. John's and Grand Falls-Windsor and invited to participate in a two-hour community consultation about genetics research in the province. Six sessions were completed between October 2010 and April 2011, with at least two others planned. Community groups included Rotary Clubs, church groups and women's groups, representing a broad cross-section of the general public (N=90). During the sessions, participants heard a presentation highlighting some of the research of the Interdisciplinary Research Team in Human Genetics at Memorial University. The presentation was followed by a facilitated discussion designed to explore some complex issues raised by genetics research (e.g., the privacy of genetic information, funding for genetics research). Participating groups thus far expressed great satisfaction with the sessions and suggested such presentations should be held regularly. Participants engaged with many of the complex issues raised by genetics research, noting the importance of such research for improving health, but also raising concerns about privacy, access and lack of knowledge about what research is happening in the province. Results to date suggest that the richness and diversity of public opinion could be a valuable resource for critical discussion and more accountable policy-making about genetics research in Newfoundland.

1058T

Validation of open-source colorectal cancer risk assessment software compatible with the U.S. Surgeon General's My Family Health Portrait tool. *W.G. Feero[1, 2], F.M. Facio[3], E. Glogowski[4], H. Hampel[5], J. Stopfer[6], A. Linn[3], D. Barton[3], L.G. Biesecker[3].* 1) Maine-Dartmouth Family Medicine Residency, Fairfield, ME; 2) Genomic Healthcare Branch, NHGRI, NIH Bethesda, MD; 3) Genetic Disease Research Branch, NHGRI, NIH, Bethesda, MD; 4) Memorial Sloan-Kettering Cancer Center, New York, NY; 5) Division of Human Genetics, The Ohio State University, Columbus, OH; 6) Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA.

Background -Family history (FH) is an acknowledged means for identifying the heritable component of an individual's cancer risk. The U.S. Surgeon General's My Family Health Portrait (MFHP) tool has been validated for the collection of family history information, but currently does not provide cancer risk assessment. Here we report on a newly developed open-source software tool designed to screen for heritable colorectal cancer (CRC) risk that is compatible with MFHP. Methods- A CRC risk assessment tool was developed using 2009 NCCN and USPSTF CRC risk assessment guidelines. The tool was designed to use patient-entered family history information to dichotomize individuals into not-elevated or elevated CRC risk categories, and provides basic age and risk-appropriate recommendations for CRC screening to all groups. The tool was validated on 150 pedigrees consecutively derived from the ClinSeqTM population. Risk assessments derived from MFHP were compared to two alternative “gold standards” for detecting risk applied to the same pedigrees: the CDC's Family HealthwareTM (FHT) CRC risk algorithm; and evaluation by 3 expert cancer genetic counselors (GC). Results- The MFHP, FHT, and GC evaluations identified 27, 14, and 16 elevated-risk probands, respectively, in the cohort. There was substantial agreement between GC CRC risk evaluations (average weighted kappa = 0.69 ± 0.04); two of three counselors had to agree that risk was elevated for a pedigree to be scored as such. Using different “gold standards” for the identifying CRC risk, sensitivities, specificities, positive predictive values (PPV) and negative predictive values (NPV) were: MFHP versus FHT, 100%, 90%, 52% and 100%; MFHP versus GC, 81%, 90%, 48% and 98%; FHT versus GC, 75%, 98%, 86% and 97%. Conclusions- The newly created software compatible with MFHP provides a tool for screening for heritable CRC risk. The sensitivities and specificities for MFHP derived using GC pedigree review as the “gold standard” for risk detection are not dissimilar from those of other types of screening tools used in primary care. The new tool has a higher sensitivity but lower specificity than FHT when compared to expert GC evaluation of CRC risk. The apparent “false positive” rate of the software is high; further research is needed to determine if this is a result of actual false positives or differences in risk assessment guidelines used by MFHP, FHT and cancer GCs.

1059T

ACTN3 R577X genotype and sport performance in Roma/Gypsies. *D. Gabrikova[1, 2], D. Hronska[2], S. Macekova[1, 2], J. Bernasovska[1, 2], A. Sovicova[1, 2], A. Bozikova[1, 2].* 1) Excellence Centre of Animal and Human Ecology, University of Presov, Presov, Slovakia; 2) Department of Biology, Faculty of Humanities and Natural Sciences, University of Presov, Presov, Slovakia.

In Europeans the XX genotype of ACTN3 gene polymorphism R577X (rs1815739) is less common among sprint/power athletes compared to general population. Previous studies have shown association between ACTN3 genotype and sprint/power or endurance performance, although not consistently. We examined the distribution of ACTN3 R577X genotypes and relationship between this polymorphism and physical performance in the Roma/Gypsy children. We tested 212 children from a community with very low socioeconomic status and with very little to absent sport activity, training and motivation. A sport performance of children aged 7-14 was tested in 4 sprint/power and 3 endurance disciplines. We did not find any association of the ACTN3 R577X genotype with performance in sprint/power disciplines. Out of endurance tests, the association was significant in flexed arm hang test. We found that the frequency of the XX genotype (13,3%) was significantly lower in Roma population than in general European population.

1060T

Clinical Genomics Data Infrastructure and ClinVar. *U. Geigenmüller[1], D. Maglott[2], S. Aradhya[3], S. Bale[3], P.R. Billings[4], C. Braastad[5], M. Eisenberg[6], M.J. Ferber[7], K. Fuentes Fajardo[8], M. Hegde[9], B. Kattman[2], S.F. Kingsmore[10], I.S. Kohane[11], D.H. Ledbetter[12], K. Lee[11], E. Lyon[13], C. Lese Martin[9], N.A. Miller[10], J. Ostell[2], J. Paschall[2], H.L. Rehm[14], G. Riley[2], C.J. Saunders[10], S.T. Sherry[2], E.D. Trautman[6], V. Zvereff[6], D.M. Margulies[15].* 1) Channing Laboratory, Brigham and Women's Hospital, Boston, MA; 2) National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; 3) GeneDx, Gaithersburg, MD; 4) LIFE Technologies, Carlsbad, CA; 5) Athena Diagnostics, Worcester, MA; 6) LabCorp/CMBP, Raleigh, NC; 7) Mayo Clinic, Rochester, MN; 8) NIH/NHGRI/OCD/Undiagnosed Diseases Program, Bethesda, MD; 9) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA; 10) Children's Mercy Hospital, Kansas City, MO; 11) Center for Biomedical Informatics, Harvard Medical School, Boston, MA; 12) Geisinger Health System, Danville, PA; 13) University of Utah Pathology Department/ARUP Laboratories, Salt Lake City, UT; 14) Harvard Medical School, Boston, MA; 15) Correlagen Diagnostics, Waltham, MA.

Both cost and technical difficulty of sequencing large regions of the genome are dropping to a level that enables its routine use in patient care. Limiting the adoption of large-scale sequencing in molecular diagnostics is the difficulty of assessing the clinical significance of detected variants. Each testing laboratory typically performs its own interpretation based on local primary data and expertise in addition to publicly available information. This approach prevents laboratories, physicians, and patients from capitalizing on community expertise and experience and may lead to inconsistent interpretations between different laboratories. Data on variants detected in testing laboratories should be made readily available to the community. Furthermore, it would benefit patients and their physicians to have ready access to each laboratory's interpretation for any given variant. Convergence among interpretations from different laboratories would increase confidence in the consensus interpretation, while divergence would indicate uncertainty and offer opportunity to sharpen the scoring algorithms. A group of major commercial and academic laboratories and bioinformatics groups have come together to support NCBI's ClinVar database as a `pre-competitive' repository of DNA sequence variation detected in the course of clinical diagnostics. The group is establishing procedures and technologies for the sharing of variants, defining critical data parameters required to evaluate pathogenicity of these variants, and working with NCBI to ensure these data can be stored and freely exchanged through ClinVar. Also, a code of conduct is being developed to assure priority for matters relating to patient safety. By making all evidence that underlies variant interpretation freely available, participants in this initiative hope that one of the major barriers to widespread adoption of extensive DNA diagnostic sequencing will be reduced.

1061T

Assessment of clinical usefulness of genetic diagnosis in neurology department of hospital. *Y. Ichikawa, H. Ishiura, J. Mitsui, T. Matsukawa, Y. Takahashi, H. Naruse, M. Taira, S. Tsuji, J. Goto.* Dept Neurology, Univ Tokyo, Tokyo, Japan.

Background: Molecular genetics has revealed many disease genes for neurological diseases, and genetic diagnosis contributes largely to clinical practice in neurology. The more disease genes are identified, the more the role of genetic diagnosis expands in neurology. **Objectives:** To assess the usefulness of genetic diagnosis of neurological diseases **Patients and Methods:** A total of 1392 hospitalized patients of the neurological division in the University of Tokyo Hospital during the period 2006 and 2010 were reviewed. After excluded the patients suffering from stroke or spondylosis or infectious disease, and the admissions for clinical trials, totally 942 subjects were analyzed retrospectively. We applied genetic analysis for subjects as follows; patients with positive family history or those with consanguinity, including spinocerebellar ataxias, spastic paraplegia, myopahty, juvenile parkinsonism or early onset dementia (< 60 yr.). The analysis methods included dideoxy-nucleotide sequence analysis, microarray-based resequncing, array CGH, fragment analysis, and SNP homozygosity mapping. **Results:** The total number of subjects who were analyzed by molecular genetic analysis were 165 (17.5%) among the 942 subjects. Eighty four patients, approximately half of the 165 subjects (50.9%), were diagnosed by genetic analysis. The highest number of the 84 genetically diagnosed patients were spinocerebellar ataxia patients (18 patients: 21.4%) which consisted of 17 autosomal dominant cerebella ataxia (ADCA) patients and one Nepalese Friedreich's ataxia patient. The second share of the genetically diagnosed patients was adrenolekodystrophy (9 patients: 10.7%), followed by Huntington disease (HD, 6 patients: 7.1%), spastic paraplegia (SPG) and Parkinson disease 2 (respectively 5 patients: 6%). Rare diseases such as neuroferritinopathy, vanishing white matter disease, Alexander disease and Hutchinson-Gilford progeria were also diagnosed. Among 27 genetically diagnosed autosomal dominant disease patients (ADCA, HD, SPG), 5 patients' family histories were unknown and had been considered to be sporadic (18.5%). **Conclusion:** Genetic analysis was needed to be applied to 17.5 % of the 942 subjects, and diagnosis of the half of them was determined genetically. Among sporadic cases, there were often patients suffering from hereditary diseases. Rare hereditary diseases were also experienced. Contribution of genetic diagnosis to practice of neurology is important.

1062T

A nine-year experience with the genetic testing of the rare disease acrodermatitis enteropathica. *S. KURY[1], S. SCHMITT[1], M. GIRAUD[1], C. TESSON[1], F. AIRAUD[1], B. DRENO[2], M. KHARFI[3], S. BEZIEAU[1].* 1) CHU Nantes, Service de Génétique Médicale, Nantes, France; 2) CHU Nantes, Service de Dermatologie, Nantes, France; 3) Charles Nicolle Hospital, Service de Dermatologie, Tunis, Tunisia.

Background: Acrodermatitis enteropathica (AE; MIM #201100) is a rare and severe zinc deficiency disorder, transmitted in an autosomal recessive mode. It is characterized by three pathognomonic symptoms occurring at birth or after weaning: acral and periorificial dermatitis, alopecia and diarrhea. In 2002, we and others identified SLC39A4 as the AE-causing gene, the mutation of which induced a defective intestinal zinc transport. Since then, a free routine testing of SLC39A4 was proposed in our laboratory to patients suspected of having an AE. Purpose: We report here the results of nine years of genetic testing in patients diagnosed worldwide with AE. We present the main difficulties inherent to the management of a rare disease, and the perspectives offered by the increasing knowledge on zinc transport. Method: In the first place, germinal mutations and rearrangements were screened in patients' genomic DNA by Sanger sequencing and QMPSF of the twelve exons and their splicing sites. Then anomalies of the untranslated and intronic regions were examined. Additional analysis of other candidate genes (SLC30A2, SLC39A5, SLC304A4 or BTD) were performed in selected cases. Results: Up today, we have tested 200 DNA samples, including 97 index cases. Twelve analyses are ongoing or suspended because of a lack of signed informed consent. In the 85 remaining patients, a mutation or a very likely deleterious variant was found in 41 patients: 34/85 (40%) patients were found homozygotes or compound heterozygotes, whereas 7/85 (8.2%) patients were found heterozygotes only. In 44/85 (51.8%) patients no mutation was found at all; misdiagnosis could explain a few negative results, and a few other cases are probably acquired acrodermatitis, but for most cases the influence of a genetic factor cannot be definitively ruled out. Conclusions: The genetic basis of AE is not fully elucidated, because the autosomal mode of inheritance can be questioned by segregation analysis in certain families. In addition, the observation of cases very suggestive of AE with no mutation identified in SLC39A4 lends credence to the hypothetical inovolvement of regulating factors and/or other genes in the pathogenic mechanism of AE. A comprehensive exome sequencing strategy is considered on genes participating in zinc metabolism.

1063T

Establishment and application of a standard diagnostic procedure for epidermolysis bullosa in China. *Z. Lin, Q. Chen, M. Lee, Y. Tan, Y. Yang.* Dermatology, Peking University First Hospital, Beijing, China.

Background: Epidermolysis bullosa(EB) represents a group of inherited skin disorders with recurrent mechanically-induced skin or mucosa bullae. With the great progress achieved in the molecular genetics during the past 20 years, nearly all the subtypes of EB can be diagnosed through genetic testing. However, in China, where the genetic testing is not widely performed, accurate diagnosis of EB under the molecular level still needs to be established. Object: To set up a standard diagnostic procedure for EB in China and to adopt it in diagnosis of EB under a molecular level. Methods: We set up an electronic database for managing the EB patients' information. Transmission electron microscopy and antigen mapping were performed to determine the level of cleft and potential defective components of dermo-epidermal basement membrane zone. Mutation analysis was followed to confirm the final diagnosis. Results: With this standard procedure, 19 out of 21 patients with EB were diagnosed based on an accurately gene mutation analysis during the past two years, including 4 cases of EB simplex, 7 cases of junctional EB, 6 cases of dystrophic EB and 2 cases of Kindler syndrome. We detected 23 novel mutations involving 7 different causative genes. Conclusions: A standard diagnostic procedure for EB is necessary in China which may notably improve the accurate diagnosis rate under a molecular level.

1064T

The Genetic Testing Registry (GTR): Genetic Tests and More. *D. Maglott[1], B.L. Kattman[1], A. Malheiro[1], J. Lee[1], M. Ovetsky[1], V. Hem[1], V. Gorelenkov[1], W. Rubinstein[1,2], C. Fomous[3], J. Ostell[1].* 1) Natl Ctr Biotech Info, NIH/NLM, Bethesda, MD; 2) University of Chicago Medical Center, Chicago IL; 3) Office of the Director, NIH, Bethesda, MD.

Scientific advances—particularly in the last decade—have expanded our understanding of the genomic and genetic factors involved in health and disease. This increased knowledge has been accompanied by a rapid rise in the number and complexity of genetic tests. Laboratory tests for more than 2,000 genetic conditions are now available, but there is no comprehensive public resource that provides detailed information about the scientific basis of these tests. Enhancing access to detailed test information is important to enable informed decision-making by health care providers, patients, caregivers, clinical laboratory professionals, payers, and policymakers; to assist regulators; and to facilitate research. To address the information gap about genetic tests, NIH developed the Genetic Testing Registry (GTR).

The GTR (http://www.ncbi.nlm.nih.gov/gtr) is a public, online resource integrating information from voluntary submissions by testing entities. After consultation with multiple advisory groups and interested stakeholders, we developed a list of the minimal and optional fields necessary to describe each submitted test. The minimal fields include what the test measures, the methods used, and contact information for the submitting laboratory. The GTR user interface combines flexible search functions with links to related medical and molecular resources. Searches may be general, or may focus on conditions (including drug responses), genes, test methods, analytes, or laboratories. Tests retrieved by a search can be compared by condition, measure, or methodology to facilitate identification and selection of a single orderable test. Search functions are integrated with well-known medical genetic resources such as GeneReviews, OMIM®, PubMed, PubMedCentral, ClinicalTrials.gov, as well as more recent sites at NCBI, namely MedGen, and ClinVar. Both MedGen and ClinVar support GTR, the former to organize information about genetic disorders and the latter to represent the clinical significance of rare variations. Submitters to GTR can register their tests using tools that integrate (1) information they may have supplied to GeneTests (http://www.ncbi.nlm.nih.gov/sites/GeneTests) with (2) information about medically relevant variations accessioned in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar) and (3) pertinent practice guidelines. This presentation will demonstrate content and navigability of the GTR.

1065T

EuroGentest Clinical Utility Gene Cards - concise guidelines for genetic testing in 300 diseases. *J. Schmidtke, A. Dierking.* Dept Human Gen, Medizinische Hochschule, Hannover 61, Germany.

EuroGentest, an EU-funded project for the harmonization of genetic testing, has started its second funding period (2011-2013). Unit 2, entitled “Genetic testing as part of health care”, holds amongst others the initiative for the establishment of Clinical Utility Gene Cards (CUGCs). CUGCs are disease-specific and expert-authored guidelines dealing with the risks and benefits of the application of genetic tests in the clinical setting. Clinical utility is an important aspect of genetic testing, but it is also difficult to define. Based on the ACCE framework the here presented guidelines give a balanced summary of the analytical and clinical validity, the clinical utility and cost-benefit issues of genetic tests. Hereby it is important that the specific requirements for a test are evaluated in the context of their impact on the clinical setting and that the laboratory genetic test is only one of the components of an overall intervention. The documents contain following information: disease characteristics (name, mutational spectrum and analytical methods), test characteristics (analytical and clinical sensitivity and specificity) and clinical utility (disease management, cost effectiveness and risk assessment). Details about the clinical setting are presented in four sections: differential diagnostics, predictive testing, risk assessment in relatives and prenatal testing. Each CUGC is authored by a multinational expert team. Potential authors are identified based on their practical experience and their publication record. Subsequent to the completion the documents are peer-reviewed and published by the European Journal of Human Genetics (EJHG). EuroGentest commissions the establishment as well as the annual update of the guidelines. Due to the standardised and concise format, CUGCs offer quick guidance to all stakeholders, including clinicians, clinical geneticists, referrers, service providers and payers. To ensure that all published documents include new developments and findings and reflect the state-of-the-art, all published CUGCs are annually revised. Each CUGC is freely available on the websites of EuroGentest, the EJHG, the European Society of Human Genetics and Orphanet. Until now 40 CUGCs have become citable publications, by the end of 2013 at least 300 CUGCs are intended to be published.

1066T

Patients' feelings and experience towards predictive genetic testing for primary open angle glaucoma. *E. Souzeau[1], K. Burdon[1], A. Dubowsky[2], J.E. Craig[1].* 1) Dept of Ophthalmology, Flinders University, Bedford Park, SA, Australia; 2) Institute of Medical & Veterinary Science, Flinders Medical Centre, Bedford Park, SA, Australia.

Rationale: Primary open angle glaucoma (POAG) is a sight threatening condition, affecting 3% of the population over the age of 50 years. Myocilin mutations are identified in 3-4% of POAG cases and are inherited in an autosomal dominant fashion. Unaffected relatives of Myocilin patients face a very high risk of carrying the familial mutation and developing glaucoma blindness. Cascade testing for relatives of individuals with Myocilin mutations has been shown to be feasible and effective in identifying at risk individuals and preventing glaucoma blindness. However, little is known about individuals' experience and impact of predictive testing for a sight threatening, but treatable condition like glaucoma. **Methods**: Index cases were recruited through The Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG). Predictive testing is offered to family members of individuals with a Myocilin mutation identified through ANZRAG. Individuals who had cascade testing and obtained their genetic result were sent a questionnaire, regardless of their result. The questionnaire focused on pre-test perceived glaucoma and genetic result risks, motivations for being tested and negative and positive feelings about being tested. **Results**: The questionnaire was sent to 30 individuals who underwent predictive genetic testing, 15 of whom were found to carry the familial Myocilin mutation. Our results show that, before being tested, the majority of our participants evaluated their risk of developing glaucoma and their risk of carrying the familial mutation as being high. This provides an insight into their decision to be tested. Most participants were motivated by the ability to take actions based on their result and the ability to provide information to their children regarding their glaucoma risk. Participants discussed their decision to be tested and their results with their family as well as their children if they were old enough. Regardless of their result, all the participants found predictive testing for glaucoma to be beneficial and none of them expressed regrets about being tested. **Conclusion**: These findings give health professionals some insight about patients' motivations and feelings for glaucoma predictive testing as well as some guidance for providing adequate support to these individuals in families carrying Myocilin mutations.

1067T

Tracing ancestral depth of families diagnosed with hereditary gastrointestinal polyps and cancers. *T.M. Tuohy[1], M.C. Done[1], N. Sargent[2], D.W. Neklason[1, 2], R.W. Burt[1, 3].* 1) High Risk Cancers Clinic, Huntsman Cancer Inst, Salt Lake City, UT; 2) Department of Oncological Sciences, Huntsman Cancer Inst, Salt Lake City, UT; 3) Department of Medicine, Univerisity of Utah, Salt Lake City, UT.

Heritable diseases are commonly the results of familial mutations, tracking back through several generations of the same family, to the “founder” in whom the mutation first arose. In conditions where the majority of cases derive from inherited rather than new mutations, it is possible to “tag” the allele on which the mutation arose, using linked markers such as STRs or SNPs. The cohort chosen for this study was ascertained over a ten-year period and included individuals enrolled both before and after clinical genetic testing became increasingly routine, as well as those who do not pursue clinical genetic testing. A two-step method of tagging the suspect locus, accompanied by cross-referencing it to a database of similarly tagged, clinically genetically tested alleles may provide a cost-effective way to enrich the cohort of uncharacterized samples for site-specific candidates carrying individual mutations. Research PCR and sequencing of the candidate matches may then be verified by clinical testing for the indicated site-specific mutation. The additional benefit of connecting distant relatives, and by extension previously unknown branches of such families serves simultaneously as a clinical ascertainment tool, for referral for standard of care, and as a resource for continued research with cohorts of patients and participants who share common genetic origins. We characterized 451 samples from clinically and/or genetically diagnosed individuals in 210 kindreds at 4 highly polymorphic STR markers spanning 3 Mb of the APC locus associated with familial adenomatous polyposis coli (FAP). A genetic diagnosis was previously known for 62 kindreds (database), while only a clinical diagnosis of FAP, AFAP, “>6 adenomas”, “diagnosed with colon cancer”, “hyperplastic polyps”, “family hx of colon cancer”, or “undefined polyposis” was available for the remaining 148 kindreds (test cases). Results were analyzed for either 3 or 4 contiguous STR matches, and samples from the unknown kindreds with perfect or close matches to known kindreds were sequenced for the relevant site-specific mutation. Matches between independently ascertained kindreds harboring the same mutations were also sought to verify or rule out common ancestry. Both genetically and genealogically verifiable, and false-positive, matches between database and test cases were obtained, demonstrating both the utility and the limitations of this approach for APC. Results and analysis will be presented.

1068T

Whole genome sequencing in clinical practice: The first year. *D. Dimmock[1,2], R. Veith[3], M. Gutzeit[3], S. Leuthner[1], R. Willoughby[1], T. May[4], M. Tschannen[2], T. Hambuch[5], P. North[6], H. Jacob[2], E. Worthey[1,2], D. Bick[1,2].* 1) Pediatrics, Med College Wisconsin, Milwaukee, WI, United States; 2) Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI, United States; 3) Children's Hospital of Wisconsin, Milwaukee, WI, United States; 4) Institute for Health and Society/Bioethics, Medical College of Wisconsin, Milwaukee, WI, United States; 5) Illumina Inc, San Diego, CA, United States; 6) Pathology/Pediatrics, Medical College of Wisconsin, Milwaukee, WI, United States.

We have demonstrated the power of genomic sequencing to identify the molecular basis of disease, thus modifying managment in a number of clincial cases (Worthey et al; Goh et al). The use of whole genome sequencing (WGS) in routine clinical practice was the logical extension of this approach. However, using WGS in routine clinical practice presents significant challenges. In our institution's clinical whole genome sequencing (WGS) program we have defined protocols for data evaluation, informed consent and data return. The steps in case management include: (1) Identification by physicians within a specialty of a case where genome sequencing is clinically warranted and where all other standard clinical assessments have been exhausted or where the aggregate cost of further testing is in favor of WGS(2) Case review by a committee with expertise in the disease area, genetics, informatics, genetic counseling, and ethics to evaluate whether genomic sequencing can advance the clinical decision-making process (3) Funding (4) Genetic counseling and consenting of the family (5) WGS in a CAP/CLIA certified laboratory (6) WGS data analysis and reporting through our institution's CAP/CLIA laboratory (7) Result reporting to families. At the time of abstract submission 43 clinical cases had been evaluated through our institutional WGS program. Although only 14 cases initially meet full criteria and were approved for WGS, the majority of cases will become eligible after further testing or information from referring physicians. To date only 5 cases have been declined for sequencing either because there was insufficient material available or no clear evidence that genomic testing would benefit care. Simular to pilot studies with Exome sequecing, WGS has acheived actionable clinical results in a subset of patients.

1069T

THM1-null Mouse Model of Ciliopathy Manifests Renal Cysts, Retinal Defects and Obesity. *P.V. Tran[1], D.R. Beier[2].* 1) Anatomy and Cell Biology and The Kidney Institute, The University of Kansas Medical Center, Kansas City, KS; 2) Genetics Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Primary cilia are antenna-like structures that extend from the plasma membrane of most vertebrate cells and are proposed to mediate signaling pathways. Defects in cilia physiology are linked to a growing class of human disorders, termed ciliopathies. Though individually rare genetic diseases, ciliopathies manifest clinical features that are common in the general population, such as renal cysts, retinal degeneration and obesity. As such, studying the pathogenesis of ciliopathies may provide critical insights into the molecular mechanisms of common medical burdens. We have identified a novel ciliary protein, THM1 (Tetratricopeptide Repeat Containing Hedgehog Modulator 1, also termed TTC21B or IFT139), which negatively regulates Hedgehog (Hh) signaling. *THM1* pathogenic alleles contribute to more than 5% of patients with ciliopathies, rendering *THM1* the most commonly mutated gene in these disorders (Davis *et al., Nat. Genet.*, 2011). We report that in conditional knock-out mice, genetic deletion of *Thm1* during late embryogenesis results in renal cysts and retinal defects in the adult mouse, while deletion of *Thm1* during adulthood leads to increased body weight and overall growth. Given the role of THM1 as a negative regulator of Hh signaling, we are investigating a possible role of enhanced Hh activity in these postnatal phenotypes. In support of this hypothesis, cyst formation in an embryonic kidney explant assay was prevented by genetic deletion of *Gli2*, the main transcriptional activator of Hh signaling, and by treatment with small molecule Hh inhibitors. To determine whether increased Hh signaling plays a causal role in the postnatal phenotypes of the conditionally deleted mutants, we are currently generating double *Thm1, Gli2* conditional knockout mice. Hh signaling remains largely unexplored in renal cystogenesis, retinal degeneration and obesity, and exploration of this pathway may reveal novel molecular mechanisms and therapeutic targets for these common illnesses.

1070T

Expansion of phenotypic characteristics of *NPHP5*-related disease. *A. Vincent[1], D. Chitayat[2, 3], R. Weksberg[2], E. Heon[1].* 1) OPHTHALMOLOGY, HOSPITAL FOR SICK CHILDREN, TORONTO, ONTARIO, Canada; 2) CLINICAL AND METABOLIC GENETICS, HOSPITAL FOR SICK CHILDREN, TORONTO, ONTARIO, CANADA; 3) PRENATAL DIAGNOSIS AND MEDICAL GENETICS PROGRAM, MOUNT SINAI HOSPITAL, TORONTO, ONTARIO, CANADA.

AIM:The nephrocystin 5 gene (*NPHP5*) originally associated with a clinical phenotype of Senior-Loken syndrome has recently been implicated to cause Lebers congenital amaurosis (LCA). We have comprehensively defined the clinical phenotype of a molecularly confirmed case of *NPHP5* who had atypical features that included hemi-hyperplasia. METHODS:Systemic and ophthalmic evaluation was performed on a child being assessed for hemihyperplasia. Karyotyping and microarray were performed. The region of chromosome 11p15 associated with Beckwith-Wiedemann syndrome was tested for methylation deficits, deletions and mutations. The *PTEN* gene associated with Proteus syndrome and 11 known LCA genes were also sequenced. RESULTS:The child, born to consanguineous parents, showed bilateral syndactyly of 2nd and 3rd toes. Hyperplasia of the right side of the body including face, upper limb and lower limb was first noted at 6 weeks of age. No evidence of visceral embryonal tumors has been noted to-date. The child has developmental delay and parents have noted nyctalopia since 1 year of age. Horizontal nystagmus noted at 3 years, has progressively worsened. At 4.5 years, the best corrected visual acuity was 6/60 in either eye (+6.00 D Sph). Contrast sensitivity was decreased to 0.15 log units. Fundus evaluation showed normal posterior pole with an equatorial concentric band of confluent white retinal lesions and deposits. Round atrophic scars were noted anterior to this. The optical coherence tomography revealed deposition of hyper-echoic material on the retinal pigment epithelium in affected regions. Electroretinogram showed non-detectable rod and cone responses. Ultrasonogram revealed cysts in the kidney and spleen and also showed hyperechoic lesion in the liver. Brain MRI and echocardiography were reported normal. Karyotype and microarray were normal. No mutations or methylation abnormalities were noted in chromosome 11p15 region. No mutations were identified in *PTEN* gene. A homozygous nonsense mutation (c. 1363C>T; p.R455X: previously reported) was identified in *NPHP5*. CONCLUSION:This case with molecularly confirmed *NPHP5*-related disease presented with unusual feature of hemi-hyperplasia and an atypical retinal phenotype. Renal and hepatic abnormalities described in this case are usually seen in *NPHP5*-related disease. All the findings including hemihyperplasia are likely *NPHP5*-related, as body curvature abnormalities have previously been recognized in animal models.

1071T

Exome sequencing to identify genetic causes of primary ciliary dyskinesia with outer dynein arms defects. *M.A. Zariwala[1], M.W. Leigh[2], L.E. Ostrowski[3], S.D. Davis[2], J.S. Berg[4], L. Huang[3], W. Yin[3], J.L. Carson[3], M. Hazucha[3], E.H. Turner[5], A. MacKenzie[5], M. Bamshad[5], D.A. Nickerson[5], J. Shendure[5], M.R. Knowles[3], Genetic Disorders of Mucociliary Clearance Consortium (GDMCC).* 1) Department of Pathology & Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; 2) Department of Pediatrics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; 3) UNC School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; 4) Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; 5) Department of Genome Sciences, University of Washington, Seattle, WA, USA.

We utilize exome sequencing to investigate genetic etiologies of primary ciliary dyskinesia (PCD)/Kartagener syndrome, an autosomal recessive genetically heterogeneous disorder. PCD is caused by defective structure and/or function of cilia and flagella. The phenotype is characterized by neonatal respiratory distress in full term neonates, recurrent otitis media, male infertility and chronic oto-sinu-pulmonary disease including bronchiectasis. ~50% patients have situs inversus and ~6% have situs ambiguus. Currently, mutations in 12 genes explain ~50% of PCD, but genetic basis in other patients is elusive. For most laboratories, the “gold standard” diagnostic test is ciliary ultrastructural analysis, but this test poses technical challenges, and there is heterogeneity of ultrastructure in patients, e.g. the presence/absence of outer dynein arms (ODA), inner dynein arms, radial spokes and/or central pair. To identify additional PCD-causing genes, we did exome sequencing in 7 unrelated, PCD patients with “classic” ODA defects (absent or truncated ODA). Sanger sequencing for biallelic mutations in *DNAH5* and *DNAI1*, genes commonly mutated in patients with ODA defects, had previously been negative for all 7 individuals. Three of the 7 patients harbored a homozygous stop mutation in a rare, but previously known PCD-causing gene, *DNAI2*. These patients were of Jewish ethnicity and haplotype analysis indicated a founder effect. Additionally, we found biallelic loss-of-function mutations in *DNAH5* in a patient who was previously negative by Sanger sequencing. Further analysis revealed lack of amplification of the mutant allele due to the presence of a rare polymorphism within the primer target, thus causing allele drop-out. We also found two rare, missense variants on highly conserved residues in *DNAH5* in another patient. Finally, we identified a homozygous splice mutation in a novel gene in one patient, and confirmation analyses and mutation profiling in an additional 93 patients is ongoing. In conclusion, we have successfully defined the genetic basis for PCD with ODA defects in 6 of 7 “unsolved” patients using exome sequencing, including a mutation missed by Sanger sequencing, a novel founder mutation in *DNAI2* that may be prevalent in subjects of Jewish ethnicity and a novel PCD-causing gene. This abstract was funded by MO1RR00046, UL 1 RR025747, 5 R01HL071798, 5 U54 HL096458-06, 5 R01HL094976-02 and 5UC2HL102923-02.

1072T

Disruption of CBP gene and decreased expression of CREB, NF2Bp65, c-JUN and c-FOS, BCL2 and c-MYC in a case of Rubinstein-Taybi Syndrome. *L.D. Kulikowski[1], L.C. Torres[2], P.L. Ramos[2], S.M.M. Sugayama[2], C.A. Moreira-Filho[2], M. Carneiro-Sampaio[2].* 1) Department of Pathology, HC FMUSP, LIM 03 Universidade de São Paulo, São Paulo, Brazil; 2) Department of Pediatrics, Instituto da Criança HC-FMUSP, LIM 36, Universidade de São Paulo, Brazil.

Rubinstein-Taybi syndrome (RTS) is commonly caused by microdeletions and point mutations in one copy of the gene encoding CREB-binding protein (*CBP*), although chromosomal translocations and inversions have been identified in a few cases. Here we present clinical and molecular studies of a patient with RTS who has a *CBP* gene disruption revealed by CGH-array and FISH. The patient presents a very severe RTS phenotype and immune dysregulation. Further investigation with Western Blot techniques demonstrated decreased expression of CREB, NF2B, c-Jun and c-Fos and also of BCL2 and c-MYC in peripheral blood mononuclear cells. Given that the patient represents a natural model for inactivation of *CBP* gene, we suggested that this gene is essential for normal expression of these transcriptional factors in peripheral blood mononuclear cells. Furthermore, the presence of structural genomic alterations in *CBP*, as well as gene dosage imbalance, may affect not only locus but also gene regulation in RTS patients.

1073T

Exploring the Intrinsic Functional Gene Dose of Recessive Mendelian Mutations with a Computational Visualizing Approach. *L. Li[1,2,4], M. McGuffin[5], W. Foulkes[1,2,3].* 1) Cancer Genetics Program; 2) Department of Oncology; 3) Department of Human Genetics; 4) Systems Biology Training Program, McGill university; 5) Department of Software and IT Engineering, École de technologie, Montreal, Canada.

Recessive Mendelian disorders are caused by the inactivation of both copies of the disease gene and are often diagnosed among young children with broad variation in phenotype severity. Known contributors to phenotype variation include mutation genotypes, genetic modifiers and environmental factors. Mechanistically, the retention of partial gene function resulting in phenotype attenuation has been demonstrated for several genes. For mutations interfering with gene expression, a partial retention of function may be achieved through the expression of the wild type or in-frame transcripts. Mutations of this category span the spectrum of mis-sense, non-sense, splicing and silence mutations, at both exonic and intronic sequences. Identifying mutations with residual gene function attributed to the expression of intact or in-frame transcripts represents a challenge for molecular diagnostics: it requires sensitive, quantitative gene expression analysis, which is not always practical in the laboratory due to the high cost of the assay, the complexity of the gene expression profile, or the scarceness of RNA material. An alternative approach is to identify mutations with residual function in a computational visualizing lab, by integrating phenotype patterns with genotype information. As a proof of principle, we visualized the phenotypic severity in patients carrying biallelic PMS2 mutations. Mutations were set into two groups according to prior knowledge of gene expression profiles: biallelic truncation or biallelic attenuation, the latter expressing lower levels of wild type or in-frame transcripts compared to wild type alleles. Then the phenotype severity of the two groups was computed and visualized. It appeared that the severity pattern precisely classified the two groups of genotypes, illustrating the feasibility of this approach in identifying genotypes with residual function. A larger dataset on Constitutive Mismatch Repair Deficiency Syndrome, Fanconi Anemia and Ataxia Telangiectasia was further tested for optimizing the visualization method. A novel diagnostic model will be formed from this study: a special group of patients, who are potential beneficiaries of restoring gene function by increasing the expression level of functional transcripts, can be identified in a computational lab with an integrated approach of phenotype clustering , visualization and transcript prediction.

1074T

Clinical and Molecular Characterization of Mircovillous Inclusion Disease (MVID) in four Arab Families. *B. Meyer[1], D. Monies[1], H. Al Zaidan[2], A. Mehaidib[3], M. Al Edreesi[4], E. Naim[1], O. Khashoggi[1], M. Faqih[5], Z. Rahbeeni[2].* 1) Department of Genetics, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 2) Department of Medical Genetics, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 3) Department of Pediatrics, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 4) Department of Pediatrics, Dhahran Health Center, Dammam, Saudi Arabia; 5) Department of Pathology & Clinical Laboratory Medicine, King Fahd Medical City, Riyadh, Saudi Arabia.

Four Arab families were included in our study. All five patients exhibited typical clinical features for Microvillous Inclusion Disease including an intractable watery diarrhea with onset near birth. The clinical diagnosis was supported by histopathological investigation. MVID is an autosomal recessive disorder resulting from mutations in *MYO5B*. All patients were from consanguineous families and we confirmed homozygosity in the *MYO5B* locus prior to sequencing this large gene (40 exons). Sequencing identified three novel homoallelic mutations (two nonsense and one missense mutation). Nonsense mutations were present in exons 24 and 36 and are consistent with loss of function. The missense mutation was present in exon 12 and was predicted to be pathogenic by the *PolyPhen-2* package. This was further confirmed by the absence of this allele in 500 control chromosomes. In one family with two affected individuals (monozygotic twins) no mutation was found in the entire coding and flanking region of *MYO5B*. Homozygosity screening did not exclude *MYO5B* and disease may be the result of mutation in a non-coding region of this gene or at a novel locus.

1075T

NPHS2, NPHS1 and WT1 mutations in Indian children with initial steroid resistant nephrotic syndrome. *S. sharma, A.K. Dinda, A. Bagga, M. Kabra.* Department of Pediatrics, All India Institute of medical sciences, New Delhi, India.

Objectives Mutations in NPHS2, NPHS1 and W11 genes is a frequent cause of steroid resistant nephritic syndrome (SRNS) and occur in 10-28% of children. The frequency and spectrum of mutations in these genes is unknown for the Indian population. We investigated the prevalence of mutations in these three genes among Indian patients with initial SRNS and their association with clinical outcomes. Methods 120 patients showing initial resistance to 4 weeks of treatment with daily prednisolone were included. Eighty patients with steroid sensitive nephrotic syndrome served as controls. Mutation analysis was performed by conformation sensitive gel electrophoresis (CSGE) of all exons of NPHS2, NPHS1 and WT1 genes. If an aberrant band was detected by CSGE, the corresponding sample was sequenced. Results We screened 120 patients (82 boys and 38 girls) belonging to different parts of India. The mean age of onset of disease was 4.36 years. Of 120 patients screened, 16 (13%) showed 10 different mutations in the NPHS2 gene. Homozygous and compound heterozygous mutations were seen in 8 out of 120 (6.6%) patients. Of 8 patients 3 who were compound heterozygous for R229Q showed complete response to CI (TAC) and 5 did not responded to CP/CI. Of 14 patients with onset of NS in infancy, only one showed a homozygous NPHS2 mutation (p.P175fsX178). Single heterozygous variants were found in 8 out of 120 (6.6%) patients. All heterozygous carriers showed complete response to CP/CI. We detected six novel mutations in the NPHS2 gene and none of the novel mutation was found in SSNS controls. Screening of WT1 gene showed three mutations IVS9+4C>T, IVS9+5G>A and R394W in three girls. The first two mutations were present in intron 9 of the WT1 gene and associated with complete sex reversal with 46,XY genotype (Frasier syndrome) and the third mutation was found in a girl with Wilm's tumor and nephrotic syndrome (Denys Drash syndrome). No mutations were found in NPHS1 gene. Conclusion The incidence of NPHS2 mutations in Indian patients with SRNS is considerably lower than that among European childern (10-30%). All WT1 mutations were present in girls with FSGS 3/12 (25%). Therefore screening of WT1 gene in all females with FSGS is necessary. No clear genotype-phenotype correlation was established. Further genetic studies using more sensitive techniques like direct sequencing and screening for other podocyte genes may help in further understanding the pathogenesis SRNS in Indian children.

1076T

Exome sequencing and analysis of split-hand/foot malformation and long-bone deficiency families with non-Medelian inheritance. *R. Uppala[1], U. Ratnamala[2], M. Naveed[3], M.T. Al-Ali[3], N. Al-Khaja[3], A. Bashamboo[4], K. McElreavey[4], S.K. Nath[5].* 1) Surgery-Transplant, University of Nebraska Medical Center, Omaha, NE; 2) Department of Pharmcology, Creighton University, Omaha, NE, USA; 3) Center for Arab Genomic Studies (CAGS), Dubai, United Arab Emirates; 4) Human Developmental Genetics, Institut Pasteur, Paris, France; 5) Arthritis and Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, USA.

Split hand/foot malformation with long-bone deficiency (SHFLD) is a rare, severe limb deformity characterized by tibia aplasia with or without split-hand/split-foot deformity with an incidence of ~1 per million live births. Genomic regions with evidence of linkage for SHFLD were identified at three chromosomal regions including our earlier identified loci at 1q31.1, 1q42.3, 4q34.3, 1q42.2-q43, 6q14.1, and 17p13.1 in a large Arab consanguineous family (UR-078) (Am.J.Hum.Genet; 80: 105-11,2007), which most likely contain novel susceptibility genes for SHFLD. In order to identify the genes responsible for these SHFLD loci, we performed exome sequencing using the SOLiDTM system at x200 coverage followed by high-resolution array CGH analysis. We prioritized our mutation search within the linkage region between SNP markers rs1124110/rs535043, and rs623155/rs1547251 respectively. In addition, we have recently ascertained several multigenerational SHFLD families of different geographic region. Genome-wide linkage analysis of three selected large families (UR-079, UR-080 and UR-083) using high-density SNP array excluded previously identified SHFLD1 and SHFLD2 and SHFLD3 loci and yielded novel digenic loci each in two distinct families (UR-080 and UR-083) indicating that SHFLD is highly heterogeneous. Additional clinical details of the affected individuals from newly studied families, the detailed results of our linkage scan with genes of interest in the novel linked chromosomal regions and exome sequencing data of SHFLD1 and SHFLD2 in selected individuals in Arab family will be presented.

1077T

Gene regulatory mutations as a cause of human limb malformations. *J.E. VanderMeer[1,2], T. Laurell[3,4,5], A.M. Wenger[6], G. Bejerano[6,7], G. Grigelioniene[4,8], A. Nordenskjold[9,10], M. Arner[3,5], A. Nordgren[4,8], N. Ahituv[1,2].* 1) Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, CA 94143, USA; 2) Institute for Human Genetics, University of California San Francisco, San Francisco, CA 94143, USA; 3) Department of Clinical Science and Education, Södersjukhuset, Karolinska Institutet, Stockholm, Sweden; 4) Department of Molecular Medicine and Surgery and Center of Molecular Medicine, Karolinska Institutet, Stockholm, Sweden; 5) Department of Hand Surgery, Södersjukhuset, Stockholm, Sweden; 6) Department of Computer Science, Stanford University, Stanford, CA 94305, USA; 7) Department of Developmental Biology, Stanford University, Stanford, CA 94305, USA; 8) Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden; 9) Department of Women and Children Health and Center of Molecular Medicine, Karolinska Institutet, Stockholm, Sweden; 10) Department of Paediatric Surgery, Astrid Lindgren Children Hospital, Karolinska University Hospital, Stockholm, Sweden.

Congenital limb malformations are the second most common human malformation. Many of these malformations appear to have genetic causes, however, mutations that cause isolated limb malformations have been difficult to discover. This could be due to the fact that not all limb malformations are caused by gene coding mutations. Another possible cause could be regulatory mutations - sequence or copy number variations that affect only noncoding regulatory regions - which are known to cause at least three types of limb malformations. As our understanding of noncoding regulatory DNA increases, we will see more cases of malformations caused by this type of mutation. In order to identify additional regulatory mutations that cause human limb malformations, we are collecting DNA from patients with isolated limb malformations and have enrolled over 300 patients to date. Using candidate region sequencing and linkage analysis in combination with computational and in vivo assays to detect regulatory sequences that harbor mutations, we are able to identify genetic lesions and understand how they could affect gene expression. As an example of this analysis, we describe a novel type of mutation in the Sonic Hedgehog limb enhancer (ZRS). Single base mutations in the ZRS have previously been shown to cause preaxial polydactyly and large duplications encompassing the entire ZRS region cause human polysyndactyly phenotypes, but no insertion or deletion mutations entirely within the ZRS have been reported. By analyzing a Swedish family with preaxial polydactyly and triphalangeal thumb, we found a 13 base pair insertion within the ZRS. Computational analysis for transcription factor binding sites suggests that this insertion creates sites for several key transcription factors involved in limb development. Using a mouse enhancer assay, we show that this insertion causes the ZRS to misdirect gene expression to an ectopic anterior region of the developing limb. This is the first report of a small insertion within an enhancer that causes a human limb malformation and suggests a potential mechanism that could explain the ectopic expression caused by this mutation. This and future work on regulatory mutations that cause limb malformations will result in increased knowledge about limb development and the pathogenesis of human limb malformations. In addition, this work provides a model for studying the role of regulatory elements in other isolated disease phenotypes.

1078T

Pitx1 Haploinsufficiency Causes Clubfoot in Humans and Mice. *D.M. Alvarado[1], K. McCall[1], H. Aferol[1], M.J. Silva[1], J.R. Garbow[2], W.M. Spees[2], T. Patel[1], M. Siegel[2,3], M.B. Dobbs[1,5], C.A. Gurnett[1,3,4].* 1) Washington University in St. Louis Departments of Orthopaedic Surgery; 2) Radiology; 3) Pediatrics and; 4) Neurology, St Louis, MO; 5) St. Louis Shriners Hospital for Children, St. Louis, MO.

Clubfoot affects 1 in 1000 live births, though little is known about its genetic or developmental basis. We recently identified a missense mutation in the PITX1 bicoid homeodomain transcription factor in a family with a spectrum of lower extremity abnormalities, including clubfoot. Because the E130K mutation reduced PITX1 activity, we hypothesized that PITX1 haploinsufficiency could also cause clubfoot. Using copy number analysis, we identified a 241kb chromosome 5q31 microdeletion involving PITX1 in a patient with isolated familial clubfoot. The PITX1 deletion segregated with autosomal dominant clubfoot across 3 generations. To study the role of PITX1 haploinsufficiency in clubfoot pathogenesis, we began to breed Pitx1 knockout mice. Although Pitx1+/- mice were previously reported to be normal, clubfoot was observed in 20 of 225 Pitx1+/- mice, resulting in an 8.9% penetrance. Clubfoot was unilateral in 16 of the 20 affected Pitx1+/- mice, with the right and left limbs equally affected, in contrast to right-sided predominant hindlimb abnormalities previously noted with complete loss of Pitx1. Peroneal artery hypoplasia occurred in the clubfoot limb and corresponded spatially with small lateral muscle compartments. Tibial bone volumes were also reduced. Skeletal muscle genes expression was significantly reduced in Pitx1-/- E12.5 hindlimb buds compared to wild-type, suggesting that muscle hypoplasia was due to abnormal early muscle development and not disuse atrophy. Our morphological data suggest that PITX1 haploinsufficiency may cause a developmental field defect preferentially affecting the lateral lower leg, a novel theory that accounts for similar findings in human clubfoot.

1079T

Aberrant firing of replication origins explains nonrecurrent rearrangements in the human genome. *A. Ankala[1], A.R. Hegde[2], A. Meka[3], E.L.H. Chin[1], S.H. Askree[1], S. Bhide[1], M.R. Hegde[1].* 1) Human Genetics, Emory University School of Medicine, Atlanta, GA 30322; 2) Biochemitry and Molecular Biology, University of Georgia, Athens, GA 30602; 3) Georgia Institute of Technology, Atlanta, GA 30332.

Nonallelic homologous recombination (NAHR), nonhomologous end joining (NHEJ) and fork stalling and template switching (FoSTeS) have been put forward as mechanisms to explain DNA rearrangements associated with genomic disorders. However, many nonrecurrent rearrangements in humans still remain unexplained. To further explore the mutational mechanisms of these rearrangements and the resultant copy number changes (CNCs), we investigated 62 clinical cases with intragenic deletions either in the human dystrophin gene (DMD, 50 cases) or in other known disease-causing genes (1 PCCB, 1 IVD, 1 DBT, 3 PAH, 1 STK11, 1 HEXB, 3 DBT, 1 HRPT1 and 1 EMD cases). Breakpoint junction sequence analysis of these deletions revealed presence of repetitive elements in 4 individual cases including 3 DMD and 1 HEXB, microhomologies (2-10 bp) at breakpoint junctions in 35, and insertions ranging from 1 to 48 bp in 16 of the total 62 cases. Among these insertions, we observed tandem repetitions (5-20 bp long) of the template region proximal to deletion breakpoint in six individual DMD cases (6 repeats in 1, 4 repeats in 3, 2 repeats in 1 and 1 repeat in 1 case). These tandem repetitions represent series of replisome slippage and re-replication events. Successful replication of the entire DMD gene is known to be completed by the participation of at least five active origins and six termination regions. Replisome slippage events observed in our current study suggests reversal of replication fork direction across termination regions due to failure of certain replication origins to fire. With a deeper insight into the complex process of replication brought about by recent studies, we propose that CNCs involving DNA deletions and duplications are a result of aberrant firing of active and inactive replication origins coupled with incomplete rescue of replication.

1080T

Analysis of X chromosome copy number variations in Brazilian men with idiopathic intelectual disability. *N. Fintelman-Rodrigues, M. Campos Jr, J.M. Santos, M.M.G Pimentel, C.B Santos-Rebouças.* Departament of genetics, State University of Rio de Janeiro, Rio de Janeiro, Brazil.

The evolution of molecular technology has uncovered a number of structural variants in the human genome, mainly in the form of copy number variations (CNVs), corresponding to submicroscopic microdeletions and -duplications. In fact, several studies have already correlated CNVs in distinct genes with different degrees of predisposition to disease, which can influence gene expression affecting genes directly or by changing their dosage. Nevertheless, for many CNVs it is unclear whether they are associated with disease or just represent benign variants. X-linked mental retardation (XLMR) or intellectual disability (ID) is a common, clinically complex and genetically heterogeneous disease arising from many mutations along the X chromosome, which affects between 1/600-1/1000 males. In this study, we analyzed CNVs for 16 genes located at X chromosome (*PQBP1*, *TM4SF2*, *ARX*, *IL1RAPL1*, *RPS6KA3*, *OPHN1*, *FACL4*, *DCX*, *PAK3*, *AGTR2*, *ARHGEF6*, *FMR1*, *FMR2*, *GDI1*, *SLC6A8* and *HUWE1*), with the purpose of evaluating if this new genomic mechanism could be related to cognitive function. The screening was conducted in 92 probands with idiopathic ID (82 from families with a possible history of XLID and 10 from ID affected sib pairs). All patients had normal karyotypes and mutations in *FMR1*, *FMR2* and *MECP2* genes were ruled out. DNA was extracted from peripheral blood and the presence of CNVs was evaluated through Multiplex Ligation-dependent Probe Amplification (MLPA), a method capable of detect CNVs in up to 50 different genomic DNA sequences. The MLPA analysis revealed two independent duplications involving probes for the *HUWE1* gene and the *GDI1* gene, confirmed by quantitative Real Time PCR. The clinical phenotypes of the patients bearing the duplications were not remarkable. The segregation analysis of HUWE1 gene shows that, although the duplication was maternally inherited, it not occurred on the ID affected brother of the proband, leading us to classify this CNV as benign. Conversely, the *GDI1* duplication was considered to be pathogenic and was also seen in a heterozygous state in the mother of the patient. Our results suggest that even though MLPA represents a reliable, easy and low cost effective method for evaluating CNVs, pathogenic CNVs do not appear to be a common etiological factor among our group of ID Brazilian patients. The more widespread use of array-CGH with increased resolution will make possible the identification of additional causal CNVs related to XLID.

1081T

Molecular Characterization of Autosomal Recessive Hyper IgE Syndrome in Saudi Arabia. *A. Hawwari, Z. Alsum, S. Al-Hisi, E. Borrero, H. Khalak, O. Alsmadi, R. Arnaout, A. Al-ghonaium, S. Al-Muhsen, H. Al-Dhekri, B. Al-Saud, H. Al-Mousa.* Genetics, Research Center, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia.

DOCK8 is a large protein that is highly expressed in the immune system especially in lymphocytes and has been recently associated with autosomal-recessive hyper-IgE syndrome (AR-HIES). Clinical data of 27 patients diagnosed with AR-HIES were collected from the pediatric clinic at KFSH&RC. Twenty two patients screened for STAT3, Tyk2 and Dock8 mutations. Two novel DOCK8 mutations that resulted in stop codons were found in nine patients. In addition, 4 patients from two separate families were found with 2 gross deletions. In one family the deletions spanned the entire Dock8 gene and the surrounding genes. In the other family, the deletion extended from somewhere at the tip of chromosome 9 to the middle of DOCK8 gene. No mutations were found in STAT3 or TYK2 genes in any of the patients screened. These mutations will be described and correlated to their clinical presentations.

1082T

De novo copy number variants associated to intellectual disability have a paternal origin and age bias. *J.Y. Hehir-Kwa[1], B. Rodríguez-Santiago[2], L.E. Vissers[1], N. de Leeuw[1], R. Pfundt[1], L.A. Pérez-Jurado[2], J.A. Veltman[1].* 1) Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; 2) Unitat de Genètica, Universitat Pompeu Fabra, and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain.

De novo mutations and structural rearrangements are a common cause of intellectual disability (ID) and other disorders with reduced or null reproductive fitness. Insight into the genomic and environmental factors predisposing to the generation of these de novo events is of significant clinical importance. In this study we used information from SNP microarrays to determine the parent-of-origin of 118 rare de novo CNVs detected in a cohort of 3,443 patients with ID. The large majority of these CNVs (76%, $p=1.14x10^{-8}$) originated on the paternal allele. This paternal bias was independent of CNV length and CNV type. Interestingly, the paternal bias was less pronounced for CNVs flanked by segmental duplications (64%), suggesting that molecular mechanisms involved in the formation of rare de novo CNVs may be dependent on the parent-of-origin. In addition, a significant increased paternal age was only observed for those CNVs which were not flanked by segmental duplications (p=0.009). This indicates that rare de novo CNVs are increasingly being generated with advanced paternal age by replication-based mechanisms during spermatogenesis.

1083T

Unprogrammed presentation number

1084T

Mitotic microhomology-mediated replication-based mechanisms underly non-recurrent pathogenic microdeletions of the *FOXL2* gene or its regulatory domain. *H. Verdin[1], B. D'haene[1], Y. Novikova[1], D. Beysen[2], P. Lapunzina[3], J. Nevado[3], C. Carvalho[4], J.R. Lupski[4], B. Menten[1], E. De Baere[1].* 1) Center for Medical Genetics, Ghent University, Ghent, Belgium; 2) Dept of Pediatrics, Ghent University, Ghent, Belgium; 3) Instituto de Genética Médica Y Molecular (INGEMM), Hospital Universitario La Paz, Universidad Autónoma de Madrid, Madrid, CIBERER, U783-ISCIII, Spain; 4) Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA.

Genomic disorders are often caused by recurrent pathogenic copy number variations (CNVs), with meiotic, non-allelic, homologous recombination as underlying mechanism. A recent study by Vissers et al. 2009 suggested that rare pathogenic CNVs spread throughout the genome are microhomology-mediated and stimulated by local genomic architecture. Here, it was our aim to study whether such mitotic mechanisms and local genomic architecture contribute to a series of non-recurrent, pathogenic, locus-specific CNVs. To this end, we fine-mapped 44 rare pathogenic microdeletions encompassing the *FOXL2* gene and neighbouring region (34), or its regulatory domain (10) respectively, both leading to blepharophimosis syndrome (BPES). For the breakpoint mapping we used targeted arrayCGH, qPCR, long-range PCR and sequencing of the junction products. Microhomology, ranging from 1 bp to 34 bp, was found in 86,7% of the breakpoint junctions, being significantly enriched in comparison with a random control population. This suggests that microhomology-mediated repair mechanisms, such as fork stalling and template switching (FoSTeS), microhomology-mediated break-induced replication (MMBIR), or alternative non-homologous end-joining (MMEJ), underly these microdeletions. Moreover, genomic architectural features, like sequence motifs, non-B DNA conformations and repetitive elements, were found in all breakpoint regions. In conclusion, we propose that the majority of these deletions is not caused by meiotic homology-based mechanisms but by microhomology-mediated replication-based mechanisms like FoSTeS or MMBIR, and thus have a mitotic origin instead. Finally the genomic architecture might stimulate the formation of these rare deletions by increasing the susceptibility for DNA breakage or promote fork stalling.

1085T

A *de novo* paradigm for intellectual disability. *L. Vissers, J. de Ligt, B. van Bon, C. Gilissen, M. Willemsen, I. Janssen, J. Schuurs-Hoeijmakers, M. Steehouwer, W. Nillesen, P. de Vries, K. van der Donk, B. van Lier, P. Arts, H. Scheffer, N. Wieskamp, M. del Rosario, A. de Brouwer, A. Hoischen, T. Kleefstra, B. de Vries, H. Brunner, J. Veltman.* Dept Human Genetics 855, Radboud University Nijmegen Medical Centre, Nijmegen Centre for Molecular Life Sciences, Nijmegen, Netherlands.

Recent studies have indicated that humans have an exceptionally high per-generation mutation rate of 7.6×10^{-9} to 2.2×10^{-8}. These spontaneous germ line mutations can have serious phenotypic consequences when affecting functionally relevant bases in the genome. In fact, their occurrence may explain why diseases with a severely reduced fecundity remain frequent in the human population, especially when the mutational target is large and comprised of many genes. This would explain a major paradox in the evolutionary genetic theory of cognitive disorders.

Previously, we have shown a family-based exome sequencing approach to test this *de novo* mutation hypothesis in 10 patients with unexplained intellectual disability. Unique non-synonymous mutations were identified and validated in nine genes. Six of these, identified in different patients, were likely pathogenic based on gene function, evolutionary conservation and mutation impact. These findings provided a strong experimental support for a *de novo* paradigm for intellectual disability.

The clinical relevance and ultimate proof for disease-causality of these novel genes lies in the identification of *de novo* mutations in additional patients of similar phenotype. As such, we are currently screening ~600 patients with unexplained intellectual disability for mutations in *YY1*, representing one of these newly identified genes. Moreover, we are extending our family-based exome sequencing approach to 100 patients to establish the diagnostic yield for *de novo* mutations in patients with unexplained intellectual disability.

1086T

A Cell-Based Translocation Assay System for Screening Plant Extracts for Fibroblast Growth Factor Receptor 3 (FGFR3) Modulators: Therapy of FGFR3-Related Skeletal Dysplasias and Cancers. *Y. Lee[1], C. Tai[1], C. Ko[1], R. Wu[2], K. King[2], Y. Chen[1].* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Development Center for Biotechnology, Taipei County, 221, Taiwan.

Activating germline mutations of fibroblast growth factor receptor 3 (FGFR3) cause various skeletal dysplasias. In somatic tissues, these mutations are associated with human cancers, making FGFR3 an excellent therapeutic target. Natural products have proved to be the most consistently successful source of drug leads. To identify potential therapeutic natural compounds for the treatment of diseases associated with FGFR3 activation, we established a cell-based protein translocation system. The U2OS cell line, which endogenously expresses FGFR-1, -2 and -4, but not 3, was chosen as the cell platform. We established that the GFP-tagged SH2 domain of the SH2-B) protein specifically interacted with and co-internalized with activated FGFR3, and is thus suitable as a probe for FGFR3 activation in U2OS cells. Proof-of-concept data from a pilot screen using PKC412, a tyrosine kinase inhibitor, as a control are presented. Four plant extracts that inhibited FGFR3 activation (hits) were identified. Furthermore, plant extracts from two closely related species of one hit also showed inhibitory activity against activated FGFR3. Our results show that this system offers a practical strategy for more definitive selectivity-evaluation and counter-screening in the early stages of drug discovery, and demonstrates therapeutic potential in the treatment of diseases associated with dysregulation of FGFR3 activation.

1087T

Molecular analysis of 246 patients with oculocutaneous albinism. *B. Arveiler[1,2], E. Lasseaux[2], C. Rooryck-Thambo[1,2], A. Rouault[2], D. Cailley[2], C. Plaisant[2], D. Lacombe[1,2], A. Taieb[3], F. Morice-Picard[1,2].* 1) Rare Diseases: Genetics and Metabolism, Univ Victor Segalen Bordeaux 2, Bordeaux, France; 2) Service de Genetique Medicale, Bordeaux Universitary Hospital, Bordeaux, France; 3) Service de Dermatologie, Bordeaux Universitary Hospital, Bordeaux, France.

Oculocutaneous albinism (OCA) is a rare autosomal recessive disease affecting 1/20000 person in the general population. OCA is caused by mutations in the TYR (OCA1), P (OCA2), TYRP1 (OCA3), and SLC45A2 (OCA4) genes. Mutations of GPR143 are responsible for X-linked ocular albinism (OA1). Due to extensive phenotypic heterogeneity and lack of genotype-phenotype correlations, molecular investigations are necessary to establish the diagnosis and to evaluate the relative frequency of the different forms of albinism. We analysed 246 patients from various origins (mainly Europe). Point mutations were analysed by DHPLC/HRM and sequencing of exons, intron-exon junctions and promoter regions. Gene dosage anomalies were investigated by semi-quantitative PCR (QMF-PCR) and, more recently, by high resolution (100 bp) array-CGH using a custom array covering the OCA1-4 and OA1 genes. The molecular analysis identified 141 point mutations (missense, nonsense, splice site) in TYR, 88 in P, 8 in TYRP1, 25 in SLC45A2 and 11 in OA1. We identified 3 deletions in TYR, 28 deletions and 2 duplications in P, and 2 deletions in SLC45A2. The relative frequencies of OCA1-4 were: 46.4% OCA1, 35% OCA2, 2.4%OCA3, and 16.2% OCA4. Despite the extensive analysis of the OCA1-4 and OA1 genes, a single heterozygous mutation was found in 28 patients (11.4%), and no mutation was found in 40 patients (16.3%). The thorough analysis of the OCA1-4 and OA1 genes in 246 patients allowed us to establish a diagnosis in 178 patients (72.3%).Intragenic microrearrangements accounted for 10.5% of the OCA1-4 alleles. It is worth noting that microrearrangements represented as many as 25.4% of all OCA2 alleles (including the common exon 7 deletion). This shows that microrearrangements should be searched for systematically in a diagnostic set up. Despite the thorough analysis performed, 27.7% of the patients remained undiagnosed. Mutations may therefore hide in unexplored regions of the genes (deep in introns, or in regulatory elements), or in other genes, which are either involved in syndromic forms of albinism or which still have to be discovered.

1088T

The LMNA c. 1968+5G>C transversion leads to progerin generation and Hutchinson-Gilford progeria syndrome. *V. Benoit[1], P. Hilbert[1], I. Maystadt[1], C. Gaspard[1], S. Castedo[2], T. Kay[3].* 1) IPG - Institut de Pathologie et de Génétique, Biologie Moléculaire, Charleroi, Belgium; 2) GDPN - Genética Médica e Diagnóstico Pré-Natal, Porto, Portugal; 3) Hospital Dona Estefania, Department of Clinical Genetics, Lisboa, Portugal.

Hutchinson-Gilford progeria syndrome (HGPS) is a very rare fatal genetic disorder characterized by precocious ageing in children. Children with HGPS appear healthy at birth and distinctive clinical features appear within the first years of life (including growth retardation, alopecia, lipodystrophy, and atherosclerosis). Most of them die from coronary artery disease at an average age of 13 years. Mutations in the Lamin A/C encoding gene (LMNA) are described to be responsible for the disease, most HGPS patients carrying the 1824C>T mutation (G608G) in exon 11. This transversion activates a cryptic donor splice site. Lamin A products in these patients then consist of mature Lamin A and progerin, a 50 amino-acids deleted Lamin A protein that exerts a dominant negative effect. In a 6 year-old patient affected by HGPS, we have identified a 1968+5G>C variation in the intron 11 of the LMNA gene. This mutation was not present in the DNA of the patient's parents, indicating that it arose de novo. This variant has already been evoked in the LOVD database; the question of its implication in HGPS has been raised but let unsolved with unknown pathogenicity. It seems that no further study has been undertaken as the question of a possible modified splicing still remained. We performed RNA studies and clearly showed that our patient's RNA generated different splicing products, including the normal one and at least one shorter form. By sequencing these products, we were able to demonstrate that this shorter form corresponds to a truncated RNA lacking 150 nucleotides and that the sequence perfectly matched the sequence of progerin. Taken together, our data unequivocally demonstrates that the c.1968+5G>C transversion is a causal mutation of HGPS. Moreover this work reinforces the concept of progerin involvement as a crucial actor in HGPS physiopathology.

1089T

Novel EVC and EVC2 mutations in Ellis-van Creveld syndrome and Weyers acrofacial dysostosis. *A. De Luca[1], M.C. D'Asdia[1,2], M. Magliozzi[1], R. Ferese[1,2], F. Consoli[1,2], V. Guida[1,2], L. Bernardini[1], M.C. Digilio[3], B. Marino[4], B. Dallapiccola[3], I. Torrente[1].* 1) Casa Sollievo della Sofferenza Hospital, IRCCS, San Giovanni Rotondo, Italy; 2) Department of Experimental Medicine, “Sapienza” University, Rome, Italy; 3) Bambino Gesù Children Hospital, IRCCS, Rome, Italy; 4) Division of Pediatric Cardiology, Department of Pediatrics, “Sapienza” University, Rome, Italy.

Ellis-van Creveld syndrome (EvC, MIM ID 225500) and Weyers acrofacial dysostosis (MIM ID 193530) are two allelic conditions that differ in the pattern of inheritance and severity of the phenotype. EvC is inherited as an autosomal recessive trait with variable expression, and Weyers acrofacial dysostosis is an autosomal dominant disorder. Two genes, EVC (MIM ID *604831) and EVC2 (MIM ID *607261), are found mutated in EvC patients. In patients with Weyers acrofacial dysostosis, convincing mutations have been identified in the last coding exon of EVC2. Here we present our experience with the direct analysis of the whole EVC and EVC2 coding regions in 26 independent families with clinical diagnosis of EvC and 2 families with clinical diagnosis of Weyers acrofacial dysostosis. We identified mutations in 21/26 (81%) cases with EvC and 2/2 cases with Weyers acrofacial dysostosis. Of the EvC cases, 16/26 (61%) had mutations in EVC and 5/26 (19%) were mutated in EVC2. The 2 cases with diagnosis of Weyers acrofacial dysostosis had a heterozygous mutation at the 3' end of the last exon of EVC2. We have not identified mutations in 5/26 cases who met our clinical criteria of EvC. SNP-array analysis of 3 cases in which we had not identified mutations in either gene revealed no pathogenic alterations in the DNA copy number. In EvC cases, 16 mutations in EVC and 4 mutations in EVC2 were novel. In patients with Weyers acrofacial dysostosis, one out of the 2 mutations was novel. Current findings expand the EvC and Weyers acrofacial dysostosis mutation spectra and provide further evidence that the 3' end of the last exon of EVC2 is a hot spot for Weyers mutations. Thus, it appears that EVC2 exon 22 should be analyzed with priority in patients with a phenotype consistent with Weyers acrofacial dysostosis. Failure to identify EVC and EVC2 mutations in 5 patients with EvC makes further genetic heterogeneity a possibility. A. De Luca and M.C. D'Asdia contributed equally to this work and should be considered co- first authors.

1090T

Screening for microdeletion/duplication syndromes and subtelomeric deletion/duplications in patients with unexplained mental retardation using MLPA analysis. *F.B. Essop, C.A. Robinson, A. Krause.* National Health Laboratory Service, Johannesburg, Division of Human Genetics, the National Health Laboratory Service and the University of the Witwatersrand; Johannesburg, Gauteng, South Africa.

BACKGROUND Mental retardation (MR) affects ~2-3% of the population. Subtelomeric rearrangements are reported to be the cause in 5-10% of idiopathic MR cases. Primary screening of such patients is done by karyotyping. When no abnormality is detected by these methods, multiplex ligation probe-dependent amplification (MLPA) analysis has been used. **OBJECTIVES** To evaluate MLPA as a diagnostic tool in patients with an unknown cause of MR. **METHODS** The P036 kit (MRC-Holland) probe mix consists of one probe for each subtelomere. The P245 probe mix simultaneously screens 21 loci associated with a microdeletion syndrome. Patients with known chromosomal abnormalities were used to validate the kits. A second kit, P070 was used to confirm a subtelomeric deletion/duplication found on the P036 kit. A total of 231 patients presenting with unexplained MR, referred over a 13 month period (July 2009 to Oct 2010) were tested. **RESULTS** 11/231 (4.76%) patients were found to have a subtelomeric deletion or duplication. 12/231 (5.19%) patients were found to have a microdeletion/duplication. 19/231 (8.23%) patients were found to have an interesting result in which the pathological significance of the deletion/duplication is unknown. **CONCLUSION** MLPA is a reliable and valuable method. The efficacy of this was demonstrated when 231 patients were tested and 9.96% abnormal cases were detected. Screening for microdeletion/duplication syndromes and for subtelomeric deletions/duplications by MLPA can be offered to all developmentally delayed patients, although clinical preselection increases the percentage of anomalies detected. Follow-up tests such as sequencing, FISH or a second MLPA kit must be done to avoid false positive results.

1091T

Detection of inherited mutations for Alport syndrome using Next Generation Sequencing. *P. Hilbert[1], M. Lizon[1], D. Goossens[2], J. Del-Favero[2], K. Dahan[1].* 1) Dept Molec Biol, Inst Pathologie et Génétique, Gosselies, Belgium; 2) Multiplicom NV, Waterfront Research Park, Galileïlaan, B-2845 Niel, Belgium.

Alport Syndrome is a progressive renal disease with cochlear and ocular involvement. The most common form (approximately 80%) is inherited in an X-linked pattern. The autosomal recessive and dominant forms constitute about 15% of the cases. X-linked Alport Syndrome is caused by mutations in the type IV collagen alpha chain 5 (COL4A5) whereas the type IV collagen alpha chain 3 (COL4A3) and 4 (COL4A4) are responsible for recessive and dominant Alport syndrome. Genetic testing for mutations in the respectively 52, 48 and 51 exons of COL4A3, COL4A4 and COL4A5 has become an integral part of clinical practice but the use of conventional Sanger sequencing is time-consuming and expensive. To determine whether amplicon based Next Generation Sequencing (NGS) would enable accurate, thorough, and cost-effective identification of Alport syndrome, we set up a project with Multiplicom (www.multiplicom.com) to design a two step multiplex PCR for the amplification of all the coding regions of the three responsible genes. 149 amplicons ranging from 270 Bp to 510 Bp were amplified in 4 multiplex PCR reactions. Genomic DNA from 24 patients with known inherited mutations or polymorphisms were studied. To differentiate patients within a single run, Multiple Identifiers (MIDs) were introduced during the PCR. The sequences were run on a GS-FLX 454 (Roche) (titanium chemistry) and analyzed with the SeqNext module of Sequence Pilot software (JSI medical). All the variants (21 mutations plus frequent SNPs) were confirmed and there were zero false positive calls. Our data confirm that amplicon based NGS will allow widespread genetic testing and genetic counseling for Alport syndrome.

1092T

Spinal Muscular Atrophy (SMA) in Singapore - an eleven-year experience in a diagnostic laboratory. *H.Y. Law, I.S.L. Ng, G.P. Tan, C.S. Yoon, E.S. Tan, S.S. Jamuar, A.H.M. Lai.* Dept Pediatrics, KK Women's & Children's Hosp, Singapore, Singapore.

Purpose: Spinal muscular atrophy (SMA) is one of the most common inherited childhood disease characterized by progressive muscle weakness, paralysis and eventually death. Our lab has been offering DNA analysis to confirm SMA since year 2000. This is to summarize our findings for SMA diagnosis in this major Children's Hospital in Singapore and to estimate the local carrier frequency. **Methods:** Homozygous deletion of *SMN1* gene was detected by restriction enzyme digest of PCR amplified exon 7 and 8 products of both *SMN1* and *SMN2* genes using forward primers modified to introduce *Hinf* I site for *SMN1* in the case of exon 7, and *Dde* I site for *SMN2* in the case of exon 8. Multiple Ligation-dependent Probe Amplication (MLPA) kit (MRC-Holland SALSA P021-SMA) was used to estimate gene copy number to confirm carrier status of parents of affected children with homozygous *SMN1* gene deletion. **Results:** SMA diagnostic test was carried out from year 2000-2010 for 101 children aged 2 days to 16 years and 23 adults 19 - 65 years at external adult neurologists' requests. Homozygous deletion of *SMN1* gene was confirmed in 45 children and 2 adults (19 and 29 year old). Age of onset and diagnosis for children were between 2 days to 6 months for 14 (30% Type I), 6 to 8 months for 7 (15% Type II), 10 months to 6 years for 23, and one child was diagnosed at 15 years old. This shows that 55% of patients had Type III SMA. With the ethnic composition of 74% Chinese, 13% Malay, 9% Indian and 3% other ethnicity in Singapore, our finding shows 29 SMA patients were Chinese (62%), 7 Malay (15%), 5 Indian (11%) and 6 others (13%). Carrier testing done for 14 couples whose children had homozygous deletion of *SMN1* found single copy *SMN1* gene in all individuals tested except for 2 spouses from 2 separate couples, who probably had “2+0” genotype (8%). Prenatal diagnosis was carried for 18 pregnancies of which 3 were diagnosed to have homozygous deletion of *SMN1*. **Conclusion:** Data collected over 11 years show an average of 4.2 SMA cases diagnosed per year. For over 35,000 deliveries each year, we approximate that the carrier frequency in Singapore is at least 1 in 50. Since our lab is only one of the 2 centres offering SMA test, the actual number of SMA diagnosed is likely to be more. Analysis of *SMN1* copy number in parents of SMA patients showed that carrier screening is feasible in this population.

1093T

Molecular screening of Ichthyosis Vulgaris in Iranian patients. *S. MATOO, N. HATAMNEJADIAN, A. YASARI MAZANDARANI, A. TAVAKOLI TAMEH, B. SEDAGHATI KHAYAT, M. MAHDAVI, SH. ABADPOUR, A. EBRAHIMI.* Dr.Ahmad Ebrahim Molecular Genetics ,PhD Parseh Medical Genetic Counseling center, Floor 7,NO.75,Royan Alley,Keshavarz Bolv. Tehran,Iran Tel-Fax:+98 21 88966579,88996889 E-mail:ae35m@yahoo.com.

Abstract Hereditary autosomal dominant Ichthyosis Vulgaris (IV), the most common form of ichthyosis, is a genetic disorder that can be caused by mutation in the Filaggrin gene (FLG) on 1q21.3. Although the prevalence of disorder depends on the location but there is a frequency about 1:250 - 1:5,000. Some common mutations were reported such as R501X and c.2282del4 among white individuals of European descent or 3321delA in Southern Chinese and Japanese cases. Methods: Fifty affected patients clinically diagnosed as IV were selected. The blood samples were collected and DNA was extracted. All 3 coding regions of FLG gene were amplified using intronic primers and the PCR products were analyzed by direct sequencing. Result: We found some novel sequence variations but the frequency of common reported mutations is lower than previously studied populations. According to the result, the molecular screening is recommended in order to clinical differential diagnosis. Keywords :Ichthyosis Vulgaris , FLG , PCR.

1094T

Identification of 15 novel mutations in Sotos syndrome. *S. Boulanger[1], M. D'amico[1], A. Destree[1], I. Maystadt[1], D. Lederer[1], H. Van Esch[2], J. Van Den Ende[3], N. Van Der Aa[3], K. Segers[4], P. Hilbert[1].* 1) Human Genetics Center, Institute of Pathology & Genetics, Charleroi, Belgium; 2) Human Genetics Center, K.U.Leuven, Belgium; 3) Department of Medical Genetics, University of Antwerp, Belgium; 4) Department of Human Genetics, CHU University of Liege, Belgium.

Sotos syndrome (MIM 117550) is a genetic disorder characterized by three cardinal features; distinctive facial appearance, overgrowth and learning disability. Moreover, major features can also occur ; behavioral problems, cardiac anomalies, renal anomalies, scoliosis and seizures. This syndrome is inherited in an autosomal dominant manner but more than 95% of patients have a de novo mutation. NSD1 (nuclear receptor SET domain1) is the only gene known to be associated with Sotos syndrome and a mutation in that gene is found in about 80-90% of affected individuals. The gene contains 23 exons encoding a 2696 amino acids protein. Depending on the criteria defined for patient screening, 27-93% (among non-Japanese individuals) of point mutations are found, approximately 5% of partial or total gene deletion and about 10% have a 5q35 microdeletion that encompasses NSD1 gene. The experience in our Human Genetics Center is based on a total of 86 patients presenting at least the 3 cardinal features but referred by different physicians working anywhere in Belgium. The entire coding region of NSD1 gene was sequenced and large rearrangements were investigated by MLPA (Multiple Ligation dependent Probe Amplification from MRC-Holland). We here describe the clinical features for 18 patients (~21%) for whom an intragenic mutation has been found. Among the mutations detected (6 nonsense, 7 missense and 5 frameshifts), 15 are novel . Up to now, no large rearrangements have been found in our population.

1095T

Detection of a mutation in Lenz microphthalmia family by exome sequencing. *T. Kaname[1], K. Yanagi[1], Y. Muramatsu[2], T. Tohma[3], H. Hanafusa[1], K. Morita[4], S. Ikematsu[4], Y. Itagaki[5], K. Kurosawa[6], S. Mizuno[2], K. Yoshiura[7], K. Naritomi[1].* 1) Dept Med Gen, Univ Ryukyus, Nishihara, Japan; 2) Dept Pediatr, Centl Hosp, Aichi Hum Serv Centr, Aichi, Japan; 3) Wanpaku Clinic, Okinawa, Japan; 4) Dept Biores Engr, ONCT, Okinawa, Japan; 5) Suita Municipl Hosp, Osaka, Japan; 6) Divn Med Genet, Clin Res Inst, Kanagawa Child Med Centr, Kanagawa, Japan; 7) Dept Hum Genet, Nagasaki Univ Grad Schl Biomed Sci, Nagasaki, Japan.

We present a large family with microphthalmia in Okinawa. In all male patients, bilateral microphthalmos, cardiovascular malformations, renal abnormalities, and mental retardation were observed. The pedigree showed X-linked recessive inheritance. Patients were diagnosed as having Lenz microphthalmia according to their clinical findings and the form of inheritance. Lenz microphthalmia syndrome was first described as a type of X-linked microphthalmia in 1955. It is known to exhibit genetic heterogeneity at this time, and two loci, Xq27-q28 and Xp11.4, have been mapped to be associated with the syndrome. In order to identify a mutation in the family, we performed exome sequencing by using a next generation sequencer and TruSeq Exome Enrichment system (illumina). To increase the sensitivity of detecting the mutation, pooled DNA with four affected males in the family was used for one exome sequencing. Of 552 called SNPs or indels on chromosome X, 51 were novel (not registered in SNP131). Then, four hemizygous (not detected as heterozygous) changes were found in exons. After comparison of exome data between affected and unaffected males, one substitution was identified and was confirmed by conventional direct sequencing in all the patients. The exome sequencing using pooled DNA of patients is effective and sufficient for mutation screening in families with X-linked recessive disorders.

1096T

Homozygous and heterozygous *IGFALS* mutations are detected in a significant fraction of patients with primary IGF-I deficiency and postnatal growth deficit. *A. Campos-Barros[1], S. de Frutos[1], E. Barroso[1], A. Gómez[1], R. Gracia-Bouthelier[2], J. Sánchez del Pozo[3], E. Gallego[3], K.E. Heath[1].* 1) Institute of Medical & Molecular Genetics (INGEMM), Hospital Univ. La Paz, UAM, IdiPaz, CIBERER, U753, Madrid, Madrid, SPAIN; 2) Pediatric Endocrinology, Hospital Universitario La Paz, Madrid, SPAIN; 3) Pediatric Endocrinology, Hospital Universitario 12 de Octubre, Madrid, SPAIN.

Introduction: Approximately 25% of proportionate short stature patients present with IGF-I deficiency in the face of normal or supranormal GH response tests, a condition defined as primary or idiopathic IGF-I deficiency. Dysfunctional *GH1* variants, mutations in the GHR coding gene, *GHR*, or in *STAT5b*, implicated in the post-receptor signalling pathway, explain a very low fraction of cases with primary IGF-I deficiency. On the other hand, recent reports have shown that mutations in *IGFALS* (16p13.3), encoding the acid-labile subunit (ALS) of the IGF-I/IGFBP-3/ALS ternary complex, may play a role in the etiology of primary IGF-I deficiency and short stature, indicating that ALS plays an important role regulating IGF-I bioavailability during postnatal growth. *Aim*: To investigate the frequency of *IGFALS* mutations in children with primary IGF-I deficiency and short stature without a known molecular defect. *Subjects*: 92 children with postnatal growth deficit (height <-2.0 SDS); decreased IGF-I (<-1.5 SDS) and normal or supranormal GH response tests. *Methods:* Mutation screening of coding sequences, intron/exon boundaries and known regulatory regions of *IGFALS*, *GHR*, *IGFBP3* and *IGF1* by HRM, DNA sequencing and MLPA. *Results*: We identified a total of 12 families with *IGFALS* mutations presenting 9 different mutations, 6 of which have not been previously described (p.S274F, p.R63G, p.L15dup p.R493H, p.T66K and p.L124P). Five unrelated families, sharing a common haplotype in the mutated allele, presented with the same mutation, N276S, previously described (Heath et al., 2008). Eight out of 9 mutations affect conserved residues, all located in leucine-rich repeat motifs of the mature ALS protein, whereas p.L15dup alters the hydrophobic signal peptide domain sequence of the precursor protein. None of the mutations were detected in 384 chromosomes from healthy controls. Two patients presented with a heterozygous *GHR* mutation, p.R229H, previously described (Goddard et al. 1995). ***Conclusions***: *IGFALS* mutations represent the most frequent molecular defect (13.0%) in the studied cohort of primary IGF-I-deficient patients with postnatal growth deficit. The detection of heterozygous *IGFALS* mutations in patients with decreased IGF-I, IGFBP-3 and ALS levels and postnatal growth deficit supports the hypothesis that ALS haploinsufficiency may be a factor involved in the etiology of moderate postnatal growth deficit associated with partial IGF-I deficiency.

1097T

TSHR is the main causative locus in autosomal recessively inherited thyroid dysgenesis. *H. Cangul[1], O. Tarim[2], Z. Aycan[3], H. Saglam[2], T. Yakut[4], Y. Cesur[5], E. Bober[6], G.A. Kirby[1], M. Karkucak[4], E. Eren[2], V. Bas[3], S. Cetinkaya[3], K. Demir[6], S.A. Yuca[5], S. Pasha[1], J.R. Forman[7], M. Kendall[8], A. Karthikeyan[9], N. Shaw[9], J. Kirk[9], W. Hogler[9], T.G. Barrett[9], E.R. Maher[1].* 1) Centre for Rare Diseases and Personalised Medicine, University of Birmingham, Birmingham B15 2TT, United Kingdom; 2) Department of Paediatric Endocrinology, School of Medicine, Uludag University, Bursa 16059, Turkey; 3) Division of Paediatric Endocrinology, Dr Sami Ulus Woman Health, Children Research Hospital, Ankara, Turkey; 4) Department of Medical Genetics, School of Medicine, Uludag University, Bursa 16059, Turkey; 5) Division of Paediatric Endocrinology, Faculty of Medicine, Yuzuncu Yil University, Van, Turkey; 6) 6 Division of Endocrinology, Department of Paediatrics, Faculty of Medicine, Dokuz Eylül University, Izmir, Turkey; 7) Institut Pasteur, Structural Bioinformatics Unit, 75015 Paris, France; 8) Exeter University, United Kingdom; 9) Department of Endocrinology and Diabetes, Birmingham Children's Hospital, Birmingham, UK.

We have analysed 215 cases with congenital hypothyroidism (CH) born to consanguineous families using microsatellite and sequencing analyses and found twelve mutations (seven novel) in TSHR and a mutational hotspot in exon 10 of the gene. Although the rate of mutation detection was low, our results revealed the TSHR as the major known-causative gene for the development of thyroid dysgenesis (CHNG) in consanguineous families. In silico analyses of the predicted structural effects of TSHR mutations indicated a good degree of correlation between the genotype and the disease phenotype. In addition we investigated the existence of the mutations we detected in fifty sporadic cases with CHNG, and found that these did not exist in non-familial cases. This study provides a clear evidence that TSHR is the primary known-locus for CHNG in consanguineous community, and that other Mendelian loci responsible for the development of the disease in the majority of cases remain unknown. Genome-wide autozygosity mapping studies we currently conduct in our cohort might help to identify novel genes causing congenital hypothyroidism.

1098T

Short stature and mutations in the ghrelin receptor (GHSR) gene. *M. Legendre[1,2], J. Pantel[2], C. Lazea[3], S. Cabrol[4], N. Collot[1], F. Dastot[1,2], P. Duquesnoy[2], L. Hilal[5], A. Kadiri[6], C. Dupuis[7], S. Nivot[8], S. Rose[1,2], A. Rothenbuhler[9], M.-L. Sobrier[2], M.-P. Vie-Luton[2], Y. Le Bouc[4], M. Polak[10], J. Leger[11], S. Amselem[1,2,12].* 1) Génétique moléculaire, Hôpital Trousseau, APHP, Paris, France; 2) Inserm U933, Hôpital Trousseau, Paris, France; 3) Clinica Pediatrie I, Cluj-Napoca, Romania; 4) Explorations fonctionnelles endocriniennes, Hôpital Trousseau, APHP, Paris, France; 5) Laboratoire de Génétique et Biologie moléculaire de la Faculté des Sciences, Kenitra, Morocco; 6) Service d'Endocrinologie, CHU Ibn Sina, Rabat, Morocco; 7) Département de Pédiatrie, CHU de Grenoble, Grenoble, France; 8) CHU de Caen, Service de Pédiatrie A, Caen, France; 9) Service d'Endocrinologie pédiatrique, Hôpital Saint-Vincent-de-Paul, APHP, Paris, France; 10) Service d'Endocrinologie pédiatrique, Hôpital Necker, Paris, France; 11) Service d'Endocrinologie pédiatrique, Hôpital Robert-Debré, APHP, Paris, France; 12) Université Pierre et Marie Curie, Paris, France.

Ghrelin, a ligand of the growth hormone (GH) secretagogue receptor (GHSR), is mainly known as the sole orexigenic hormone and as a strong stimulator of GH secretion. Surprisingly, murine models in which the ghrelin axis - i.e. ghrelin or its receptor - is disrupted do not show a pronounced lean and short phenotype, but mainly display subtle changes in glucose and lipid metabolism. So far, very few *GHSR* mutations have been implicated in short stature with or without altered GH secretion tests.

The objective if this study is (i) to further test the contribution of GHSR to isolated GH deficit (IGHD) and idiopathic short stature (ISS) and (ii) to provide a most complete description of the phenotype associated with each *GHSR* variation.

We screened the *GHSR* in 290 independent children with ISS or IGHD. We assessed the phenotype (height, weight, somatotrope investigation) of subjects carrying a *GHSR* variation. Constitutive activity, ghrelin response and cell surface expression of the new variants were assessed through appropriate in vitro assays.

Nine molecular defects were identified: in addition to three previously described mutations (A204E, W2X and R237W), we found six novel variations in the heterozygous state in six probands presenting with ISS (n=1) or IGHD (n=5). According to the functional studies that are underway, those *GHSR* mutations should account for 3 to 5% of the short stature phenotype in the study group. Except for W2X and R237W, short stature was transmitted in a dominant manner with incomplete penetrance. Variable expressivity of the disease phenotype was noticed. Several of the new variants so far studied showed an impaired constitutive activity associated or not with decreased cell surface expression.

This study, which strengthens the importance of GHSR constitutive activity in human growth, shows that GHSR mutations are responsible for at most 5% of short stature in humans.

1099T

Nonclassic Thyrotropin Resistance: *TSHR* Mutation Carriers with Discrepantly High Thyroidal Iodine Uptake. *S. Narumi[1], K. Nagasaki[2], T. Ishii[1], K. Muroya[3], Y. Asakura[3], M. Adachi[3], T. Hasegawa[1].* 1) Dept of Pediatrics, Keio Univ School of Med, Tokyo, Japan; 2) Dept of Pediatrics, Niigata Univ Medical and Dental Hospital, Niigata, Japan; 3) Dept of Endocrinology and Metabolism, Kanagawa Children's Medical Center, Yokohama, Japan.

Biallelic inactivating mutations in the thyrotropin (TSH) receptor gene (*TSHR*) are the chief molecular mechanism underlying end-organ insensitivity to TSH (*i.e.,* TSH resistance). Most patients with TSH resistance have low to normal thyroidal radioiodine uptake, which is consistent with the physiological knowledge that TSH stimulates iodine uptake via the Gs/cAMP pathway. To date, only one *TSHR* mutation-carrying family with discrepantly high thyroidal ^{123}I uptake has been reported[1].

In this study, we aimed to test whether *TSHR* mutation carriers with high ^{123}I uptake are observed in a cohort of Japanese patients with congenital hypothyroidism. We enrolled 24 Japanese patients having congenital hypothyroidism and high ^{123}I uptake, and sequenced *TSHR*. As a result, we identified two patients with biallelic *TSHR* mutations: p.[T145I]+[R450H] in one and p.[R450H]+[I661fs] in the other. The two subjects had slightly high ^{123}I uptake (41.8 and 43.0%, reference 8-40) but had a normal-sized thyroid. To verify the pathogenicity of the three mutations, we evaluated the capacities of mutant TSHRs to activate Gs- and Gq-coupled signaling pathways *in vitro*. Expression experiments revealed that T145I-TSHR retained partial ability to transduce both Gs- and Gq-coupled pathways, whereas I661fs-TSHR could transduce neither of them. R450H-TSHR had partial ability to transduce Gs-coupled signaling but had abrogated ability to transduce Gq-coupled signaling, indicating that coupling to Gq was dominantly affected.

In summary, we showed that 8% of Japanese congenitally hypothyroid patients with high ^{123}I uptake has inactivating *TSHR* mutations. Expression of this apparently discrepant phenotype, which we propose to term nonclassic TSH resistance, is presumably associated with the characteristic signaling property of the mutant TSHR, namely the Gq-dominant coupling defect.

References

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1100T

Whole exome sequencing in *SCN1A*-negative Dravet syndrome reveals novel candidate genes and suggests genetic heterogeneity. *G.L. Carvill[1], S.C. Yendle[2], J. McMahon[2], J. Cook[1], S.F. Berkovic[2], I.E. Scheffer[2], H.C. Mefford[1].* 1) Division of Genetic Medicine, Department of Pediatrics, University of Washington, Seattle, WA; 2) Epilepsy Research Center and Department of Medicine, University of Melbourne, Australia.

Dravet syndrome is an early-onset epileptic encephalopathy characterized by a distinct seizure pattern and progression, as well as patient decline and ultimately severe disability. The molecular etiology of _75% of Dravet syndrome patients can be attributed to *SCN1A* heterozygous mutations, with 90% of mutations arising *de novo*. In addition, *PCDH19* mutations have been identified in a Dravet-like syndrome; single cases have been described with dominant *GABRG2* and recessive *SCN1B* alterations. Consequently, a quarter of Dravet syndrome patients lack a definitive molecular diagnosis. In order to identify novel candidate genes for Dravet syndrome, we performed exome sequencing in six *SCN1A* mutation-negative patients, including two parent-proband trios and four unrelated probands. A *de novo* disease model revealed an unexpected, previously unidentified *SCN1A* acceptor splice-site mutation in the proband of the first trio, thus validating this approach for disease-gene identification. The patient of the second trio carries a *de novo*, rare, missense mutation in *GABRA1*, a GABA receptor subunit, which was not present in 1200 control exomes. Given the established inhibitory neurotransmitter properties of GABA and associated receptors, along with previous reports of *GABRA1* variants in two unrelated cases of milder, generalized epilepsy, this gene is an excellent candidate for Dravet syndrome. Mutation screening in additional patients with Dravet syndrome is ongoing. Analysis of the four probands revealed no putative mutations in *SCN1A*, *GABRA1*, nor any other known Dravet gene. Moreover, there was no gene in which each of the remaining four probands carried a rare variant. These results suggest that non-*SCN1A* Dravet syndrome may be marked by diverse genetic heterogeneity. Two candidate genes have been identified based on the presence of rare (but distinct) variants in two of the four probands; familial segregation studies of these variants are underway. We are investigating additional rare, highly conserved (GERP>3.5) variants in a strategic manner based on the likelihood of the variant's deleterious effect. Genes harboring *de novo* variants will be assessed in additional patients. In conclusion, whole exome sequencing to detect *de novo* disease-causing mutations in a severe epilepsy syndrome is an effective approach and has identified at least one candidate gene for follow up in Dravet syndrome.

1101T

Refinement of the benign familial infantile seizures (BFIS) chromosome 16 epilepsy locus in a large Utah family. *N. Singh[1], R. Mao[1,4], E. Lyon[1,4], S. Shetty[1,4], M. Dixon[2], M. Leppert[2], F. Filloux[3].* 1) Pathology, Univ Utah, Salt Lake City, UT; 2) Human Genetics, Univ Utah, Salt Lake City, UT; 3) Pediatrics, Univ Utah, Salt Lake City, UT; 4) ARUP Laboratories, Salt Lake City, UT.

Current estimates suggest that up to 40% of childhood epilepsies are caused by inherited genetic defects. Information about the precise defect that causes such epilepsies reduces the need for invasive procedures to determine the cause of seizures and can provide a precise target for tailored therapies, both of which greatly reduce the burden to children and their family members. One such childhood epilepsy is the early-onset benign familial infantile seizures (BFIS). In these patients, seizures typically begin around 3-8 months, and are characterized by psychomotor arrest, cyanosis, head and eye deviation to one side, diffuse tonic contraction and bilateral clonic jerks. Seizures in BFIS often occur in clusters but are short-lived and disappear within a few days, weeks or months in all patients. The later development of children with BFIS is generally normal. However, other complications are known to occur in some families with BFIS, and these include debilitating migraine headaches and an involuntary movement disorder known as paroxysmal dyskinesia (PD). We phenotypically characterized and collected DNA samples from a large four-generation Utah family with BFIS. Of the 41 family members collected, 14 exhibited at least one seizure beginning as early as 2 months with the majority of individuals experiencing multiple seizures. Extensive follow up on affected individuals demonstrated that no individuals had seizures past early childhood, and adult patients did not experience migraines or paroxysmal dyskinesia. A genome-wide linkage analysis using an autosomal dominant inheritance model with 70% penetrance gave a maximum LOD score of 4.99 for the D16S746 marker on chromosome 16p11.1. Adjacent polymorphic markers localized a 0.67cM region of no recombination at 16p11.2-q12.1. Four obligate carriers and six non-penetrant individuals were also identified. A larger overlapping region on chromosome 16 has been previously shown in multiple studies to harbor a gene for both BFIS and BFIS with PD. While the genetic region identified in our study appears small, the physical region is approximately 16Mb and contains 54 UCSC genes. An 11Mb human genome sequence gap that flanks the chromosome 16 centromere contributes to the paucity of known genes in this large physical region. Further examination of this critical linkage region in affected individuals will include an Affy 6.0 SNP analysis and next generation sequencing.

1102T

Exclusion of known lymphedema genes in two families with severe congenital lymphedema. *M. Amyere[1], S. Greenberger[2], D. Chitayat[3], E. Pras[4], K. Chong[3], T. Uster[3], H. Reznik-Wolf[4], D. Marek-Yagel[4], L. Boon[5], M. Vikkula[1].* 1) Human Molecular Genetics, de Duve Institute, Brussels, Belgium; 2) Vascular Biology Program and Department of Surgery, Children's Hospital Boston and Harvard Medical School, Boston, MA 02115, USA; 3) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital; University of Toronto, Toronto, Ontario, Canada; 4) Danek Gartner Institute of Human Genetics, Sheba Medical Center, Ramat-Gan, Israel; 5) Centre for Vascular Anomalies, Division of Plastic Surgery, Cliniques Universitaires Saint-Luc, Brussels, Belgium.

Lymphedema is a soft tissue swelling resulting from abnormal accumulation of interstitial fluid containing high molecular weight proteins due to abnormal drainage of lymph by the lymphatic vasculature. Primary lymphedema is due to an abnormal lymphangiogenesis which usually starts in utero. Some of the cases are inherited and in most cases have autosomal dominant or recessive mode of inheritance. Some of the autosomal dominant cases have incomplete penetrance and variable expression. Both syndromic and non-syndromic cases have been caused by mutations in genes with major role in lymphangiogenesis including FOXC2, VEGFR3, SOX18, CCBE1, PTPN14 and GCJ2. We report two consanguineous families with recurrence of an autosomal recessive form of congenital lymphedema. One of the families is of Iranian-Jewish and Israeli descent and the other of Iraqi descent. Direct sequencing of the known lymphedema genes including coding sequences and intron-exon boundaries, did not identify any mutation. Whole genome scan using Affymetrix SNP-Chip 250K was thus performed in both families and no copy number change was identified in the affected members. Autozygosity mapping and parametric linkage analysis also excluded the candidate lymphedema-genes. Three overlapping autozygous regions were identified in the two families and were confirmed by linkage analysis. Our findings provides evidence for the existence of new causative gene or genes associated with the autosomal recessive form of congenital lymphedema. (E-mail: Miikka.vikkula@uclouvain.be).

1103T

Genetic Linkage Analysis to Identify Susceptibility Loci for Primary Biliary Cirrhosis (PBC) in British Columbia's First Nations Peoples. *S. Asuri[1,2], S. McIntosh[1], L. Fields[1], L. Arbour[1,2].* 1) Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Division of Medical Sciences, University of Victoria, BC, Canada.

Introduction: While rare in most populations, PBC is highly prevalent in the First Nations (FN) population in British Columbia reflecting a referral rate for liver transplantation eight times higher than non-FN. PBC is a chronic autoimmune liver disease progressing from destruction of interlobular bile ducts to cirrhosis, often necessitating liver transplantation. It mainly affects women, in their 40-60s. Other autoimmune diseases frequently co-exist with PBC. Both genetic and environmental factors are considered to likely influence the pathogenesis. Genetic factors that predispose to PBC are continuously being elucidated and indicate population specificity. Our goal was to identify susceptibility loci that may contain genes predisposing to PBC in this population using genome wide family based linkage analysis. **Methods:** To date 130 FN participants from 31 families had enrolled in the study (45 affected and 85 without PBC) Two of three diagnostic criteria (positive AMA, increased liver enzymes, liver biopsy with defining features) were necessary for inclusion as a case. After review of medical records, anyone with inconclusive diagnosis, lack of DNA sample were excluded. The final whole-genome linkage which included 32 “affected” and 35 informative “unaffected family controls” from 26 families was performed using one array of the Affymetrix 5.0 set. *Merlin* was used to perform multipoint parametric and nonparametric linkage. Linkage disequilibrium was controlled for to prevent inflation of LOD scores. **Results:** The maximum LOD score of 2.3 was seen at chromosome 19p13 and LOD scores >2.0 were seen at 1q23, 6q21, 9q21, 17p13. Genes residing in these loci were identified as plausible candidates, including *ICAM-1, Netrin-1* (previous association with PBC), *RUNX-2*, *CDKAL1*, *STX-8*, *DNMT-1*, *TYK2* (association with other autoimmune disease), *Netrin-1*, *TrkB*, *TJP2*, *laminin(4*, *GAS7*, *Pin-1* (involved in apoptosis regulation), *PBX-1*, *CD2AP*, *WASF-1*, *Fyn* (regulation of immune signaling pathways such as *TGF-*) , *NF*2) , *IFN* pathway). **Conclusion:** As with other recent genome-wide analysis multiple loci were recognized to possibly contribute to genetic susceptibility for PBC. Although some loci contain genes previously implicated in PBC and other autoimmune diseases such as SLE, arthritis and psoriasis, unique loci harboring genes that may play a role in PBC pathogenesis were also identified. Further evaluation of this association is underway.

1104T

Linkage and homozygosity mapping identifies a 14 bp deletion in *CHX10* in families with microphthalmia and brain atrophy. *K.K. Selmer[1], G.D. Gilfillan[1], P. Strømme[2], O.J. Kanavin[2], T. Hughes[1,3], K. Brandal[1], R. Lyle[1], D.E. Undlien[1,3].* 1) Dept. of Medical Genetics, Oslo University Hospital, Oslo, Norway; 2) Dept. of Pediatrics, Oslo University Hospital, Oslo, Norway; 3) Institute of Medical Genetics, University of Oslo, Oslo, Norway.

Anophthalmia and microphthalmia (AM) are common causes of blindness, and birth prevalence is approximately 30 per 100 000 individuals. More than half of the patients have a syndromic form with other clinical symptoms in addition to the eye anomalies.

The purpose of this study was to find the genetic cause of an autosomal recessive form of microphthalmia in three Pakistani families with one affected child each. All three patients were also severely affected by progressive and widespread brain atrophy. The families all originated from a small Pakistani village founded by eight brothers in the 1820's. Consanguineous marriages were common in this community and the parents in one of the three families were cousins. Linkage analysis in combination with homozygosity mapping was performed by genotyping SNPs genome-wide. The longest homozygous stretch shared by the patients was 2.4 Mb and reached the maximum possible LOD score for these families. This region was a strong candidate region, as it contained one of the genes known to be responsible for non-syndromic AM; the *CHX10* gene. This gene was sequenced and a 14 bp deletion in exon 3 was identified in all three patients. The deletion interrupted the reading frame and created a premature stop codon downstream from the deletion. As severe forms of brain atrophy have not been described in other patients with homozygous *CHX10* null mutations, we hypothesized that another linked gene was also homozygously mutated. Targeted DNA capture of the candidate region was performed and the DNA sequenced with the use of high throughput sequencing techniques. The sequencing of this region did not reveal any likely disease mutations.

We have identified a 14 bp deletion in three Pakistani families with microphthalmia and brain atrophy. Learning difficulties and autism have previously been described in patients homozygous for *CHX10* null mutations, so it is possible that our patients represent a severe form of this clinically heterogeneous disease. However, the severe brain involvement which led to death in all three patients, could also suggest that an additional, unknown disease locus is involved.

1105T

Role of Fragile X Related Proteins in mammalian circadian behaviors and glucose homeostasis. *J. Lumaban, D. Nelson.* Molec & Human Gen, Baylor College Med, Houston, TX.

Fragile X syndrome, the most common form of inherited developmental disability, results from the absence of the *fragile X mental retardation 1* (*FMR1*) gene product FMRP. *FMR1* has two paralogs in vertebrates: *fragile X related gene 1* and *2* (*FXR1* and *FXR2*). One of the behavioral symptoms observed in fragile X patients is the increased occurrence of sleep disorders. *Fmr1* or *Fxr2* knockout (KO) mice display a shorter free-running period of locomotor activity in total darkness (DD) compared to wild-type mice, while mice lacking both *Fmr1* and *Fxr2* (double knockout) exhibit complete loss of rhythmic activity in both the light:dark cycle (LD) and DD. DKO mice also display significant alterations in the cyclical patterns of abundance of core clock component messenger RNAs in the liver, but not in the suprachiasmatic nucleus. These findings suggest that Fmrp and Fxr2p are acting downstream of the central clock to control rhythm in mice, but it is unclear how the FXRs affect the peripheral outputs, such as in the liver.

Locomotor assays with restricted feeding demonstrate that the *Fmr1/Fxr2* DKO mice were able to adjust their rhythm to food availability. Remarkably, these mice also had a significantly higher mortality rate (~60%) in the first three days of restricted feeding, which suggests a difference in their physiological response to food restriction. Circadian cycling in the liver is known to contribute to glucose homeostasis, and we are testing for the specific involvement of FXRs in this system. In mice of similar body weight, body fat is significantly lower in the *Fmr1/Fxr2* DKO mice compared to WT. They had consistently low levels of glucose, cholesterol, and leptin over a 24hr cycle, while the cycling of the other metabolic markers in the blood- adiponectin, glycerol, free fatty acids, and triglycerides, but not insulin- is out of phase. The DKO mice also exhibited exaggerated clearance of a bolus of glucose, and hypersensitivity to insulin, even as both insulin production and gluconeogenesis appear normal. The close association of food input to the circadian system and the timing of sleep and wakefulness, together with the typical disturbances of circadian behavior and sleep in Fragile X syndrome, open up a new perspective for the investigation and treatment of patients suffering from this disorder.

1106T

Evaluation of a PCR-based assay using Sizing PCR in combination with CGG Repeat Primed (RP) PCR for FMR1 screening in a clinical setting. *K. Storm, N. Peeters, W. Wuyts, F. Kooy.* Department of Medical Genetics, University & University Hospital of Antwerp, Edegem, Antwerp, Belgium.

Abnormal expansion of the CGG repeat (> 200 CGGs) in the 5' untranslated region of the FMR1 gene (Xq27.3) causes Fragile X syndrome, a common cause of inherited mental retardation. This CGG expansion causes hypermethylation of the FMR1 promotor region resulting in FMR1 gene silencing. Smaller unmethylated CGG expansions have been associated with premature ovarian insufficiency (POI) and Fragile X tremor/ataxia syndrome (FXTAS). The CGG repeat lengths can be divided in four diagnostic categories: 1) normal alleles with ≤ 49 CGG repeats 2) gray zone alleles (50-58 CGGs) 3) premutation alleles (59-200 CGGs) and 4) full mutation alleles with more than 200 methylated CGG repeats. In our lab's routine Fragile X testing, the length of the CGG repeat is first sized by PCR with primers flanking the CGG repeat. If no normal allele is amplified in males or no heterozygosity for normal alleles is observed in females, further investigation by Southern blot (SB) analysis is required to detect/exclude larger CGG repeats and to examine the methylation status of the FMR1 gene. Although regarded as the golden standard, SB analysis is laborious, time consuming and requires large amounts of high quality DNA. We now evaluated a commercially available assay, Asuragen's CE-marked AmplideX FMR1 PCR Kit, by analyzing a heterogeneous set of DNA samples (from different matrices) including a whole range of FMR1 expansion types (normal, gray zone, premutation, full mutation and mosaic samples of both genders) that were previously characterized by PCR and SB in our lab. We were able to detect retrospectively all known expansions, including mosaic patterns. Homozygous, heterozygous (resolution of 1 repeat) and expanded allele carrier status could reliably be determined in females. Exact sizing was possible for a spectrum of premutation alleles. The assay is also sensitive to size mosaicism and AGG interruptions. In conclusion, this PCR-based assay provides a final conclusion for the large majority of referred cases and reduces the need for SB testing. For a minority of samples, a methylation-sensitive test is still required to unequivocally distinguish (large) premutation alleles from (small) full mutation alleles where correct interpretation still depends on methylation status.

1107T

Functional Characterization of novel variants in Noonan Syndrome. *J.J. Lee[1], B.H. Lee[1,2], J.M. Kim[1], G.H. Kim[1], J.H. Choi[2], H.W. Yoo[1,2].* 1) Medical Genetics Center, Asan Medical Center, Seoul, Korea; 2) Dept. pediatrics, Asan Medical Cetner Children's hospitol, Seoul, Korea.

Noonan syndrome (NS) exhibits functional alterations of the Ras-mitogen-activated protein kinase (MAPK) pathway. Mutations in *PTPN11* (39.0%), *SOS1* (20.3%), *RAF1* (6.8%), *KRAS* (5.1%), and *BRAF* (1.7%), encoding the members of the Ras-MAPK pathway, were identified in 59 Korean patients with NS. Among these, p.P261T and p.S259T of *RAF1* and p.K170E of *SOS1* were not previously reported. All these mutations are located on highly conserved sites. In silico analyses also predicted these variants as mutations. p.P261T and p.S259T are located in conserved region 2 (CR2) domain of RAF1. The phosphorylated status of p.S259 in the CR2 domain suppresses RAF1 activation in the resting state; when GTP-Ras binds RAF, this residue becomes dephosphorylated, leading to a partial disinhibition or activation of RAF1. In particular, the mutations in two variants, p.S259T and p.P261T, were located at or near the p.S259 residue. Accordingly, their in vitro activities were higher than those of wild-type RAF1 in the presence of growth factor. On the other hand, p.K170E in *SOS1* is located in the HF domain, where NS mutations have rarely been reported. The HF domain is predicted to interact with the pleckstrin-homology (PH)-RAS-exchange motif (Rem) domain linker or helical linker. The ionic interactions between p.R552 or p.S548 in the PH-Rem linker and p.D140 and p.D169 in the HF domain are critical for stabilizing this autoinhibitory conformation of SOS1. p.K170E is located near these ion-pair connections and expected to affect this pairing, partially disrupting the autoinhibitory conformation to expose the Ras-binding site, even in the resting state. Consistent with this inference, endogenous expression of the Ras-GTP complex was higher in the COS-7 cells expressing p.K170E than in those expressing wild-type SOS1. In conclusion, our study helps to understand molecular pathogenic mechanisms underlying NS.

1108T

Congenital diarrheal disorders: translating whole exome sequencing to the clinic. *M. Yourshaw, D. Liu, S.F. Nelson, M. Martín.* University of California Los Angeles, Los Angeles, CA.

Selective and generalized nutrient malabsorption causes chronic diarrhea and may require life-long total parenteral nutrition (TPN). Although several mutations have been identified that cause generalized malabsorptive diarrhea (GMD), many cases have an unknown genetic etiology. Metabolic profiles and histology may be insufficient to indicate appropriate genetic testing and to diagnose and prescribe treatment for such cases. We selected a number of patients for unbiased whole exome sequencing. Sequencing libraries were prepared from sheared DNA that had been extracted from saliva samples. The libraries were enriched for protein-coding regions of the genome by in-solution capture with RNA probes. Libraries were sequenced on either the SOLiD or Illumina platforms. The short sequencing reads were aligned to the reference human genome. Mean oversampling of bases was ~40X and ~25% of the ~45Mb targeted exome was read at least 10X. Variants were identified and filtered on sequencing and mapping quality. We filtered for alleles that would adversely affect protein coding or splicing, and that were not found in dbSNP or control exomes. Using custom software, under a recessive model, we searched for potentially harmful variants that were homozygous or compound heterozygous. In instances where DNA from affected siblings was available, we required that both siblings have the same alleles. In addition to identifying a number of alleles that require further functional validation, we identified a single plausible variant genome-wide in one patient: a novel nonsense mutation in proprotein convertase subtilisin/kexin type 1 (PCSK1), a calcium activated serine endoprotease, A deficiency in PCSK1 can lead to a diverse combination of symptoms including severe enteropathic diarrhea and diabetes insipidus. This information was instrumental in diagnosing previously unsuspected diabetes in this patient and led to appropriate treatment.

1109T

Demonstration of the interactions of genes and environment in determining the phenotype of a genetic disease using a mouse model of a fatty acid oxidation disorder. *W. Wang[1], A.-W. Mohsen[2], J. Palmfeldt[3], M. Barmada[1], Y. Wang[2], N. Gregersen[3], J. Vockley[1,2].* 1) Department of Human Genetics , Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA; 2) Department of Pediatrics,School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA; 3) Research Unit for Molecular Medicine, Institute of Clinical Medicine, Aarhus University Hospital and Faculty of Health Sciences, University of Aarhus, Aarhus, Denmark.

Objective:Gene-environment interactions are typically measured in epidemiological studies. Ascertaining reproducible cellular change in genetically susceptible individuals to environmental exposure remains a challenge. Restricting the search for interactions to gene products that interact in relevant biological pathways is promising. Very long chain acyl-CoA dehydrogenase (VLCAD) deficiency identified through newborn screening is a clinically heterogeneous fatty acid) -oxidation disorder without clear genotype-phenotype correlations. Acute symptoms are often induced by stress. We studied fasting effects using a genetic mouse model to explore changes in gene-environment interaction and identify gene-gene interactions in associated pathways. Methods:The mitochondrial proteome of VLCAD deficient mice was quantitatively compared to wild type mice in fed and fasted states. iTRAQ labeling with nano-LC/LTQ-Orbitrap mass spectrometry was employed to identify the changes in both deficient and wild type groups and the differences in response to fasting. The affected pathways were evaluated using Ingenuity Pathway Analysis and a regression model was applied to assess the changes associated with ACADVL gene and fasting. Results: We identified numerous proteomic changes associated with gene deficiency and fasting as well as gene-gene interactions within relevant pathways. Most of changes induced by fasting were different and few changes were shared in the mutant and wild type mice. The pattern of changes in chaperone proteins including HSPD1 and HSPE1 upon fasting was different in them. Two consensus proteins NUDT7 and NLTP were altered in deficient mice regardless of feeding status. Fasting reversed the compensatory increase in oxidative phosphorylation in fed deficient mice. Secondary changes in fasted deficient mice were involved in cardiac dysfunction. Conclusion: Fasting induced different proteomic changes in deficient mice indicate differences in the interactions of genes and gene-environment, and important roles of these interactions in the pathogenesis of VLCAD deficiency. Particularly, fasting in mutants reversed the protective response in specific pathways induced by genetic deficiency alone. Our system presents an approach to explore gene-environment interaction and their contribution to gene-gene interactions. Future characterization of functional interactive genes and association studies in humans will provide further insight into disease mechanism.

1110T

Identification of a Novel Gene for Autosomal Dominant High-grade Myopia Using Whole Exome Sequencing. *T. Young[1,2,3], A. Powell[2], T. Klemm[3], S. Rozen[3], V. Soler[2], T. Yanovitch[1], E. Nading[1], F. Hawthorne[2], L. Goh[3], K-N. Tran Viet[2].* 1) Pediatric Ophthalmology, Duke Univ Eye Ctr, Durham, NC; 2) Duke Center for Human Genetics, Duke University Medical Center, Durham NC; 3) Duke- National University of Singapore, Singapore.

Purpose: To identify a gene for autosomal dominant high-grade myopia in a large Caucasian pedigree using deep sequencing technology. Methods: A 15-member family with high-grade myopia (8 affected, mean affected dioptric spherical equivalent of -12.00) was ascertained. Next-generation sequencing at 50X using the Illumina Hi Seq technology and whole exome capture processing was performed. Sequence reads were aligned, and processed for single nucleotide variant and insertion/deletion calls. In-house pipeline filtering analyses along with comparisons using the 1000 Genomes and dbSNP databases were performed. Expression studies using RT-PCR of extracted mRNA converted to cDNA of systemic and ocular tissues were performed using exon-specific primers. GAPDH primers were designed for housekeeping expression studies. Results: A novel premature stop codon mutation Q53X was discovered for the gene SCO2 which maps to chromosome 22q13.33. This followed segregation in all affected family members, and was not found in 800 control DNA samples. Expression of SCO2 was determined in the retina, retinal pigment epithelium, and sclera of 24 week fetal and adult eye tissues. Conclusions: An SCO2 gene mutation was found to segregate with high-grade myopia in a large autosomal dominant pedigree. The gene product is implicated in cellular copper homeostasis. Copper deficiency has historically been associated with myopic refractive error development. A biologic mechanism for this gene may involve poor collagen and elastin cross-linking of the scleral wall, and/or subtle retinal photoreceptor alteration. This is the first identification of a novel causative gene for autosomal dominant high-grade myopia.

1111T

Interferon gamma impact on Epidermolysis Bullosa Simplex in patients with keratin 14 mutations. *T. Farez[1], M. Bchetnia[1], M. Tremblay[1], G. Leclerc[2], A. Dupérée[2], J. Powell[3], C. McCuaig[3], C. Morin[2], V. Legendre-Guillemin[1], C. Laprise[1].* 1) Dept Sciences fondamentales, universite du Quèbec à Chicoutimi, Saguenay, Canada; 2) Centre de santé et de services sociaux de Chicoutimi, Saguenay, Canada; 3) Hôpital Sainte-Justine, Montréal, Canada. Hôpital Sainte-Justine, Montréal, Canada. Hôpital Sainte-Justine, Montréal, Canada.

Scientific context: Epidermolysis bullosa simplex (EBS) is a rare genetic disease characterized by basal keratinocytes cytolysis, intraepidermal blister formation and skin fragility. EBS is classified into different phenotypes according to the severity: EBS-localized (EBS-loc), EBS-generalized (EBS-gen) and EBS-Dowling-Meara (EBS-DM). It is generally inherited in an autosomal dominant manner and caused by mutations in keratin 5 (KRT5) or keratin 14 (KRT14) genes. In normal cells, both keratin types are associated to form an intermediate filaments network that allows maintaining skin integrity. In EBS, mutations lead to disruption of intermediate filaments assembly. In addition to type I keratin 5 (K5) and type II keratin 14 (K14), the epidermis basal layer produces keratin 15 (K15), that also belongs to type II keratin. Radoja and al. (2004) have suggested that stimulating K15 production could remedy to cytoskeleton defects in EBS harboring K14 mutations by forming heterodimers with K5. More therapeutic molecules need to be tested in order to reduce the symptoms or cure this disease and modulation of K15 could be a promising avenue. Objectives: Based on literature data, our interest focused on the effect of interferon gamma (IFN-.) on cultured keratinocytes from EBS KTR14 mutants by analyzing K15 production level. Methods: Samples consisted of 3 immortalized keratinocyte cell lines isolated from patients (1 EBS-DM and 2 EBS-loc) and 3 isolated from controls. A dose-response with IFN-. was performed for 48 hours. The amount of K15 was measured by Western blot. KRT15 gene expression level was measured by qRT-PCR. Results: The amount of K15 is decreased with increasing amount of IFN-. . This decrease is more important in patient's cell lines than in control ones. The qRT-PCR data validates these results. Indeed, KRT15 RNA expression is decreased in presence of IFN-. but tendency did not reach significance. Conclusion: These results suggest that IFN-. reduces K15 production in EBS.

1112T

Potential therapeutic target of 185delAG, 5382insC, as two types of mutations in BRCA1, in breast cancer. *H. Fiuji[1], A. Avan[2, 3], M. Maftouh[3], R. Mirhafez[4], A. Avan[5].* 1) Department of Biochemistry, Payam Nour University, Mashhad, Iran; 2) 3. Department of Clinical Genetics, Section Oncogenetics, Medical Faculty, VU Medical Center Amsterdam, Amsterdam, The Netherlands; 3) Department of Medical Oncology, VU Medical Cancer Center Amsterdam, VUmc, PO Box 7057, 1007 MB, Amsterdam, The Netherlands; 4) 4. Department of Modern Sciences and Technologies, Faculty of Medicine, Mashhad University of Medical Science, Mashhad, Iran; 5) Clinic of Wilson's disease, Imam Reza Hospital, Tabriz University of Medical Sciences, Tabriz, Iran.

BRCA1 is one of the most important genes that is interfering with other genes particularly BRCA2, p53, which can be crucial for breast and ovarian cancer. In this abstract we have shown two significant mutations and its role on protein encoding. Investigation of these prevalence mutations in BRCA1, 185delAG, 5382insC, can give a clue for curing breast and ovarian cancers.massive activity of BRCA1 and taking part in cell as maintaining genome by engaging in DNA repair, cell cycle check point control and also mitotic or cell division steps show the significant role of this gene. 185delAG, 5382insC which have located on exon2 and exon 20, respectively, bring about to produce of deficient protein (BRAt). These are prevalence mutations among major population in the world, however, these mutations has found essentially in the Jewish- Ashkenazi with high frequency in the family breast and ovarian cancers, resulting in interbreeding among the group. This group has ancestors from Eastern and central Europe and 1% of this population are carriers of the most common founder mutation, the BRCA1 185delAG and 5382insC truncation. These are frame shift mutation that results in a premature stop signal at codon 39, 1829Ter, for 185delAG and 5382insC, respectively in the BRCA1 protein. This mutation is also carried by significant numbers of non-Jewish Spanish, Spanish Gypsy, and women of mid-eastern decent. Women carrying these mutations have an approximate 65% lifetime risk for developing breast cancer and 15%-54% lifetime risk for developing ovarian cancer.taken together, 185delAG and 5382insC have critical points for BRCA1 gene structure and produce of truncations protein causing to influence on breast and ovarian cancers. These mutations are founded among the other different country populations and it shows global prevalence of them. Furthermore, our results also suggest the presence of these two mutations in some of the Iranian patients and also, highlight its potential as a therapeutic target for treatment of breast and ovarian cancers.

1113T

Deletion of Porcn in mice confirms that defective WNT signaling causes the features of human Goltz syndrome (Focal Dermal Hypoplasia). *W. Liu[1], T. Shave[2,4], X. Wang[1], A. Balasa[3], S. Wen[1], H. Nguyen[2,4], I. Van den Veyver[1,5,6].* 1) Dept OB/GYN, Baylor Col Med, Houston, TX; 2) Dept MCB, Baylor Col Med, Houston, TX; 3) Dept Pediatrics, Baylor Col Med, Houston, TX; 4) Stem Cells and Regenerative Medicine Center, Baylor Col Med, Houston, TX; 5) Dept MHG, Baylor Col Med, Houston, TX; 6) The Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston TX.

Focal Dermal Hypoplasia (FDH) is caused by dominant loss-of-function mutations in X-linked PORCN. PORCN orthologues in Drosophila and mice encode endoplasmic reticulum proteins required for secretion of Wnt proteins. Hence, the pleiotropic features of FDH likely result from defective WNT signaling, but molecular proof and animal models have been lacking. We generated conditional Porcn mutant mice by introducing intronic loxP sites and a neomycin gene in the mouse Porcn locus for conditional inactivation. Porcn-ex3-7flox mice have no apparent developmental defects, but chimeric mice retaining the neomycin gene (Porcn-ex3-7Neo-flox) have limb, skin and urogenital abnormalities. Conditional Porcn inactivation by EIIa-driven Cre-recombinase results in increased embryonic lethality. Prx-Cre driven inactivation produces FDH-like limb defects, while Krt14-Cre driven inactivation produces alopecia and abnormal dentition. Cell-based assays confirm that human PORCN mutations can reduce WNT3A secretion. Combined with findings on the Porcn animal model, this demonstrates that human FDH indeed results from defective WNT-signaling.

1114T

Increased oxidative stress in Nijmegen Breakage Syndrome results from PARP hyperactivation after DNA damage. *M. Digweed, H. Krenzlin, I. Demuth, B. Salewsky.* Institute of Medical and Human Genetics, Charité - Universitätsmedizin Berlin, Berlin, Germany.

The NBN gene codes for the protein nibrin, an integral member of the MRE11/RAD50/NBN (MRN) complex essential for the processing of both physiological DNA double strand breaks and those resulting from exposure to ionizing radiation. Hypomorphic mutation of the NBN gene underlies Nijmegen Breakage Syndrome (NBS), an autosomal recessive genetic disease. The cardinal features of NBS can be attributed to a failure in DSB repair/processing: immunodeficiency, radiosensitivity, meiotic failure and an extremely high incidence of malignancies. The occurrence of cancer is particularly high in NBS compared to other genetic instability syndromes and is even increased amongst heterozygous relatives. In order to understand this tumor predisposition we have been examining effects of NBN mutation beyond the DSB repair defect itself. Such studies in conditional NBN null mutant mice have indicated disturbances in redox homeostasis. Clearly this could contribute to DNA damage, an increased mutation rate and cancer. Indeed, several hours after exposure of mouse cells to a mutagen, the complete absence of nibrin results in high levels of reactive oxygen species. The same is true, if not so pronounced, for NBS patient cells. This increased oxidative stress is caused by depletion of NAD+ due to hyperactivation of the strand-break sensor, Poly(ADP-ribose) polymerase. Indeed, specific inhibition of PARP reverses the oxidative stress phenotype of both null mutant nibrin mouse cells and NBS patient cells. We conclude that the increased mutation rate in NBS patient cells and the extremely high incidence of malignancy amongst patients is due to the combination of a primary DSB repair deficiency with secondary oxidative DNA damage.

1115T

Dominant mutations in RP1L1 are responsible for occult macular dystrophy (Miyake's Disease). *T. Iwata[1], M. Akahori[1], K. Tsunoda[1], Y. Miyake[1, 2], Y. Fukuda[3], H. Ishiura[3], S. Tsuji[3], T. Usui[4], T. Hatase[4], M. Nakamura[5], H. Ohde[6].* 1) National Institute of Sensory Organs, Tokyo, Japan; 2) Aichi Medical University, Aichi, Japan; 3) University of Tokyo, Tokyo, Japan; 4) Niigata University, Niigata, Japan; 5) Nakamura Eye Clinic, Nagoya, Japan; 6) Keio University, Tokyo, Japan.

Occult macular dystrophy (OMD, Miyake's Disease) is an inherited macular dystrophy characterized by progressive loss of macular function but normal ophthalmoscopic appearance. Typical OMD is characterized by a central cone dysfunction leading to a loss of vision despite normal ophthalmoscopic appearance, normal fluorescein angiography, and normal full-field electroretinogram (ERGs), but the amplitudes of the focal macular ERGs and multifocal ERGs are significantly reduced at the central retina. Linkage analysis of two OMD families was performed by Affymetrix SNP 6.0 array and the SNP High Throughput Linkage analysis system (SNP HiTLink). Coding exons of four protein coding genes in the candidate region were analyzed for sequence variations by direct DNA sequencing. Immuno-staining for RP1L1 was carried out on frozen sections of normal cynomolgus and paraffin section of normal marmoset. Genome wide linkage analysis localized the disease locus to chromosome 8p22-p23. Among the 128 genes in the associated region, 22 genes were expressed in the retina, and four candidate genes were selected. No mutations were found in the first three candidate genes, methionine sulfoxide reductase A (MSRA), GATA binding 4 (GATA4), and pericentriolar material 1 (PCM1), however, amino acid substitution of p.Arg45Trp in retinitis pigmentosa 1-like 1 (RP1L1) was found in three OMD families and p.Trp960Arg in a remaining OMD family. These two mutations were detected in all affected individuals but none in 876 controls. Immunohistochemistry of RP1L1 in the retina section of cynomolgus and marmoset monkey revealed expression in the inner segment of rod and cone photoreceptor, supporting a role of RP1L1 in the photoreceptors that, when disrupted by mutation, leads to OMD. Identification of RP1L1 mutations as causative for OMD has potentially broader implications for understanding the differential cone photoreceptor functions in the fovea and the peripheral retina. The function of RP1L1 is being investigated.

1116T

Exome sequencing in patients with DOOR (Deafness, Onycho-Osteodystrophy, and Mental Retardation) syndrome. *D. Kasperaviciute[1], A. Tostevin[1], V. Plagnol[2], S. Mead[3], S. Nampoothiri[4], E. Blair[5], H. Cross[6], R.C.M. Hennekam[7], S.M. Sisodiya[1].* 1) Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, Queen Square, London, WC1N 3BG, UK; 2) UCL Genetics Institute, London, UK; 3) MRC Prion Unit, Department of Neurodegenerative Diseases, UCL Institute of Neurology, London, UK; 4) Amrita Institute of Medical Sciences and Research Centre, Cochin, Kerala, India; 5) Department of Clinical Genetics, Oxford Radcliffe Hospitals National Health Service (NHS) Trust, Churchill Hospital, UK; 6) Neurosciences Unit, UCL Institute of Child Health, London, UK; 7) Department of Pediatrics, Academic Medical Center, University of Amsterdam, The Netherlands.

DOOR syndrome is a rare disorder characterised by sensorineural hearing loss, hypoplastic or absent nails on hands and feet and intellectual disability. Additional clinical features include seizures from infancy, optic atrophy, polyneuropathy, and, sometimes, dental, cardiac and renal abnormalities. Autosomal recessive inheritance has been suggested, although de novo dominant mutations cannot be excluded as causal. We have sequenced exomes of three unrelated patients with DOOR syndrome. Agilent SureSelect Exome capture followed by 90bp pair-end sequencing on Illumina HiSeq platform was used to sequence the exomes to >40x average read depth. The reads were aligned using Novoalign software, and single nucleotide variants and indels were identified and annotated using a combination of Samtools, Dindel, Annovar software and in-house scripts. Since DOOR syndrome is a very rare condition with just over 30 patients having been described in the literature so far, we used strict filtering to reduce the number of potential candidate causal variants. Filtering included removing variants described in dbSNP build 130, 1000 Genomes Project, 46 controls genomes released by Complete Genomics and an in-house database of 224 sequenced exomes, as well as removing genes in which loss-of-function variants are frequent. To identify candidate causal variants, we then investigated both autosomal recessive and de novo dominant mutation models. We used homozygosity mapping in a patient from a family with known consanguinity to identify 11 identical-by-descent candidate genes harbouring non-synonymous coding variants. None of the other two unrelated patients had rare homozygous or compound heterozygous variants in these genes. No other gene had homozygous or compound heterozygous rare variants in all three patients. To investigate a de novo dominant mutation model, we looked for the genes that had rare loss-of-function, non-synonymous or essential splice-site variants. No genes had such changes in all three patients, and 37 genes had such changes in two patients. Further evaluation in family members and unrelated patients is underway.

1117T

Mapping of a lethal disorder with pulmonary hypertension and limb deficiency. *D.C. Lynch[1], K.M. Boycott[2], D.E. Bulman[3], J.S. Parboosingh[1], A.M. Innes[1].* 1) Medical Genetics, Alberta Children's Hospital, Calgary, Alberta, Canada; 2) Children's Hospital of Eastern Ontario, Ottawa, Ontario; 3) Ottawa Hospital Research Institute, Ottawa, Ontario, Canada.

The Hutterite population of North America is a genetic isolate and provides a powerful opportunity for mapping genetic diseases. Several Mendelian disorders are present in the population, some of which are unique to or overrepresented in the Hutterites. Alveolar capillary dysplasia (ACD) is a rare and invariably lethal form of persistent pulmonary hypertension of the newborn. A distinct form of ACD has been reported in the literature associated with bilateral tibial agenesis and missing digits, which we have termed alveolar capillary dysplasia with limb deficiency (ACD-LD). The objective of this study is to map a locus and identify a mutation for ACD-LD, which is presumed to follow an autosomal recessive pattern of inheritance. We studied one Hutterite family which includes two children with a disorder resembling ACD-LD and two unaffected siblings. Due to the endogamous nature of the population, a homozygosity mapping approach was used. A 27 Mb region of shared homozygosity in the patients was identified on chromosome 10 using an Affymetrix Genome-Wide Human SNP Array 6.0 and genotyping with microsatellite markers. Two genes within the region, NRP1 and BAMBI, were prioritized for sequencing due to their potential involvement in lung vascular and/or limb development, but no mutations were identified. Within our lab, whole exome sequencing in as little as one Hutterite patient has been shown to be an efficient method of disease gene identification when paired with homozygosity mapping. Whole exome sequencing using the Agilent SureSelect platform is underway; analysis of the genes within the linkage interval is expected to reveal a putative mutation.

1118T

Whole exome sequencing in a consanguineous pedigree reveals 2 novel RYR1 mutations in patients with complete external ophthalmoplegia. *S. Shaaban[1,2,3], C. Andrews[1,2,3,4], J. Demer[5], E. Engle[1,2,3,4,6,7,8].* 1) Neurology, Children's hospital Boston, Boston, MA; 2) FB Kirby Neurobiology Center, Boston, MA; 3) Harvard Medical School, Boston, MA; 4) Howard Hughes Medical Institute, Chevy Chase, Maryland; 5) Ophthalmology, Neurology, Bioengineering and Neuroscience Interdepartmental Programs, Jules Stein Eye Institute, University of California, Los Angeles, California; 6) Medicine (Genetics), Children's hospital Boston, Boston, MA; 7) Ophthalmology,Children's hospital Boston, Boston, MA; 8) Manton Center for Orphan Disease Research,Children's hospital Boston, Boston, MA.

Three affected children (2 sibs and a first cousin once removed) of a consanguineous pedigree of Mexican ethnicity presented to ophthalmology with complete ophthalmoplegia, ptosis and facial diplegia and were also noted to have developmental delay and hypotonia. The presentation was consistent with atypical Moebius syndrome, while MRI revealed normal cranial nerves in the subarachnoid space and hypoplastic extraocular muscles (Dumars et al.J AAPOS. 2008 Aug;12(4):381-9). Array-based linkage and homozygosity analysis highlighted a 7.8 Mb region of shared homozygosity on chromosome 19 between the three affected children. Whole exome sequencing of one of the sibs and the cousin revealed 2 novel missense mutations at RYR1 gene which fell within the region of homozygosity. The 2 mutations segregated with the affection status in the pedigree, altered evolutionary conserved residues, and were predicted to be damaging by Polyphen 2 and Pmut. RYR1 encodes a ryanodine receptor found in skeletal muscle and RYR1 mutations have been previously reported in malignant hyperthermia susceptibility, central core disease, and minicore myopathy with external ophthalmoplegia; the latter seems to be the diagnosis in our patients. Utilizing linkage, homozygosity analysis and the new technology of next generation sequencing helped redefine the phenotype and delineated the genetic etiology underlying the phenotype in our pedigree.

1119T

PKD1 and PKD2 gene variation in Italian patients affected by Autosomal Dominant Polycystic Kidney Disease (ADPKD). *P. Carrera[1, 2], F. Rigo[1], S. Calzavara[2], C. Montrasio[2], P. Messa[5], A. Edefonti[5], R. Magistroni[6], F. Scolari[7], P. Manunta[4], A. Boletta[3], M. Ferrari[1, 2, 4].* 1) San Raffaele Scientific Institute, Center for Translational Genomics and Bioinformatics, Milano, Italy; 2) Diagnostica e Ricerca San Raffaele, Milano, Italy; 3) San Raffaele Scientific Institute, Division of Genetics and Cell Biology, Milano, Italy; 4) University Vita-Salute San Raffaele, Milano, Italy; 5) IRCCS Policlinico, Milan, Italy; 6) University of Modena, Italy; 7) Spedali Civili di Montichiari, University of Brescia, Italy.

ADPKD is the most common genetic nephro-pathology in humans, affecting about 1/1000 individuals. Aim of the present work was to identify variants of PKD1 and PKD2 genes in Italian patients affected by ADPKD. Analysis of PKD1 and PKD2 variation would allow: to confirm the diagnosis in clinically uncertain/atypical cases; to offer genetic counseling in at risk families; to exclude the presence of a mutation in related donors for kidney transplantation; to define the molecular spectrum of PKD1 and PKD2 in Italian patients. A semi-automated Sanger direct sequencing protocol has been developed for detection of variants in PKD1 and PKD2. Specific amplification of PKD1 was achieved either following previously published protocols or by using newly designed PCR/sequencing primers. By this protocol we analyzed a total of 278 subjects as follows: 190 unrelated patients belonging to families, 66 relatives and 22 patients with no reported familiarity. In the majority of patients (191/212, 90%) variants were present. In 94% (178/190) of familial cases variants were found in PKD1 and/or PKD2. In sporadic cases, 59% (13/22) were positive. In 20 patients, variants were present in both genes and 83 had more than one variant in PKD1. In addition, a high level of genetic variability (55 SNPs) in these genes was observed with an average of about 12 SNPs/patient in PKD1 and 2 SNPs/patient in PKD2. This is further attested by the evidence that 84.5% of the identified variants have never been described before. By combining results for truncating and known variants we identified pathogenic mutations in 61% (117/191) of patients with gene variants, 100 (85,5%) with a mutation in PKD1, 17 (14,5%) mutated in PKD2. For variants not yet determined with respect to their pathogenicity, a prediction was attempted according to PKDB criteria (Rossetti et al., 2010). Concordance of the results obtained with SIFT, AGVGD and PolyPhen2 allowed the calling of 18 likely-neutral and 12 highly-likely-pathogenic variants. In many cases there's no concordance between the softwares and the interpretation of additional information becomes relevant. Segregation in members of the family increases the score for pathogenicity/neutrality of a variant; otherwise the concurrent presence of a known mutation could lean to consider the indeterminate change more likely as neutral. Combining our previous data with these results, allowed us to identify pathogenic mutations in 67% (129/191) of patients.

1120T

A novel nucleotide change in the intron 12 of the SPAST gene might produce an aberrant protein transcript. *A. Magariello[1], M. Liguori[1], A. Patitucci[1], L. Citrigno[1], R. Mazzei[1], FL. Conforti[1], C. Ungaro[1], W. Sproviero[1,2], D. Bosco[3], M. Plastino[3], A. Gambardella[1,2], M. Muglia[1].* 1) Institute of Neurological Sciences, CNR, Mangone (CS), Italy; 2) Institute of Neurology, University `Magna Graecia', Catanzaro, Italy; 3) U.O. of Neurology “S. Giovanni di Dio” Crotone, Italy.

Hereditary spastic paraplegia (HSP), also known as Strümpell-Lorrain syndrome, is a large group of inherited and heterogeneous neurologic disorders caused by degeneration of the corticospinal axons. All modes of inheritance (autosomal dominant, autosomal recessive and X linked) have been reported; however autosomal dominant (AD) inheritance is the main mode, accounting for about 70%-80% of all HSPs. Mutations in the SPAST gene are the most common causes of ADHSP since it has been described for up to 40% of cases. To date, more than 300 SPAST mutations have been reported; they are mostly single nucleotide changes or small deletions/insertions, but large deletions and duplications have also been identified. No significant genotype-phenotype correlation were found. We report about a 58 -year-old man who complained for a progressive ambulation impairement started at 50 years. At the time of the neurological examination he showed a moderate spastic paraparesis with hyperreflexia in the lower limbs, spontaneous clonus in his legs and a modest hypoesthesia at the lower extremities; he also complained for urinary incontinence. Electrophisiological examination and RMI scans of brain and spinal cord resulted within the normal range. In the evaluation of his family tree, one brother and 2 second-degree cousins were only reported affected by autism-like behavioural symptoms. Genomic DNA was extracted after receiving informed consent from the patient. All 17 coding exons of the SPAST gene were amplified by standard conditions of polymerase chain reaction and analysed by direct sequencing of the amplicons utilizing the ABI 3130 genetic analyzer (Applied Biosystems). A heterozygous nucleotide change c.1493+18 g>t in the intron 12 of the SPAST gene was identified. The identified nucleotide change c.1493+18 g>t was not reported in the SNPs databases. The silico analysis predicted that the transition g>t at position +18 from the exon 12 generates a novel cryptic donor splice site. Therefore, the reported change might produce an alternative splicing corresponding to an aberrant transcript. To date different nucleotide changes in intronic sequences not directly involving but near the splice site of the SPAST gene were already reported as aberrant. The c.1493+18 g>t is probably the first pathological chance lying far from the splice site. However a mRNA expression study is in progress in order to verify the real functional effect of this nucleotide change.

1121T

Assay design for detection of the SMN1 “2+0” genotype in carriers of Spinal Muscular Atrophy (SMA). *G. Pont-Kingdon[1], A. Wilson[1], E. Lyon[1,2].* 1) Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, UT; 2) Department of Pathology, University of Utah, Salt Lake City, UT.

Spinal Muscular Atrophy is a severe neuromuscular degenerative disease due to the absence of the survival motor neuron protein encoded by the SMN genes. The disease is autosomal recessive and necessitates mutation in both alleles of the SMN1 gene. In 95% of cases, the disease is due to homozygous deletion of the SMN1 gene. A second gene SMN2 is partially active and influences the severity of the disease from extremely fatal to mild. When combining all SMAs with different degrees of severity, the disease has an incidence of 1/10 000 live births and a carrier frequency (number of individual with a chromosome with no copies of the SMN1 gene) around 1/40. The large majority of carriers (95%) have one chromosome with one SMN1 gene and the other chromosome with zero (“1+0” genotype) while non carriers have two copies of the SMN1 gene (one on each chromosome, “1+1” genotype). Four to five percent of the normal population has three copies of the SMN1 gene indicating the existence of chromosomes with two SMN1 genes in cis (on the same chromosome). Carrier screening methods establish the number of SMN1 gene in a genome. If an individual carries one chromosome with two copies of SMN1 and one chromosome with no SMN1 genes (“2+0” genotype), the result would be two SMN1 copies, erroneously classifying this individual as a non carrier. These carriers are missed by any technology used currently. Several quantitative assays have been designed for determination of the SMN1 gene copy number. We are adapting and testing previously developed quantitative real time PCR assays that distinguishes carriers “1+0“ and non carriers “1+1” to also distinguish the ”2+0” carrier genotype. Mathematical modeling and estimation of error rate due to sampling and experimental errors allowed us to predict that both genotypes ”1+1” and “2+0” will provide a different distribution of data if samples are tested in multiple replicates. This method could identify carriers independent of the gene copy per chromosome.

1122T

Molecular analysis of *ZEB2* responsible for the Mowat-Wilson syndrome. *Y. Yamada[1], K. Yamada[1], S. Mizuno[2], E. Nishi[2], N. Ishihara[3], N. Akimura[4], K. Matsuda[4], N. Okamoto[4], Y. Hiraki[5], N. Wakamatsu[1].* 1) Dept Gen, Inst Dev Res, Aichi Human Service Ctr, Kasugai, Aichi, Japan; 2) Ctrl Hosp, Aichi Human Service Ctr, Kasugai, Aichi, Japan; 3) Nagoya Univ Sch Med, Nagoya, Japan; 4) Res Inst, Osaka Med Ctr Maternal Child Health, Osaka, Japan; 5) Hiroshima City Children's Ctr Health Dev, Hiroshima, Japan.

The mutations of *ZEB2* (*ZFHX1B*) cause an autosomal dominant disorder of Mowat-Wilson syndrome associated with profound mental retardation, delayed motor development and specific facial features such as hypertelorism, often with microcephaly, epilepsy, congenital heart disease, and Hirschsprung disease. We performed molecular analysis of *ZEB2* to confirm diagnosis of Mowat-Wilson syndrome. To screen for mutation, DNA fragments including each exon of *ZEB2* from patients and normal controls were amplified by PCR and subjected to direct sequencing analysis. In this study, we report 4 novel frame-shift mutations were found in the typical patients with Mowat-Wilson syndrome, three mutations deleted one base (164delC, 55fs74X; 647delG, 216fs223X; 1493delC, 498fs515X), and a 4-bp duplication (639insCCTG, 213fs238X). Further, the nonsense mutations of R695X and R921X reported previously were found from new patients, respectively. In other 5 typical cases, we observed deletion of whole or partial *ZEB2*. We have identified 50 mutations in *ZEB2* up to the present. The nonsense mutation R695X, which is considered a hot spot mutation of *ZEB2*, was identified in 9 independent cases. In 48 typical cases, 20 nonsense mutations and 28 frame-shift mutations were detected. These mutations generated truncated protein without the zinc finger domain located in the C-terminal region. The other two cases with milder mutation (one amino acid deletion and splicing error mutant with small amounts of normal mRNA) result in atypical clinical features including moderate mental retardation. On the other hand, we observed chromosomal deletions including *ZEB2* in 20 cases with typical or more severe features.

1123T

Genetic Profile for Patients with Familial Hemophagocytic Lymphohistiocytosis Type 5. *K. Zhang[1], JA. Johnson[1], D. Kissel[1], U. Zur Stadt[3], AH. Filipovich[2].* 1) Div Human Gen, Cinncinnati Children's Hosp, Cincinnati, OH; 2) Division of Bone Marrow Transplant and Immune Deficiency, Cincinnati Children's Hospital Medical Center USA; 3) , Research Institute, Children's Cancer Center, Hamburg Germany and Department Hematology and Oncology, University Medical Center Hamburg Eppendorf, Hamburg, Germany.

Familial hemophagocytic lymphohistiocytosis (Familial HLH or FHL) is an autosomal recessive disorder of immune regulation, characterized by defects in cell-mediated cytotoxicity that results in fever, hepatosplenomegaly and cytopenias. It typically occurs in early childhood, but many adult cases have also been reported. It is often rapidly fatal unless treated with chemotherapy, immune suppression and follow by bone marrow transplantation.Several genes are specifically associated with FHL including PRF1 (FHL2), UNC13D (FHL3), STX11 (FHL4) and STXPB2 (FHL5). The purpose of this study is to characterize STXBP2 mutations in North American patients with FHL5. PCR based sequencing of the entire coding regions and exon/intron boundaries of the STXBP2 gene were undertaken in a total of 329 unrelated patients with the clinical diagnosis of HLH. The significance of missense variants were evaluated with a lab developed algorithm including database reviews (HGMD, NCBI etc.) as well as in silico analyses (PolyPhen, SIFT, Grantham Scale, etc.). This study was performed with the approval of Cincinnati Children's Hospital Medical Center IRB. Bi-allelic STXBP2 mutations were found in 32 unrelated families. Heterozygous mutation and sequence variants were also identified in 22 additional symptomatic patients; the significance of these variants in the development of HLH in the heterozygous state is currently unknown. Fourteen novel mutations and 9 sequence variants were found in this cohort of patients. There are a few common mutations were observed. P477L is a mutation found frequently in patients with Arabic descent. 1247-1G>C and G541S are the two common mutations in Caucasian patients. G541S can be observed by itself or cosegregated with the 795-4C>T variant. While L130S and T345M are almost always observed together, T345M by itself also presented in about 2% of the local control population, and is likely a benign polymorphism. Thirty-five other sequence variants are also found in this cohort of patient and do not appear to result in aberrant protein. Bi-allelic STXBP2 mutations are identified in approximately 10% of North American patients with FHL and sequencing analysis of STXBP2 gene should be included as part of the standard genetic evaluation of these patients. Further studies are necessary to characterize the role in the disease development of the novel missense and splicing site variants observed in this study.

1124T

Comprehensive analysis of novel disease-causing copy number variants in syndromic obesity. *C.S. D'Angelo, C.P. Koiffmann.* Dept Genetics, Univ Sao Paulo, Sao Paulo, Brazil.

There are numerous reports of dysmorphic syndromes including obesity and developmental delay as presenting clinical features. Many have an overlapping phenotype that represents a diagnostic challenge. The most prevalent of these conditions is Prader-Willi syndrome (PWS), which is due to lack of paternal expression of imprinted genes within chromosome 15q11q13. Other syndromes with obesity have been associated with deletions of chromosomes 1p36, 2q37, 6q14.1q15, 6q16, 9q34, 11p14.1, 17p11.2. In addition, several rare copy number variants (CNVs) have been detected in obese patients with no known genetic diagnosis but demonstrating complex “obesity-plus” phenotypes. After microarray analysis in a series of patients with dysmorphic syndromes that include obesity and developmental delay, we identified several cryptic chromosomal imbalances representing new aetiologies of syndromic obesity and, hence, novel obesity loci for targeted follow-up. We developed a set of custom-designed MLPA probes to rapid screen for regions of copy number gains and losses that might be overrepresented in a cohort selected for syndromic obesity. Most obese patients had normal methylation test for PWS or had other common causes of syndromic obesity, such as 1p36 and 17p11.2 deletions, ruled out. To date, we have found six of over 150 patients carrying chromosomal deletions at 15q11.2 (three), 2q37 (two), and 9q34 (one). The latter were further characterized by a customized oligonucleotide-based DNA microarray (SurePrint G3 Human 8x60K from Agilent). Haploinsufficiency of HDAC4 and EHMT1, located at the critical region for 2q37 and 9q deletion syndromes, have been suggested as being involved in the regulation of body weight and critical for brain function. The two del (2)(q37.3) patients have a similar sized terminal deletion of ~6 Mb encompassing HDAC4, whereas the 9q deletion is only 600 kb in size extending from the telomere into EHMT1. We detected a single-copy of the SNORD116 snoRNAs to patients with a PWS or PWS-like phenotype. Deletion of only these genes is sufficient to cause PWS. As of now, we have not confirmed any of the newly discovered obesity loci to be recurrent. Overall, these data suggest that despite extensive overlap of phenotypes, very few obese patients share identical aetiologies. It warrants screening them for novel genomic regions involved in the cause of obesity syndromes as the same genes are likely to be implicated in common obesity. CEPID-FAPESP, CNPq.

1125T

New gene variants in Idiopathic Scoliosis. *K.F Fendri[1, 3], S.A.P Patten[1, 3], P.E Edery[2], F.M Moldovan[1, 3].* 1) Orthopaedic Molecular Biology Laboratory, Sainte-Justine Hospital Research Centre, 3175 Cote-Sainte-Catherine Rm 4731, Montreal, H3T 1C5 (Quebec), Canada; 2) Cytogenetics service, Civil Hospice of Lyon, East Hospital Bloc, CBPE 6th floor, 59 Boulevard Pinel, Bron Cedex 69677, France; 3) Faculty of Dentistry, University of Montreal, PO Box 6128 Stn CV, Montreal, H3C 3J7 (Quebec), Canada.

Adolescent Idiopathic Scoliosis (AIS) is the most common form of spinal deformity. It occurs primarily in girls and progresses during pre-pubertal and pubertal growth. The aetiology and molecular mechanisms of AIS are not clear; currently the consensus is that AIS has a multifactor aetiology, with multiple genetic factors. Many loci for AIS have been mapped to different chromosome regions but no genes have yet been clearly identified as responsible for AIS, and most importantly, the resulting protein defects remain to be demonstrated. Recently, a collaboration study using genetic linkage analysis to the mapping of a dominant disease-causing gene to two critical genomic intervals 3q12.1 and 5q13.3 in a large multiplex French family. We aim to identify the gene(s) that could be involved in AIS and to validate their involvement by both genetic and functional analyses. A large multiplex AIS French family was chosen for this study based on clinical and radiological data. Two strategies were carried out to discover mutations causing the AIS disease, whole-exome sequencing using high-throughput technology and candidate regions genes' sequencing using sanger technology. Sequence data were analyzed through a customized computational pipeline to identify variants in 4 family patients. Biological activity of mutant proteins is under evaluation by in vivo functional studies in zebra fish. The sequencing of the candidate genes' coding sequences was conducted on all family members. Sequence analysis revealed 2 rare SNPs located on the coding regions of a gene suggesting its possible association to AIS disease in this family. These 2 SNPs are located on the C-terminal region of the protein and affect its structure and probably its cellular activity leading to the disease. This region interacts with the mRNA of a gene which defects cause scoliosis as a secondary phenotype. Whole-exome sequencing of family patients identified new genetic variants that could be responsible for the AIS disease in this family. The pathogenic nature of all these variants is currently under investigation in the zebra fish model. Identifying susceptibility genes for AIS will facilitate the understanding of underlying biochemical pathways (functional studies) and ultimately the development of specific therapies (pharmacological studies). This is likely to have important implications, given that AIS aetiology is unknown. Supported by the Fondation Yves Cotrel, Institut de France.

1126T

Identification of novel genes for frontotemporal lobar degeneration using whole genome sequencing. *I. Gijselinck[1,2], S. Philtjens[1,2], T. Van Langenhove[1,2], S. Engelborghs[2,3], J. van der Zee[1,2], R. Van Den Berghe[4], P. Santens[5], G. Maes[1,2], K. Peeters[1,2], M. Mattheijssens[1,2], P.P. De Deyn[2,3], C. Van Broeckhoven[1,2], M. Cruts[1,2].* 1) Neurodegenerative Brain Diseases Group, Department of Molecular Genetics, VIB, Antwerpen, Belgium; 2) Institute Born-Bunge, University of Antwerp, Antwerpen, Belgium; 3) Department of Neurology and Memory Clinic, Hospital Network Antwerp Middelheim and Hoge Beuken, Antwerpen, Belgium; 4) Department of Neurology, University Hospitals Leuven and University of Leuven, Leuven, Belgium; 5) Department of Neurology, University Hospital Ghent and University of Ghent, Gent, Belgium.

In genetically complex adult-onset diseases, sampling sufficient individuals of a Mendelian multiplex family informative for genetic linkage studies is often complicated due to e.g. the late-onset of disease and the variable disease onset and penetrance. Frontotemporal lobar degeneration (FTLD) is a complex neurodegenerative brain disease with a positive family history in up to 50% of patients of which today we can explain only 30 to 40% by a causal mutation in one of four of the known genes. The emerging technology of whole genome sequencing (WGS) might be an alternative solution allowing for direct identification of disease-causing genetic variants in selected patients and nuclear families. We are using this approach to identify novel FTLD genes that might identify new disease pathways and biological insight in the disease process. We obtained the whole genome sequence of 15 unrelated, mainly early-onset, familial FTLD patients and of an affected sib of four of the selected patients. Analysis of the genome sequences resulted in about 4 million variants per genome. We obtained an average coverage of 70-fold genome sequence and captured on average 96% of both alleles. All DNA variants were annotated and prioritized using multiple variant filtering procedures including sequence quality, genomic location, allele frequency, zygosity, predicted functional consequences and presence in the sequenced patients. In this way, the number of variants was reduced to a manageable number of possible candidate variants, with approximately 250 missense or splice variants segregating in each sib pair. We are genetically validating the selected variants and determining their frequency in control individuals and extended patients cohorts. Identification of a shared variant in multiple patients might indicate a founder mutation, while a gene with different deleterious variants in multiple patients might indicate a major FTLD gene. In-depth analysis will show whether the WGS approach is successful in indentifying coding or non-coding, rare high-penetrant disease mutations in complex diseases. The identification of novel FTLD genes will provide valuable insights into the pathogenesis of the disease and will eventually lead to the development of disease models, therapeutic targets and more efficient molecular diagnostics.

1127T

Whole-genome sequencing to identify disease causing mutations in hereditary sensory and autonomic neuropathy. *C. Gonzaga-Jauregui[1], W. Wiszniewski[1], J. Reid[1,2], I. Kurth[3], R.A. Gibbs[1,2], J.R. Lupski[1,4,5].* 1) Molec & Human Gen, Baylor College Med, Houston, TX, USA; 2) Human Genome Sequencing Center, Baylor College Med, Houston, TX, USA; 3) Institute of Human Genetics, University Hospital Jena, Jena, Germany; 4) Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA; 5) Texas Children's Hospital, Houston, Texas, USA.

Next-generation sequencing technologies and targeted capture have enabled high-throughput sequencing of exomes to discover pathogenic variants and identify new genes involved in mendelian diseases. However, exome sequencing is based in capture and enrichment of only the ~2% coding fraction of the genome to identify simple nucleotide variants (SNVs). Whole-genome sequencing allows the unbiased detection of all different types of variation in a given individual, from SNVs to copy-number variants (CNVs) that may be responsible or contribute to a disease phenotype. Hereditary and sensory autonomic neuropathies (HSAN) affect primarily the peripheral nerves and are characterized by progressive loss of sensitivity in the extremities; the different subtypes vary in the degree of autonomic involvement. Several genes have been identified to cause the different subtypes of HSAN but the genetic cause of the disease in some individuals remains unknown. We have used massively parallel sequencing by oligo ligation/detection technology, SOLiD, to perform whole-genome sequencing at ~30x average depth of coverage of a female patient affected by hereditary sensory and autonomic neuropathy (HSAN) who tested negative for mutations in known HSAN genes and other neuropathy associated genes. Comparison of the genomic sequence obtained to the human genome reference assembly identified 3.2 million single nucleotide polymorphisms (SNPs); 19,151 of these occur in coding regions of which 9,814 cause non-synonymous substitutions. Filtering of these variants for high quality calls and common variants present in dbSNP and observed by the 1000 Genomes Project, in order to look for novel variants, reduced the list of potential candidate variants to 232 nonsynonymous changes. Considering that the patient is the daughter of a first cousin marriage, we undertook a recessive model of inheritance for her disease, focusing on homozygous variants, narrowing our list of candidate genes to nine. Whole-genome sequencing can be applied to identify the genes and mutations responsible for mendelian phenotypes for which the molecular basis remains unknown, even if they are genetically heterogeneous. This technology will soon be performed routinely to achieve accurate genetic diagnosis and aid in clinical practice.

1128T

High-Throughput Sequencing for Causal Gene Discovery in Mendelian Diseases. *S.N. Jhangiani[1], M.N. Bainbridge[1], D.R. Murdock[1], F.J. Probst[2], J.L. Jeffries[3], I.F. Newsham[1], Y.Q. Wu[1], M. Wang[1], D.M. Muzny[1], R.A. Gibbs[1].* 1) Human Genome Sequencing Center, Baylor College of Medicine, Houston, Texas, USA; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA; 3) Department of Pediatrics (Cardiology), Baylor College of Medicine and Texas Children's Hospital, Houston, Texas, USA.

Discovering the causative alleles of the approximately 3,000 Mendelian diseases that as yet have no associated genes will elucidate key pathways, facilitate diagnostics and help guide therapy. The HGSC Mendelian Diseases Project, in collaboration with investigators in the Texas Medical Center, has generated more than 1Tb of high quality sequencing data from more than 150 subject samples representing greater than 35 disorders with autosomal recessive, autosomal dominant, *de novo* and X-linked inheritance patterns. These disorders affect a wide range of systems and processes including: heart, immune, mitochondrial, neuronal, skeletal, tumor formation, development and others.

We used a high-throughput, multiplexed capture sequencing pipeline capable of generating 1,500 capture libraries per month, and a fully automated bioinformatics pipeline that integrates multiple functional, gene model and allele frequency databases for variant analysis. This pipeline combined with high-throughput sequencing (HTS) has proven useful and cost effective in associating genes with diseases. We have implicated novel genes in eight Mendelian disorders, including a single family with Multiple Lipomatosis. Novel mutations have also been discovered in known causative genes in four disorders including: three novel mutations implicated in *MYH7* to cause Left Ventricular Non-compaction, a congenital heart disorder with over 18 associated genes. *MYH7* is not commonly sequenced in LVNC thus highlighting the value of whole exome sequencing for diagnostics in genetically heterogeneous diseases. This work demonstrates that the future of high-throughput sequencing will play in integral role in clinical management with diagnostics and treatment.

1129T

Exome Sequencing of Four Dyschromatosis Universalis Hereditaria Patients and One Unaffected Person from a Chinese Pedigree. *Y. Li[1], H. Liu[2], H. Liany[1], F. Zhang[2], J. Liu[1].* 1) Human Genetics, Genome Inst Singapore, 60 Biopolis Street, #02-01, Genome, Singapore, Republic of Singapore; 2) Shandong Provincial Institute of Dermatology and Venereology, Shandong Academy of Medical Science, P R China.

Dyschromatosis universalis hereditaria (DUH) and dyschromatosis symmetrica hereditaria (DSH) are pigmentary dermatoses that are most commonly seen in Japanese and Chinese. They may show autosomal dominant or recessive inheritance modes, and recent evidence indicated that DSH and DUH were genetically distinct disorders. DSH has been mapped to chromosome 1q21.3 [Zhang et al 2003, Miyamura et al 2003, He et al 2004] with autosomal dominant inheritance in Asian families. Chromosome 6q24.2-q25.2 with autosomal dominant inheritance in two Chinese families [Xing et al 2003] and chromosome 12q21-q23 with autosomal recessive inheritance in an Arabian consanguineous family [Stuhrmann et al 2008] were reported to be implicated in DUH. We investigated the genetic architecture of DUH by target sequencing 50 Mb of exomes for each of the four DUH patients and one unaffected person from a Chinese pedigree with 31 members using Illumina GAII platform. The mean coverage depth is 45.4, and on average, 93.7% of targeted bases were covered by >=5x, 86.7% of targeted bases were covered by >=10x. 68276 high quality SNPs and 19666 short indels were discovered among the 5 persons. Analyses assuming autosomal dominant and recessive inheritance modes were carried out. Several implicated genes and further validation strategies will be discussed.

1130T

Whole Exome Sequencing in a single nuclear family finds novel compound heterozygous changes in KCTD7 causing late infancy-childhood neurodegeneration. *T.C. Markello[1,2], D.A. Adams[1,2], L. Wolfe[2], M. Sincan[2], K. Fuentes Fajardo[2], MP. Jones[3], U. Harper[3], S. Chandrasekharappa[3], C.J. Tifft[2], C. Boerkoels[2], W.A. Gahl[1,2], Nisc Comparative Sequencing Program.* 1) Med Gen Branch, NIH/NHGRI, Bethesda, MD; 2) Undiagnosed Diseases Program, NIH/NHGRI, Bethesda, MD; 3) NHGRI/IR/CGB/CGS, Bethesda, MD.

Two of four siblings who were normal at birth developed late infantile ataxia with slowly progressive myoclonus and severe degenerative neurological dysfunction. Four grandparents, both parents and all four siblings underwent traditional linkage analysis with a maximum LOD score of 0.82 at 17% of the genome, which corresponded to the same regions located by Boolean logic SNP chip linkage. The parents and four siblings underwent whole exome sequencing with Agilent Sure select 38MB exon capture and sequencing on an Illumina GIIx platform with an average 150x coverage. For the proband 79% of the UCSC coding bases were covered with high quality, most probable genotype MPG score ⁄ 10. A combined 112,936 variants were identified in the 6 genotypes. Using standard frequency, linkage, Mendelian consistency and deleterious prediction filters biallelic mutations in a single gene *KCTD7* were detected. This gene has been linked to the similar but non-identical phenotype myoclonic epilepsy in two previous families by homozygosity mapping. Our family extends the phenotype of recessive loss of function in *KCTD7*. It also demonstrates that SNP based linkage from simple Boolean logic can produce the same chromosome linkage map as traditional linkage programs run with microsatellite markers.

1131T

Mutations in the Amiloride-Sensitive Epithelial Sodium Channel in African patients with cystic fibrosis-like disease. *L. Mutesa[1, 2], C. Verhaeghe[2], J.F. Vanbellinghen[2], V. Dhennin[3], V. Bours[2, 3].* 1) Medical Genetics, National University of Rwanda, Kigali, Rwanda; 2) Center for Human Genetics, University of Liege, Liege, Belgium; 3) GIGA-Genomics Facility, University of Liege, Liege, Belgium.

BACKGROUND The defect in chloride and sodium transport in cystic fibrosis (CF) patients is a consequence of CFTR loss of function and/or an abnormal interaction between cystic fibrosis transmembrane conductance regulator (CFTR) and amiloride sensitive epithelial sodium channel (ENaC). Apart from the defective chloride secretion, loss of functional CFTR results in increased sodium absorption through the ENaC channel in CF patients. A few patients were described with CF-like symptoms with a single CFTR mutation and/or an ENaC mutation. OBJECTIVE To study African patients with CF-like symptoms and to relate the disease to gene mutations of ENaC genes. METHOD We investigated whether mutations in the genes that code for the different subunits of ENaC gene (SCNN1A, SCNN1B and SCNN1G) might result in cystic fibrosis (CF)-like disease in patients in whom only one CFTR gene is mutated, or that carry no mutations at all in the CFTR coding region and its exon/intron junctions. We extensively performed ENaC genes sequencing in these CF-like patients and established the frequency of identified ENaC mutations in a cohort control. RESULTS In total, 66 sequence variants in ENaC genes were found in 60 CF-like patients. Several novel ENaC gene mutations were identified and some of them were located in highly conserved domains and consistent with a pathophysiological role. Only three novel mutations including p.V348M and p.W423R in SCNN1B subunit and p.R180W in SCNN1G were observed once in our patients, but not in controls. The preliminary functional studies performed using expression in Xenopus laevi oocytes showed that the p.V348M is a gain of function mutation with a high amounts (2-30%) functional ENaC activity. CONCLUSION Our data suggest that CF-like syndrome in Africa could be associated with ENaC mutations. The combination of ENaC and CFTR mutations may play a hitherto unrecognized role in lung diseases.

1132T

Identification of genes for nonsyndromic hearing impairment using haplotype sharing and exome sequencing. *M. Schraders[1], K. Neveling[2], J. Oostrik[1], C.F.H.A. Gilissen[2], R.J.C. Admiraal[1], J.A. Veltman[2], H.P.M. Kunst[1], E.H. Hoefsloot[2], R.J.E. Pennings[1], H. Kremer[1,2].* 1) Department of Otorhinolaryngology, Head and Neck Surgery, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; 2) Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

Linkage analysis and homozygosity mapping are valuable tools for the discovery of genes mutated in autosomal recessive nonsyndromic hearing impairment (arNSHI), which have been successfully used for the identification of the majority of the currently known arNSHI genes. Insight in the causes of arNSHI in western Europe and the US, however, remained limited. In our group, we demonstrated that homozygosity mapping can be successfully employed in mixed populations such as that of the Netherlands. Additionally, we recently identified a 5th gene by this approach. However, in about 70% of the families with arNSHI no significant shared homozygous regions could be found. Therefore, we initiated whole exome sequencing in the proband of 20 families using the SureSelect human exome kit (Agilent) and the SOLiD sequencing technology (Life Technologies). On average 25,018 genetic variants were identified per patient. After exclusion of known variants using dbSNP130 and variants detected in a in-house database on average 2,124 variants remained per patient, 557 of which were located in the coding regions or the canonical dinucleotides of splice sites. For each patient we selected the variants with at least 5 variant reads and which were consistent with the recessive inheritance pattern of the disease. Moreover, haplotype sharing by affected individuals of the same family as derived from the SNP genotyping was taken into account. This procedure resulted in 2-8 candidate genes per family. Subsequently, segregation analysis was performed in families for which DNAs of parents or unaffected family members were available. So far we have selected two novel promising candidate genes for further analysis. Also, in three families hearing impairment could be attributed to novel compound heterozygous mutations in the known deafness genes *LOXHD1*, *TMC1*, and *MYO15A*.

1133T

Identification of a frameshift mutation in HMGB3 (MIM 300193) in a family with Lenz microphthalmia (MCOPS1; MIM 302300). *A.F. Scott[1], D.W. Mohr[1], L.M. Kasch[1], J.A. Barton[1], B. Craig[1], B.A. Marosy[1], K.F. Doheny[1], W.C. Bromley[2], T. Roderick[2], E.W. Jabs[3].* 1) McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins Univ Sch Med, Baltimore, MD 21287; 2) Ctr for Human Genetics, P.O. Box 770 Bar Harbor, ME 04609; 3) Dept of Genetics and Genomic Sciences, Mount Sinai Sch Med, NYC, NY 10029.

Goldberg and McKusick (1971) reported a kindred from Maine with X-linked kyphoscoliosis, microphthalmos, mental retardation, and microcephaly similar to that first described by Lenz (1955). Forrester et al (2001) mapped the gene, MCOPS1 (MIM 309800) to Xq27-28. We obtained an affected proband and his mother from the Maine pedigree. The proband was subject to whole genome sequencing using Complete Genomics, Inc. and to Illumina paired-end sequencing following X-exome capture (Agilent). Both methods confirmed a frameshift mutation in the fourth coding exon of the evolutionarily conserved high mobility group protein HMGB3 (MIM 300193; Phastcons score of 1) resulting in a readthrough that produces VADYISRKESLMVQRVLLKLPGKRWKRKMKKRRRKKRRRRRRRRMN-KETVYLSPCEYLES* in place of VADYKSKGKFDGAKGPAKVARKK-VEEEDEEEEEEEEEEEEEDE*. Subsequently, Sanger sequencing on the mother confirmed her carrier status. Morpholino knock down of the Xenopus ortholog, Xhmgb3, was reported to have a significant role in retinal progenitor proliferation during eye development and to produce reduced eye and brain sizes in developing embryos (Terada et al, 2006). Confirmation in additional pedigrees will validate the first association of a human disorder with HMGB3 mutations.

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1134T

Replication evidence that constituents of the apical plasma membrane contribute to Meconium ileus in Cystic Fibrosis. *X. Li[1], H. Corvol[2,3], W. Li[4,1], T. Chiang[1], F. Lin[5], P-Y. Boelle[3,6], M. Drumm[7], G. Cutting[8], M. Knowles[9], P. Durie[10], J. Rommens[5,11], L. Sun[4,12], L. Strug[1,4].* 1) Program in Child Health Evaluative Sciences, Hospital for Sick Children, Toronto, ON, Canada; 2) AP-HP, Hôpital Trousseau, Pediatric Pulmonary Department; Inserm U938, Paris, France; 3) Université Pierre et Marie Curie-Paris 6, Paris, France; 4) Dalla Lana School of Public Health, University of Toronto, Toronto, ON, Canada; 5) Program in Genetics and Genome Biology, Hospital for Sick Children, Toronto, ON, Canada; 6) AP-HP, Hôpital St Antoine, Biostatistics Department; Inserm UMR-S707, Paris, France; 7) Case Western Reserve University Departments of Pediatrics and Genetics, Cleveland, Ohio, USA; 8) Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; 9) Cystic Fibrosis-Pulmonary Research and Treatment Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; 10) Program in Physiology and Experimental Medicine, Hospital for Sick Children, Toronto, ON, Canada; 11) Department of Molecular Genetics, University of Toronto, ON, Canada; 12) Department of Statistics, University of Toronto, Toronto, ON, Canada.

Cystic Fibrosis (CF) is a life-shortening recessive disease caused by mutations in the CFTR gene. Lung disease and the occurrence of common comorbidities such as intestinal obstructions vary in CF patients, even across individuals with the same CFTR mutations. This suggests the involvement of genes other than CFTR, which we aim to identify. The intestinal complication, Meconium Ileus (MI), which occurs in ~16% of newborn CF patients, is >88% heritable. A genome-wide association study (GWAS) by the North American (NA) Consortium for CF Modifiers (n=3763) identified two genes (SLC6A14, p=9.88E-09 and SLC26A9, p=1.28E-12) associated with MI accounting for ~5% of the MI phenotypic variation. To increase power and identify more phenotypic variance, we performed a hypothesis driven GWAS (GWAS-HD) (Strug et al., 2010), which incorporates knowledge of CF to prioritize GWAS results. We hypothesized that variation in other gene products that reside in the apical membrane of epithelial tissue where CFTR resides may influence residual or adapted epithelial functions, and could modify CF disease phenotypes. GWAS-HD provided evidence that multiple constituents of the apical plasma membrane were associated with MI (permutation p=0.0002, testing 157 apical genes jointly). SLC9A3 and ATP2B2, now also reached genome-wide significance after prioritization by the hypothesis (q-value < 0.05). SNPs in SLC6A14 and SLC26A9 were replicated (p=0.001 and p=0.0001, respectively) in a combined sample of an independent NA collection (n=1,140 genotyped at 14 SNPs) and a French population-based cohort with genome-wide data (n=1,232). The French cohort also provided replication of SLC9A3 in a gene-based analysis (p= 0.017) and replication of the apical hypothesis (p=0.022, testing the 157 apical genes jointly), which required genome-wide data. The replicated association evidence for the apical membrane candidates in MI indicated that (1) GWAS-HD uncovered additional association evidence for genes with MI that would not otherwise have been shown to reach statistical significance with the available patient group; (2) current therapeutic paradigms aimed at improving delivery of the mutated CFTR gene product could be complemented by modulation of epithelial function as determined by apical solute transporter activities; and (3) GWAS-HD can be applied to studies of other Mendelian disorders or complex traits when additional information is available.

1135T

Looking for regulators of dystrophin Dp71 gene expression in hepatic cells. *C. Becerril[1], E. Blancas-Sánchez[2], P. Zapata-Benavides[1], J. Dávila-Velderrain[1], B. Cisneros[3], M. Bermúdez de León[2].* 1) Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México; 2) Laboratorio de Biología Molecular, Centro de Investigación Biomédica del Noreste, Instituto Mexicano del Seguro Social, Monterrey, Nuevo León, México; 3) Departamento de Genética, Centro de Investigación y de Estudios Avanzados del I.P.N., México, D.F., México.

Mutations on DMD gene lead to Duchenne muscular dystrophy, the most common and severe dystrophy in children (1/3,500 live born males). DMD gene has at least 7 different promoters, which give products with independent regulation tissue- and time-specific. Dystrophin Dp71, the smallest product, is transcribed from intron 62 and expressed in all non-muscular tissues. However, Dp71 function still remains unclear. To evaluate the molecular mechanism that underlined the Dp71 expression, different cell models have been employed, including muscle and neuronal cells. In muscular cells, Dp71 expression is down-regulated during myogenesis by the lack of activating factors Sp1 and Sp3; otherwise, the induction of Dp71 expression in neuronal differentiation is caused by activation of Sp1 and the relief of repression by AP2-alpha. Previously, we reported that beta-naphthoflavone, a polyaromatic xenobiotic, down-regulate the Dp71 mRNA and protein expression in hepatic cells. Hence liver is the key organ involved in xenobiotic metabolism, we considered that the knowledge of the regulation of Dp71 in liver cells could contribute to identify and understand its function in this organ. To elucidate the transcriptional mechanism of Dp71 in hepatic cells by beta-naphthoflavone, we analyzed the Dp71 expression and identified transcriptional factors and elements involved in its regulation. Dp71 mRNA half-life was determined in hepatic cells by quantitative RT-PCR, and no changes were observed in beta-naphthoflavone-treated cells with respect to control cells (9.11 ±2.9h versus 9.22± 1.6h, respectively). Dp71 promoter sequence analysis, using TRANSFAC database, showed that -224 to +65 region contains several Sp1/Sp3 and YY1 binding sites. Probes spanning basal promoter region were designed and used to analyze specific transcriptional complexes by Electrophoretic Mobility Shift Assays (EMSA). EMSAs revealed the interaction of Sp-family transcriptional and YY1 factors to their respective DNA elements. These results were confirmed by supershift assays with specific anti-Sp1 and anti-YY1 antibodies. Functional analyses to demonstrate Sp1/Sp3 and YY1 factors on Dp71 gene expression in hepatic cells are in progress.

1136T

Studying familial hypercholesterolemia by means of real-time pcr expression. *T.Yu. Komarova, A.S. Golovina.* Genetics, Institute of Experimental Medicine, St.-Petersburg, Russian Federation.

Aims. Familial hypercholesterolemia (FH) is a common (1:500) monogenic disease caused by low density lipoporotein (LDL) catabolism lowering due to dysfunction of the specific LDL receptor (LDLR). We aimed to investigate if the LDLR mRNA level in freshly isolated peripheral venous blood leukocytes was inversely correlated with plasma level cholesterol in patients with heterozygous FH. **Methods.** Fresh leukocytes were prepared from peripheral venous blood by means of centrifugation at 1500g with the Lymphosep cushion. Leukocytes from the interphase were collected and washed three times with RPMI medium. Viability of leukocytes was tested by Trypan blue dye exclusion. Total RNA was prepared from 1-2 millions of viable white blood cells by means of AquaPure RNA Isolation Kit (Bio-Rad Laboratories, USA) and 1-2 mkg of total RNA was used for first strand cDNA synthesis with iScriptTM cDNA Synthesis Kit (Bio-Rad Laboratories). Approximately 100 ng of cDNA was used in real-time PCR mRNA quantitation test. TaqMan technology was used with R6G labeled TaqMan probe to quantitate the reference gene - UBIC (ubiquitine C) mRNA and fluorescein labeled TaqMan probe was used to amplify LDLR mRNA exon 4 fragment in the same tube using CFX96 device (Bio-Rad Laboratories). Normalized relative expression of the LDLR gene in FH samples was compared to expression of the LDLR gene in healthy individual and plotted versus blood plasma total cholesterol figures.

Results. We found LDLR mRNA level to be highly variable in FH blood leukocytes ranging from 0.2 to 1.8 times content of LDLR mRNA in the control individual. We found that most possible mechanism of LDLR gene dysfunction in common deltaG197 (also known as G218del or FH-Lithuania mutation) variant heterozygous leukocytes is due to mRNA level lowering and not to defects in the intracellular protein maturation and transport. Patients taking up high dose of statins usually demonstrate the elevated level of LDLR mRNA.

Conclusions. We found no inverse correlation between LDLR mRNA level in peripheral blood FH leukocytes and plasma cholesterol level. So far, quantitation of LDLR mRNA in leukocytes cannot be used for prediction of disease severity and efficacy of hypocholestorolemic drug treatment in heterozygous FH.

1137T

Genotype- phenotype correlation in beta thalassaemia. *Z. Abdul Latiff[1], M. Sivalingam[2], ML. Looi[2], ND. Husin[2], FZ. Mohd Radin[2], R. Mohd Isa[2], SZ. Syed Zakaria[1], NH. Hussin[3], H. Alias[1], H. Ibrahim[4], R. Jamal[2].* 1) Department of Paediatrics, UKM Medical Centre, Kuala Lumpur, Malaysia; 2) UKM Medical Molecular Biology Institute, UKM Medical Centre, Kuala Lumpur, Malaysia; 3) Department of Pathology, UKM Medical Centre, Kuala Lumpur, Malaysia; 4) Institute of Paediatrics, Hospital Kuala Lumpur, Malaysia.

Thalassaemia is the commonest single gene disorder in Malaysia with an estimated 5% carrier rate for) -thalassaemia. The clinical manifestation of) -thalassaemia disease depends on the type of) gene mutations, (-gene interaction and the Xmn1 polymorphism. This study was aimed at identifying the) -gene mutations in local thalassaemia patients and the complementary effect of co-inheritance of (-gene mutations and Xmn1 polymorphism in) -thalassaemia patients. A total of 264 samples was studied, consisting of thalassaemia major (n = 104), thalassaemia intermedia (n= 29), HbE) thalassaemia (n=130), and HbE (n=1). Detection of (and) gene mutations and characterization of the secondary modifier Xmn1 polymorphism in .-globin gene were performed by Multiplex PCR ((SEA, (20.5, (FIL, (3.7, (4.2, (MED), ARMS, sequencing and RFLP PCR respectively. A total of 19) mutations were characterized and 91% of alleles were identified. Alleles of 12 patients failed to be identified. CD26 and CD41/42 were the most common) -gene mutations found in Malay and Chinese population respectively. Patients were then genotypically classified into thalassaemia major () °/) °), n=55; thalassaemia intermedia () °/) $^{+}$ or) $^{+}$/) $^{+}$), n=68; HbE) thalassaemia (CD26/) ° or CD26/) $^{+}$), n=95; HbE homozygous (CD26/CD26), n= 11 and) thalassaemia heterozygous with one affected allele () °/) or) $^{+}$/)), n=23. The sensitivity of clinical diagnosis of thalassaemia major, intermedia and HbE) thalassaemia was 97.8%, 18.5% and 88.5% respectively. HbE homozygous patients exhibited high HbA2 value (median value 70%). Thalassaemia major and intermedia patients required more frequent transfusion compared to other genotypically classified groups (χ2=37.012, p=0.0001). Xmn1 polymorphism in heterozygous [+/-] state was most common in HbE) thalassaemia patients. Of 37 HbE) thalassaemia patients, those with Xmn1 polymorphism [+/-] were observed to have high HbA2 value; required less frequent transfusion compared to those without the Xmn1 polymorphism. Six) thalassaemia patients were found to co-inherit (thalassaemia, namely (SEA and (3.7. Co-inheritance of (- thalassaemia did not significantly alleviate the severity of HbE) thalassaemia. In conclusion, molecular analysis should be used for accurate diagnosis and to help personalize the management of thalassaemia. Analysis of Xmn1 polymorphism may help in the management as heterozygous state of Xmn1 polymorphism alleviate the severity of thalassaemia.

1138T

Increased rate of nontruncating mutations in the *NF1* gene among individuals with *NF1* related disorders and pulmonic stenosis: A novel genotype-phenotype correlation and insight. *S. Ben-Shachar[1,2], S. Constantini[2], E. Sach[3], D.G. Evans[3], S.M. Huson[3].* 1) Genetic Institute, Tel Aviv Sourasky Medical Center, Tel-Aviv, Israel; 2) The Gilbert Israeli Neurofibromatosis center, Tel Aviv Sourasky Medical Center, Tel-Aviv, Israel; 3) Genetic Medicine Research Group, Manchester Biomedical Research Centre, Manchester Academic Health Sciences Centre, University of Manchester and Central Manchester Foundation Trust, St Mary's Hospital Manchester, United Kingdom.

Background: Neurofibromotosis type 1 (NF1) is caused by heterozygous mutations in the *NF1* gene. Pulmonic stenosis (PS) appears in NF1 more often than expected, Particularly in patients with the Neurofibromatosis-Noonan (NFNS) and Watson syndrome (WS) phenotypes. **Objective:** To test whether specific types of mutations in the gene are associated with PS in WS and NFNS. **Methods:** We have tested PS rates and genotype-phenotype correlation among individuals with NFNS/WS and PS, based on previously published cases as well as a new cohort of patients. **Results and conclusions:** Comparing to NF1, NFNS patients (regardless of gene mutation) had higher rates of PS (32% vs. 1.1%). When stratifying patients with NFNS according to the mutation type the increased PS rates appear to be driven by the NFNS group with non-truncating mutations: 66.7% PS in NFNS with non-truncating *NF1* mutation vs. 1.1% in NF1, p<0.001, LR= 55; while NFNS patients with truncating mutations were not different from NF1 patients. Moreover, of 5 individuals with WS phenotype and well characterized mutations, only one had a truncating mutations, while three had non-truncating mutation and one had a large intragenic deletion. We conclude that PS in *NF1* related disorders is clearly associated with non-truncating mutations in the NF1 gene providing a new insight of genotype-phenotype correlation associated with NF1 gene. Hence, we recommend cardiac evaluation for any individual diagnosed with NF1 related disorders having a non-truncating mutations.

1139T

TRIM50 forms a complex with HDAC6 and p62/SQSTM1 that localizes to aggresomes. *C. Fusco[1], L. Micale[1], M. Egorov[2], M. Monti[3], E.V. D'Addetta[1], B. Augello[1], G. Cotugno[1], F. Cozzolino[3], A. Calcagni[1], M.N. Loviglio[1], R. S. Polishchuk[2], P. Pucci[3].* 1) Medical Genetics, Casa Sollievo della Sofferenza, San Giovanni Rotondo, Foggia, Italy; 2) Telethon 3 CEINGE Advanced Biotechnology and Department of Organic Chemistry and Biochemistry, Federico II University, 80131 Naples, Italy; 3) CEINGE Advanced Biotechnology and Department of Organic Chemistry and Biochemistry, Federico II University, 80131 Naples, Italy.

TRIM50 is hemizygous in patients affected by Williams Beuren syndrome, a microdeletion neurodevelopmental genomic disorder. TRIM50 encodes a cytoplasmic E3-ubiquitin ligase that catalyzes the binding of specific substrates to the ubiquitin leading them to the degradation by activating processes as Proteasome and/or autophagy. Here we showed that TRIM50 forms highly motile, labile and dynamic cytoplasmic bodies that are precursors of the aggresome. In fact we showed that upon proteasome inhibition, TRIM50 localizes to the aggresome in a microtubule dependent manner. In addition, we identified HDAC6 as a new binding partner of TRIM50, an association that is strengthen under conditions of proteasome impairment. Using HDAC6-deficient mouse fibroblasts we demonstrated that HDAC6 is required for the proper localization of TRIM50 within the aggresome. Moreover, among a number of putative TRIM50-interacting proteins identified by nano LC-MS/MS, we found that TRIM50 interacts with p62/SQSTM1, a multifunctional adaptor protein implicated in various cellular processes including autophagic clearance of aggregation-prone polyubiquitinated proteins. Of note TRIM50 modulates the protein level of HDAC6 and p62, mainly increasing their insoluble aggresome fractions. Our work showed that TRIM50 bodies, by its E3-ubiquitin ligase are aggresome precursors. When TRIM50 fails, by its E3-ubiquitin ligase, to drive its proteins target to proteasomal degradation, an alternative route is taken to ensure their sequestration transporting them to the aggresome via the association with HDAC6 and possibly their subsequent removal by p62-mediated autophagy. Since aggresomes are cytoprotective by sequestering and eliminating deleterious aggregated proteins, we can suggest that haploinsufficiency of TRIM50 might play a role in the determination of Williams Beuren syndrome phenotypes resulting in the accumulation of ubiquitinated and aggregated proteins sensitizing cells to proteotoxic stressors.

1140T

Molecular Analysis of NPHS2 gene in a Brazilian cohort with childhood Nephrotic Syndrome. *M.S. Guaragna[1], A.C.G.B. Lutaif[2], S.Z.P. Rigazzo[2,4], V.M.S. Belangero[2,4], G. Guerra-Júnior[3,4], M.P. de Mello[1].* 1) Centro de Biologia Molecular e Engenharia Genética, CBMEG, Universidade de Campinas, UNICAMP, São Paulo, Brazil; 2) Centro Integrado de Nefrologia, CIN, Universidade de Campinas , UNICAMP, São Paulo, Brazil; 3) Grupo Interdisciplinar de Estudos da Determinação e Diferenciação do Sexo, GIEDDS, Universidade de Campinas, UNICAMP, São Paulo, Brazil; 4) Departamento de Pediatria, Faculdade de Ciências Médicas, FCM, Universidade de Campinas, UNICAMP, São Paulo, Brazil.

Nephrotic Syndrome (NS) is defined by edema, massive proteinuria, hypoalbuminemia and hyperlipidemia. Clinically, NS has been divided into two categories based on the response to steroid therapy: steroid-sensitive (SSNS) and steroid-resistant (SRNS). Among the SRNS, the renal histology typically shows focal segmental glomerulosclerosis (FSGS) with a high rate of development of end stage renal disease (ESRD). Inherited impairments of the glomerular filtration barrier (GFB) have been identified as important causes of NS. The GFB is composed of a fenestrated endothelium, the glomerular basement membrane and podocyte foot processes. The *NPHS2* gene encodes a protein called podocin, which has an important role in GFB. Mutations in *NPHS2* are frequently the cause of early onset autosomal recessive form of the disease (AR SRNS). In this study we report the molecular evaluation of *NPHS2* gene of 40 Brazilian patients (n=80 alleles) with infantile NS. The 8 exons, promoter region and intron/exon boundaries of *NPHS2* were direct sequenced. A total of seven already described missense mutations (7/80, 8.75%) have been identified in 6 heterozygous patients: p.P20L, in 2 patients (2.5%); p.A242V, in 1 patient (1.25%); p.E264Q, in 1 patient (1.25%); p.R229Q variant, in 2 patients (2,50%), one of them also carry the p.A284V mutation (1.25%). It has been shown that the p.R229Q is a polymorphism that in compound heterozygousis with another *NPHS2* mutation contributes to AR-FSGS. The significance of familial or sporadic heterozygous cases with only one mutation is still unclear. The *NPHS2* mutation may interact with other genes producing an additive effect in the frame of a multigenic inheritance. Or, there could be a sort of susceptibility to develop proteinuria conferred by the genetic background where other factors may represent the breaking event. These results are presented for the first time in a Brazilian cohort and emphasize the relevance of the molecular analysis of NS in childhood to define its etiology. SRNS is a clinically heterogeneous disease and the genotypic information may guide further treatment for these patients. Additionally, since SRNS patients with homozygous or compound heterozygous mutations in NPHS2 have reduced risks for recurrence of FSGS in a renal transplant compared with children without mutations, living related donor transplantation might be considered more promptly.

1141T

A novel mutation in the AMELX gene and multiple crown resorptions. *J.W. Kim[1,2], S.K. Lee[1], K.E. Lee[1], S.E. Jung[1], S.J. Song[1], S.H. Cho[1].* 1) Pediatric Dentistry, Seoul National University School of Dentistry, Seoul, Korea; 2) Molecular Genetics, Seoul National University School of Dentistry, Seoul, Korea.

Amelogenesis imperfecta (AI) is a heterogeneous group of genetic disorders with regard to genetic etiology and clinical phenotype and affects tooth enamel with no other non-oral syndromic conditions. X-linked AI is caused by mutations in the AMELX gene the only AI candidate gene located on the X chromosome. To date, 15 mutations in the AMELX gene have been found to cause AI. We identified a proband associated with generalized hypoplastic enamel with unusual multiple crown resorption in premolars and molars. Pedigree analysis suggested an X-linked hereditary pattern. We performed mutational analysis for the AMELX gene based on the candidate gene approach. Sequencing analysis revealed a novel mutation in exon 6 (g.4090delC, c.517delC, p.Pro173LeufsX16). This frameshift mutation produces a premature stop codon within exon 6 and is predicted to replace 33 amino acids at the C-terminus with 15 novel amino acids if the mutant mRNA escapes the nonsense-mediated decay system. Although crown resorption in AI patients occurs frequently in hypoplastic type, an association with the AMELX mutation has not been previously reported. We believe that these findings will broaden our understanding of the clinical phenotype and pathogenesis of X-linked AI. This work was supported by a Science Research Center grant to the Bone Metabolism Research Center (20110001025) and a grant (20100020542) from the National Research Foundation of Korea (NRF), both funded by the Ministry of Education, Science, and Technology (MEST) of the Republic of Korea.

1142T

Molecular characterization of thalassemia: Effect of Alpha gene Number on phenotype. *R. Kumar, S. Agarwal.* Department of Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India.

Introduction: Co-inheritance of (thalassemia reduces (/) chain imbalance and the amount of redundant alpha globin, with an ameliorating effect, while the presence of extra (globin genes will have an adverse effect. Aim: To find to frequency of alpha gene deltion in triplication and their effect on phenotype on beta thalassemia Material and Methods: 241 thalassemia cases who visited OPD, Department of Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow during the period of 2007-2010 were studied. Beta thalassemia mutations were detected by ARMS-PCR whereas GAP-PCR technology was used to detect alpha gene nubmer Results: The overall prevalence of alpha gene deletion was found 12.5% and that of alpha triplication was 5.8%.The frequency of alpha gene deletion in homozygous thalassemia intermedia group was found significantly higher than homozygous beta thalassemia major group (p value=0.029). Similarly the alpha gene triplication was found higher in heterozygous thalassemia intermedia group than thalassemia trait group (p value=0.000). The phenotypes of 5 cases out of 12 homozygous thalassemia intermedia cases were explained on the basis of coinheritance of alpha thalassemia. 13 cases of thalassemia trait were co inherited with alpha triplication and out of these 13, 9 cases were presented as thalassemia intermedia. Conclusion: alpha gene number plays an important role in modulating the phenotype of thalassemia and thus alpha gene number should also be analysed along with beta thalassemia mutations during prenatal daignosis of thalassemia.

1143T

Analysis of the polymorphism -930 A/G (rs9932581) in the CYBA gene in pediatric patients with sickle cell anemia. *M.B. Melo[1], G.P. Gil[1], G. Ananina[1], M.B. Oliveira[1], M.J. Silva[1], D. Stancato[1], F. Menaa[1], M.N.N. Santos[2], A.S. Araujo[3], M.A. Bezerra[3], F.F. Costa[4].* 1) CBMEG, Univ Campinas, Campinas SP, Brazil; 2) Department of Clinical Pathology, Univ Campinas, Campinas, SP, Brazil; 3) Hemope, Recife, PE, Brazil; 4) Hemocentro - Department of Clinical Medicine, Univ Campinas, Campinas, SP, Brazil.

Sickle cell anemia (SCA), although resulting from the homozygosity of a single mutation at position 6 of the) -globin locus, displays heterogeneous phenotypes. The severity of this threatening-life disease can be estimated using an online calculator (e.g. Sebastiani et al., 2007 with scores ranging from 0 to 1) or based on clinical evaluation (e.g. Xandra et al., 2010 with scores ranging from 0 to 285), but this severity estimation is not sufficient to explain the molecular mechanisms associated to the observed phenotypes. Indeed, one mechanism that may help to explain the heterogeneity of the disease might be the oxidative stress, resulting from vaso-occlusive ischemia-reperfusion as well as hemolysis. The NADPH is an important source of superoxide anion (O2.-) in vascular cells. One of its subunits p22phox is encoded by the CYBA gene (16q24), which has several allelic variants, such as -930 A/G (rs9932581). Studies suggest an association between the allele G frequency of this variant and (i) hypertension, (ii) increased expression of the CYBA gene. However, the allele A frequency is less encountered in hypertensive patients and is associated with lower expression of the gene. These data indicate the dominance of the allele G compared to the allele A. Here, we hypothesized the association of the alleles with the severity of SCA pediatric patients using Mann-Whitney test. From 92 SCA pediatric patients with age ranging from 2 to 17 years old, direct sequencing of the fragment containing the variant -930A/G was performed. The Hardy-Weinberg equilibrium was tested by Chi-square test and the severity scores were calculated using both P. Sebastiani's online calculator and Xandra's clinical evaluation method. We observed that the genotype distribution is in Hardy-Weinberg equilibrium. From the severity scores using Xandra's method (mean ± SD: 53 ± 36 - range: 0 to 120), the genotype group AA was significantly ($p<0.05$) associated with low severity. However, this finding was not confirmed with Sebastiani's method (mean scores ± SD: 0.46 ± 0.39 - range: 0.028 to 0.997). Due to the complexity of the disease and also to the age of the patients, the methods used to evaluate severity scores may reveal different results and consequently genotype-phenotype associations are difficult to establish. In this work, the method by Xandra and colleagues was able to detect association between the AA genotype and low severity of the disease in SCA pediatric patients.

1144T

Functional Consequences and Structural Interpretation of Mutations of Human Choline Acetyltransferase. *X. Shen[1], T. Crawford[2], J. Brengman[1], G. Acsadi[3], S. Iannaconne[4], E. Karaca[5], C. Khoury[6], J. Mah[7], S. Edvardson[8], Z. Bajzer[9], D. Rodgers[10], A. Engel[1].* 1) Dept. of Neurology, Mayo Clinic, Rochester, MN, USA; 2) Dept. of Neurology and Pediatrics, John Hopkins Hospital, Baltimore, MD, USA; 3) Children's Hospital of Michigan, Detroit, MI, USA; 4) Dept. of Neurology, University of Texas Southwestern Medical Center, Dallas, TX, USA; 5) Dept. of Medical Genetics, Ege University, Izmir, Turkey; 6) Dept. of Neurology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA; 7) Dept. of Pediatrics, University of Calgary, Calgary, Alberta, Canada; 8) Dept. of Pediatric Neurology, Hadassah University Hospital, Jerusalem, Israel; 9) Dept. of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA; 10) Dept. of Molecular and Cellular Biochemistry and Center for Structural Biology, University of Kentucky, Lexington, KY, USA.

Choline acetyltransferase (CHAT; EC 2.3.1.6) catalyzes synthesis of acetylcholine from acetyl-CoA and choline in cholinergic neurons. *CHAT* mutations cause potentially lethal congenital myasthenic syndromes associated with episodic apnea (CHAT-CMS) (MIM ID 254210). Here we analyze the functional consequences of 12 missense and 1 nonsense mutations of CHAT in 11 patients. Nine of the mutations are novel. We examine expression of the recombinant missense mutants in Bosc 23 cells, determine their kinetic properties and thermal stability, and interpret the functional effects of 11 mutations in the context of the atomic structural model of human CHAT. With amino acid changes based on the 83 kDa M type CHAT (NP_065574), 5 mutations (p.Trp421Ser, p.Ser498Pro, p.Thr553Asn, p.Ala557Thr, p.Ser572Trp) reduce enzyme expression to <50% of wild-type. Mutations with severe kinetic effects are located in the active-site tunnel (p.Met202Arg, p.Thr553Asn and p.Ala557Thr) or adjacent to the substrate binding site (p.Ser572Trp), or exert their effect allosterically (p.Trp421Ser and p.Ile689Ser). Two mutations with milder kinetic effects (p.Val136Met, p.Ala235Thr) are also predicted to act allosterically. One mutation (p.Thr608Asn) below the nucleotide binding site of CoA enhances dissociation of AcCoA from the enzyme-substrate complex. Two mutations introducing a proline residues into an (-helix (p.Ser498Pro and p.Ser704Pro) impair the thermal stability of CHAT.

1145T

Heme oxygenase-1 gene promoter polymorphism is associated with reduced incidence of acute chest syndrome in children with sickle cell anemia. *C. Bean[1], S. Boulet[1], D. Ellingsen[1], M. Pyle[1], E. Barron-Casella[2], J. Casella[2], S. Ofori-Acquah[3], W.C. Hooper[1], M. DeBaun[4].* 1) Division of Blood Disorders, Centers for Disease Control, Atlanta, GA; 2) Division of Pediatric Hematology, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD; 3) Division of Hematology/Oncology/BMT, Emory University, Atlanta, GA; 4) Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN.

Sickle cell anemia (SCA) is a common hemolytic disorder with a broad range of complications, including anemia, vaso-occlusive episodes, acute chest syndrome (ACS), pain and stroke. Despite the clinical importance of the role of vaso-occlusive episodes in pain and ACS, there remains limited insight into their underlying etiology. Sickle erythrocytes have a high rate of lysis, releasing large amounts of hemoglobin into the plasma. Free heme released from hemoglobin induces vascular inflammation and is a major source of oxidative stress in patients with SCA.

Heme oxygenase-1 (*HMOX1*) is the inducible, rate limiting enzyme in the catabolism of heme and may attenuate the severity of outcomes from vaso-occlusive and hemolytic crises. A $(GT)_n$ dinucleotide repeat located in the promoter region of the *HMOX1* gene is highly polymorphic, with long repeat lengths linked to decreased activity and inducibility. We examined this polymorphism to test the hypothesis that short alleles are associated with decreased risk of adverse outcomes (pain requiring hospitalization and ACS) in a cohort of 949 children with SCA.

Allele lengths varied from 13 to 45 $(GT)_n$ repeats and showed a trimodal distribution, with peaks at 23, 30 and 39 repeats. Compared with children with longer allele lengths, children with two shorter alleles (4%; ≤25 repeats) had lower rates of hospitalization for ACS (IRR 0.28, 95% CI: 0.10-0.81), after adjusting for gender, age, asthma, and percent fetal hemoglobin. No relationship was identified between allele lengths and pain rate. To our knowledge, this is the first study to examine the role of genetic variation in *HMOX1* in a population of patients affected with a congenital hemolytic disorder and, we demonstrate that genetic variation in the inducible form of heme oxygenase is associated with ACS. Specifically, patients homozygous for short $(GT)_n$ repeat alleles have a significantly lower rate of hospitalization per patient year for ACS, a leading cause of death in patients with SCA. Greater understanding of the role of the heme oxygenase response in these patients may be a key to improved prediction and treatment strategies.

1146T

Homozygous c.369_370delAG and c.374_376delCGC Mutation in STX11 Gene in a Patient with Familial Hemophagocytic Lymphohistiocytosis. *H. Onay[1], D. Yilmaz Karapinar[2], A. Aykut[1], S. Gokce[2], B. Karapinar[2], F. Ozkinay[1,2].* 1) Department of Medical Genetics , Ege University Faculty of Medicine, Izmir, Turkey; 2) Department of Pediatrics, Ege University Faculty of Medicine, Izmir, Turkey.

Familial hemophagocytic lymphohistiocytosis (FHL) is a rare autosomal recessive lethal condition characterized by fever, cytopenia, hepatosplenomegaly, and hemophagocytosis. Upto date three genes that cause FHL have been identified. Perforin gene (PRF1) mutations have been identified in %20-50 of the patients with FHL. Mutations in MUNC13-4 and Syntaxin 11(STX11) genes also cause FHL. A 2-year old boy was referred to Pediatric Intensive Care Unit for hepatic failure, hypotension, hematochezia, and coma. Glasgow coma score was 12. He had splenomegaly, fever (unresponsive to antibiotheraphy), pancytopenia, bleeding diathesis, and low fibrinogen levels. Bone marrow biopsy was performed. Bone marrow was hypocellular, major cell type was histiocytes, and contained hemophagocytic macrophages. Due to consanguinity between parents and child age being under two years, the diagnosis was considered as familial (primary) hemophagocytic lymphohistiocytosis (FHL). The patient was referred to Medical Genetic Department for mutation analysis. Sequencing of the STX11 gene revealed a homozygous c.369_370delAG and c.374_376delCGC mutation. This rare mutation was solely described in a Turkish family. The age of onset of our patient was similar to the patients in this previous report. Although HLH 2004 treatment regimen was applied, the patient deceased due to cardiac arrest at the end of 4 days. Herein we present a case with FHL with a very rare 5 bp deletion mutation in STX11 gene.

1147T

STK4 deficiency: A novel primary immunodeficiency affecting both innate and adaptive immunity and including cardiac defects. *A.A. Schaffer[1], H. Abdollahpour[2], G. Appaswamy[2], R. Beier[2], E.M. Gertz[1], A. Schambach[3], H.H. Kreipe[4], D. Pfeifer[5], K.R. Engelhardt[6], N. Rezaei[7], B. Grimbacher[6], S. Lohrmann[8], R. Sherkat[9], C. Klein[1,10].* 1) National Center for Biotechnology Information, National Institutes of Health, DHHS, Bethesda, MD, USA; 2) Department of Pediatric Hematology/Oncology, Hannover Medical School, Hannover, Germany; 3) Department of Experimental Hematology, Hannover Medical School, Hannover, Germany; 4) Institute of Pathology, Hannover Medical School, Hannover, Germany; 5) Department of Hematology/Oncology, Core Facility II Genomics, Freiburg University Medical Center, Freiburg, Germany; 6) Department of Immunology and Molecular Pathology, Royal Free Hospital & University College London, London, UK; 7) Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran; 8) Department of Pediatric Cardiology, Hannover Medical School, Hannover, Germany; 9) Department of Infectious Diseases, Isfahan University of Medical Sciences, Isfahan, Iran; 10) University Children's Hospital Munich, Dr. von Haunersches Kinderspital, Munich, Germany.

We describe an unusual clinical phenotype associating intermittent neutropenia, progressive T- and B-cell lymphopenia, and atrial septal defects in three members of a consanguineous kindred. Homozygosity mapping and candidate gene sequencing revealed a mutation in the gene STK4 (formerly MST1). STK4 is the human ortholog of Drosophila Hippo, the central constituent of a highly conserved pathway controlling cell growth and apoptosis. STK4-deficient lymphocytes and neutrophils exhibit enhanced loss of mitochondrial membrane potential and increased susceptibility to apoptosis.

1148T

Mutations in *KIF7* link Joubert syndrome with Sonic Hedgehog signaling and microtubule dynamics. *H.J. Bolz[1,2], C. Dafinger[1,3], M.C. Liebau[3,4], S.M. Elsayed[5,6], Y. Hellenbroich[7], E. Boltshauser[8], G.C. Korenke[9], F. Fabretti[3], A.R. Janecke[10], I. Ebermann[2], G. Nürnberg[11,12], P. Nürnberg[11,12], H. Zentgraf[13], F. Koerber[14], K. Addicks[15], E. Elsobky[5,6], T. Benzing[3,12], B. Schermer[3,12].* 1) Center for Human Genetics, Bioscientia, Ingelheim, Germany; 2) Institute of Human Genetics, University Hospital of Cologne, Cologne, Germany; 3) Renal Division, Department of Medicine and Centre for Molecular Medicine, University of Cologne, Cologne, Germany; 4) Department of Pediatrics, University of Cologne, Germany; 5) Medical Genetics Center, Cairo, Egypt; 6) Children's Hospital, Ain Shams University, Cairo, Egypt; 7) Institute of Human Genetics, University Hospital of Schleswig-Holstein, Campus Lübeck, Germany; 8) Department of Paediatric Neurology, University Children's Hospital of Zurich, Zurich, Switzerland; 9) Klinikum Oldenburg, Zentrum für Kinder- und Jugendmedizin, Elisabeth Kinderkrankenhaus, Neuropädiatrie, Oldenburg, Germany; 10) Department of Pediatrics II, Innsbruck Medical University, Innsbruck, Austria; 11) Cologne Center for Genomics and Centre for Molecular Medicine, University of Cologne, Cologne, Germany; 12) Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, University of Cologne, Cologne, Germany; 13) Department of Tumor Virology, German Cancer Research Center Heidelberg, Germany; 14) Department of Radiology, University of Cologne, Cologne, Germany; 15) Department of Anatomy, University Hospital of Cologne, Cologne, Germany.

Joubert syndrome (JBTS) is characterized by a specific brain malformation, the molar tooth sign, and various additional pathologies. It results from mutations in any one of at least 10 different genes. JBTS has been linked to dysfunction of primary cilia, since the gene products known to be associated with the disorder localize to this evolutionarily ancient organelle. Here we report the identification of a novel locus for Joubert syndrome, *JBTS12*, on chromosome 15q25.3-q26.3, with mutations in the *KIF7* gene in a consanguineous JBTS family and subsequently in other patients with JBTS of different severity. One patient carried a heterozygous *KIF7* mutation together with two hypomorphic *TMEM67* mutations, suggesting that disease in this patient may be oligogenic, resulting from the mutational load with three mutant alleles in two JBTS genes. KIF7, an ortholog of the *Drosophila* kinesin *Costal2*, is a known regulator of Hedgehog signaling and a putative ciliary motor protein. We found that KIF7 co-precipitated with nephrocystin-1 (NPHP1), another protein mutated in JBTS and related ciliopathies. Knockdown of *KIF7* expression in cell lines caused defects in cilia formation and induced abnormal centrosomal duplication and fragmentation of the Golgi network. The Golgi phenotype affected all three compartments of the Golgi apparatus, and could be rescued by over-expression of full-length KIF7. In contrast, over-expression of a KIF7 truncation lacking the predicted motor domain led to the same Golgi phenotype as the knockdown of *KIF7*. We observed an increase of tubulin acetylation and changes in the cell shape. Consistently, KIF7 was found to interact with the histone deacetylase HDAC6 and the polarity complex protein PAR3. In conclusion, loss of KIF7 function affects microtubule stability and growth direction which may impact cilia, centrosomes and Golgi and represent a disease mechanism for JBTS. *KIF7* represents a prime candidate for mono- and oligogenic forms of other ciliopathies related to JBTS, namely Meckel-Gruber-, Senior Loken- and Bardet-Biedl syndrome, Leber congenital amaurosis and nephronophthisis.

1149T

FORGE Canada: A nation-wide effort to identify genes causing Mendelian disorders. *K.M. Boycott[1], C.L. Beaulieu[1], J. Marcadier[1], J. Michaud[2], J. Friedman[3], M. Samuels[2], B. Knoppers[4], B. Fernandez[5], F. Bernier[6], S. Scherer[7], M. Brudno[8] FORGE Canada Consortium.* 1) Children's Hospital of Eastern Ontario Research Institute, Ottawa, ON, Canada; 2) Centre de Recherche de l'Hôpital Ste-Justine, Montréal, QC, Canada; 3) University of British Columbia, Vancouver, BC, Canada; 4) Centre of Genomics and Policy, McGill University, Montreal, QC, Canada; 5) Discipline of Genetics, Memorial University, St. John's, NFLD, Canada; 6) Department of Medical Genetics, University of Calgary, Calgary, AB, Canada; 7) The Center for Applied Genomics, The Hospital for Sick Children, Toronto, ON, Canada; 8) Department of Computer Science and Donnelly Centre, University of Toronto, Toronto, ON, Canada.

Single-gene disorders, while individually rare, have, in aggregate, an enormous impact on the well-being of families. The majority of the estimated 10,000 genes that cause these conditions remain unknown and with the introduction of massively parallel next-generation sequencing techniques a unique opportunity exists to identify the remaining genes causing Mendelian disease. It is estimated that 500,000 Canadian children are affected with a rare genetic disorder. The objective of the FORGE Canada Consortium - Finding of Rare Disease Genes in Canada - initiative is to rapidly identify disease genes for over 100 childhood monogenic disorders, in an 18 month time-frame with established funding through Genome Canada and the Canadian Institutes of Health Research. To this end we have developed a collaborative national consortium of clinicians and scientists - with over 150 members and including clinical site-leads from all 21 Clinical Genetics Centres in Canada. Our clinical coordination has enabled identification of sufficient patient resources for the study of many disorders which individually would not have been possible through purely local ascertainment. We have established a pipeline for discovery that includes resources for over 175 disorders; each disorder has an identified team lead to guide the research for that particular project. We are using whole-exome, and in some cases whole-genome, re-sequencing at the three Science and Technology Innovation Centres in Canada. We have established a National Data Coordination Centre to facilitate data analysis, data sharing and development of improved analysis tools. Once potential causative variants are identified through this pipeline, team leads are responsible for validation and further study of these genes in their research laboratories. Guidelines for the application of these new technologies for the investigation of childhood disease are being created through an integrated GE^{3}LS research component, including knowledge translation to clinical practice. Examples of gene identification successes will be highlighted including Hadju-Cheney syndrome and other disorders currently in progress. The wealth of knowledge this effort will generate will immediately translate to improved patient care and will provide pathways for further study and potential therapeutic investigation. Through the FORGE Consortium, Canada is emerging as an international leader focused on the study of rare genetic disease.

1150T

Whole-exome sequencing in a single proband reveals causative mutation in autosomal recessive peeling skin syndrome. *R.M. Cabral[1], M. Kurban[1], M. Wajid[1], Y. Shimomura[1, 2], A.M. Christiano[1, 3].* 1) Dermatology, Columbia University, New York, NY; 2) Epidemiology, Columbia University, New York, NY; 3) Genetics & Development, Columbia University, New York, NY.

We ascertained a large consanguineous family segregating peeling skin syndrome noninflammatory type A (OMIM 270300) as a recessive trait. Autozygosity mapping using the Affymetrix 10K genotyping chip revealed a 15Mb region with strong evidence for allele sharing among affected individuals, with a LOD score of 10.9. We then performed whole-exome sequencing using the SOLiD 4 platform on a single affected family member. We identified a total of 1,093 variants that were not present in any public databases or our internal database of exome variants derived from ethnically-matched unrelated samples. Only one homozygous variant was present in the autozygous region, 229C>T, located within the gene CHST8. We validated this finding with Sanger sequencing in all members of the pedigree and demonstrated co-segregation with the trait. The CHST8 gene encodes a Golgi membrane carbohydrate sulfotransferase, N-acetylgalactosamine-4-O-sulfotransferase 1 (GalNAc4-ST1), known to be highly expressed in pituitary, cerebellum and brain. Immunohistochemistry on frozen normal skin sections with a polyclonal antibody raised against GalNAc4-ST1 showed that this enzyme is also expressed throughout the epidermis, predominantly in the granular and cornified layers. To investigate the effect of the 229C>T mutation (R77W) on the function of GalNAc4-ST1, we performed a colorimetric assay for sulfated glycosaminoglycan (GAGs) quantification, and compared total amounts of sulfated GAGs between human keratinocytes (CCD1106 KERTr cell line) transfected with wild type and mutant CHST8 cDNA constructs. We observed decreased levels of total sulfated GAGs in cells transfected with the mutant CHST8 construct compared to wild type, suggesting loss of function of mutant GalNAc4-ST1. Both wild type and mutant GalNAc4-ST1 co-localized with the Golgi membrane marker GM130, as assessed by immunofluorescence staining of CCD1106 cells. However, Western blot analysis revealed lower expression levels of the full length GalNAc4-ST1 protein, as well as an additional lower molecular weight immunoreactive band in cells transfected with the mutant CHST8 construct compared to wild type CHST8. These results demonstrate that the R77W mutation leads to decreased expression levels of GalNAc4-ST1, possibly by leading to decreased protein stability and increased degradation, which results in loss of function.

1151T

Deficiency of adaptor protein complex 4 causes autosomal recessive intellectual disability with absent speech, shy character, progressive spastic paraplegia and short stature. *L. Colleaux[1], O. Philippe[1], A. Raas-Rothschild[2], S. Eck[3], E. Graf[3], R. Buchert[4], G. Borck[1], A. Ekici[4], F.F. Brockschmidt[5,6], M.M. Nöthen[5,6], A. Munnich[1], T.M. Strom[3,7], A. Reis[4], R. Abou Jamra[4].* 1) INSERM U781,Fondation IMAGINE, Département de Génétique, Hôpital Necker-Enfants Malades, PARIS, France; 2) Department of Human Genetics and Metabolic Diseases, Hadassah Hebrew University Medical Center, Jerusalem, Israel; 3) Institute of Human Genetics, Helmholtz Center Munich, German Research Center for Environmental Health, Neuherberg, Germany; 4) Institute of Human Genetics, University of Erlangen, Erlangen, Germany; 5) Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany; 6) Institute of Human Genetics, University of Bonn, Bonn, Germany; 7) Institute of Human Genetics, Klinikum rechts der Isar, Technische Universität München, München, Germany.

Intellectual disability of autosomal recessive inheritance represents an important fraction of severe cognitive dysfunction disorders. Yet, the extreme heterogeneity of these conditions markedly hampers gene identification. Here, we report on eight affected individuals from three consanguineous families that all presented with severe intellectual disability with absent speech, shy character, stereotypic laughter, muscular hypotonia that progressed to spastic paraplegia, microcephaly, feet deformity and decreased muscle mass of the lower limbs, inability to walk, and growth retardation. Using a combination of autozygosity mapping and either Sanger sequencing of candidate genes or next generation exome sequencing, we identified one mutation in each of three genes encoding adaptor protein complex 4 (AP4) subunits: a nonsense mutation in *AP4S1* (NM_007077.3:c.124C>T,p.Arg42*), a frameshift mutation in *AP4B1* (NM_006594.2: c.487_488insTAT,p.Glu163_Ser739delinsVal), and a splice mutation in *AP4E1* (NM_007347.3, c.542+1_542+4delGTAA,r.421_542del,-p.Glu181Glyfs*20). Adaptor protein complexes (AP1-4) are ubiquitously expressed evolutionarily conserved heterotetrameric complexes that mediate different types of vesicle formation and the selection of cargo molecules for inclusion into these vesicles. Interestingly, two mutations affecting *AP4M1* and *AP4E1* have recently been found to cause cerebral palsy associated with severe intellectual disability. Our findings illustrate the power of combining systematic autozygosity mapping with large-scale sequencing for unraveling the molecular bases of autosomal recessive ID. More importantly, combined to previous observations, these results support the hypothesis of a crucial role of AP4- mediated trafficking in brain development and functioning and demonstrate the existence of a clinically recognizable AP4 deficiency syndrome.

1152T

Mutation in *STXBP5L* in a novel neurodegenerative disorder with severe axonal peripheral neuropathy, neurogenic muscular atrophy and optic atrophy. *M. Corbett[1], R. Sharma[1], K. Smith[2], A. Hoischen[3], J. Veltman[3], M. Bahlo[2,4], E. Haan[5,6], J. Gecz[1,5,7].* 1) Genetics and Molecular Pathology, SA Pathology, North Adelaide, Australia; 2) The Walter and Eliza Hall Research Institute of Medical Research, Parkville, Australia; 3) Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 4) Department of Mathematics and Statistics, University of Melbourne, Parkville, Australia; 5) School of Paediatrics and Reproductive Health, The University of Adelaide, Adelaide, Australia; 6) South Australian Clinical Genetics Service, SA Pathology, Adelaide, Australia; 7) School of Molecular and Biomedical Sciences, The University of Adelaide, Adelaide, Australia.

We describe a consanguineous pedigree with two siblings affected by a novel neurodegenerative disorder associated with severe axonal peripheral neuropathy, neurogenic muscular atrophy, hypotonia, muscle weakness, optic atrophy and developmental delay. Homozygousity mapping identified an identical by descent (IBD) region on chromosome 3, 110459136 bp-123879723 bp (hg18 March 2006 build) between the single nucleotide polymorphisms (SNPs) rs2673391 and rs1355563, with a LOD score of 2.31. All coding exons, microRNA and conserved sequences (based on elements with LOD > 50 from the 28 way Vertebrate Multiz alignment track from the UCSC genome browser) totaling approximately 1 Mbp of the IBD interval were enriched from genomic DNA of the proband using array based sequence enrichment (*Roche*). The enriched DNA was sequenced using *Roche GS FLX* pyrosequencing and resulting data mapped to the human genome. 1171 homozygous sequence variants were identified in the IBD interval and those localized to coding sequences were filtered against dbSNP130 to reveal a single, unique, missense variant in syntaxin binding protein 5 like (*STXBP5L*). The product of *STXBP5L* is an as yet uncharacterized protein expressed in the central and peripheral nervous system. The closely related STXBP5 (or tomosyn) protein is known to inhibit neurotransmitter release through inhibition of the formation of the SNARE complexes between synaptic vesicles and the plasma membrane. This suggests a potential role of STXBP5L in neurotransmitter transport or release. On aggregate, our clinical, genetic and molecular data suggest that mutation in *STXBP5L* provides a plausible explanation for this novel, progressive neurodegenerative disorder.

1153T

BLOC-1 mutation screening in Hermansky-Pudlak Syndrome reveals a new HPS subtype, HPS-9, associated with mutations in PLDN (pallidin) and a novel BLOS3 (HPS-8) mutation. *A.R. Cullinane[1], J.A. Curry[1], C. Carmona-Rivera[1], G. Golas[2], C.G. Summers[3], C. Ciccone[1], N.D. Cardillo[1], H. Dorward[1], R.A. Hess[1], J.G. White[4], D. Adams[1,2], M. Huizing[1], W.A. Gahl[1,2].* 1) Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda MD 20892, USA; 2) Intramural Office of Rare Diseases Research, Office of the Director, National Institutes of Health, Bethesda MD 20892 USA; 3) 3. Departments of Ophthalmology and Pediatrics, University of Minnesota, Minneapolis, MN 55455 USA; 4) 4. Department of Laboratory Medicine, University of Minnesota, Minneapolis, MN 55455 USA.

Hermansky-Pudlak Syndrome (HPS) is an autosomal recessive condition characterized by oculocutaneous albinism and a bleeding diathesis due to absent platelet delta granules. HPS, a genetically heterogeneous disorder of intracellular vesicle biogenesis, and has 8 known subtypes, 7 of which are associated with genes encoding different Biogenesis of Lysosome-related Organelles Complex (BLOC) proteins. We screened patients with HPS-like symptoms for mutations in HPS1-6 and found no functional mutations in 38 individuals. We then examined these individuals for all 8 genes encoding the BLOC-1 proteins. We identified homozygous nonsense mutations in PLDN, encoding the BLOC-1 subunit, pallidin, in a single individual with characteristic features of HPS. No PLDN mutations have previously been described in humans, although Pldn is mutated in the HPS mouse model pallid. The pallidin protein interacts with the early endosomal t-SNARE syntaxin-13. We could not detect any full-length pallidin protein in our HPS-9 patient's fibroblasts or melanocytes despite normal mRNA expression of the mutant transcript. An alternative transcript was detected in the patient that would skip the exon that harbors the mutation; however, we demonstrate that, if this transcript were translated into protein, it would not interact with syntaxin-13 although it correctly localizes to early endosomes. We also identified only the second mutation to date in BLOS3, causing HPS-8. In this infant's melanocytes, BLOS3 mRNA expression was significantly reduced compared to control, suggesting nonsense-mediated decay was occurring for this mutation. Absence of either PLDN or BLOS3 causes instability of the BLOC-1 protein complex, and in both HPS-9 and HPS-8 melanocytes, the melanogenic protein TYRP1 showed aberrant localization, increased plasma-membrane trafficking, and failure to reach melanosomes. These results help explain the patients' severe albinism and establish a common cellular defect within patients having BLOC-1 gene mutations.

1154T

Mutations in *MEGF11* cause juvenile open angle glaucoma. *B.J. Fan[1], D.Y. Wang[1], X. Chen[1], K. Linkroum[1], E.A. DelBono[1], J.L. Haines[2], J.L. Wiggs[1].* 1) Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA; 2) Center for Human Genetic Research, Vanderbilt University School of Medicine, Nashville, TN.

Juvenile open-angle glaucoma (JOAG) is a rare disorder that is typically inherited as an autosomal dominant trait. Family linkage studies have suggested 5 genetic loci harboring genes responsible for JOAG, including *GLC1A* (1q24.3-q25.2), *GLC1J* (9q22), *GLC1K* (20p12), *GLC1M* (5q22.1-q32) and *GLC1N* (15q22-q24). *Myocilin* (*MYOC*) was identified as the causative gene at *GLC1A*, whereas the responsible genes at the other loci remain to be identified. Our fine mapping study identified 6 adjacent SNPs at *GLC1N* associated with JOAG in a Chinese case-control sample. In the present study, we further refined the association using an independent sample of Caucasian populations and identified *multiple EGF-like-domains 11* (*MEGF11*) as a potential causative gene for JOAG. A total of 579 unrelated patients with glaucoma, including 40 patients with JOAG and 539 patients with adult-onset primary open angle glaucoma (POAG), and 365 control subjects were genotyped for the 6 SNPs at *GLC1N* by ABI TaqMan assays. The SNP rs441949 in *MEGF11* showed a significant association with JOAG in the Caucasian sample ($p = 0.0027$, Bonferroni-corrected $p = 0.016$; OR = 2.47, 95%CI: 1.40-4.33). The other 5 SNPs were not associated with JOAG ($p > 0.07$). None of these SNPs were associated with POAG ($p > 0.25$). All exons and splicing junctions of *MEGF11* were screened for sequence alterations in the JOAG patients and a subset of controls. Twenty-two sequence alterations were identified in *MEGF11* in the JOAG patients, ten of which were novel including 2 nonsynonymous changes (G238D and D899N) which were absent in the control group (n = 65). Both G238D and D899N are evolutionarily conserved and predicted to have pathological effects using PolyPhen-2. Overall our results support *MEGF11* at the *GLC1N* locus as a novel disease-causing gene for JOAG. **Grant support:** NEI Grants R01EY015872 and P30EY014104, Research to Prevent Blindness and The Massachusetts Lions Eye Research Fund.

1155T

Genetic heterogeneity in the Brittle Cornea Syndrome (BCS) - New evidence that *ZNF469* and *PRDM5* regulate extracellular matrix development and maintenance within the same pathway. *C. Giunta[1], F. Manson[2], E.M.M. Burkitt Wright[2], H.L. Spencer[2], N. Zoppi[3], A.R. Janecke[4], C. Bürer-Chambaz[1], H. Al-Hussain[5], M. Wilson[6], M. Bakshi[6], D. Sillence[6], M. Colombi[3], B. Steinmann[1], M. Rohrbach[1], G.C.M. Black[2].* 1) Division of Metabolism, University Children's Hospital and Children's Research Center, Zurich, Switzerland; 2) 2Genetic Medicine Research Group, Manchester Biomedical Research Centre, Manchester Academic Health Sciences Centre, University of Manchester and Central Manchester Foundation Trust, St Mary's Hospital Manchester, UK; 3) Division of Biology and Genetics, Department of Biomedical Sciences and Biotechnology, Medical Faculty, University of Brescia, Brescia, Italy; 4) Division of Clinical Genetics, Innsbruck Medical University, Innsbruck; 5) King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia; 6) Department of Clinical Genetics, Children's Hospital at Westmead, Sydney, Australia.

The Brittle Cornea Syndrome (BCS; MIM 229200) is an autosomal recessive connective tissue disorder caused by mutations in *ZNF469* (Abu et al., 2008) and is characterized by extreme thinning and fragility of the cornea that may rupture even in the absence of adequate trauma and can lead to blindness. Keratoconus or keratoglobus, high myopia, blue sclerae, hyperelasticity of the skin without excessive fragility, and hypermobility of the small joints are additional features of BCS. In a cohort of thirteen BCS affected families (Al-Hussain et al., 2004), genome-wide SNP-homozygosity mapping and sequencing identified *ZNF469* mutations in only nine families, thus suggesting genetic heterogeneity in BCS. Thereafter, autozygosity mapping in two newly identified large BCS families of Pakistani origin allowed us to identify the transcription factor PRDM5 as the second gene mutated in BCS, thus confirming genetic heterogeneity (Burkitt Wright et al., 2011). Screening of further patients identified homozygous mutations in *PRDM5* in five additional BCS families, including three from Al-Hussain et al. 2004, who had tested negative for *ZNF469* mutations, as well as heterozygous *PRDM5* mutations in individuals homozygous for *ZNF469* mutations. The phenotypic spectrum of BCS appears similar, if not identical, in patients with mutations in either *ZNF469* or *PRDM5.* Heterozygous carries for a *PRDM5* mutation present with a clear intermediate phenotype. It remains to be investigated if the presence of *ZNF469* together with P*RDM5* mutations in the same individual is associated with a more severe phenotype. In our patient cohort consisting meanwhile of 20 families, we have identified distinctive syndromic features of BCS, including hypercompliant tympanic membranes, hip dysplasia, and severe hypermobility also of the large joints, thus being reminiscent of the hypermobility type of Ehlers-Danlos syndrome (MIM 130020). PRDM5 is a widely expressed transcription factor that modulates many aspects of tissue development and maintenance, such as cell fate and cell adhesion, via mechanisms that include Wnt signaling. Our study indicates that regulation of expression of extracellular matrix components, particularly fibrillar collagens, by PRDM5 and ZNF469 is a key molecular mechanism that controls normal corneal and other connective tissue development and maintenance and, if disturbed, underlies the generalized connective tissue disorder in BCS including the devastating corneal fragility.

1156T

Comparative study of mutation spectra in North-West Russia familial hypercholesterolemia: St. Petersburg versus Petrozavodsk. *A.S. Golovina[1,2], T.Y. Komarova[2].* 1) Biochemistry, SPbSU, Saint-Petersburg, Russian Federation; 2) Institute of Experimental Medicine of the NorthWest Branch of the Russian Academy of Medical Sciences (IEM NWB RAMS).

Introduction Familial hypercholesterolemia (FH) is a common monogenic disease leading to premature coronary and cerebral atherosclerosis. FH is caused by mutations in the low density lipoprotein receptor (LDLR) gene or in the APOB gene coding for the LDLR major ligand -apolipoprotein B-100. Aim of the present study was to compare spectra of mutations leading to FH development in patients from two major cities in North-West Russia: St. Petersburg and Petrozavodsk.

Methods

We used venous blood of 60 Petrozavodsk FH patients to extract DNA and different methods of molecular biology including PCR, SSCP-analysis, molecular cloning in Escherichia coli, and DNA sequencing. Data were compared with mutation spectra in 100 St.Petersburg FH patients.

Results

1. R3500Q APOB mutation that is frequent variant in European populations does not occur in FH patients from both cities. 2. None of FH patients from Petrozavodsk carried mutation FH-North Karelia in the LDLR gene, that is typical for the residents of eastern Finland but found only once in St. Petersburg. 3. C139G and G197del LDLR gene mutations that are recurrent in St. Petersburg FH do not occur in Petrozavodsk sample. 4. We have identified new mutation c.192del10/ins8 in two unrelated patients from Petrozavodsk. This mutation represents a complex rearrangement in the 5 `part of the LDLR gene third exon.

Conclusion

New mutation c.192del10/ins8 results in shift of the reading frame and creates a premature stop codon in the LDLR gene fourth exon. Therefore, this mutation is a likely cause of FH in both heterozygous patients.

1157T

Adams-Oliver syndrome, identification of a putative causal gene using exome sequencing. *S.J. Hassed[1], G.B. Wiley[2], S. Wang[2], J.Y. Lee[1], S. LI[1], Y. Xu[1], Z.J. Zhao[3], J.J. Mulvihill[1], J. Robertson[2], P.M. Gaffney[2].* 1) Dept Pediatrics/Gen, Univ Oklahoma Med Ctr, Oklahoma City, OK; 2) Oklahoma Medical Research Foundation, Oklahoma City, OK; 3) Dept. Pathology, Univ Oklahoma Med Ctr ,Oklahoma City, OK.

Adams-Oliver syndrome (AOS) is a rare genetic disorder characterized by vertex scalp defects resembling cutis aplasia and terminal limb malformations. Other features include congenital heart defects, agenesis of corpus callosum, capillary hemangioma, club foot, cryptorchidism, syndactyly, and cutis marmorata. All features are congenital, show a high degree of variability, and do not progress with age. The mode of inheritance is likely autosomal dominant. Pathogenesis remains unknown, but may involve abnormal vasculogenesis followed by vascular disruption that impairs tissue formation distally: the critical site appears to be the small vessels. Array comparative genomic hybridization was performed using the Roche/NimbleGen chip with normal results in 6/7 patients, suggesting that AOS is not the result of a microdeletion or microduplication. In an attempt to identify a susceptibility gene, the exomes of multiple members from two unrelated families with AOS were enriched and sequenced. Polymorphisms identified in each exome sequence were then screened to isolate functional mutations. Screening criteria include non-synonymous amino acid changes, absence from both dbSNP build 130 and the 1000 Genome Project pilot data, removal of mutations within segmental duplication regions, and the requirement that remaining variations be within regions annotated as Most Conserved Elements among vertebrates. Comparison of the sequence data both within and between the two families identified a single putatively causal gene harboring two mutations concordant with the disorder and with each mutation being unique to one family. Both mutations occur in the same highly conserved functional site despite being located in separate exons. Protein studies were undertaken to confirm the functional role of these mutations in the etiology of AOS.

1158T

Novel X-linked Disorder Identifies the Phenotype of a Germline Mutation in PIGA: The Gene Somatically Mutated in Paroxysmal Nocturnal Hemoglobinuria. *J.J. Johnston[1], A.L. Gropman[2], J. Martin[2], J.C. Sapp[1], J.K. Teer[1], R.A. Brodsky[3], L.G. Biesecker[1,4], NIH Intramural Sequencing Center.* 1) Genetic Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA; 2) Division of Neurology, Children's National Medical Center, Washington, DC, USA; 3) Division of Hematology, Department of Internal Medicine, Johns Hopkins University, Baltimore, MD, USA; 4) NIH Intramural Sequencing Center, National Human Genome Research Institute, Rockville, MD, USA.

Phosphatidylinositol glycan class A (PIGA, MIM 311770) is involved in the first step of glycosylphosphatidylinositol (GPI) biosynthesis. Many proteins are anchored to the cell by GPI and in the absence of GPI anchors, these proteins are degraded intracellularly. The loss of CD55 and CD59 on erythrocytes causes complement-mediated lysis in paroxysmal nocturnal hemoglobinuria (PNH), a disease that manifests after clonal expansion of hematopoietic cells with somatic PIGA mutations. While somatic PIGA mutations have been identified in many PNH patients, it has been proposed that germline mutations are lethal. We report a family with an X-linked lethal disorder with cleft palate, neonatal seizures, contractures, CNS structural malformations, and other anomalies. The family presented with three deceased male children and two obligate carrier females. Samples were unavailable for two of the three children. X chromosome inactivation studies in carrier females showed 100% skewing supporting X-linkage. A single sample from an obligate carrier female was included in an X exome next generation sequencing screen to identify the causative alteration for this phenotype. The sequencing yielded 18,509,569 reads. 52.1% of the reads could be uniquely aligned to the X exome target with an overall coverage of 107X. We found 1,271 substitutions and 89 insertions/deletions. We filtered variants for nonsynonymous, splice, frame-shift, and stop alleles as the mutation caused a lethal phenotype. Variants were also filtered for heterozygosity because the test subject was an unaffected carrier female. Variants present in eight control samples sequenced with the same methodology, dbSNP or the ClinSeq™ cohort (401 control individuals) were excluded. This left six variants, including a single nonsense mutation, c.1234C>T in PIGA, which predicts p.Arg412X. This variant segregated with affection/carrier status in the family. Frameshift, nonsense, or splice mutations in PIGA were not identified in over 400 controls. This mutation is very similar to mutations known to cause dysfunction of PIGA in PNH. The genetic data show that the c.1234C>T p.Arg412X variant is a severe change in the gene, is rare in the population, is present in an affected child, and is linked to the affected chromosome in this family. We conclude that this variant in PIGA caused the phenotype in this family, and this family presents the long-sought phenotype for PIGA germline mutations.

1159T

Insulin-like growth factor-1 polymorphism and cancer risk: a systematic review and meta-analysis. *M.S. Haerian[1], B.S. Haerian[2].* 1) Research Center for Gastroenterology and Liver Disease, Shaheed Beheshti University, M.C., Tehran, Iran; 2) Department Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

Objective: Insulin receptor substrate-1 (IRS-1) protein plays an important role in the IGF-1 signaling pathway. It is involved in development and progression of various cancers. In this study, we assessed the relationship between of the IRS-1 G972A gene polymorphism with the risk of breast, colorectal, and prostate cancers. Materials and Methods: We meta-analyzed the related studies that evaluated the role of G972A polymorphisms with cancer risk under alternative genetic models. Results: Meta-analysis of the pooled data showed no allelic and genotypic association of the G972A polymorphism with cancer risk. Sub-analysis by cancer type supported this result in each type of cancer. Conclusion: Our data suggested that the IRS-1 G972A polymorphism is not a risk factor for breast, colorectal, and prostate cancers.

1160T

Detection of High Levels of Urinary Apoptotic Nucleic Acids and Promoter Hyper-methylation of Multiple Tumour Suppressor Genes in Prostate Cancer. *A. Haj-Ahmad, M. Abdalla, Y. Haj-Ahmad.* Norgen Biotek Corp, Thorold, Ontario, Canada.

Prostate cancer is the most frequently occurring cancer and is the second highest cause of cancer mortality in males. Serum prostate specific antigen (PSA) is currently used as an indicator for the diagnosis and management of prostate cancer. Patients with a serum PSA level between 2.5 ng/mL and 10 ng/mL will often undergo prostate biopsies to confirm prostate cancer. However, <30% of these men will biopsy positive for cancer; meaning that the majority of men underwent an invasive biopsy with little benefit. A non-invasive biomarker, which can detect prostate cancer at an early stage, is therefore urgently needed. Urinary Apoptotic Nucleic Acids (uaNAs) were isolated from urine obtained from 3 groups of individuals (Prostate Cancer patients, Benign Prostatic Hyperplasia patients and healthy control individuals). By analyzing the levels of the uaNAs among the three groups we found that the uaNAs levels were significantly higher in the Prostate Cancer group when compared to the uaNAs levels observed in the BPH and control groups. Based on the aforementioned results, we decided to investigate the potential of using elevated levels of uaNAs for the early diagnosis of PCa. Toward this, we investigated whether the appearance of epigenetic changes that contribute to the development of cancer correlated positively with uaNA levels. This association was explored based on the following reasoning: during carcinogenesis, as the number of aberrant epigenetic mutations rises, cellular proliferation also rises which also means higher rates of apoptosis as cells compete for minimal resources; this shows up as elevated levels of apoptotic bodies in circulation and hence in urine. Therefore, the epigenetic silencing of known tumor suppressor genes was investigated with the purpose of correlating these aberrant events with the observed increase in uaNA levels in prostate cancer patients. Based on this hypothesis, we have established a correlation between the number of hyper-methylated tumour suppressor genes and the levels of uaNAs that together could be used for the early detection of PCa.

1161T

Genetic study of Tunisian familial hematological malignancies. *w.s. HAMADOU[1], R. ELABED[1], Y. BEN YOUSEF[3], A. KHELIF[3], H. SOBOL[2], Z. SOUA[1].* 1) U.R :biology of leukemias and lymphomas (03/UR/08-19) laboratory of Biochemistry, Faculty of Medicine, Sousse, Tunisia; 2) Department of Oncology Genetics, Prevention and Screening, IPC, Marseille, France; 3) Clinical Hematology Department, CHU F. Hached, Sousse, Tunisia.

Several genes associated with hematological malignancies have been described in the literature, but the genetic basis of familial aggregations remain poorly known. Among the genes that predispose to hematologic malignancies, there are ARTLS (regulation and expression of apoptotic proteins), PRF1 (lytic function and T lymphocytes immunosuveillance), CASP8 (apoptosis), CEBPA (control of cell proliferation and differentiation), FAS (cell necrosis and apoptosis). To determine the involvement of these genes, we conducted this preliminary study in searching of recurrent germline mutations in 8 Tunisian patients related in pairs by direct sequencing of the coding regions of five candidate genes: ARTLS1, PRF1, CASP8, CEBPA and FAS. Patients were dignosed for different types of hematologic malignancies: Hodgkin's disease (HD), non-Hodgkin lymphoma (NHL), myelodysplastic syndrome (MS), acute lymphoblastic leukemia (ALL) and chronic myelogenous leukemia (CML). This strategy has identified 4 mutations in the gene FAS including 3 silent mutations and missense mutation (c.365C> T p.Thr122Ile). For ARTLS1 gene, an heterozygous silent mutation (c.570C> T p.Arg190Arg) is found in 2 related patients and an homozygous, heterozygous missense mutation (c.442T> C p.Cys148Arg) in 5 patients. The CEBPA gene revealed a single silent mutation (c.690G> T p.Thr230Thr). For the gene PRF1 two silent mutations were identified. For the CASP8 gene a silent homozygous mutation was detected (c.1011G> A p.Lys337Lys) in 2 related patients and a homozygous missense mutation (c.904 C> G p.Asp302Glu) was detected in one patient. This study will be continued on other familial cases, while including other candidate genes for better understanding familial aggregations of hematologic malignancies and consists of reliable diagnostic tools to improve medical care.

1162T

Extending the Benign and Malignant Phenotypes Associated with Germline *DICER1* Mutations. *N. Hamel[1,2], M. Tischkowitz[1,3], A. Bahubeshi[1,3], B. Pasini[4], S. Asioli[5], G. Baynam[6], A. Overkov[7], R.P. Frieder[8], M. Dishop[9], N. Graf[10], M. Ekim[11], D. Bouron-Dal Soglio[12], J. Arseneau[13], R.H. Young[14], N. Sabbaghian[1,3], A. Srivastava[1,3], J.R. Priest[15], W.D. Foulkes[1,2,3].* 1) Program in Cancer Genetics, Departments of Oncology and Human Genetics, McGill University, Montreal, Canada; 2) The Research Institute, McGill University Health Center, Montreal, Canada; 3) Lady Davis Institute, Segal Cancer Center, Jewish General Hospital, Montreal, Canada; 4) Department of Genetics, Biology and Biochemistry, University of Turin, Italy; 5) Department of Biomedical Sciences and Human Oncology, University of Turin, Italy; 6) Genetic Services of Western Australia; 7) School of Paediatrics and Child Health, University of Western Australia, Australia; 8) Cancer Risk Assessment and Prevention, Santa Monica & Department of Obstetrics and Gynecology, David Geffen School of Medicine at UCLA, USA; 9) Department of Pathology, The Children's Hospital, Aurora, USA; 10) Department of Pathology, The Children's Hospital at Westmead, Sydney, Australia; 11) Department of Pediatric Nephrology, School of Medicine, Ankara University, Ankara, Turkey; 12) Department of Pathology, CHU Sainte Justine, Montreal, Canada; 13) Department of Pathology, McGill University, Montreal, Canada; 14) Department of Pathology, Massachusetts General Hospital, Harvard University, Boston, USA; 15) The International Pleuropulmonary Blastoma Registry, Children's Hospitals and Clinics of Minnesota, Minneapolis, USA.

DICER1 is crucial for embryogenesis and early development. Thus far, 40 different germ-line *DICER1* mutations have been reported in 42 probands world-wide. Although the range of attributable phenotypes continues to be described, germ-line mutations are definitely associated with pleuropulmonary blastoma, cystic nephroma, ovarian sex cord stromal tumors (especially Sertoli-Leydig cell tumor) and multi-nodular goiter. We report here *DICER1* mutations predicted to be deleterious in seven additional families who, in addition to the above phenotypes, also manifested uterine cervix embryonal rhabdomyosarcoma (cERMS, four cases), Wilms tumor (WT, three cases), cervical primitive neuroectodermal tumor (cPNET, one case), pulmonary sequestration (one case) and hamartomatous intestinal polyp (one case). Taken together, these findings show that cERMS, WT and likely PS, cPNET and hamartomatous intestinal polyps fall within the spectrum of *DICER1*-related diseases. Undifferentiated pleomorphic sarcomas may also rarely occur in mutation carriers. Moreover, *DICER1* appears to be the first gene implicated in the etiology of both cERMS and cPNET.

1163T

Direct Detection and Enumeration of Circulating Tumor Cells (CTC) by castPCR Analysis of Digitally Enriched Samples. *T. Hartshorne, D. Deng, S. Sproul, Y. Bao, Y. Fawn, D. Merrill, P. Brzoska, C. Chen.* Life Technologies, 850 Lincoln Centre Dr. Foster City, CA94404, USA.

The molecular characterization and enumeration and of circulating tumor cells (CTCs) promises to be valuable for cancer diagnosis, survival prognosis, and therapeutic guidance. However, current CTC analysis methods first require extensive enrichment of rare CTCs in human blood. We report here a new approach for direct detection of CTCs in whole blood samples using digital sample enrichment and highly sensitive competitive allele-specific TaqMan PCR (castPCR) to detect rare amounts of somatic mutations and quantitative reverse transcription PCR (qRT-PCR) to detect cell type-specific gene expression. Whole blood samples from lung cancer patients, or samples from normal individuals mixed with characterized lung cancer cell line cells, were partitioned in aliquots of 2.5-50 µL in 96- or 384-well plates, such that each well contained either one or no cancer cells in the presence of 2x104-4x105 normal white blood cells and 1x107-2x108 red blood cells. The sample partition process resulted in a 20-400x digital enrichment of the relative ratio of CTC to normal cells in CTC-positive wells. castPCR clearly identified somatic mutations and CK19 tumor cell biomarker expression in whole blood samples containing small numbers of spiked-in cancer cells, but not in normal whole blood samples. Furthermore, the CK19 marker and KRAS mutation were identified together in the same sample wells, indicating that the identified mutation was specifically derived from the cancer cells. In blood samples from two lung cancer patients, all CK19-positive CTC-containing wells were also positive for the EGFR p.L858R mutation. Approximately 1/3 of the circulating lung tumor cells in a patient with the EGFR p.L858R mutation also were positive for the EGFR p.T790M mutation, which is an inducible drug-resistant CTC marker. All sample wells that were negative for EGFR mutations were shown to contain corresponding wild type allele sequences. In conclusion, our data suggest that the combination of digital sample enrichment and castPCR plus RT-qPCR technologies could be used to directly detect cancer mutations and enumerate CTCs in whole blood without prior biophysical sample enrichment. This new approach may pave the way for noninvasive CTC monitoring and individualized therapy.

1164T

Investigating the function of microseminoprotein-beta in prostate cancer. *J.E. Hayes[1,2], X. Xu[1,3], H. Lilja[4,5], R.J. Klein[1].* 1) Cancer Biology and Genetics , Memorial Sloan-Kettering Cancer Center, New York, NY 10065 USA; 2) BCMB Program, Weill Cornell Graduate School of Medical Sciences, New York, NY 10065, USA; 3) Immunology & Microbial Pathogenesis Program, Weill Cornell Graduate School of Medical Sciences, New York, NY 10065, USA; 4) Department of Clinical Laboratories, Surgery (Urology) and Medicine (GU-Oncology), Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA; 5) Department of Laboratory Medicine, Lund University, Skåne University Hospital, 205 02 Malmö, Sweden.

Background: Multiple genome-wide association studies on prostate cancer (PrCa) have identified a single nucleotide polymorphism, rs10993994, that is associated with inherited PrCa risk. This SNP is of particular interest as it lies in the proximal promoter region of microseminoprotein-beta (*MSMB*), a gene that encodes for a major secreted protein of the prostate () -MSP) which was previously suggested to have an anti-proliferative effect on PrCa cells. Previous work from our lab suggests that rs10993994 may be a functional SNP and play a role in determining physiological levels of) -MSP in the serum and semen of healthy, young men. As of yet, the physiological function of) -MSP remains unknown.

Objective: We hypothesize that) -MSP acts to hinder the growth of PrCa cells, acting as a putative tumor suppressor. Therefore, it is our objective to elucidate the function of) -MSP and to determine the mechanism by which it acts.

Results: We have performed a series of *in vitro* and cell-based experiments to determine the physiological function *MSMB*. Adding exogenous) -MSP to PrCa cell lines reproducibly decreased cell viability over time course studies. Though a previous study visually observed apoptosis upon the addition of exogenous) -MSP, we did not observe any caspase 3/7 activity in our assays. We then followed up to determine if exogenous) -MSP acted to inhibit cellular proliferation and/or alter the cell-cycle. Finally, initial studies provide evidence that the function of) -MSP is conserved between mouse and humans, despite being evolutionarily divergent.

Conclusions: Our initial results further support the anti-proliferative effects that) -MSP has on PrCa cells, though the exact mechanism remains elusive.

1165T

Whole-exome sequencing of a rare case of familial childhood acute lymphoblastic leukemia. *J. Healy[1], V. Saillour[1], M. Bourgey[1], A. Alter[1], M. Larivière[1], J.F. Spinella[1], C. Richer[1], S. Busche[2,3], B. Ge[3], A. Montpetit[3], P. Awadalla[1,4], T. Pastinen[2,3], D. Sinnett[1,4].* 1) Sainte-Justine UHC Research Center, University of Montreal, Montreal, Qc, Canada; 2) Department of Human Genetics, McGill University, Montreal, QC, Canada; 3) McGill University and Genome Quebec Innovation Centre, Montreal, Qc, Canada; 4) Department of Pediatrics, Faculty of Medicine, University of Montreal, Montreal, Qc, Canada.

Acute lymphoblastic leukemia (ALL) is the most common cancer in children, accounting for approximately 25% of all pediatric cancer cases. However familial childhood ALL is extremely rare. Few families with multiple non-twinned siblings diagnosed with childhood ALL have been reported, and to date no highly penetrant leukemia susceptibility gene(s) has been identified to explain this uncommon occurrence. We postulated that pure (nonsyndromic) familial childhood ALL could result from the accumulation of disadvantaging rare DNA variants in predisposing genes or biological pathways. To address this hypothesis, we used next-generation sequencing technologies to capture and re-sequence the whole-exomes of a family comprising the mother, father and two male non-twinned affected siblings (sibling A and sibling B). Both brothers were diagnosed with the identical ALL subtype, pre-B hyperdiploid childhood ALL, three years apart. The similar clinical and molecular characteristics of the siblings suggest shared etiologic factors. Using the Agilent SureSelect All Human Exon Kit and the SOLiD 3 Plus system, we captured and sequenced a total of 20.7 Gb of exonic sequence for the entire family, with a mean coverage of 47X. For each individual, approximately 96% or 36.4 Mb of the targeted bases were covered⁄ 1X and 68%; of the targeted bases or 25.7 Mb passed our thresholds for variant calling. We identified 48,688 positions at which the called allele(s) differed from the reference genome in at least one of the four family members. In total, we identified 20,260 germline variants in sibling A and 30,752 in sibling B, of which 347 (1.7%) and 477 (1.6%), respectively, were rare (MAF<5%) in dbSNP. We investigated non-synonymous homozygous variant and compound heterozygous positions shared between the siblings. Based on several criteria (PolyPhen annotation, known allele frequency, etc.), we identified variants that are strong functional candidates to explain this case of pure familial childhood ALL. In parallel, high-density genotyping was also performed (Illumina Omni 2.5M) for quality control and structural variant detection. We identified shared copy number variants among the brothers that could also be involved in leukemogenesis. Although these genetic and structural variants require independent validation and functional assessment, this is the first study to identify genetic factors involved in pure familial childhood ALL.

1166T

Lobular endocervical glandular hyperplasia found in a Peutz-Jeghers syndrome patient positive for STK11 mutation. *A. HIRASAWA[1,2], T. AKAHANE[1], T. TSURUTA[1], K. BANNO[1], N. SUSUMU[1], K. SUGANO[2], D. AOKI[1].* 1) Dept Gyne/Obst, Sch Med, Keio Univ, Tokyo, Japan; 2) Genetic Clin, Keio Univ Hosp, Tokyo, Japan.

Peutz-Jeghers syndrome (PJS) has been frequently associated with adenoma malignum, a subtype of cervical adenocarcinoma. Lobular endocervical glandular hyperplasia (LEGH) has recently been proposed to be a possible precursor of adenoma malignum. It has been reported that many cases previously diagnosed as adenoma malignum now would be diagnosed as LEGH. However, only a few reports have focused on a possible link between LEGH and glandular tumor of the uterine cervix associated with PJS. Herein, we report a case of LEGH found in a PJS patient positive for STK11 mutation. The patient was a 42-year-old woman with a diagnosis of PJS during childhood. She noticed her 7-year-old son to have similar pigmentation of the lips, the oral cavity, and the fingers to hers, raising her concern that the son might have PJS like her. She brought him to our outpatient genetic counseling clinic. She had ever undergone a total of 6 laparotomies for repeated intussusception due to small-intestinal polyps since her childhood, but had never consulted detailed genetic counseling or examination. After obtaining her informed consent, mutation analysis of STK11 gene was performed in the present mother, and deletion was identified in the exon. We gave explanation during genetic counseling that the mother should be screened for cervical lesions for surveillance purposes, and thus, she visited our gynecological department. At her first visit, gynecological examination revealed excessive watery vaginal discharge and polycystic mass of the uterine cervix. Then, the patient received diagnostic laser conization of the cervix, and LEGH was pathologically diagnosed. However, considering that the presence of adenocarcinoma could not be ruled out and that she did not require fertility preservation, the patient underwent modified radical hysterectomy and bilateral salpingo-oophorectomy. Pathological examination of the surgical specimen revealed the presence of LEGH, but there was no evidence of adenoma malignum. In women with PJS, not only periodic observation of the intestinal tract but also surveillance of the uterine cervix are essential, keeping in mind the presence of cervical LEGH and adenoma malignum.

1167T

Differential methylation in pancreatic cancer and the associated effects on gene expression. *J. Hoskins[1], J. Jinping[1], H. Parikh[1], I. Collins[1], S. Hussain[2], L. Amundadottir[1].* 1) Laboratory of Translational Genomics, National Cancer Institute, Gaithersburg, MD; 2) Laboratory of Human Carcinogenesis , Center for Cancer Research, National Cancer Institute, Bethesda, MD.

Pancreatic cancer ranks among the most deadly cancers, with a mortality rate nearly equal to its rate of incidence. Previous works have reported significantly different DNA methylation profiles between tumor and normal pancreatic samples across the genome, suggesting epigenetic effects on gene expression. However, only one other group thus far has compared methylation changes with transcriptome-wide changes in gene expression. In this study we have attempted to expand on this work by using a customized NimbleGen 720K CpG Island Plus RefSeq Promoter Array in methyl-DNA immunoprecipitation (MeDIP) analysis of 10 pancreatic cell lines (1 normal and 9 tumor) and 10 normal/tumor paired tissue samples. By using a Bonferroni-corrected "Probe Sliding Window" ANOVA analysis, we found over 9000 peaks of significant ($p<0.01$) differential methylation between tumor and normal pancreatic samples, of which 7114 were associated with nearly as many genes. Previous studies have reported less than 2000 differentially methylated genes in pancreatic tumor samples. Of these gene regions, 3503 were hypermethylated, and 3611 were hypomethylated in tumor samples. In order to associate these methylation differences to changes in gene expression, we performed RNA sequencing on 7 of the pancreatic cell lines (1 normal and 6 tumor). There was good correlation between hypermethylation and down-regulation of gene expression. However, hypomethylation was a much poorer predictor of increased transcription. Several hypermethylated and down-regulated genes from this analysis are in agreement with another recent study, but many associations identified are novel. Further analysis and validation of this data should yield plenty of new information on DNA methylation changes in pancreatic cancer, and the effects of these changes on gene expression.

1168T

CNVs AND CN-LOH IN MDS AND AML: A STUDY OF 140 CASES. *X. Hu[1], A. Iqbal[2], A. Ahmd[2], G. Raca[3], X. Xu[3], D. WolFF[4], R. Burack[2], B. Kipphut[2], D. Mulford[2], M. Li[1].* 1) Cancer Genetics Lab, Baylor College of Medicien, Houston, TX; 2) Dept of Pathology and Laboratory Medicine, Univ of Rochester Med Ctr, Rochester, NY; 3) Univ of Wisconsin-Madison, WI; 4) Dept Pathology and Laboratory Medicine, Med Univ of South Carolina, Charleston, SC.

Myelodysplastic syndromes (MDS) are a group of heterogeneous myeloid neoplasms with high risk of progression to acute myeloid leukemia (AML). The molecular pathogenic mechanisms that underlie the transformation of MDS to AML are largely unknown. About 50% of MDS patients show no cytogenetic abnormalities, making monitoring disease progress in these patients difficult. We hypothesize that some cryptic genomic copy number variations (CNVs) and regions with copy number neutral loss of heterozygosity (CN-LOH) may be or harbor genetic events responsible for the disease transformation. We studied 140 patients with newly diagnosed MDS (n=80) or AML (n=60) to evaluate genomic alterations using a custom designed cancer specific CGH microarray that targets over 500 cancer genes and more than 100 cancer-associated genomic regions. Thirty six MDS cases were studied for CN-LOH using a SNP array or a combined CGH/SNP array platform that detects both CNVs and CN-LOH. CNVs were identified in all patients including 66 patients with normal cytogenetic results. The CNVs were enriched in genomic regions containing cancer genes TP73, CSF1R, NOTCH1, AKT1, BLM, and BUB1B in both MDS and AML. However, heterozygous deletion of MSH2 and CDKN1B were more commonly seen in patients with MDS; and heterozygous deletion of CNBP, ABL1, and PTEN were more prominent in patients with AML. Twenty-five of the 36 MDS cases showed CN-LOH of⁄ 10Mb in 63 genomic regions. Although CN-LOH was distributed throughout the whole genome, 1p and 14q were more frequently involved. These results demonstrate the utility of arrays that detect both CNVs and CN-LOH in cancer analysis. Our study revealed many previously unrecognized CNVs and CN-LOH in patients with MDS and AML, which may play important roles in the transformation of MDS to AML and be or contain biomarkers for cancer diagnosis and/or appropriate targeted therapies.

1169T

Combined Analysis with Copy Number Variation Identifies Hotspot-located Risk Loci in Lung Cancer. *L. Hu, X. Chen, X. Li, Y. Liu, Z. Zhang, P. Wang, X. Yi, J. Zhang, Y. Zhu, Z. Wei, F. Yuan, X. Kong.* Molecular genetics, Institute of Health Sciences, Shanghai, Shanghai, China.

Lung cancer is the most important cause of world-wide cancer death, and its occurrence is susceptible to inheritance and environment. However, the mechanisms mediating genetic factors and environment are far from fully understood. Copy number variations (CNVs) are prevailing genetic variations in the genome, and current studies have proved that CNVs will be a promising target affected by tumor and cancer. To further test this hypothesis, we systematically analyzed the CNV-association in two datasets of lung cancer, which were about 2000 cases (and 2000 controls) from Environment And Genetics in Lung cancer Etiology (EAGLE) and 850 cases (and 850 controls) from the Prostate, Lung, Colon and Ovary Study Cancer Screening Trial(PLCO). We firstly used a prefound association method to test the datasets separately, and then tested the association-accordance through CNV-type distribution comparison between the both datasets. Finally, we identified seven CNV-loci associated with lung cancer. Three of these loci could be validated through previous physiological studies or association publications about cancer. Interestingly, our further analysis showed that shared associated CNVs are linked with recombination hot-spot while significant CNVs in separate testing are far from hot-spots, which implicated that some hot-spots are harmful to lung cancer in different populations. We searched the physiological function of the separate significant CNVs, and found EGFR-signaling related genes (and other genes) around these CNVs were reported with cancer or lung cancer, which proved that critical genes might be targeted in lung cancer by rare CNV-mutations. In a word, our analysis provided more new cues in cancer studies, especially prompted the affected mode of CNV with different origins.

1170T

Genetic variants in sex hormone metabolic pathway genes and risk of esophageal squamous cell carcinoma. *P.L. Hyland[1,2], N. Hu[1], W. Wheeler[3], K. Yu[4], T. Ding[5], J-H. Fan[6], S.M. Dawsey[7], C.C. Abnet[7], A.M. Goldstein[1], Z-Z. Tang[5], Y-L. Qiao[5], N.D. Freedman[7], P.R. Taylor[1].* 1) GEB, DCEG, NCI, NIH, Rockville, MD; 2) CPFP, CCT, NCI, NIH, Rockville, MD; 3) IMS, Inc., Silver Spring, MD; 4) BB, DCEG, NCI, NIH, Rockville, MD; 5) Shanxi Cancer Hospital,Taiyuan, People's Republic (PR) China; 6) Dept of Epidemiology, Cancer Institute (Hospital), Chinese Academy of Medical Sciences, Beijing, PR China; 7) NEB, DCEG, NCI, NIH, Rockville, MD.

Esophageal cancer (EC) is the sixth leading cause of cancer-related deaths worldwide and exhibits a dramatic geographic distribution in incidence and histological subtype. In China, EC is the fourth leading cause of cancer death, where essentially all cases are histologically esophageal squamous cell carcinoma (ESCC) as opposed to adenocarcinoma which is more common in the Western world. Recently, it has been suggested that sex hormones may be associated with the risk of ESCC. Therefore, we examined the association between genetic variants in sex hormone metabolic genes and ESCC risk in a high risk population from north central China. We also investigated jointly the association between risk of ESCC and multiple genetic variants within an overall sex hormone metabolic pathway (and/or subpathways) defined according to current biological knowledge. A total of 1026 ESCC cases and 1452 controls were genotyped for 1250 tagged single nucleotide polymorphisms (SNPs) in 51 sex hormone metabolizing and sex hormone-related genes such as transporters and signaling receptors (collectively referred to here as sex hormone metabolic genes). Pathway-, subpathway-, gene- and SNP-based associations with ESCC risk were evaluated using the adaptive rank-truncated product (ARTP) method and the additive model within unconditional logistic regression adjusted for age and sex. We also conducted stratified analyses by gender. Statistical significance was determined through use of permutation. No associations were observed for genes involved in the overall sex hormone metabolic pathway (P=0.14) or in the subpathways (androgen synthesis: P=0.30; estrogen synthesis: P=0.15; and estrogen removal: P=0.19) with risk of ESCC. However, six genes (including *SULT2B1*, *CYP1B1*, *CYP3A5*, *CYP3A7*, *SHBG* and *CYP11A1*) were significantly associated with ESCC risk (P<0.05). Our comprehensive examination of 51 genes in the sex hormone metabolic pathway is consistent with a potential association with risk of ESCC, and the identified six genes in particular merit confirmation in future studies.

1171T

Frequencies of common splice variants of prognostically important fusion oncogenes in Pakistani Leukemia patients: Possible implication in Leukemia biology, differential diagnosis, prognosis and treatment.

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Introduction: Leukemia is a heterogeneous disease involving many fusion oncogenes(1), which are resulted due to genetic abnormalities like translocations, deletions, are helpful in differential diagnosis and prognostic stratification and are directly involved in Leukemogenesis(2).As environmental factors coupled with natural genetic variations drive the genetic abnormalities and hence the fusion oncogenes, frequencies of fusion oncogenes and their splice variants can vary in different geographical regions. Methods: Bone marrow samples were collected from 501 Leukemia patients. RNA was extracted by TriZol method(3).RT-PCR was used to study the different leukemic fusion oncogenes(3). Interphase-FISH was employed to confirm the results of RT-PCR(4). Frequencies of fusion oncogenes and their splice variants were compared to western populations using SPSS version 17. Results: Significant differences between Pakistani and western populations with respect to frequencies of BCR-ABL splice variants b2a2 & b3a3 in CML, SIL-TAL1(del 1),TEL-AML1 (t 12;21) oncogenes in adult ALL and BCR-ABL (t 22;9),E2A-PBX1 (t 1;19), TEL-AML1 (t 12;21), MLL-AF4 (t 4;11) in paediatric ALL (p=0.01). This shows that cumulative frequencies of fusion oncogenes related with poor prognosis (BCR-ABL (t 22;9),SIL-TAL1 (del 1), E2A-PBX1(t 1;19), MLL-AF4 (t 4;11) are higher in Pakistani pediatric ALL patients than western counterparts(74.2% vs 27%, p=0.01) while reverse is the case of TEL-AML (16.5% vs 25%, p=0.01) associated with good prognosis(Table 1). Conclusions/Discussion: Significant difference between Pakistani and western populations in terms of splice variants of different fusion oncogene frequencies indicate ethnic differences in genetics of leukemia, in accordance with previous reports (5,6). These results explain the molecular genetic basis of poor prognosis and survival of paediatric ALL and adult AML patients in Pakistan(7). Our studies have implications in clinical management of Leukemia at healthcare policy-making bodies and clinical centers.References:1:Pallisgaard et al. Blood 1998;92(2):574-88.2:Vardiman,et al. Blood. 2009, 114(5):937-51.3:Van-dongen et al, Leukemia (1999) 13, 1901-1928.4: Kajtar et al., Cytometry 2006,69A:506-514.5:Daniel-Cravioto et al., Leukemia & Lymphoma 2009, 50(8):1352-1360.6:Siraj et al.,Leukemia 2003;17:1192-3.7:Zaki et al.,2002. J. Pak Med. Assoc., 52 (6):247-9.

1172T

NQO1 expression correlates inversely with NF2B activation in human breast cancer.

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Introduction NQO1 participates in cellular defense against oxidative stress and regulates apoptosis via p53- and NF-kappaB-mediated pathways. We have previously found that homozygous missense variant NQO1*2(rs1800566) predicts poor survival among breast cancer patients, particularly after anthracycline-based adjuvant chemotherapy. Here we investigated NQO1 and NF2B protein expression and global gene expression profiles in breast tumors with correlation to tumor characteristics and survival after adjuvant chemotherapy. **Methods** We used immunohistochemical analysis of tissue microarrays to study NQO1 and NF2B expression in two series of tumors: 1000 breast tumors unselected for treatment and 113 from a clinical trial comparing chemothepapy regimens after anthracycline treatment in advanced breast cancer. We used gene expression arrays to define genes co-expressed with NQO1 and NF2B. **Results** NQO1 and nuclear NF2B were expressed in 83% and 11% of breast tumors, and correlated inversely (p=0.012). NQO1 protein expression was associated with estrogen receptor (ER) expression (p=0.011) whereas 34.5% of NF2B-nuclear/activated tumors were ER negative (p=0.001). NQO1 protein expression and NF2B activation showed only trends, but no statistical significance for patient survival or outcome after anthracycline treatment. Gene expression analysis highlighted 193 genes that significantly correlated with both NQO1 and NF2B in opposite directions, consistent with the expression patterns of the two proteins. Inverse correlation was found with genes related to oxidation/reduction, lipid biosynthesis and steroid metabolism, immune response, lymphocyte activation, Jak-STAT signaling and apoptosis. **Conclusion** The inverse relationship between NQO1 protein expression and NF2B activation, underlined also by inverse patterns of association with ER and gene expression profiles of tumors, suggests that NQO1 - NF2B interaction in breast cancer is different from several other tissue types, possibly due to estrogen receptor signaling in breast cancer. Neither NQO1 nor NF2B protein expression appear as significant prognostic or predictive markers in breast cancer.

1173T

Comprehensive characterization of BRCA1/2 mutational spectrum in breast/ovarian cancer patients from Lithuania. *R. Janavicius[1, 2], V. Rudaitis[3], L. Griskevicius[1].* 1) Hematology and oncology center, Vilnius University Hospital Santariskiu clinics, Vilnius, Lithuania; 2) State Research institute Innovative Medicine Center; 3) Dept. of Gynecology, Vilnius University Hospital Santariskiu clinics, Vilnius, Lithuania.

BACKGROUND. The germline mutations in BRCA1/2 genes are the most significant and well characterized genetic risk factors for breast and/or ovarian cancer. Detection of mutations in these genes is an effective method of cancer prevention and early detection. Different ethnic and geographical regions may have different BRCA1 and BRCA2 mutation spectrum and prevalence due to founder effect. The population of Lithuania has over several centuries undergone limited mixing with surrounding populations and is mostly of indigenous Baltic origin. The aim of our study was to asses full BRCA1/2 mutational spectrum in Lithuanian population. METHODS. We performed comprehensive mutation analysis of BRCA1/2 genes in 380 unrelated breast and/or ovarian cancer patients (with/without family history) and predictive unaffected patients (with family history) using high resolution melting (HRM) screening (on Light Cycler 480/Light Scanner 384) followed by direct sequencing (ABI 3500) and MLPA for large genomic rearrangements (LGR). RESULTS. Overall, we have identified 19 different mutations (12 in BRCA1 and 7 in BRCA2 genes). 5 frequent pathogenious mutations in BRCA1 gene (c. 4035delA, c. 5266dupC, c.181T>G, c.1687C>T and c. 5258G>C) comprised 48%, 28%, 6%, 3% and 3% respectively of all BRCA1 gene mutations; a single BRCA2 mutation (c.658delGT) comprised 43% of all mutations in this gene. Two novel BRCA1 (c.4516delG, c.2481delA) and 3 novel BRCA2 genes mutations (c.6999insT, c.5697_5710del14, c.6410delA) were identified; no LGRs were found. The most common c.4035delA appears to be true Lithuanian (Baltic) founder mutation and haplotype genotyping was initiated. CONCLUSIONS. Characterization of BRCA1/2 mutational spectrum in Lithuania enabled to develop screening protocol for 6 common BRCA1/2 mutations, which are prevalent in 88% of all mutations. This knowledge will provide more efficient approach for the individualization of genetic testing affordable for all breast/ovarian patients and their relatives, which will be of utmost importance in the era of targeted therapy.

1174T

Linkage study suggests common genetic determinants for chronic lymphocytic leukemia and monoclonal B-cell lymphocytosis. *T.M. Jarvinen[1], S. Liyanarachchi[1,2], I. Comeras[1,3], L. Senter[1,3], G. Lozanski[4], E. Hertlein[5], J.C. Byrd[5], A. de la Chapelle[1,2].* 1) Human Cancer Genetics Program, Ohio State University, Columbus, OH, USA; 2) Department of Molecular Virology, Immunology and Medical Genetics, School of Biomedical Science, Ohio State University, Columbus, OH, USA; 3) Department of Internal Medicine, Ohio State University Comprehensive Cancer Center, Columbus, OH, USA; 4) Department of Pathology, Ohio State University Medical Center, Columbus, OH, USA; 5) Department of Internal Medicine, Division of Hematology, Ohio State University Comprehensive Cancer Center, Columbus, OH, USA.

Chronic lymphocytic leukemia (CLL) characterized by an accumulation of mature B-cells is the most common adult leukemia in the Western world. Monoclonal B-cell lymphocytosis (MBL), a suggested precursor lesion of CLL, is an indolent hematologic condition with low levels of circulating B-cell clones (<5000/mm^3). Advancing age and male sex are known risk factors, but genetic determinants are unknown. Previous linkage studies conducted in CLL have identified multiple putative disease loci, but no predisposing gene has been identified to date. We conducted a genome-wide linkage scan in a multi-generational family. Altogether 17 individuals were genotyped: 3 with CLL, 6 with MBL and 8 unaffected. Greater than 250000 SNPs were typed using the Illumina Human CytoSNP-12 platform. The number of SNPs analyzed was reduced to 5000 by selecting the most highly heterozygous marker in every 500 kb segment. The overall genotyping success rate was 97% and Mendelian inheritance errors were negligible (0.03%). Linkage analysis was performed with Merlin software by applying both nonparametric and parametric approaches, using either a dominant or recessive model. Considering the 3 CLL cases as affected and 6 MBL cases as unaffected, a region in chromosome 15q gave a peak with nonparametric linkage (NPL) score of 0.48 (the maximum predicted attainable score being 0.58). Considering the 6 MBL cases as affected and the 3 CLL cases as unknown, the same region in chromosome 15q gave a peak NPL score of 0.54 (maximum predicted 0.85). Considering the CLL and MBL cases as affected, the genome-wide maximum NPL score of 0.86 (maximum predicted 1.36) was obtained in the same region in chromosome 15q. The other strongest peaks when considering CLL as affected were observed in chromosomes 3q and 20pq. To our knowledge, this is the first linkage study to address genetic determinants for MBL. However, the study was confined to a single pedigree. None of the observed linkage peaks in CLL or MBL coincided with previously identified candidate loci nor did they reach statistical significance in terms of standard thresholds. To date, a single association study has directly assessed the inherited genetic susceptibility to MBL showing that genetic predisposition is at least partly shared between CLL and MBL. In line with this, our results suggest that CLL and MBL share genomic regions conferring susceptibility to both phenotypes. Predisposing genes in these regions remain to be identified.

1175T

Genome-wide association analysis of lymphoma. *V. Joseph[1], T. Kirchhoff[1], J. Brown[2], D.B. Yehuda[3], A. Dutra-Clarke[1], N. Hansen[1], J. Przybylo[1], V. Devlin[1], A. Viale[1], R. Klein[1], A. Zelenetz[1], K. Offit[1].* 1) Dept. Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY; 2) Dana Farber Cancer Center, Boston, MA,; 3) Hadasaah University, Jerusalem, Israel.

Background: The incidence of lymphoma has doubled over the past two decades in the U.S., the etiology of the most common types of the disease remains unknown. There is increasing evidence that genetic predisposition has a significant impact on the development of lymphomas. Genome-wide association analysis (GWAS), have proven to be useful tool for the identification of inherited susceptibility loci for human cancers. Objective: Discovery of novel germ line variants predisposing to lymphoma and lymphoma subtypes using GWAS. Methods: We have carried out a genome wide association study to discover genetic loci conferring risk for development of lymphomas using Affymetrix 6.0 SNP arrays on 959 kindreds of lymphoma. These consisted of 204 Hodgkins disease and 752 Non-Hodgkins disease cases. Amongst the NHL subtypes, we had 37% follicular, 36% diffuse large B-cell, 9% CLL/SLL, 9% mantle and 3% marginal zone lymphomas. As controls, we have used publically available data acquired from dbGAP initiative on 4059 individuals genotyped by Affymetrix 6.0 as part of schizophrenia and diabetes studies. To reduce population heterogeneity, both cases and controls chosen were only of European (EU) ancestry. Results: We performed logistic regression of each lymphoma subtype using factors such as age and four principal components of the population variation of samples on 5018 sample. After QC and QA test, 597831 SNPs were passed for analysis. In the pan-lymphoma analysis, we have discovered a SNP with in a gene that was recently reported as member of DNA repair pathway(p=5.48E-08). In the follicular subtype, we have confirmed the HLA 6q region that harbor the HLA-DQ1 locus (p=1.0E-08; rs2621416) as an important lymphoma susceptibility locus. Genomic inflation 3 was 1.05. We also discovered novel loci on chr11 upstream of ZBTB16, a zinc finger transcription factor implicated previously in leukemia. Our data also suggests the first evidence for the role of transcription factor KLF12 in Hodgkins lymphoma. We are currently performing a replication stage on 1250 lymphoma cases and 1900 controls, all of EU ancestry to confirm these early findings. Conclusion: There is preliminary evidence of germline variants in humans increasing risk for lymphocytic malignancies. Our findings provide new insights into the molecular pathogenesis of lymphoma.Acknowledgement:Lymphoma Foundation and Barbara Lipman Research Fund.

1176T

Elevated Levels of Oxdatively Damaged DNA in Patients with Selenoprotein Deficiency. *M. Karbaschi[1], E. Schoenmakers[2], M.D. Evans[1], K. Chatterjee[2], M.S. Cooke[1].* 1) University of Leicester, Leicester, United Kingdom; 2) Institute of Metabolic Science, Cambridge, United Kingdom.

Selenium (Se) is a vital dietary element for eukaryote and prokaryotic cells. In humans, its biological role is mediated principally by incorporation of selenocysteine, into selenium containing proteins. About 30 different selenoproteins have so far been observed in human cells and tissues, performing a variety of different functions including removal of cellular reactive oxygen species, reduction of oxidised methionines in proteins, metabolism of thyroid hormones, transport and delivery of selenium to peripheral tissues, protein folding and ER stress, plus proteins whose precise function is unknown [1].One of the best-known functions of selenoproteins is displayed by the family of selenium-dependent glutathione peroxidases which reduces hydrogen peroxide, and damaging lipid and phospholipid hydroperoxides to harmless products such as water and alcohols [2]. This function helps to reduce the risk of induction of further oxdatively generated damage to biomolecules and accordingly reduces risk of a variety of damage-related pathological conditions. In this study, we examined the baseline levels of oxdatively damaged DNA in two individuals, one adult and one child with compound heterozygous defects in the selenocysteine insertion sequence-binding protein 2 (SECISBP2) gene. As part of a complex phenotype, using hOGG1-modified comet assay, we noted a significant (P<0.0001) increased baseline levels of 8-oxoguanine and alkali labile sites in dermal fibroblasts, vs. age/gender match controls in the presence and absence of exogenous H2O2 (50 μM, 30 min). Using the same assay, we demonstrated that whilst DNA repair processes were unaffected, antioxidant defences were impaired, the likely source of the elevated baseline levels. Similar results were noted following exposure to 10 J/cm2 UVA (P<0.0001), accounting for the pronounced photosensitivity seen in these subjects. These results demonstrate the central role of selenoprotein in cellular antioxidant defence.

1177T

Genes in the inflammatory and innate immunity pathway and prostate cancer. *R. Kazma[1], J.A. Mefford[1], I. Cheng[2], S.J. Plummer[3], B.A. Rybicki[4], G. Casey[3], J.S. Witte[1].* 1) Department of Epidemiology and Biostatistics and Institute for Human Genetics, University of California San Francisco, San Francisco, CA; 2) Epidemiology Program, Cancer Research Center of Hawai'i, University of Hawai'i, Honolulu, HI; 3) Department of Preventive Medicine, Noris Comprehensive Cancer Center Keck School of Medicine, University of Southern California, Los Angeles, CA; 4) Department of Biostatistics and Research Epidemiology, Henry Ford Hospital, Detroit, MI.

Prostate cancer is the leading cancer in terms of incidence and second leading cancer in terms of mortality among men in developed countries. Several observations support the hypothesis that the inflammatory and innate immunity responses might play a role in prostate cancer initiation and progression. To determine whether genes coding for proteins involved with inflammatory and innate immune responses are associated to an increase in risk or to a higher rate of progression of prostate cancer, we undertook a comprehensive analysis of 318 single nucleotide polymorphisms (SNPs) in 46 candidate genes controlling inflammation and innate immunity. Our sample comprised 878 aggressive prostate cancer cases and 536 controls recruited from the major health care providers in Cleveland Ohio and the Henry Ford Health System in Detroit. First, we investigated the potential associations with single marker tests, gene-gene and gene-environment interaction tests. Non-genetic factors considered in gene-environment interaction tests included smoking, family history of prostate cancer, body mass index, non-steroidal anti-inflamatory drugs, omega-3 fatty acids, trans-fatty acids, red meat, and a high glycemic index diet. Second, we used a kernel-based logistic regression to study sets of SNPs within each gene and across pathways. The kernel-based logistic regression corrects for linkage disequilibrium patterns and can account for SNP-SNP interactions within each SNP set. Finally, we evaluated association with prostate cancer progression using a Cox proportional hazards model for cases only. All analyses were adjusted for age, ethnicity, genetic ancestry, institution, and initial PSA level.

1178T

COX-2 gene polymorphisms (rs20417 and rs5277) and risk of Colorectal cancer. *F. Khorshidi, M. Mohebbi, M. Montazer Haghighi, M. Yaghoob Taleghani, B. Damavand, M. Vahedi, M.R. Zali.* Shahid beheshti University, The research center of gastroenterology and liver, Tehran, Tehran, Iran.

Background: The incidence of colorectal cancer (CRC) is lower in Iran than in Western countries, being the fifth and third most common cancer in men and women. However, its incidence in Iran has increased recently. Evidence derived from several lines of investigation suggest that prostaglandins, metabolites of arachidonic acid, play an important role in colon cancer development.Cyclooxygenase-2 (PTGS2) overexpression has been implicated in various cancers. Aim: To determine the possible modulating effect of the COX-2 polymorphisms, rs20417 and rs5277, on the risk of colorectal cancer (CRC) in a Iranian population. Methods: This case-control study includes 100 patients with CRC and 100 control subjects with no polyps who underwent total colonoscopy. Genotypes of the COX-2 polymorphisms (rs20417 and rs5277) were determined by polymerase chain reaction-based restriction fragment length polymorphism. COX-2 genotypes were analyzed and odds ratios with 95% confidence intervals were estimated by logistic regression. Result: A significant difference was observed in the distribution of the COX-2 polymorphism -765G→C (rs20417) between cases and controls (P=0.014). The -765GC genotype was more prevalent in this study in both groups. Furthermore, the distribution of the COX-2 polymorphism, rs5277, was not significantly different between CRC cases and controls (P= 0.983). Conclusion: Our results suggest that -765G→C polymorphism in COX2 could be a good prognostic indicator for patients with CRC. Genotyping this polymorphism may be useful for predicting the clinical outcome of sporadic CRC. Keywords: Colorectal cancer; Cyclooxygenase-2; Genetic polymorphism.

1179T

Sorting Nexin 3 Overexpression Disrupts EGFR and MET Endosomal Trafficking Promoting Cell Proliferation and Tumorigenicity in Pediatric Glioblastoma. *D. Khuong Quang[1], H. Al-Halabi[2], T. Haque[2], D. Faury[3], B. Meehan[3], J. Rak[3], S. Albrecht[4], N. Jabado[1,2,3].* 1) Department of Human Genetics, McGill University, Montreal, Canada; 2) Department of Experimental Medicine, McGill University, Montreal, Canada; 3) Department of Pediatrics, Montreal Children's Hospital, Montreal, Canada; 4) Department of Pathology, Montreal Children's Hospital, Montreal, Canada.

Introduction: Amplification/mutation of receptors-tyrosine-kinases (RTK) plays a major role in gliomagenesis. Using microarray data we generated, we identified overexpression of Sorting Nexin 3 (SNX3), a protein involved in the endosomal trafficking of RTK including EGFR. Our hypothesis is that dysregulated expression of SNX3 may delay RTK degradation and promote sustained intracellular activation through these receptors, mimicking RTK amplification seen in adult-GBM events in a subset of pediatric-GBM (pGBM). In addition, we assessed the effect of SNX3 overexpression on oncosome production. Microvesicles and exosomes allow the sharing of oncogenic and transforming signals between neighbouring cells and have an important role in adult gliomagenesis. *Methods:* We stably overexpressed cMyc-tagged-SNX3 in pGBM (SF188 and SJG2) and aGBM (U87) cell lines. Parallel SNX3 knock-down experiments were performed in cell lines. Effects of overexpression/silencing of SNX3 were investigated on EGFR and MET activation, cell signaling and cell proliferation in vitro and in vivo (xenograft model NOD/SCID mice). We quantified the level of microvesicles and exosomes produced in SNX3 overexpressing cells in comparison to empty vector controls. *Results:* SNX3 overexpression delayed EGFR and MET degradation following RTK engagement. This increased and sustained activation of Ras and JNK pathways and cell proliferation in vitro. Importantly, it promoted tumour formation in NOD-SCID mice. Experiments done on SNX3-knocked down cell lines led to a decreased intracellular signalling. In vitro, SNX3 overexpression was associated with increased production of microvesicles and exosomes compared to controls. *Conclusion:* Our results indicate that SNX3-overexpression disrupts physiological trafficking of multiple membrane receptors including EGFR and MET leading to sustained activation of signalling pathways that promote cellular proliferation and tumorigenicity. We also show increased oncosome formation in association with SNX3 overexpression, highlighting this as a potentially novel mechanism of enhancing tumorigenesis in pGBM.

1180T

Targeted second generation sequencing of genes with allele-specific gene expression in pediatric acute lymphoblastic leukemia cells. *A. Kiialainen[1,7], P. Wahlberg[1], J. Nordlund[1], O. Karlberg[1], L. Milani[1,8], U. Liljedahl[1], T. Flaegstad[2,9], G. Jonmundsson[3,9], J. Kanerva[4,9], K. Schmiegelow[5,9], G. Lönnerholm[6,9], A-C. Syvänen[1].* 1) Department of Medical Sciences, Uppsala University, Uppsala, Sweden; 2) 2Department of Pediatrics, University and University Hospital, Tromsø, Norway; 3) Department of Pediatrics, Landspitalinn, Reykjavik, Iceland; 4) Division of Hematology/Oncology and Stem Cell Transplantation, Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland; 5) Pediatric Clinic II, Rigshospitalet, and the Medical Faculty, the Institute of Gynecology, Obstetrics and Pediatrics, the University of Copenhagen, Copenhagen, Denmark; 6) Department of Women's and Children's Health, University Children's Hospital, Uppsala, Sweden; 7) Current address: Pharma Research and Early Development, F. Hoffmann-La Roche Ltd., Basel, Switzerland; 8) Current address: Estonian Genome Centre, University of Tartu, Estonia; 9) For the Nordic Society of Pediatric Hematology and Oncology.

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer in the developed countries. With modern treatment protocols children with ALL have a good prognosis, but for unknown reasons, approximately 20% of patients do not respond to treatment. Thus, there is a need for improved stratification of ALL patients into groups for alternative treatment protocols. We have performed a survey of allele-specific gene expression (ASE) of over 8,000 genes in RNA samples from 197 ALL patients whose in vitro response to ten anti-cancer drugs is known (Milani *et al*, Genome Res. 2009, 19(1):1-11). Our hypothesis was that ASE could be used as a guide to identify genes with cis-acting regulatory genetic variants that could explain the differences in treatment response between ALL patients and serve as biomarkers for stratification of the patients for treatment. We used ASE to select 56 genes that displayed ASE in a large number of the patients for detection of regulatory genetic variants by second generation sequencing. A target region of 3.1 megabases in size, including all exons, introns, UTRs and 5 kb of flanking sequences of the 56 gene regions, was enriched from genomic DNA of 63 ALL samples using hybrid-capture methods and sequenced on an Illumina Genome Analyzer. In this region, we called 8,384 SNPs that were present in at least two samples. From these we selected 768 putative regulatory SNPs for genotyping in a set of 1,000 samples from Nordic ALL patients and in controls matched for country of origin. The genotype data was subjected to association tests between different patient groups and between ALL patients and controls to detect putative functional regulatory genetic variants.

1181T

Multiplex sequencing of targeted genes for GWAS fine mapping. *D. Klevebring[1,2], M. Neiman[1,2], J. Lindberg[1,2], S. Sundling[1], F. Wiklund[1], H. Grönberg[1], K. Czene[1], P. Hall[1].* 1) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; 2) Science for Life Laborarory, Karolinska Institutet, Stockholm, Sweden.

Aim To identify potential causative genetic variants in genomic regions that have been associated with cancer risk through genome wide association studies (GWAS). To investigate whether these potential causative variants have larger effect sizes than the tag SNPs identified by GWAS. **Background** GWAS has been successfully used to identify loci associated with risk in several diseases, including breast and prostate cancer. However, SNPs identified are commonly regarded as tag-SNP, not themselves mediating the functional effect. In order to identify putative functional germline variants, large-scale sequencing of selected loci will be a valuable tool. This study aims to identify potential causative genetic variants in linkage regions around three tag-SNPs identified through GWAS using an in-house developed highly multiplexed sequencing approach. This approach allows for sequencing genomic regions of up to 500 kilobases in hundreds of cases and controls. For each sample, the full genomic sequence of the captured regions will be acquired. **Methods** We have developed a strategy for highly multiplexed sequencing of a limited genomic region (<500 kb) based on a two-tagging approach. From a sample set of breast cancer cases and controls, where mammographic density is available, we have performed sequencing of three genomic loci associated with breast cancer risk, where one of them is also associated with mammographic density. A custom sequence capture kit is used to perform multiplexed capture of the targeted regions, after which sequencing is carried out. **Preliminary results**We have developed a sequence capture-based low-cost sample preparation for highly multiplexed barcoding of hundreds of samples in order to sequence a specified genomic region smaller than 500 kilobases. Sequencing of a 96-sample multiplexed library been successfully carried out and for the majority of samples coverage is sufficient (>10x) to identify germline varation.

1182T

Identification of lung adenocarcinoma driver mutations by pooled exome sequencing and network reconstruction. *X. Kong, S. Yin, B. Lin, L. Hu.* Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

Recent advances in high-throughput sequencing technologies provide opportunities to examine the cancer genome in a comprehensive and unbiased manner. However, such studies are typically limited to few individuals and pinpointing the driver mutations that contribute to tumorigenesis is rarely straightforward. Here we report pooled whole-exome sequencing and a network construction approach for rapid and cost-effective detection of pathogenic mutations in lung adenocarcinoma (ADC). We created eight pools of DNA from a cohort of 39 ADC tumor samples and 66 normal controls for exome sequencing and after data filtering and function arguments, we identified 1551 rare mutations in 1278 genes that were predicted to affect protein function in ADC. We then applied a unified network reconstruction to identify functionally related modules that are highly targeted by ADC rare mutations. These ADC mutations were further stratified according to their interaction with previous known cancer genes and background mutations identified in normal subjects. We subsequently resequenced and confirmed 662 high likely driver mutations which we suggest act in concert to carry out specific functions that are causally linked to tumorigenesis. We find evidence for important driving roles of immune genes, kinases and miRNAs. Our study illustrates how mutation network reconstruction can be used to identify causal mutations in individual cases even in the presence of incomplete sequencing coverage.

1183T

Cancer specific promoter methylation in tubulocystic and papillary renal cell carcinomas. *M. Korabecna[1,2], P. Steiner[1], T. Vanecek[1], E. Pazourkova[2], Z. Musil[2], M. Hora[3], O. Hes[1].* 1) Sikl's Institute of Pathology, Faculty of Medicine in Pilsen, Charles University in Prague; 2) Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University in Prague; 3) Department of Urology, Faculty of Medicine in Pilsen, Charles University in Prague.

BACKGROUND: Tubulocystic renal cell carcinoma (TC RCC) belongs to the rare recently described renal tumors. Pathologic and cytogenetic findings support the evidence for the close relatedness of TC RCC to papillary renal cell carcinoma (PRCC) but the data concerning the methylation status of tumor related genes in TCRC are missing. We performed this pilot study to obtain the methylation profiles for comparison between TC RCC and PRCC. METHODS: 16 cases of TCRCC and 480 cases of PRCC were retrieved from cohort of 16.000 primary kidney tumors from Pilsen Tumor Registry. We selected suitable frozen samples of tumors (2 TCRC and 4PRCC) and their adjacent morphologically normal tissues for DNA isolation. The extent of promotor methylation of a 48 genes panel (tumor suppressors and genes involved in epithelial to mesenchymal transition) was examined using the Methyl Profiler DNA Methylation PCR Array Systems (SABiosciences, Qiagen). Cluster analysis (SABiosciences, Qiagen) was used for data evaluation. RESULTS: We provide the evidence that the methylation profiles of TC RCC contain the same hypermethylated genes (e.g. PDLIM4, RASF1, RUNX3, SOCS1) as PRCC. The VHL gene, which is often hypermethylated in clear cell renal carcinomas and whose status seems to be important with regard to response to sunitinib therapy, was unmethylated in all samples examined by us. CONCLUSION: The character of methylation profiles provides the new helpful tool for evaluation of biological relatedness of rare renal tumors with the high potential to bring new insights in their complex pathogenesis and therapy response. Supported by the grant no. I/328 of the Ministry of Industry and Trade of the Czech Republic and the grant no. 12010-4 of the Ministry of Health of the Czech Republic.

1184T

Loss of FADS2 function at 11q13 cancer hot spot region causes synthesis of unusual butylene-interrupted fatty acids. *K.S.D. Kothapalli, W.J. Park, P. Lawrence, J.T. Brenna.* Division of Nutritional Sciences, Cornell University, Ithaca, NY.

Several human cytogenetic and fine mapping studies have pin-pointed HSA 11q13 region as a major hotspot for a number of human cancers. Many mechanisms including 11q13 deletions, loss of heterozygosity, translocations and allelic amplification have been reported. The fatty acid desaturase cluster, encoded by genes FADS1, FADS2, and FADS3, localize within a 100 kb region on human chromosome 11q12-13.1. FADS2 and FADS1 encode for critical enzymes for long chain polyunsaturated fatty acid (LCPUFA) biosynthesis, introducing double bonds between specific carbon atoms. Omega-3 (=3 or n-3) and omega-6 (=6 or n-6) PUFA are key nutrients linked to most of the diseases of humans, specifically cardiovascular (CVD), cancer, diabetes and metabolic syndrome, and are key structural components of neural tissue. The LCPUFA DGLA (20:3n-6), ARA (20:4n-6), EPA (20:5n-3) and DHA (22:6n-3) are precursors for cell signaling eicosanoids and docosanoids that are very valuable drug targets. In several cancer cells the critical rate limiting step catalyzed by delta 6-desaturase (FADS2) enzyme is non-functional. The reason for this defect has been suggested to be due to extensive chromosomal deletions, but no molecular evidence is available. The Δ8-desaturation substrates 20:2n-6 and 20:3n-3 are often detected in these cancer cells, thus suggesting that the elongation step is functional. Recently, as an alternative LCPUFA synthetic route we demonstrated delta-8 desaturation pathway catalyzed by a FADS2 product. Here we present using MCF7 breast cancer cells a) evidence of metabolic restoration of delta 6-desaturase and delta 8-desaturase activities by heterologous expression of FADS2, b) in the substantial absence of FADS2 activity, FADS1 compensates by producing unusual butylene-interrupted 5,11,14-20:3 and 5,11,14,17-20:4 fatty acids and c) competition between FADS1 and FADS2 for the same substrates. Cyclooxygenase (COX) normally acts on the double bonds at positions 8 and 11, thus the absence of the double bond at position 8 renders them inactive as substrates for prostaglandin biosynthesis. The loss of FADS2-encoded activities in cancer cells completely shuts down the classical and alternative LCPUFA pathways, eliminating eicosanoid and docosanoid precursor biosynthesis and thus further limiting cell-cell signaling. Our present results provide an impetus to better understanding the role of fatty acid desaturases, especially FADS2 as a tumor suppressor in neoplastic disorders.

1185T

Genomic characterization of familial lung cancer patients. *Y. Kukita[1], K. Taniguchi[1], J. Okami[2], M. Higashiyama[2], Y. Suzuki[3], S. Sugano[3], R. Matoba[4], J. Kato[5], N. Kato[5], I. Nakamae[5], T. Kawabata[6], K. Kodama[2,7], K. Kato[1].* 1) Research Institute, Osaka Medical Center for Cancer and Cardiovascular Diseases, Japan; 2) Department of Thoracic Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Japan; 3) Graduate School of Frontier Sciences, the University of Tokyo, Japan; 4) DNA Chip Research Inc., Japan; 5) Graduate School of Biological Sciences, Nara Institute of Science and Technology, Japan; 6) Institute for Protein Research, Osaka University, Japan; 7) Yao Municipal Hospital, Japan.

Massively parallel sequencing enables identification of causative mutations of genetic diseases by analyzing small family members. In our hospital, several familial cancer cases have been collected, and we analyzed genomes from an affected sib pair with familial lung cancer using SNP array and exome-sequencing. Although these subjects had no unusual large insertions/deletions, we detected many large homozygous regions (more than 200 Mb of the genome per person), and they were thought to be the products of consanguineous union. By exome-sequencing, we identified more than 500 novel non-synonymous variants per person after subtracting known polymorphic variants. Combining results from both assays leaded identification of a homozygous CHEK2 mutation that caused unstable structure of the gene-product. Because CHEK2 is known as a multi-organ cancer susceptibility gene, additional causative mutations may involve for the phenotype of our subjects. We are investigating association of remaining novel variants with lung cancer.

1186T

C-Kit and Bcl-2 Expression in Testicular Cancer. *S. Lai[1,2], LK. Green[1,2].* 1) Pathology, Baylor College of Medicine, Houston, TX; 2) Pathology, Michael E. DeBakey VA Medical Center, Houston, TX.

Recent developments in understanding the molecular biology of testicular cancer implicated a number of key players including p53, pRb, pINK protein, c-kit and bcl-2 protein. Expression and gain of function mutation of the c-kit gene that encodes a receptor tyrosine kinase (KIT) have been reported in gastrointestinal stromal tumor and seminoma. It is suggested that activated mutation of c-kit occurs early in germ cell development and that gonocytes with an activated c-kit receptor are restricted in their differentiation, escaping normal cellular development. Genes in apoptosis pathway such as Bcl-2 play an important role in tumorigenesis of cancers. We studied c-kit and Bcl-2 expressions by immunohistochemistry in 18 testicular cancers including 8 pure seminomas, 7 mixed germ cell tumors, 1 metastatic adenocarcinoma, 1 leiomyosarcoma and 1 large cell lymphoma. We found that c-kit was diffusely and strongly expressed in pure seminomas and metastatic adenocarcinoma, while only focal and weak c-kit positivity was shown in seminoma and nonseminomatous components of mixed germ cell tumor. Bcl-2 expression was seen in metastatic adenocarcinoma and lymphoma, but not in seminoma, mixed germ cell tumor or leiomyosarcoma. We analyzed the prognostic or predictive significance of expression of both markers.

1187T

PTGER4 is regulated by ETV6, a transcription factor implicated in childhood leukemia. *J. Larose[1], C. Malouf[1,2], S. Langlois[1], D. Sinnett[1,3].* 1) Division of Hemato-Oncology, Research Center Sainte-Justine University Health center, Montreal, Canada; 2) Department of Biochemystry, Faculty of Medecine, University of Montreal, Montreal, Canada; 3) Department of Pediatrics, Faculty of Medecine, University of Montreal, Montreal, Canada.

Pre-B acute lymphoblastic leukemia (pre-B ALL) is the most prevalent pediatric cancer in Western countries. The translocation t(12;21), which produces an ETV6-AML1 chimera, is observed in ~25% of pre-B ALL cases and is one of the most frequent genetic alterations found in childhood ALL. However, this chromosomal rearrangement is insufficient to initiate leukemogenesis alone. At diagnosis, the second copy of ETV6 is deleted in most pre-B ALL patients carrying the t(12;21), thereby leading to its inactivation. ETV6, a transcriptional repressor of the Ets family, is essential for haemopoiesis. We postulated that ETV6 plays a role in childhood leukemogenesis through the deregulated expression of its transcriptional targets. Using a combination of expression microarray analysis and qRT-PCR experiments in patients with leukemia we identified several putative transcriptional targets of ETV6. One of these targets, PTGER4, encodes a G-protein-coupled receptor for prostaglandin E2 (PGE2). To determine whether PTGER4 is a direct target of ETV6, we performed gene reporter assays and chromatin immunoprecipitation in three human cell lines, including a lymphocytic cell line. We showed that ETV6 regulates the transcription of PTGER4 by targeting a region close to the basal promoter. The observed repression is mediated by both ETV6 functional domains: the pointed domain involved in oligomerization and protein interactions and the ETS DNA-binding domain. Although ETV6 was significant enriched on PTGER4 promoter, ETV6-mediated transcriptional regulation did not depend on consensus Ets-binding sites. To further dissect the impact of altered regulation of PTGER4 in leukemogenesis, we conducted functional studies. Using different constructs to regulate the expression of ETV6, we showed that PTGER4 deregulation influences cell cycle, proliferation and apoptosis. Our work highlights that PTGER4 is a transcriptional target of ETV6 and that its ETV6-mediated transcriptional regulation requires molecular mechanisms that are different from the other Ets transcription factors.

1188T

Does germline variation in the number of Glutathione S-transferase gene copies affect the risk of metachronous colorectal neoplasia? *C. Laukaitis[1, 2], C. Fuentes-Mauss[2].* 1) Department of Medicine, University of Arizona College of Medicine, Tucson, AZ; 2) Arizona Cancer Center, University of Arizona, Tucson, AZ.

Twin studies assign 1/3 of variation in colorectal cancer (CRC) risk to inherited genetic factors, but the hereditary cancer syndromes account for just 5% of CRC. Having a first-degree relative with CRC increases a person's CRC risk by 2-3 fold, but most often that risk is not inherited in a Mendelian fashion. We seek to uncover gene variations that can improve cancer risk-assessment protocols.Both single nucleotide polymorphisms (SNPs) and variation in gene copy number (CNV) can influence CRC risk, either by causing a high-penetrance hereditary cancer syndrome or through more complex mechanism. This project addresses the contribution to familial cancer risk of inherited SNPs and CNVs in Glutathione S-transferase (GST) genes. These genes vary in sequence and copy number, their gene products are important in detoxification of potential carcinogens, and variation in their copy number has shown variable association to colorectal cancer risk. Equivocal results from studies attempting to correlate CNV in the GSTM1 and GSTT1 genes could have technical or biological reasons: 1) early studies could not differentiate between individuals with one versus two GST gene copies because they relied on PCR for genotyping; 2) data have been collected in many populations with wide variation in both environmental exposures and the frequency of GST gene deletions; 3) the advanced CRC phenotype assayed may have confounded association; and/or 4) lack of a biological effect. It is worth re-visiting the hypothesis that GST gene copy number influences CRC risk using new technology that can accurately assess gene copy number, examining a large but ethnically homogenous population, and choosing an outcome early in the neoplastic process with less confounding by variables. We assessed three GST genes for SNP and CNV variation in individuals from two negative interventional trials to ask whether variants in these genes predict adenoma recurrence. SNP genotypes for the GSTM1, GSTT1, and GSTP1 genes were determined using microarray analysis and we have extrapolated gene copy number genotype from these data. We have confirmed the genotype in a subset of samples using MLPA (CNV) and TaqMan (SNP) technology. Finally, we are testing whether a specific CNV and/or SNP genotype influences risk of metachronous adenoma development.

1189T

MicroRNAs contribute to the chemoresistance of cisplatin and paclitaxel in patients with head and neck squamous cell carcinoma. *AC. Laus[1], T. Macedo[1], AC. Carvalho[2], C. Scapulatempo Neto[1], AL. Carvalho[1], EI. Palmero[1], MMC. Marques[1].* 1) Molecular Oncology Research Ct, Barretos Cancer Hospital, Barretos, Brazil; 2) Biological Science Department, Sao Paulo Federal University.

Head and neck squamous cell carcinoma (HNSCC) is a devastating disease. Despite advances in diagnosis and treatment, mortality rates have not improved significantly over the past three decades. MicroRNAs (miRNAs) are small non-coding RNAs that function as negative regulators of gene expression. They are strongly implicated in human cancers, including HNOC and the evidences for the involvement of miRNAs as important regulators of chemosensitivity and chemoresistance in HNSCC poorly understood. High mobility group A2 (HMGA2) expression has been shown to be associated with enhanced selective chemosensitivity to cisplatin, in cancer cells. Since HMGA2 expression in HNSCC is regulated in part by miRNA-98 (miR-98) and let-7i, our aim in this study was to compare the differential expression of both microRNAs and to investigate their relationship with the chemoresistance against cisplatin and paclitaxel. Forty six patients from Barretos Cancer Hospital were diagnosed with HNSCC and were treated with neoadjuvant chemotherapy using cisplatin and paclitaxel in combination. The progression free survival rate to this therapy was assessed applying RECIST criteria. After the neoadjuvant therapy, the patients were evaluated and separated into two groups: those with good response to therapy (complete or partial response) and those non-responders (progression or stable disease). Total RNA was extracted from FFPE tumor biopsies using RecoverAll Total Nucleic Acid Isolation Kit (Ambion), and quantified with NanoDrop 2000 spectrophotometer. The quality of miRNAs was assessed using Bioanalyzer Small RNA chip (Agilent). The expression levels of miR-98 and let-7i were measured by miRNA Quantitative Real Time PCR, using 7900HT Fast Real-Time PCR System (Applied Biosystems). Our results showed that both miR-98 and let-7i were down-regulated in 66% and 50% of the patients with good response to therapy, respectively. The relationship of HMGA2 protein expression with the chemosensitivity to cisplatin in HNSCC and considering that this protein is a target of miR-98 and let-7i, our results suggest that the down-regulation of both miRNAs can be related with the good response to neoadjuvant therapy.

1190T

Novel regions of suggestive linkage in African American hereditary prostate cancer families. *E.M. Ledet[1], J.E. Bailey-Wilson[2], D.M. Mandal[1].* 1) Department of Genetics, Louisiana State Univ HSC, New Orleans, LA; 2) National Human Genome Research Institute, National Institutes of Health, Baltimore, MD 21224.

Prostate cancer is a complex multi-allelic disease and the most common malignancy in men throughout the world. In the United States, incidence of prostate cancer in African American men is more than twice than that of any other race. Also, a lifetime risk of mortality from prostate cancer is 3% for white men and 4% for African-American men. Thus far, numerous disease susceptibility loci have been identified for this cancer but definite locus-specific information is not yet established due to the tremendous amount of genetic and disease heterogeneity. Additionally, despite high prevalence of prostate cancer amongst African American men, this population has been under represented in genetic studies of prostate cancer. In order to identify the susceptible locus (loci) for prostate cancer in African Americans, we have performed linkage analyses on members of 15 large high-risk families. Specifically, these families were recruited from Louisiana and represent a uniquely admixed African American population exclusive to Southern Louisiana. In addition to geographical constraints, these families were clinically homogeneous creating a well characterized collection of large pedigrees. The families were genotyped with Illumina Infinium II SNP HumanLinkage-12 panel and extensive demographic and clinical information was documented from the hospital pathological reports and family interviews. Both non-parametric and parametric allele-sharing linkage analyses were performed using Merlin, version 1.1.2. Under the dominant model, we identified two novel regions, 12q24 (HLOD=2.21) and 2p16 (HLOD=1.97), with suggestive evidence of linkage. This is the first time that chromosome 12q24 and 2p16 has been shown to be associated with prostate cancer in high-risk African American families. The results illustrate the significance of utilizing large unique, but homogenous pedigrees.

1191T

Evidence for population-based screening of BRCA1 and BRCA2. *E. Levy-Lahad[1,2], E. Gabai-Kapara[1,2], B. Kaufman[3], C. Catane[3], S. Regev[4], P. Renbaum[1], U. Beller[5], M-C. King[6], A. Lahad[7].* 1) Medical Genetics Institute, Shaare Zedek Medical Center, Jerusalem, Israel; 2) Hebrew University Medical School, Jerusalem, Israel; 3) Division of Oncology, The Chaim Sheba Medical Center, Tel Hashomer, Israel; 4) The Institute for Medical Screening, Chaim Sheba Medical Center, Tel Hashomer, Israel; 5) Department of Obstetrics & Gynecology, Shaare Zedek Medical Center, Jerusalem, Israel; 6) Department of Medicine (Medical Genetics) and Department of Genome Sciences, University of Washington, Seattle, WA; 7) Department of Family Medicine, Hebrew University Medical School, Jerusalem, Israel.

Background: Inherited BRCA1 and BRCA2 mutations are associated with high risks of breast and ovarian cancer, and acceptable prevention measures (e.g. risk reducing salpingo-oophorectomy) significantly reduce morbidity and mortality. In Ashkenazi Jews (AJ), three variants account for ~95% of deleterious mutations, with a combined population carrier rate of 2.5%. Testing for common mutations in AJ is inexpensive and highly specific, and could fulfill WHO screening criteria if carriers ascertained from the general population were shown to be at high cancer risk. Current risk estimates are based on female carriers ascertained through personal or family history and thus may be subject to selection bias. ***Aim:*** To determine cancer risk in women with BRCA1/BRCA2 mutations by identifying carrier families through healthy males in the general AJ population. ***Methods:*** AJ males with no personal cancer history were tested for BRCA1/BRCA2 mutations. Relatives of carriers were tested and cancer risk in females was assessed based on genotype and family history. ***Results:*** Among 8196 males, 175 were heterozygous for one or more BRCA1/BRCA2 founder mutations (2.2%). Among their female relatives with mutations, risks of breast and ovarian cancer were comparable to current risk estimates: ~80% by age 75 for breast and ovarian cancer combined. Indeed, of female carriers in these families, 45% had been previously diagnosed with breast or ovarian cancer. As expected, family history of breast or ovarian cancer was more common in carriers vs. non carriers ($p<0.001$), especially in BRCA1 carriers ($p=0.02$). However, 63% of families with BRCA1/BRCA2 mutations had minimal or no family history of breast or ovarian cancer. Lack of family history was related to low incidence as a result of paternal inheritance, small family size, male preponderance and few females inheriting the familial mutation. Women carriers in these families would be missed using current referral criteria. ***Conclusions:*** Risk of breast and ovarian cancer to women with BRCA1/BRCA2 mutations is high in the general population. By WHO criteria, population-based screening is justified. This is currently feasible and cost-effective in any population with common founder mutations and could be universally applicable given genomics-based advances in genetic testing. General genetic screening for a dominant adult-onset disease is a novel proposition, and its practical and psychosocial aspects should be further explored.

1192T

Glioblastoma Multiforme: genomic estimates of tumor purity and revised classification. *B. Li[1], Y. Senbabaoglu[1], W. Peng[2], J. Li[1,2].* 1) Bioinformatics, University of Michigan, Ann Arbor, MI; 2) Human Genetics, University of Michigan, Ann Arbor, MI.

Glioblastoma Multiforme (GBM) is an aggressive brain tumor with poor prognosis. Accurate definition of GBM subclasses is important for understanding its biological heterogeneity, and provides the knowledge base for better diagnosis and informed treatment. Recently, the Cancer Genome Atlas (TCGA) project characterized > 200 GBM samples, of which 128 were analyzed by both DNA genotyping and expression profiling, presenting an opportunity for integrated analyses. As primary tumors often contain both aneuploid cells and euploid cells, we used allele-specific copy number alteration (CNA) data from Illumina genotyping arrays to estimate the fraction of aneuploid cells, termed Aberrant Genome Proportion (AGP), and incorporated this measure of tumor purity in class discovery. Our genomic estimates of tumor purity correlate poorly with histopathologic report of tumor content, yet unlike the latter, are strongly correlated with principal components of total copy number data and gene expression data. Similar relationship between intratumoral heterogeneity and apparent between-tumor diversity is also seen in the ovarian cancer dataset from TCGA, suggesting that AGP is an important covariate in tumor classification. Combined analysis of expression and CNA data lead to a revised scheme of GBM classification, with two main subtypes: Typical and Atypical. Atypical GBMs are of high purity, are previously assigned to the Proneural group, carry signatures of secondary GBM such as IDH1 and TP53 mutations, are hypermethylated, and came from younger patients with better outcome. Typical GBMs span a continuum of expression profiles that parallels the purity gradient, carrying characteristic PTEN mutations, EGFR focal amplifications, gains of chr7 and losses of chr10. Importantly, proneural samples within the Typical group have significantly worse outcome than the rest of Typical samples. Using inferred AGP we extrapolated the expression profiles for pure tumor cells and found that Classical and Mesenchymal subtypes are recognizable among Typical GBMs, yet previously described Typical-Proneural and Neural subtypes are in fact not separable. We propose a step-wise diagnostic protocol for GBM that Incorporates these findings. Acknowledgement: We thank members of TCGA Research Network for sharing data and for helpful discussions.

1193T

Testing for BRCA1/2 mutations in hereditary breast cancer in Rio de Janeiro, Brazil. *M.A.F.D. Lima, K.R.L. Souza, A.C.E. Santos, C.H. Costa, A. Moreira, M. Moreira, F.R. Vargas.* Instituto Nacional do Câncer, Rio de Janeiro, Rio de Janeiro, Brazil.

Breast cancer (BC) is the second most frequent cancer among women in the world, and it is the most common neoplasia among women in Brazil (INCA, 2010). It is estimated that approximately 10% of BC and ovarian cancer (OC) cases are related to hereditary cancer syndromes (Lynch et al., 2008). We present our experience with testing for BRCA1/2 mutations in 141 BC and OC patients referred to our cancer genetic counseling unit. All patients included in this cross-sectional study fulfilled ASCO 1997 or NCCN 2008 criteria, and pre-test evaluation included medical geneticist and psychologist consultations. Probability models (Penn II, Myriad II and BRCAPro) were used to assess likelihood of BRCA1 or BRCA2 germ-cell mutations. Of a total of 141 families (138 female and 3 male probands) bilateral BC was observed in 14.1% patients; unilateral BC in 76.5%; bilateral OC in 3.5% and unilateral OC in 1.4%. BC was associated to other tumors (not OC) in 2 patients (kidney and gastrointestinal stromal tumor). Mean age for diagnosis of BC and OC was 42.1 years (range 20-74 years). Germ-cell BRCA1/2 disease-causing mutations were identified in 12 probands. There was no difference in groups with and without mutation regarding age of first tumor. Sensitivity and specificity of Penn II, Myriad II and BRCApro models were calculated. Penn II seems to be the most sensitive and BRCA-pro the most specific.

1194T

Integrated analysis of prostate cancer for the identification of biomarkers correlated to recurrent disease. *J. Lindberg[1,2], D. Klevebring[1,2], W. Liu[3], O. Laurin[4], M. Neiman[1,2], J. Xu[3], P. Wiklund[4], F. Wiklund[1], L. Egevad[5], H. Grönberg[1].* 1) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; 2) Science for life laboratory, Stockholm, Sweden; 3) Center for Cancer Genomics, Wake Forest University School of Medicine, NC, USA; 4) Department of Urology, Division of Surgery, Karolinska Hospital, Stockholm, Sweden; 5) Department of Pathology and Cytology, Karolinska University Hospital, Stockholm, Sweden.

Background: Prostate cancer (PC) is the most common type of cancer accounting for ~35% of annual male cancer incidents and although the five-year survival rate is 75%, PC is the most frequent cause of death from cancer in Sweden. One of the main problems with treatment of PC is the lack of informative biomarkers for diagnosis and prognosis. PSA is a biomarker present in serum that has been used to monitor and diagnose PC patients for more than a decade, but the molecule has several drawbacks with massive unnecessary treatment as a consequence. Here we report on the current progress of an analysis of a biobank of PC tissues with the overreaching goal of identifying biomarkers for aggressive disease. We have taken the approach of 1) Mapping the somatic variation landscape in PC with focus on aggressive disease. 2) Evaluating circulating tumor DNA as a personalized biomarker in PC. 3) Integrating RNAseq data with the somatic mutation data for the identification of plausible protein biomarkers. 4) Evaluating the potential protein biomarkers both in tumors and plasma. Current results: So far we have extracted RNA/DNA and proteins from 110 PCs with >3 years of follow up and >70% of tumor content. Whole-exome sequencing combined with CNV data on 21 tumors from 19 individuals demonstrates the somatic variation landscape of PC to be as heterogeneous as for other solid tumors. Although few genes were recurrently mutated, significantly overrepresented themes related to chromatin modification, protein ubiquitination and ion transport, among others, were identified. Also, in-depth analysis of multiple tumor loci from one prostate indicates the presence of multiple independent tumors, with potential implications for sampling and biomarker discovery.

1195T

A General Statistical Approach to Somatic Mutation Discovery in Cancer Genome Sequence. *P. Liu, X. Hua, H. Xu, S. Park, Y. Lu.* Dept Physiology and Cancer Center, Medical College of Winconsin, Milwaukee, WI.

Cancers are caused by the accumulation of genomic alterations. Next-generation sequencing (NGS) technologies have revolutionized cancer genomics research by providing an unbiased and comprehensive method of detecting somatic cancer genome alterations, including nucleotide substitutions, small insertions and deletions, copy number alterations, and chromosomal rearrangements. However, substantial statistical and computational challenges still exist for analyzing NGS data. Here, we proposed a general statistical model for inference of somatic mutations and estimation of DNA contamination using NGS data. The proposed new approach possesses two prominent statistical properties. First, the method can jointly call somatic variants from multiple tumor samples. Second, the DNA contamination, commonly existing in tumor sequencing, is incorporated into our statistical models. We derived an efficient EM algorithm to obtain the maximum likelihood estimates of sequencing error rate, somatic mutation status and DNA contamination rate. Simulation results showed that these estimations are unbiased in our model. When incorporating DNA contamination rate into the model, our approach further increases 5-10% power as compared with conventional methods. As a result, we developed C routines to implement our approach. The program is user-friendly and can simultaneously handle hundreds of Binary Alignment/Map (BAM) files - the compressed binary version of the Sequence Alignment/Map, with multiple threads from cancer sequencing projects. To demonstrate its utility, we applied our approach to the analysis of 201 lung cancer exomes from The Cancer Genome Atlas (TCGA).

1196T

Identification of a genetic variant associated with treatment outcome in ovarian cancer. *Y. Lu[1], S. Johnatty[1], E. Gamazon[2], J. Beesley[1], X. Chen[1], B. Gao[3], P. Harnett[3], R.S. Huang[2], E. Despierre[4], F. Heitz[5], E. Hogdall[6], C. Hogdall[6], R. Brown[7], K. Moyisch[8], P. Fasching[9], E. Goode[10], E.M. Dolan[2], S. Macgregor[1], A. deFazio[3], G. Chenevix-Trench[1], the Ovarian Cancer Association Consortium (OCAC).* 1) Queensland Institute of Medical Research, Brisbane, QLD, Australia; 2) Department of Medicine, University of Chicago, Chicago, IL; 3) Department of Gynaecological Oncology and Westmead Institute for Cancer Research, University of Sydney at the Westmead Millennium Institute, Westmead Hospital, Sydney, Australia; 4) Department of Gynaecologic Oncology, University Hospitals Leuven, Leuven, Belgium; 5) Abteilung für Gynäkologie und gynäkologische Onkologie, Ludwig-Erhardt Str. 100, 65197 Wiesbaden; 6) Dept. of Virus, Hormones and Cancer, Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark; 7) Imperial College London, Hammersmith Hospital Campus, London, United Kingdom; 8) Roswell Park Cancer Center, Buffalo, NY; 9) Division of Hematology and Oncology, Department of Medicine, David Geffen School of Medicine, Los Angeles, California; 10) Department of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN.

Cell-based models have shown that response to chemotherapy has a heritable component. We hypothesized that to identify loci associated with treatment outcome, we should focus on cases known to have had the standard chemotherapy for epithelial ovarian cancer. We therefore performed a two-stage genome-wide association study (GWAS) of progression free survival (PFS) following first line carboplatin/paclitaxel chemotherapy in ovarian cancer cases. In the first stage, we genotyped (Illumina Omni1) 183 Australian cases selected using an extreme phenotype design, and also included data on 134 cases from the TCGA and 68 from Mayo Clinic. In this stage, 260 cases had `standard' treatment (“primary” group: at least 4 cycles of carboplatin 5-6 AUC and paclitaxel 135-175 mg/m2 every three weeks) and 125 cases (“secondary”) had an unknown amount of carboplatin/paclitaxel chemotherapy. In the 2nd stage we genotyped 156 of the top ranking genotyped and imputed SNPs in 4660 cases (1080 `primary' and 1433 `secondary') from 11 sites in OCAC. The additive allelic association with the PFS was assessed in a Cox Proportional Hazards model, adjusting for study site, histological subtype, grade, stage and residual disease. For the SNPs with low minor allele frequencies, we ran permutations to correct the asymptotic p-values. One SNP clearly replicated in the 2nd stage. The associations in both stages were strongest in the “primary” group (1st stage results for this imputed SNP: hazard Ratio (HR) per minor allele=6.30, 95% CI=[4.26, 9.30], two-sided asymptotic p-value=1.88e-6, permuted p-value= 4.46e-5; 2nd stage: HR per minor allele=3.37, 95% CI=[2.48, 4.62], one-sided asymptotic p-value=5e-5, permuted p-value=2.9e-4; meta-analysis p-value corrected by permutation=1.7e-7). Furthermore, an independent cell-based GWAS conducted using HapMap lymphoblastoid cell lines showed that a more common SNP (MAF~4.5%) in moderate LD with our reported SNP (r2~0.28), was associated with carboplatin sensitivity (p-value=9e-3). Both these SNPs are located in a gene on chromosome 9 that is known to be associated with high-density lipoprotein cholesterol. We are now conducting additional experiments to evaluate the biological relevance of this gene with respect to treatment outcome, including the potential role of cholesterol metabolism as a determinant of response to chemotherapy. Our findings may provide some insight into the suggested role of statins in improving outcome for ovarian cancer.

1197T

The role of miRNAs in Cyclo-oxygenase-2 mediated breast cancer metastasis. *M. Majumder, L. Dunn, P.K. Lala.* Department of Anatomy and Cell Biology, The University of Western Ontario, London, Ontario, N6A 5C1, Canada.

Over-expression of the inflammation associated enzyme Cyclo-oxygenase (COX)-2 in breast cancer patients promotes tumor progression and metastasis by multiple mechanisms. MicroRNAs (miRNAs) are small regulatory RNAs that are emerging as biomarkers for cancer. Identification of miRNAs regulated by COX-2 will help to dissect post-transcriptional regulation. We examined the consequences of introducing COX-2 gene into COX-2-ve, ER+ve, non-metastatic MCF-7 human breast cancer cell line named MCF-7-COX-2. When MCF-7-COX-2 cells compared with empty vector-transfected MCF-7-mock cells showed (1) EMT, (2) higher proliferative, migratory and invasive abilities, (3) increased vascular mimicry, (4) increased in stem like cells population with higher expression of stem cell markers in vitro. We conducted differential gene and miRNA micro arrays in MCF-7-mock vs. MCF-7-COX-2 cells, with Affymatrix Human Gene Array 1.0 ST and Affymatrix Genechip miRNAs Array, expression micro arrays. ANOVA with alpha value 0.05 was used to determine associated probe sets followed by a Benjamini and Hochberg Multiple testing correction to reduce the false positive rate. For combined mRNA and miRNA analysis, correlation coefficient (±0.99) was fixed to determine up and down regulated miRNAs with their target gene of expression in MCF-7-COX-2 cell line compared to MCF-7-mock. We have identified two miRNAs (miR-526b and miR-655) that are up-regulated in MCF-7-COX-2. These two COX-2 up-regulated miRNAs were shown to down-regulate fourteen target genes linked with tumor-suppressor functions. We hypothesize that the COX-2-up-regulated miRNAs, are important for COX-2 mediated metastasis in human breast cancer. To test this, various COX-2 disparate human breast cancer cell lines were used to examine COX-2 functions, and to quantify miRNA expression with Taqman miRNA expression assay. COX-2 expression at mRNA level was directly related to expression of mir-655. We are now testing the metastatic abilities of MCF-7-COX-2 cell line in vivo in NOD/SCID/GUSB null mice to measure lung metastasis (identified by GUSB staining of cancer cells). We shall test whether knocking down of miRNAs in COX-2 over-expressing cells (1) can abrogate COX-2 mediated functions in vitro, (2) experimental lung metastasis and (3) find correlation of COX-2 and miRNAs expression in fresh/frozen breast cancer specimens that can personalize diagnostic and therapeutic approaches after surgery.

1198T

P53 isoforms are regulated by ETV6, a transcription factor involved in childhood acute lymphoblastic leukemia. *C. Malouf[1,2], J. Larose[1], S. Langlois[1], B. Neveu[1,2], D. Sinnett[1,3].* 1) Division of Hemato-Oncology, Research Center Sainte-Justine University Health Center, Montreal, Canada, H3T 1C5; 2) Department of Biochemistry, Faculty of Medicine, University of Montreal, Montreal, Canada, H3T 1J4; 3) Department of Pediatrics, Faculty of Medicine, University of Montreal, Montreal, Canada, H3T 1C5.

Introduction. Pre-B acute lymphoblastic leukemia (ALL) is the most frequent pediatric cancer. Around 25% of patients carry the translocation t(12;21) that leads to the expression of the ETV6-AML1 chimera. This chimera alone is not sufficient to the development of leukemia. Notably, patients carrying the t(12;21) often present a deletion of the short arm of the non-translocated chromosome 12. This results in the inactivation of ETV6, an Ets transcriptional repressor with very few known transcriptional targets. We propose that ETV6 participates in childhood leukemogenesis through the deregulated expression of its transcriptional targets. **Experimental method**. In previous studies, we identified TP53 as a putative transcriptional target of ETV6. The TP53 classic promoter is responsible for the transcription of the p53 isoforms, whereas the TP53 alternative promoter regulates the transcription of the Δ133p53 isoforms. To understand the ETV6-mediated transcriptional regulation of TP53, we used *in vitro* gene reporter assays and *in vivo* chromatin immunoprecipitations. We also measured the expression of the p53-isoform transcripts by nested PCR in a cohort of leukemia patients. **Results**. ETV6 represses the transcription of both the TP53 classic and alternative promoters. The transcriptional regulation requires a region close to the basal promoters, along with both functional domains of ETV6: the Pointed domain (PNT) implicated in oligomerization and protein interactions and the DNA-binding domain (ETS). In the TP53 classic promoter, ETV6 interacts with chromatin in a region comprising three consensus Ets-Binding Sites (EBS) which are essential to the ETV6-mediated transcriptional repression. However, the consensus EBS of the TP53 alternative promoter were not recognized by ETV6. Since we didn't observe an enrichment of ETV6 on the TP53 alternative promoter, we propose that ETV6 could sequester transcription factors involved in Δ133p53 expression. Finally, we measured the expression of p53 isoforms in patients with pre-B ALL; although the p53 isoforms were down-regulated, we observed an up-regulation of the Δ133p53 isoforms. **Conclusion**. ETV6 regulates the transcription of all p53 isoforms using molecular mechanisms that are adapted to the target promoter. This study is the first to report a deregulated expression of the p53 isoforms in childhood leukemia and to suggest the implication of Δ133p53 in leukemogenesis.

1199T

Fine needle aspiration biopsy (FNAB) and aCGH in uveal melanoma. *D. Martinet[1], A. Schalenbourg[2], B. Rapin[1], L. Zografos[2], J.S. Beckmann[1], A.P. Moulin[2].* 1) Service of medical genetics, Lausanne, Lausanne, Switzerland; 2) Pathology,Ophthalmology, Jules Gonin Eye Hospital, Lausanne, Switzerland.

Genomic alterations in uveal melanoma are of prognostic value in terms of patient survival. We evaluated in this context the reliability of fine needle aspiration biopsy (FNAB) in molecular chromosomal analysis of uveal melanoma. High resolution aCGH analysis was conducted in 21 cases of uveal melanoma following enucleation. In 16 cases, 25G FNAB was performed immediately after enucleation. The results of CGH array from FNAB perfectly correlated with those arising directly from the tumor after section of the eye. In only one case (6%), there was insufficient DNA for array CGH analysis. Isolated monosomy 3 was present in 1 case; monosomy 3, 8q gain in 5 cases;, monosomy 3, 8q gain and 1p loss in 4 cases, monosomy 3, 6p gain, 8q gain and 1p loss in 3 cases; 6p gain only in 3 cases; 6p gain and 8q gain in 3 cases; no alteration on chromosomes 1, 3, 6 and 8 in 2 cases. FNAB is a reliable technique and provides enough tissue for the analyses of the entire genome, allowing the prospective use of this technique in uveal melanoma patients without enucleation. Our data do not confirm the hypothesis that 6p gain and monosomy 3 are mutually exclusive and represent different sequences in the pathogenesis of uveal melanoma.

1200T

Clinical laboratory experience of gene expression profiling of 2,384 solid tumors. *M.J. McGinniss, A. Ghazalpour, M. Hadlock, J. Garcia, N. Marcus, Y. Hosohata, J. Zarkovic, D. Jacquin, D. Flood, K. Wiste, K. Swetel, B. Toussaint, L. Teets, R.P. Bender.* Molecular Diagnostics, Caris Life Sciences, Phoenix, AZ.

Background: Optimizing therapeutic selections for cancer patients using the molecular profile of their tumor is an unmet need. Currently, many providers use results of tumor profiling by platforms including IHC, FISH and mutational analyses to identify potential cancer treatments. Gene expression profiling with microarrays also has the potential to help classify solid tumor subtypes and assist with therapy selections based on the under or over-expression of particular transcripts. Methods: Solid tumor samples are assessed by board-certified pathologists to ensure the presence of at least 20% tumor nuclei. Total RNA is extracted from tumor tissues (FFPE, frozen and RNAlater-preserved tissues). In addition, extracted RNA is verified to be of sufficient quality (using spectrophotometric metrics and qPCR results on a housekeeping gene RPL13a). The RNA sample is then subjected to a whole genome (29,285 transcripts) microarray analysis using Illumina cDNA-mediated annealing, selection, extension and ligation (DASL) process with the HumanHT-12 v4 beadChip (Illumina Inc., San Diego, CA). After direct hybridization and scanning of the bead array, the expression of a subset of 88 transcripts are then compared to tissue-specific normal control pools and the statistical significance of the difference between the patient tumor sample and control is determined. Results: The median Ct value from the RPL13a qPCR results for both frozen samples (N=70) and RNAlater preserved samples (N=851) was 24. The median Ct value for FFPE samples (N=1463) was 27. The ability of this assay to detect expression changes is directly proportional to the amount of tumor nuclei present in the patient sample. Dilution experiments showed that samples with 20% tumor nuclei are ~65% concordant for gene expression information compared to samples with >95% tumor nuclei with an R-squared value >0.99. Our intra-assay variability (same chip; same operator) was extremely low and the R-squared value was 0.99. The inter-assay variability (different chip; different operators) was somewhat lower with R-squared value of 0.93. The expected variability with internal proficiency (different reagent lots; different months; different operators) was >0.8. Conclusions: This microarray assay has been a reliable and robust method to perform gene expression profiling on routine solid tumor samples, including FFPE, submitted for clinical molecular profiling.

1201T

Genome-wide transcriptional sequencing identifies novel mutations in metabolic genes in human hepatocellular carcinoma (HCC). *D. Meerzaman[1], C. Yan[2], B. Dunn[3], M. Edmonson[1], R. Finney[2], C. Cultraro[1], L. Dong[1], Z. Yang[1], Y. Hu[1], J. Kelley[1], H. Zhang[1], N. Park[4], K. Buetow[1,2].* 1) NCI/LPG, NIH, Bethesda, MD; 2) NCI/CBIIT, Rockville, MD; 3) NCI/DCP, Bethesda, MD; 4) University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea.

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer mortality in the world. Although the association of HCC with chronic liver disease is well established, the exact etiology of HCC remains undefined. Here, we report the results of next-generation transcriptome sequencing from three human hepatocellular carcinoma tumor/tumor-adjacent pairs. RNA sequencing was carried out using the Illumina Solexa Genome Analyzer generating approximately 240 million 75-mer reads for all six samples. Sequence reads were mapped to hg18 using BWA and Bambino, an in-house tool. Expression values were normalized with the RPKM method. We identified 4,513 up-regulated genes and 1,182 down-regulated genes in tumor compared to matched normal samples. This differential gene expression was further confirmed using a large data-set containing 434 normal liver and tumor samples. Network analysis of expression data identified the Aurora B signaling, FOXM1 transcription factor network and Wnt signaling pathways as altered in HCC. Our mutation analysis revealed 30 mutations that were validated using Sanger sequencing. In addition to known driver mutations such as TP53 and CTNNB1, our mutation analysis also identified six non-synonymous mutations in genes implicated in metabolic processes. Furthermore, five of these mutated genes are known to be involved in metabolic diseases such as diabetes and obesity: IRS1, HMGCS1, ATP8B1, PRMT6 and CLU. The data presented here strongly suggest that alteration of metabolic genes is highly associated with the development and progression of liver cancer and will provide further insight into etiology liver cancer.

1202T

Molecular Characterization of Adeno-Pituitary Adenoma: Familial Isolated Prolactinoma. *F.M. Melo, L. Bastos-Rodrigues, M.S. Sarquis, L. De Marco.* UFMG - Universidade Federal de Minas Gerais, Belo Horizonte, MG., Brazil.

Pituitary adenomas are benign neoplasms that arise from the expansion of single cells from the adeno-pituitary. These tumors comprise 10% to 15% of all intracranial tumors. The pathogenic mechanisms of these tumors can be either genetic or epigenetic changes, resulting in the deregulation of cell cycle, signaling defects or loss of tumor suppressor factors. The prolactinoma is the most frequent pituitary adenoma, and may cause infertility and amenorrhea-galactorrhea syndrome in females. However, familial cases are rare and poorly characterized. Therefore, the propose of this work is to perform molecular characterizations of a family that carries prolactinoma. For this, we surveyed mutations described in two tumor suppressor genes (AIP and MEN1). We also evaluated a specific mutation, p.Gly2019Ser, in the gene LRRK2 associated with the greater propensity of tumors in patients with Parkinson's disease. For this study, genomic DNA from two patients diagnosed with prolactinoma, and two healthy relatives were extracted from peripheral blood samples. Sequencing was performed for the six exons of AIP and the exon 41 of LRRK2. Experiments with MEN1 are underway. No mutations were detected in the exons 1, 3, 4 and 6 of the AIP gene. Exons 2 and 5 are in review process. Two polymorphisms (p.Gln165Lys, p.Gln307Pro) were identified in all samples, which demonstrates the reliability of the results with the data already published. Mutations in LRRK2 were not detected. Considering the related results, no correlations were detected between mutations in the selected genes and prolactinoma development. To complement this work we will apply, in the near future, exoma sequencing in order to identify other mutations that can be correlated to the development of prolactinomas in isolated familial cases.

1203T

Identifying women at increased risk of breast cancer: Can we use genotyping at low penetrance loci? *C. Merrick[1], J. Dunlop[2], L. Baker[1], E. Gellatly[1], A. Martin[3], P. Quinlan[1], R. Tavendale[1], A.M. Thompson[1], C. Palmer[1], M. Reis[4], J.N. Berg[1,3].* 1) University of Dundee, Ninewells Hospital, Dundee, DD1 9SY, United Kingdom; 2) East of Scotland Regional Genetics Unit, Ninewells Hospital, Dundee, DD1 9SY, United Kingdom; 3) NHS Tayside, Ninewells Hospital, Dundee, DD1 9SY, United Kingdom; 4) Tayside Breast Cancer Family Clinic, Ninewells Hospital, Dundee, DD1 9SY, United Kingdom.

Background: Most inherited predisposition to breast cancer is attributable to low penetrance susceptibility loci, a number of which have been identified through genome-wide association studies. Although individually each locus has a small effect, combining data from multiple loci would be expected to provide more risk information. We investigated the size of risk determination that can be achieved using genotyping at 18 loci. We then calculated its effect when combined with risk estimated from family history alone in terms of management under UK guidelines, where a woman who has a 10 year risk of 3% or greater requires additional breast screening from a younger age. **Methods**: Genotyping for 18 loci was carried out in 253 women at increased risk of breast cancer due to a positive family history and 118 matched controls. The relative risks conferred by genotype at the 18 loci were combined under a log-additive model and transformed into a log-polygenic risk. The BOADICEA risk estimation tool was used to calculate breast cancer risk due to family history. **Results**: Both the increased risk and control groups demonstrated a normal distribution of log-polygenic risk with similar variance. There was a significantly higher mean in the increased risk compared to the control group (mean = 0.1313 and 0.0874 respectively, P = 0.007). No significant correlation was found between polygenic risk calculated from genotype data and the family history risk estimated using BOADICEA. When polygenic risk was combined with family history risk there was significant reclassification of risk for those with a family history. 36.76% moved into a higher risk category while 3.68% moved into a lower risk category. **Conclusions**: Our data suggests that genotyping will be clinically relevant for estimating breast cancer risk. Individuals with a family history overall have a higher genotype risk than the population. The lack of correlation of genotype risk with BOADICEA risk suggests that the two risk estimates can be considered independently. By combining genotype with family history data, we demonstrated a significant reclassification of risk for individuals with a family history, with better identification of women in this group requiring intervention.

1204T

Understanding the role of TRIM8, a new p53 target gene that modulates p53 activity, in the progression of glioma. *L. Micale[1], M.F. Caratozzolo[2], C. Fusco[1], B. Augello[1], M.N. Loviglio[1], M.G. Turturo[1,5], G. Cotugno[1], T. Lopardo[3], F. Galli[3], S. Cornacchia[2], F. Marzano[2], A.M. D'Erchia[2], L. Guerrini[3], G. Pesole[4], E. Sbisà[3], A. Tullo[2], G. Merla[1].* 1) Medical Genetics Unit, IRCSS, Casa Sollievo della Sofferenza, San Giovanni Rotondo, Foggia, Italy; 2) Institute for Biomedical Technologies , ITB, Bari, Italy; 3) Department of Biomolecular Sciences and Biotechnologies, Milan; 4) 5Institute of Biomembranes and Bioenergetics, IBBE, Bari; 5) Telethon Institute of Genetics and Medicine, TIGEM, Napoli.

Impairment of p53 function has a crucial role in tumor evolution. p53 inactivation occurs through alteration of p53 function by mutations and deregulation of signaling pathways or effectors molecules that regulate p53 activity. The stabilization and activation of p53 are crucial in preventing cells from becoming cancerous. Thus, one of the most important challenges for the development of improved cancer therapies is the discovery of additional p53-inactivating pathways or p53 modulators. We describe a previously unknown function for TRIM8, as a new p53 target gene that modulates the p53-mediated tumour suppression mechanism. We showed that, under stress conditions, such as UV treatment, p53 induces the expression of TRIM8, which in turns interacts with and stabilizes p53. The stabilization of p53 was paralleled by an increase of Ser15 and Ser20 phosphorylated p53 level, a marker of the activation status of p53 for cell cycle arrest activity. Furthermore, overexpression of TRIM8 regulates the p53 transcriptional activity by increasing p21 and GADD45 mRNA levels, that result in inhibition of cell cycle progression. Concurrently, TRIM8 overexpression decreases MDM2 protein levels suggesting that TRIM8-mediated p53 stabilization occurs through MDM2 degradation. TRIM8 is highly expressed in human brain and it maps to 10q24.3, a region proposed to contain tumor suppressor genes involved in gliomas. By analyzing approximately 100 human glioma cell lines we showed that TRIM8 expression is strongly under expressed in these tumors, correlating with the tumor histological grade. Intriguingly, the restoring of TRIM8 expression level in U87 glioma cell line enhances the stabilization of endogenous p53 protein level and induces the suppression of cell proliferation, as revealed by western blot and MTT assays. Moreover we assessed the molecular mechanisms responsible for TRIM8 downregulation, revealing TRIM8 LOH and hypermethylation of CpG islands. Finally we found that in glioma samples TRIM8 mRNA level anti-correlates with miR-17 expression, a microRNA involved in glioma progression. Consistently, target prediction tools and luciferase assay showed that miR-17 targets 3'UTR-TRIM8, resulting in TRIM8 downregulation. Collectively, our observations suggest the existence of a new p53-TRIM8 feedback-loop mechanism and support the hypotheses that TRIM8 might participate to the development of gliomas through a molecular modulation of p53.

1205T

Functional Polymorphism in Promoter Survivin Gene and Risk of Urothelial Bladder Carcinoma in North Indian Population. *R.D. Mittal, P. Jaiswal, A. Mandhani, T. Mittal, R. Kapoor.* Urology & Renal Transplantation, Sanjay Gandhi PGI, Lucknow, Uttar Pradesh, India.

Survivin is a member of novel inhibitor of apoptosis protein family that is expressed in human cancers and not normal tissues. The molecular detection of bladder cancer by targeting of Survivin as a novel molecular marker may be useful as it plays an important role in the occurrence and progression of cancer. We examined whether -31G>C, -644T>C, -625G>C, -1547 A>G and -241C>T was related to the risk of developing bladder carcinoma (BC) in a case-control population from Northern India which consisted of 200 patients with BC and 200 healthy controls. This polymorphism was genotyped by Polymerase Chain Reaction- Restriction Fragment Length Polymorphism (PCR-RFLP). The genetic associations with the occurrence and progression of BC were estimated by logistic regression. We observed a statistically significant increased occurrence of BC risk associated with the variant CC genotype of Survivin-31G>C (P = 0.021; OR= 2.61; 95% CI (1.16-5.87).We also observed hetero genotype of Survivin -625G>C to be at risk (p=0.019), while variant allele GG demonstrated 1.6 fold increased BC risk. The variant genotype in other polymorphic site of Survivin (-1547 A>G) was significantly associated with BC risk (p=0.047). In case of Survivin-241C>T the risk genotype for BC risk was hetero (p=0.035). Furthermore, the haplotype analysis revealed that G-G-C-A-C haplotype had BC risk. Smoking significantly modulated the risk in patients with Survivin 1547-A>G polymorphism. A significant association was observed for the Survivin -644 T>C (p=0.012) and for Survivin- 625 G>C with GC+CC combination (p=0.003) and GC genotype (p=0.01) with the severity of BC (as measured by tumor-node-metastasis staging system). Variant as well as hetero genotype of Survivin-31G>C was associated with risk of recurrence (HR = 0.22 and 0.35) in superficial BC patients receiving BCG treatment thus showing least survival (log rank= 0.004).Our findings suggested that the functional polymorphism -31C>G,-625G>C,-1547A>G and -241C>T in the promoter of Survivin gene may play a significant role to intercede the susceptibility to BC in North Indian population Acknowledgement: Financial support from Council of Scientific and Industrial Research (CSIR) and Department of Science and Technology (DST).

1206T

CREB3 and STK11 show different gene expression profiles in benign and malignant salivary gland cancers. *N. Mohammadi Ghahhari[1], M. Kadivar[1], A.R. Kamyab[1], M.T. Khorsandi Ashtiani[2], H. Mohammadi Ghahhari[3].* 1) Department of Biochemistry,Pasteur Institute of Iran, Tehran, Iran; 2) 2. Otorhinolaryngology Research Center, Amir-Alam Hospital, Department of Otolaryngology-Head and Neck Surgery, Tehran University of Medical Sciences, Tehran, Iran; 3) 3. Department of Otorhinolaringology and Head & Neck Surgery, Imam Reza Hospital, Tabriz University of Medical Sciences, Tabriz, Iran.

Introduction:Salivary gland cancers (SGCs) are heterogeneous neoplasms showing various histopathological characteristics with uncertain prognosis for their patients. Unfortunately, because of their rarity, only few studies have been performed to investigate molecular mechanisms involved in their tumor formation and progression. In the present study, we aimed to evaluate the expression level of two genes including STK11 and CREB3 which are supposed to play roles in both pro- and anti-apoptotic pathways in the cell. Materials and methods:In the study, a total of 23 fresh tissue specimens were obtained from patients having SGCs comprising of 11 pleomorphic adenomas and one sample of each warthin's tumor (WT), benign adenomatous tumor (BAT), cystic parotid adenoma (CPA), low grade mucoepidermoid carcinoma (MEC), small cell carcinoma (SCC), clear cell carcinoma (CCC) and one with no remarkable diagnosis (Non Tumor), respectively. Five normal tissue samples (NR) were also included in the study. The mean age of patients was 34 and none of them underwent radiotherapy or chemotherapy before surgery. After RNA isolation and cDNA synthesis, we performed quantitative Real-Time PCR for all samples with specific primers. RNA polymerase-II gene was selected as an endogenous control. Subsequently, Real-Time PCR data was analyzed using the comparative threshold cycle ($\Delta\Delta$CT, ratio=2 -$\Delta\Delta$CT) and statistical methods. Results:Statistical analysis showed the CREB3 gene expression percent for NRs, PAs, WT, BAT, CPA, Not Tumor, MEC, SCC and CCC were calculated as 100.5±6.8, 101.7±7.5, 91.4±24.1, 85.6±3.1, 77.8±6.9, 105.2±3.5, 158.1±32.2, 114.1±21.7 and 114.7±7.7, respectively. Moreover, the gene expression percent for STK11 gene was 98±1.6 in normal tissues and 29.4±10.0 in PAs, 78.8±10.6 in WT, 785.3±74.7 in BAT, 10.9±8.6 in CPA, 62.7±16.9 in Not Tumor, no expression in MEC, 202.1±37.4 in SCC and 3.4±1.8 in CCC. Discussion:To our knowledge, this is the first report that the expression levels of STK11 and CREB3 have been quantified in benign and malignant SGCs using qReal-Time PCR method. Based on tumor suppressor role for CREB3 gene, we may conclude that all tumors show it by having small changes in gene expression level. STK11 gene has responsibilities as tumor suppressor and oncogene in the cell. Therefore, in PAs, WT, CPA, Not Tumor, MEC and CCC it has partially lost its tumor suppression role. However, it indicates very strong oncogenic function in BAT and SCC.

1207T

Systematic Meta-Analysis for Common Low Penetrance Genes in Colorectal Cancer. *Z. Montazeri[1], E. Theodoratou[2], J. Little[1], H. Campbell[2].* 1) Epidemiology and Community Med, Ottawa University, Ottawa, Canada; 2) centre for Population Health Science, The University of Edinburgh, UK.

To identify genetic variance influencing colorectal cancer (CRC), we conducted systematic reviews and meta-analysis for a set of genes which were identified in genome wide association studies (GWAS). To date, 15 common genetic variants influencing risk of CRC have been reported from GWAS for colorectal cancer. We identified and extracted data from published studies up to 31 March 2011. In addition, unpublished data from the Scotland GWAS (SOCCS) and/or Ontario GWAS (ARCTIC) were included in the analyses. We carried out meta-analysis to derive summary effect estimates for 18 polymorphisms in 15 genes; and obtained summary crude odds ratios and 95% CI for recessive, additive and one dominant model for variants that were identified from GWAS. We applied either the fixed effect model or in the case of heterogeneity the random effect model. Heterogeneity was quantified by calculating the Q statistic; we also calculated the I^2 heterogeneity metric. The statistical power of each meta-analysis was estimated. In assessing the credibility of genetic association, we consider the Venice criteria and the Bayesian False Discovery Probability (BFDP). We classified the genetic association in 3 categories as positive, less credible positive and negative. Furthermore, we applied the model free meta-analysis approach for those SNPs that were identified as “positive”. Two SNPs at 8q24 locus showed positive association irrespective of genetic model considered (OR: 1.45; 95% CI (1.39-1.51) for rs6983267 and OR: 1.32; 95%CI: (1.22-1.42) for rs10505477) which has been found to be associated with other types of cancer; whereas SMAD7 was inversely associated with CRC with OR: 0.75; 95%CI: (0.71, 0.79). The associations for other variants were also observed and categorized.

1208T

Genomic Copy Number Alterations in Renal Carcinoma: Associations with Case Characteristics and VHL Gene Inactivation. *L.E. Moore[1], E. Jaeger[2], M.L. Nickerson[3], P. Brennan[4], S. Devries[2], R. Roy[2], H. Li[5], D. Zaridze[6], V. Janout[7], V. Bencko[8], M. Navratilova[9], N. Szeszenia-Dabrowska[10], D. Mates[11], W.M. Linehan[12], M. Merino[13], J. Simko[2], R. Pfeiffer[1], P. Boffetta[14,15], W.H. Chow[1], N. Rothman[1], F.W. Waldman[2].* 1) Cancer Epidemiology and Geneti, U.S.National Cancer Institute, NIH, DHHS, Bethesda, MD; 2) University of California, San Francisco San Francisco, CA Comprehensive Cancer Center; 3) Cancer Inflammation Frogram, National Cancer Institute, Frederick, Maryland; 4) International Agency for Research on Cancer, Lyon, France; 5) Division of Biostatistics, Dept of Environmental Medicine, New York University, NY, NY; 6) Institute of Carcinogenesis, Cancer Research Centre, Moscow, Russia; 7) Department of Preventative Medicine, Faculty of Medicine, Palacky University, Olomouc, Czech Republic; 8) Institute of Hygiene and Epidemiology, Charles University, Prague, Czech Republic; 9) Department of Cancer Epidemioogy and Genetics, Masaryk Memorial Cancer Institute, Brno, Czech Republic; 10) Department of Epidemiology, Institue of Occupational Medicine, Lodz, Poland; 11) Institute of Public Health, Buycharest, Romania; 12) Urologic Oncology Branch, National Cancer Institute,National Cancer Institute, Bethesda, MD; 13) Support to the Division of Cancer Epipdemiology and Genetics, NCI, SAIC-Frederick, Inc, Frederick MD; 14) The Tisch Cancer Institue, Mount Sinai School of Medicine, New York, NY; 15) International Cancer Prevention Research Institute, Lyon, France.

About 90% of adult kidney cancers are renal cell carcinoma (RCC), the majority of which are clear cell adenocarcina (ccRCC) which can be further classified by genomic profiling. Comparative genomic hybridization was used to identify alterations in tumors from patients enrolled in a large case-control study of RCC in Central Europe. Associations with histopathology, occupational exposures, and genetic risk factors were evaluated based on previously observed associations. Of 1097 cases in the final study, 524 RCC cases (48%) diagnosed from hospital reports provided frozen tumor biopsies. Of these, 415 biopsies were confirmed with ccRCC. Tumors were macrodissected, DNA extracted. Scanning and OncoBAC arrays were comprised of 2464 BACs selected at approximately megabase intervals along the genome, printed in quadruplicate. Multivariate regression and polynomial models were used to identify associations between chromosomal regions and patient/tumor characteristics. The most frequent chromosome alterations were: 3p-(92.2%), 14q-(46.5%), 8p-(38.1), 4q-(35.4%), 9q-(32.2%), 9p(31.6%)-, 6q(30.6%)-, 3q-(29.2%), 10q-(25.8%), 13q-(24.3%), 1p-(23.6%) and 5q+(60%), 7q+(39.5%), 7p+(30.6%), 5p+(26.3%), 20q+(25.3%), 12q+(24.6%), 12p+(22.7%).In multivariate analyses, only VHL inactivation [through promoter hypermethylation and sequence alteration] was associated with 3p- (OR=1250(95% CI:476-3125) compared to VHL wild type cases. The frequency of clonal losses differed significantly between VHL wild type, hypermethylated and genetically altered cases at regions on 3p, distal and proximal to the FHIT gene locus (3p14.3). Grade was associated with 1p-, 4q-, 6q-, 8p-, 9p-, 9q-, 13q-, 14q-, and 5p+, 7p+, 7q+, 12q+. Stage was associated with 1p-, 9p-, 9q-, 10q-, 13q- and 12p+, 12q+, 20q+. Males had significantly more alterations compared to females on 3q-, 9p-, 9q-, 13q-, 14q- and 7q+, 12p+, 12q+, and 20q+ independently of stage or grade. Cases without a family history of cancer had significantly more alterations and breakpoints compared to those that did. This is the first study of its size to conduct multivariate analyses of chromosomal alterations with detailed information on patient/tumor characteristics and risk factors. We identified case heterogeneity by loss of clones on 3p that varied by the presence and mechanism of VHL gene inactivation. They also suggest that male cases may be more genetically instable than female cases, and may be more aggressive.

1209T

Evaluation of circulating tumor DNA as marker for decease progression in breast and prostate cancer. *M. Neiman[1,2], J. Lindberg[1,2], D. Klevebring[1,2], T. Nordström[1,3], L. Eriksson[1], L. Nygård[4], F. Celebioglu[4], K. Czene[1], P. Hall[1], H. Grönberg[1].* 1) Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Solna, Sweden; 2) Science for life laboratory, Stockholm, Sweden; 3) Danderyds Hospital, Danderyd, Sweden; 4) Stockholm South General Hospital, Stockholm, Sweden.

In the western world, breast and prostate cancer are the most common forms of cancer in women and men respectively. Of all reported cancer cases in men in Sweden 2009, 35.7 % were prostate cancer and for breast cancer in women the corresponding figure was 28.7 % . One of the biggest challenges in cancer treatment is monitoring disease progression in diagnosed patients such as tumor aggressiveness and treatment response. In cancer, circulating tumor miRNA and DNA have been correlated to both early detection and prognosis. Since the half-life of circulating DNA in blood is less than 30 min, it has been successfully used to monitor treatment outcome. Structural rearrangements in the tumor genome can be identified through whole genome sequencing. The breakpoints of these rearrangements can be used targets for a qPCR assay making it possible to detect single tumor DNA molecules in milliliters of plasma. When dissecting heterogeneous tumors, such as prostate or breast cancer tumors, it is difficult to get material consisting of more than 70% tumor cells, which is desired for tumor sequencing. To circumvent this issue, we use Laser Capture Microdissection (LCM) prior to DNA extraction. The amount of DNA that can be extracted from microdissected tumor tissue is however limited. In order to be able to perform Illumina sequencing from these small amounts of DNA it is not possible to use the standard sample preparation protocols. We have developed an alternative sample preparation protocol making it possible to prepare sequence ready libraries from minute amounts of DNA. The yield is around 30 times higher than when using the standard protocol. Using this new protocol we are able to sequence DNA from microdissected material and fine-needle biopsies. We have collected plasma and tumor tissue from prostate and breast cancer patients that underwent surgery at Karolinska Univeristy Hospital and Stockholm South General Hospital respectively. From each patient, whole genome sequencing is carried out on both tumor and germline DNA to identify tumor-specific rearrangements which can be monitored using individualized qPCR assays. Tumor DNA amounts are measured in plasma samples collected at multiple time points during treatment.

1210T

Molecular profiling of inherited colorectal cancer syndromes by genomic analysis of normal tissue. *D. Neklason[1,2], B. Milash[1], L. Frey[1,3], M. Done[1], N. Sargent[1], T. Berry[1], T. Tuohy[1], R. Burt[1,4].* 1) Huntsman Cancer Inst, Univ Utah, Salt Lake City, UT; 2) Dept of Oncological Sciences, Univ Utah, Salt Lake City, UT; 3) Dept of Biomedical Informatics, Univ Utah, Salt Lake City, UT; 4) Dept of Medicine, Univ Utah, Salt Lake City, UT.

Current technologies are only able to provide genetic diagnosis on 20 to 90% of index cases depending on the gene and syndrome. We hypothesize that a diagnostic test can be developed based on gene expression in normal tissue from the end organ tissue of a disease. The mRNA from the mutated gene may be unchanged in many cases, but the downstream effects due to a nonfunctional protein may have a detectable readout. A diagnostic test is under development for the inherited colorectal cancer syndromes based on a genome-wide analysis of mRNA expression in normal colonic epithelial biopsies obtained during endoscopy. The utility of this method is to properly classify patients where genetic diagnosis is incomplete and suggest molecular pathways that are shared and differentiate the syndromes. RNA was extracted from fresh colonic biopsies. Agilent 44K whole genome microarrays were run on 7-10 normal colon tissue samples from control, FAP, AFAP, Cowden syndrome, hyperplastic polyposis (HPP), and HNPCC patients. Microarray data were normalized and log-transformed for analysis by four methods. We applied a SAM-like method using GeneSifter software with a t-test. Diagnostic genes for each syndrome were selected based on p-value and relative expression level compared to control. Differentially regulated genes were also selected using a rank product analysis. Paired genes that create a distinct convex hull, relative to either controls or all other syndromes, were identified and selected for maximal separation between the groupings. Finally, classification models were used to create decision tree classifiers with area under the ROC curve to separate the syndromes. Gene transcripts selected by each of these methods were confirmed by RTqPCR. The mRNAs from causative genes are decreased in most normal colonic tissues from Cowden syndrome (PTEN), HNPCC (MLH1), and a small subset of AFAP and FAP (APC) patients, presumably through nonsense mediated decay. Cowden syndrome can be diagnosed by differential expression of CLIC6, MFAP5, and possibly ADRA1. HNPCC has been initially characterized by expression of KIAA1804 and KIT. For AFAP and FAP, molecular diagnosis will likely involve multiple markers, including TTC12, NKD1, ANPEP. These initial results indicate that molecular differences can be identified in normal tissue from individuals harboring a single genetic mutation. This strategy offers promise as a diagnostic tool, especially when conventional genetic diagnosis fails.

1211T

Replication of susceptibility loci for lung cancer in a French Canadian population. *J.D.U. Nguyen[1], M. Lamontagne[1], C. Couture[1], M. Laviolette[1], Y. Bossé[2], The Merck-Laval-UBC-Groningen Lung eQTL consortium.* 1) Centre de recherche Institut universitaire de cardiologie et de pneumologie de Québec, Laval University, Quebec, Canada; 2) Department of Molecular Medicine, Laval University, Quebec, Canada.

Recent genome-wide association studies (GWAS) have identified three genetic loci reproducibly associated with lung cancer: 15q25, 5p15 and 6p21. The aim of this study was first to test whether these loci are also associated with lung cancer in a French Canadian population and then to identify the causal genes in these regions using lung expression quantitative trait loci (eQTL) data. More than a million single-nucleotide polymorphisms (SNP) were genotyped in 420 patients undergoing lung cancer surgery. Genetic association tests were restricted to SNPs previously associated with lung cancer on chromosomes 15q25, 5p15, and 6p21. The allele frequencies of seven uncorrelated SNPs were compared to a control group of 3,151 individuals of European ancestry. Genome-wide gene expression profiles of 409 non-tumor lung tissues from the same lung cancer patients were measured to identify eQTLs. SNPs rs8034191 and rs8042374 on chromosome 15q25 were significantly associated with lung cancer (p = 2.91E-6 and 0.006, respectively). SNP rs2736100 on chromosome 5p15 was specifically associated with a histological subtype of cancer, adenocarcinoma (p = 0.004). The single SNP (rs3131379) tested on chromosome 6p21 was also associated with lung cancer (p = 0.032). Genetic variants that were associated with lung cancer on 15q25 were also associated with the expression levels of the gene CHRNA5 located in the same chromosomal region (p = 2.23E-22 for the strongest eQTL). This study confirms the three susceptibility loci for lung cancer in a French Canadian population and also suggests that the risk alleles on chromosome 15q25 confer susceptibility for lung cancer by modulating the expression levels of CHRNA5 in the lungs.

1212T

Early insights from whole-exome analysis of early-onset, multiple-case breast cancer pedigrees. *T. Nguyen-Dumont[1], D.J. Park[1], F. Odefrey[1], F. Hammet[1], Z.L. Teo[1], D.E. Goldgar[2], S.V. Tavtigian[3], B.J. Pope[4], A. Lonie[4], M.C. Southey[1], BCFR, BRCAX Consortium.* 1) Genetic Epidemiology Laboratory, Department of Pathology, The University of Melbourne, Victoria, Australia; 2) Department of Dermatology, University of Utah School of Medicine, Salt Lake City, Utah, USA; 3) Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah School of Medicine, Salt Lake City, Utah, USA; 4) Victorian Life Sciences Computation Initiative, Victoria, Australia.

Germline mutations in known susceptibility genes explain a relatively small proportion of the heritable risk for breast cancer (BC) (30-35%). In this study, we aim to identify genes in which variants could account for the majority of the unexplained residual familial aggregation of BC. We are applying whole-exome capture followed by massive parallel sequencing (XC-MPS) to analyze highly selected, early-onset, multiple-case BC pedigrees from international resources, which have been previously screened for mutations in known BC susceptibility genes. We have used the SeqCap EZ Human Exome Library v2.0 kit (Nimblegen), and the SOLiD4 platform (Life Technologies) to sequence coding regions and proximal intronic splice consensus sequences in pairs of affected young women more distantly related than first cousins, within each pedigree. The exomes of 20 individuals from 10 families have thus far been analyzed. We have developed an in-house bioinformatics analysis pipeline based on peer-recognized Bioscope, SAMtools, Picard, GATK, BEDtools and IGV software, and the human genome reference hg19. Our initial analysis strategy filters out variants listed in dbSNP131, and filters for SIFT-predicted damaging variants. We have developed novel data filtering approaches to markedly increase specificity over conventional pipelines, without compromising sensitivity. The great majority (86%) of candidate genetic variants identified through our XC-MPS pipeline prove to be genuine via Sanger sequencing. We have identified rare, predicted protein damaging variants in 168 genes with key roles in disease-relevant pathways such as DNA repair and replication, cell cycle checkpoints, centromere function, telomere maintenance, chromosome stability and estrogen signaling. Despite observing 6 core DNA repair genes exhibiting a protein-truncating mutation or disruption to a core splice site consensus sequence, we have not, to date, observed such a potentially “catastrophic variant” to be shared by both analyzed members of the same pedigree. XC-MPS has revealed the extensive genetic variation present in human exomes. The proportion of variation with plausible relevance to disease predisposition is considerable and further work is required to characterize the true BC predisposition genes. Our findings suggest that a substantial proportion of the currently unexplained familial risk for BC might be explained by combinations of rare genetic variants that together confer high risks for BC.

1213T

Diagnostic improvement of thyroid nodules cytology by determination of three genes expression profile. *V. Novik[1,2], G. Molina[3,4], A. Vasquez[4], R. Ceriani[4], M. Fuentes[4], C. Weisntein[4], C. Henríquez-Roldán[5], G. Arístides[6], D. Navarrete[1], F. Brusco[7], G. Lezana[7], A. Lobos[8,9].* 1) Departamento de Medicina. Escuela de Medicina, Universidad de Valparaíso, Valparaiso, Región de Valparaíso, Chile; 2) Facultad de Medicina. Universidad Nacional Andres Bello. Sede Viña del Mar; 3) Unidad de Genética Forense, Servicio Médico Legal de Valparaíso, Valparaíso, Chile; 4) Facultad de Farmacia. Universidad de Valparaíso; 5) Centro de Estudios Estadísticos. Universidad de Valparaíso; 6) Hospital Eduardo Pereira, Valparaíso; 7) Hospital Naval Almirante Nef. Viña del Mar; 8) Hospital Gustavo Fricke. Viña del Mar; 9) Departamento de Cirugia, Universidad de Valparaíso.

Thyroid cancer represents only 1% of total amount of cancer. However, thyroid nodules are frequent. 5%-10% of the total population will develop a thyroid nodule during they life. Most of these will be benign. Currently, fine-needle aspiration biopsy (FNAB), represents the most important initial test for diagnosing malignancy. The result of the FNAB cytology can be classified as benign (70% of cases), malignant (5% to 10%), indeterminate or suspicious (10% to 20%), or nondiagnostic (10% to 15%). Although nondiagnostic FNABs can be repeated, the indeterminate or suspicious group presents a dilemma for the clinician. Patients with a non diagnostic FNAB must undergo a thyroidectomy with a diagnosis objective. MET, FN1 and TPO genes have been determined as consistent differential gene expression markers between malignant and non-malignant tissue in thyroid nodules. These candidates should help to develop a panel of markers with sufficient sensitivity and specificity for the diagnosis of thyroid tumors in a clinical setting. Twelve preoperative samples of FNAB from patients with a thyroid nodule, were obtained. All patients were undergoing to a thyroidectomy with a diagnostic or therapeutic objective. An informed consent were accepted and signed by the patients. The expression of MET, FN1 and TPO genes were determined by quantitative real time PCR (Fret probe, ROCHE), using G6PDH to normalize. A general index including the expression of the three genes were determined using main components analysis. The index was compared with the histopathology of the surgery biopsy (Gold Standard). A sensitivity of 100% and a specificity of 71,4% was obtained for malignancy state. The pre-operatory diagnosis could be improved in a 36%. The thyroidectomies with diagnosis objective in these group of patients was 41,7%. These unnecessary surgeries could be avoided using molecular genetics results in the cytology of thyroid nodules. The molecular diagnosis with this 3 gene model might be a reliable test to preoperatively diagnose the malignant potential of thyroid nodules. FONIS SA07I20066.

1214T

Li-Fraumeni syndrome: retesting archival DNA samples identifies previously unrecognized germline *p53* mutations. *A. Novokmet[1,2], B. Baskin[4], P. Ray[2,3], D. Malkin[1,2].* 1) Hematology/Oncology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 3) Molecular Genetics, The Hospital for Sick Children, Toronto, Ontario, Canada; 4) Clinical Genetics, Uppsala University Hospital, Uppsala, Sweden.

Background: Li-Fraumeni syndrome (LFS) is a familial cancer predisposition syndrome that results from germline alterations in the *p53* gene. Unlike other cancer predisposition syndromes, LFS predisposes to a wide range of cancers including premenopausal breast cancer, sarcomas, brain tumors, adrenocortical carcinomas, and others. Over the past 20 years, the criteria for LFS have expanded beyond the classical definition, resulting in an increased number of individuals being tested. Additionally, technological advances have facilitated discovery of previously occult alterations. Upon review of samples received by our laboratory for *p53* analysis between 1991 and 2004, we identified 71 samples for retesting. **Methods:** We queried our extensive Cancer Genetics database, containing more than 4100 individuals, for banked blood-derived DNA samples that had undergone *p53* mutation analysis prior to April, 2004 and were found not to have an alteration. Diagnosis and/or family history were confirmed to meet the classical, Birch, or Chompret criteria for LFS. Seventy-seven patient samples were retrieved and of those, 71 were of sufficient quality and quantity for analysis. The samples were submitted to the Hospital for Sick Children's Molecular Genetics Laboratory for analysis. **Results:** Of the 71 samples submitted for *p53* analysis, 8/71 were found to harbor a *p53* germline alteration (Cys229Arg, Val157Ile, Tyr236X (n=2), Thr125Thr, Arg213Gln (n=2), and deletion exons 10-11), 1/71 was found to be wildtype, and the remaining 62/71 were found to harbor a presumed non-disease-causing codon 72 polymorphism (Arg72-Arg, Pro72Arg). Three of the five alterations (Cys229Arg, Val157Ile, Tyr236X) have previously been identified in somatic tissues (http://www-p53.iarc.fr); however, this is the first report of their occurrence in the germline. **Conclusions:** Advances in technology have allowed detection of a higher frequency of *p53* alterations and have facilitated the identification of a *p53* alteration in eight individuals who were previously found to be wildtype. Positive findings are important for affected individuals with respect to risk of development of second malignancies and for other at-risk family members, given an overall lifetime cancer risk of 85% in individuals with LFS. These findings stress the importance of banking DNA for possible future retesting in anticipation of advances in technology.

1215T

Expression of human endogenous retroviruses in childhood acute leukemia cells. *J. Nowak[1], K. Nowicka[1], J. Rembowska[1], D. Januszkiewicz[1,2].* 1) Inst Human Gen, Polish Academy Sci, Poznan, Poland; 2) Department of Paediatric Oncology, Hematology and Transplantology Poznan University of Medical Sciences, Poznan, Poland.

Human endogenous retroviruses sequences(HERVs) constitue 8% of the whole human genome. The complete structure of HERVs contains gag, pol, env and primer binding site flanked by Long Terminal repeats (LTRs). The majority of HERVs are not complete and its individual sequences are spread throughout the genome often in more than one copy. The majority of HERVs are defective due to mutations, deletions and insertions. There are reports indicating the participation of HERVs in etiopathogenesis of autoimmune diseases and cancer. In order to learn the importance of HERVs in leukemogenesis we investigated the expression of gag, pol and env genes of HERV K, H, W and E in acute leukemias in children. RNA was isolated from PBL of 120 acute lymphoblastic leukemia (ALL) patients, 40 acute myeloblastic leukemia children and 20 healthy individuals. HERVs gene expression was studied by real-time PCR and the results were normalized to reference gene expression and to expression of all studied genes in PBL. In ALL the high expression of gag, pol and env only of HERV-K has been found. The expression of other studied HERVs in ALL was similar to that in PBL. In AML cases, among studied HERVs, the expression of HERV-E differs significantly in comparison to ALL and PBL. Preferential gag, pol and env expression of HERV-K in ALL and HERW-E in AML strongly suggests the contributions of these HERVs in pathogenesis of acute leukemias in children. Determination of specific role of HERVs in leukemias warrants futher investigations. The work has been sponsored by Ministry of Science and Education grant No 401195339.

1216T

Detecting copy number aberrations in tumours using SNP array technology: A comparison of several available methods. *K. Oros Klein[1], S. Arcand[2], A. Birch[3], D. Provencher[4,5], J. Squire[6], A.M. Mes-Masson[4,5], P.N. Tonin[2,3,7], C.M.T Greenwood[1,8,9].* 1) Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, QC; 2) The Research Institute of the McGill University Health Centre, Montreal, QC; 3) Department of Human Genetics, McGill University, Montreal, QC; 4) Centre de recherche du Centre hospitalier de l'Université de Montréal, Montreal, QC; 5) Institut du cancer de Montréal, Montreal, QC; 6) : Department of Pathology and Molecular Medicine, Queen's University, Kingston, ON; 7) Department of Medicine, McGill University Montreal, QC; 8) Department of Oncology, McGill University, Montreal, QC; 9) Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, QC.

In a well-defined set of ovarian cancer tumour samples and ovarian cancer cell lines of the serous histopathological subtype, we have compared the performance of several methods for inferring copy number aberrations (CNAs) from high-density SNP data derived using the Illumina 610-Quad BeadChip array. Inference about CNAs in tumours and cell lines is made more difficult by (a) the presence of normal tissue mixed with tumour tissue, (b) clonal variation between cells in the same tumour, and (c) aneuploidy. Analysis methods have evolved with time to allow for several of these factors. We compared the performance of 4 algorithms, dChip (Zhao et al. 2004, Cancer Res), GenoCNA (Sun et al. 2009, Nucleic Acids Res), GAP (Popova et al., 2009, Genome Biol) and OncoSNP (Yau et al. 2010, Genome Biol), where each of these methods bases inferences of CNAs on both the total intensity and the balance of the signals derived from the two alleles for each SNP marker. We have analyzed serous ovarian tumours representing differences in disease from benign and low malignant potential (LMP) tumours to the more aggressive high grade carcinomas, several well characterized ovarian cancer cell lines, as well as matched blood samples from some patients. Spectral Karyotyping (SKY) analysis is available for the cell lines to validate inferences. As expected there appeared to be an increase in CNVs with more aggressive disease, as has been reported by other groups. We find that for highly-rearranged genomes, that OncoSNP and GAP accurately identified changes in ploidy and regions with copy number gains and losses, although the exact copy number appears to be difficult to estimate. However, for genomes from benign or LMP tumours, we found that these two algorithms called far too many alterations than were expected for these tumour types which are known to exhibit either no or modest levels of chromosomal instability. For all algorithms, the inference about loss of heterozygosity appeared unstable. Although algorithms for CNA inference are rapidly evolving, there is still room for further improvement.

1217T

Circulating MicroRNAs expression profiling to identify reference genes for relative quantification in Acute Lymphoblastic Leukemia (ALL) patients. *R. ORTIZ-LOPEZ[1,2], M. LUNA-AGUIRRE[1], I. GARZA-VELOZ[1], V. TREVIÑO-ALVARADO[6], F. MAR-AGUILAR[4], H. GUTIERREZ-AGUIRRE[3], O. GONZALEZ-LLANO[3], R. SALAZAR-RIOJAS[3], G.I. MALAGÓN-SANTIAGO[4], A. ROJAS-MARTÍNEZ[1,2], A. HIDALGO-MIRANDA[5], C. JAIME-PEREZ[3], D. GÓMEZ-ALMAGUER[3], H. MARTÍNEZ-RODRIGUEZ[1].* 1) Departamento de Bioquímica y Medicina Molecular. Facultad de Medicina. Universidad Autónoma de Nuevo León (UANL). Monterrey, N.L. - MÉXICO; 2) Centro de Investigación y Desarrollo en Ciencias de la Salud. UANL. Monterrey, N.L. - MEXICO,; 3) Laboratorio de Medicina Molecular. Servicio de Hematologia. Hospital Universitario (UANL). Monterrey, N.L. MÉXICO; 4) Departamento de Biología Celular y Genética, Facultad de Ciencias Biológicas, UANL, San Nicolás de los Garza, N.L. MÉXICO; 5) Instituto Nacional de Medicina Genómica, México D.F; 6) Departamento de Ciencias Computacionales, ITESM, Campus Monterrey, N.L. MÉXICO.

INTRODUCTION: MicroRNAs (miRNAs) are an important class of gene regulators, acting on several aspects of cellular function such as differentiation, proliferation, apoptosis, etc. Circulating miRNAs were found to exist in plasma in a highly stable form, and aberrantly expressed in many human diseases. The expression levels of circulating miRNAs are estimated by quantitative real-time polymerase chain reaction (RT-qPCR), however, the accuracy of the results is largely dependent on proper data normalization. Despite increasing miRNA expression studies in leukemia, specifically ALL-B, no previous report identification suitable reference genes for normalization in plasma samples have been reported. In this study we identify the most stable reference microRNAs using a high-throughput approach in ALL-B plasma samples. The candidate references miRNAs were validated using miR-223 as the target miRNA, which has been previously reported as down expressed in ALL. METHODS: Plasma samples from 32 ALL-B patients and 5 healthy controls were analyzed. RNA was isolated and miRNAs profiling was determined in 13 ALL-B patients and 5 controls by qRT-PCR TaqMan Low Density Array and data were analyzed. The most stably expressed miRNAs were obtained with the mean expression value of all miRNAs and identify those that resemble the mean. These candidate miRNAs were validated by qRT-PCR SyberGreen analysis in the plasma of 32 ALL-B patients and 5 controls and the relative expression of miR-223 was determined. RESULTS: We selected 3 miRNAs (miR-101, miR-106a and miR-26b) that were most similar to the mean expression and were the most stable based in the geNorm algorithm. We evaluated these candidate normalizers and obtained low stability values of the individual genes indicating great gene stability. Similarity index of expression across the sample set was 97% and presented significance positive correlation. We obtained a statistic significance difference between all ALL-B samples and controls for the miR-223 expression. CONCLUSION: In previous studies, miRNA expression was estimated by relative quantification using U6, RNU44, 18S and miR-16 as internal controls, however they are not miRNAs, except miR-16, and may not be representative of the miRNA fraction. Our study identified suitable reference genes for qRT-PCR analysis of circulating miRNAs in ALL-B patients. For this study, the best combination of stably expressed genes was miR-101, miR-106a and 26b in ALL-B samples.

1218T

Methylation profile analysis of DNA repair genes in hepatocellular carcinoma with MS-MLPA. *O. Ozer[1], B. Bilezikci[2], S. Aktas[3], F.I. Sahin[1].* 1) Department of Medical Genetics, Baskent University Faculty of Medicine, Ankara, Turkey; 2) Department of Pathology, Baskent University Faculty of Medicine, Ankara, Turkey; 3) Department of General Surgery, Baskent University Faculty of Medicine, Ankara, Turkey.

Hepatocellular carcinoma (HCC) is one of the rare tumours with well-defined major risk factors. Frequently seen factors include environmental agents like Hepatitis B and C, alcohol, aflatoxins and other etiological agents. The multifactorial etiology of HCC can be explained by its complex molecular pathogenesis. A wide range of genetic changes; including gene expression changes, mutations, and loss of heterozygosity and promoter hypermethylation; has been reported in HCC. Methylation specific - multiplex ligation dependent probe amplification (MS-MLPA) is a newly developed diagnostic tool which enables to detect methylation profiles of multiple genes at the same reaction. In a previous study, reduced expression of DNA repair genes has been reported in HCC samples but the mechanism was not explained. In the current study, methylation status of seven genes involved in DNA repair mechanisms, namely MLH1, PMS2, MSH6, MSH2, MGMT, MSH3 and MLH3, was investigated in formalin fixed paraffin embedded tumour tissue samples from HCC patients, using the MS-MLPA method and the results were correlated with available clinical findings. The most common etiological factor in these cases was the presence of hepatitis B alone (47.2%). Among the 56 cases that were studied, promoter methylation was detected in at least one of the genes in 27 (48.2%) cases, only in one gene in 13 (23.2%) cases and in more than one gene in 14 (25%) cases. Methylation was detected in all of the genes studied only in one (1.8%) patient. Of the seven genes investigated, methylation was the most frequently observed in MSH3, in 14 (25%) cases. Methylation of at least one gene was significantly more frequent in patients with single tumours than multifocal tumours. There were significant differences regarding hepatitis B status, Child class, tumour number, grade and TNM stage in cases where PMS2 methylation was detected. To our knowledge, this is the first study investigating the methylation profile of seven DNA repair genes in HCC samples. Our results suggest that methylation of genes involved in mismatch repair may be responsible in the pathogenesis of HCC. Evaluating changes in multiple genes in these pathways simultaneously would be more informative. Despite being a robust and relatively inexpensive method, the MS-MLPA assay could be more extensively applied with improvements in the currently intricate data analysis component.

1219T

No evidence that *FAN1 R377W* or *R507H*, identified via exome sequencing in multiple-case, early-onset breast cancer families, influence breast cancer risk. *D.J. Park[1], F. Odefrey[1], F. Hammet[1], G.G. Giles[2], L. Baglietto[2], J.L. Hopper[3], D.F. Schmidt[3], E. Makalic[3], O.M. Sinilnikova[4], D.E. Goldgar[5], M.C. Southey[1], ABCFS, MCCS.* 1) Genetic Epidemiology Laboratory, Department of Pathology, The University of Melbourne, Melbourne, Victoria, Australia; 2) Cancer Epidemiology Centre, The Cancer Council Victoria, Melbourne, Victoria, Australia; 3) Centre for Molecular Environmental Genetic and Analytical Epidemiology, School of Population Health, The University of Melbourne, Carlton, Victoria, Australia; 4) Unité Mixte de Génétique Constitutionnelle des Cancers Fréquents, Hospices Civils de Lyon/Centre Léon Bérard, Université de Lyon, Lyon, France; 5) Department of Dermatology, University of Utah School of Medicine, Salt Lake City, Utah, USA.

Massively parallel sequencing is increasingly being applied to the study of complex human disease. Our primary interest is to characterise previously undescribed contributions to the heritable component of human cancers. To this end, we applied whole-exome capture followed by massively parallel sequence analysis to the germline DNA of greater than third-degree affected relatives from multiple-case, early-onset breast cancer families. Prior testing for variants in known breast cancer susceptibility genes in these families failed to identify causal mutations. After analysing four of these pedigrees, we identified two different variants in the DNA damage repair gene *FAN1* (*R377W*, chr15:31197995 C>T and *R507H*, chr15:31202961 G>A [hg19]) which were not present in dbSNP131. *FAN1 R377W*, predicted to be damaging by SIFT and PolyPhen2, was present in all six tested members of the first family with cancer (five with breast cancer, one with malignant melanoma). *FAN1 R507H*, predicted to be damaging by SIFT but benign by PolyPhen2, was observed in one of two tested members of the second family with breast cancer. We genotyped *FAN1 R377W* and *R507H* sites across 1417 population-based cases and 1490 unaffected population-based controls (frequency-matched for age). These variants were rare in the Australian population (minor allele frequencies of 0.0064 and 0.010, respectively) and were not associated with breast cancer risk (OR=0.80, 95% CI[0.39-1.61], p=0.50 and OR=0.74, 95% CI[0.41-1.29], p=0.26, respectively). Analysis of breast cancer risks for relatives of case and control carriers did not find evidence of an increased risk. Despite the biological role of *FAN1*, the plausibility of its role as a breast cancer predisposition gene, and the possible deleterious nature of the identified variants, these two variants do not appear to be causal for breast cancer. More extensive genetic analysis of *FAN1* in future studies will further explore its possible role as a breast cancer susceptibility gene.

1220T

Analysis of *IKZF1* splicing variants, and expression of *CASP8AP2* and *H2AFZ* in childhood acute lymphoblastic leukemia. *P. Perez-Vera[1], R. Juarez-Velazquez[1], A. Reyes-Leon[1], C. Salas[1], A. Medrano[1], R. Paredes[2], R. Cardenas[2], G. Lopez-Hernandez[3], P. Navarrete[1], A. Lopez[4], A. Carnevale[5], R. Ortiz[6].* 1) Human Genetics Dept, Instituto Nacional de Pediatria, Mexico, D.F; 2) Hemato-Oncology Dept, Instituto Nacional de Pediatría, Mexico, D.F; 3) Hematology Dept, Hospital del Niño Poblano, Puebla; 4) Gastroenterology Dept, INCMNSZ, Mexico, D.F; 5) INMEGEN, Mexico, D.F; 6) Universidad Autonoma Metropolitana-Iztapalapa, Mexico, D.F.

Treatment improvement in childhood acute lymphoblastic leukemia (ALL) depends on the assessment and identification of molecular markers associated with response to therapy. Gene expression analyses of lymphoblast at diagnosis have identified several genes with potential prognostic significance. The dominant-negative isoforms of *IKZF1* and low *CASP8AP2* and *H2AFZ* expression levels are considered independent predictors of adverse outcome. The aim of this study was detecting *IKZF1* splicing variants and determining the *CASP8AP2* and *H2AFZ* expression levels, in order to know their association with conventional prognostic factors or if may influence the early response to treatment. This was a prospective study performed in a group of ALL children. Bone marrow samples were collected from 66 ALL pediatric patients at diagnosis, attended at the Instituto Nacional de Pediatría and Hospital del Niño Poblano in Mexico, between 2008 and 2010. Total RNA was extracted from mononuclear cells; nested RT-PCR was performed for analyzing *IKZF1*'s isoforms expression. Quantitative-PCR method was used for evaluating expression levels of *CASP8AP2*, *H2AFZ* and the endogenous control *ABL1*. Fifty-four patients were diagnosed with B-ALL, 18 patients showed adverse conventional prognostic factors (age <1 or >10 years old, WBC count higher than $50x10^9$/L, presence of *BCR-ABL1* fusion). Sixteen of them presented dominant-negative variants of *IKZF1* or low expression of *CASP8AP2* or/and *H2AFZ*, one of these patients died. In 36 patients conventional adverse factors were not found, however, up to date 4 have died and all of them presented adverse expression-levels or non-functional isoforms. Twelve patients showed T-ALL, in 10 cases low expression levels of *CASP8AP2* were detected; in addition all patients showed low levels of *H2AFZ*. To date, 7 out of 10 patients with low levels of both genes relapsed and died. In the studied patients, dominant-negative variants Ika6 and Ika8 were identified, as well as low expression levels of *CASP8AP2* and *H2AFZ*. Although the results suggest that these markers could be potential prognostic factors, in B-ALL long-term survival analysis is required to demonstrate it. In contrast, in T-ALL low expression of *CASP8AP2* and *H2AFZ* showed association with relapse and patient's death. Measurement of these markers at diagnosis could be able to detect patients prone to therapy failure. Acknowledgment: CONACYT SALUD-2006-C01-44402 and scholarship 165427 to RJ-V.

1221T

microRNA regulation of cell viability and drug sensitivity in lung cancer. *A. Pertsemlidis[1,4], L. Du[1], C. DeSevo[2], R. Borkowski[2], M. Baker[2], A. Gazdar[1,3,4,6], J. Minna[1,4,5,6].* 1) Simmons Comprehensive Cancer Center, UT Southwestern Medical Center, Dallas, TX; 2) Graduate School of Biomedical Sciences, UT Southwestern Medical Center, Dallas, TX; 3) Department of Pathology, UT Southwestern Medical Center, Dallas, TX; 4) Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX; 5) Department of Pharmacology, UT Southwestern Medical Center, Dallas, TX; 6) Hamon Center for Therapeutic Oncology Research, UT Southwestern Medical Center, Dallas, TX.

Lung cancer is the leading cause of cancer-related deaths, with the majority of deaths due to failed therapy from tumor drug resistance. Third-generation chemotherapeutic agents represent the standard first-line treatment for advanced small cell (SCLC) and non-small cell (NSCLC) lung cancer patients. Response rates are poor (20-40%) with a median survival of 8-10 months. In an unbiased and comprehensive approach, we have combined a high-throughput screening platform with a library of chemically synthesized microRNA mimics and inhibitors. We have used this platform to identify mimics and inhibitors that reduce cell viability in general, and those that specifically sensitize cells to paclitaxel. We have identified several miRNAs for which over-expression or inhibition has a dramatic and selective effect on cell viability or drug response. Using in vitro approaches, we have demonstrated that over-expression of miR-337-3p sensitizes NSCLC cells to taxanes. By combining in vitro and in silico approaches, we identified STAT3 and RAP1A as direct targets that mediate the effect of miR-337-3p by enhancing taxane-induced arrest in the G2 phase of the cell cycle. We have also identified an inhibitor of miR-139-5p as a potent and selective regulator of SCLC cell viability. Inhibiting miR-139-5p decreases SCLC cell viability by over 80%, but has a minimal cytotoxic effect on that of NSCLCs or immortalized human bronchial epithelial cells. Increasing levels of miR-337-3p and inhibiting miR-139-5p may therefore provide novel therapeutic tools for the treatment of NSCLC and SCLC lung tumors, respectively.

1222T

Lung adenocarcinoma and squamous cell carcinoma develop through distinct genomic and epigenomic pathways. *L. Pikor[1], W.W. Lockwood[1,2], I.M. Wilson[1], B.P. Coe[1,3], R. Chari[1,4], K.L. Thu[1], C.E. MacAulay[1], S. Lam[1], W.L. Lam[1].* 1) Integrative Oncology, BC Cancer Research Center, Vancouver, British Columbia, Canada; 2) National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA; 3) Department of Genome Sciences, University of Washington School of Medicine, Seattle, WA, USA; 4) Department of Genetics, Harvard Medical School, Boston, MA, USA.

Non-small cell lung cancer (NSCLC) has traditionally been regarded as a single disease in terms of systemic therapy; however, recent evidence suggests that adenocarcinoma (AC) and squamous cell carcinoma(SCC), the two major subtypes of NSCLC, respond differently to both molecular targeted and newly developed chemotherapies. Identifying the molecular differences between these tumor types will therefore have a significant impact on novel treatment design and stratification, with the potential to improve the dismal outcome associated with lung cancer. An integrative analysis of genome-wide DNA copy number, methylation and gene expression profiles was performed on 261 primary NSCLC tumors (169 AC and 92 SCC) to identify subtype-specific molecular alterations. Ingenuity Pathway Analysis was used to define the canonical pathways and gene networks driving subtype development. Principle Component Analysis in multiple independent datasets was performed to assess the contribution of genes to disease phenotype and survival analysis was employed to identify genes associated with NSCLC prognosis that could better determine patient outcome. Characterization of AC and SCC genomic and epigenomic landscapes revealed 778 altered genes, with corresponding gene expression changes that are selected for during lung tumor development in a subtype-specific manner. Analysis of over 200 additional NSCLCs confirmed that these genes are responsible for driving the differential development and resulting phenotypes of AC and SCC. Importantly, we identified key oncogenic pathways disrupted in each subtype that likely serve as the basis for differential behaviors in tumor biology and clinical outcomes. Downregulation of HNF4(target genes was the most common pathway specific to AC, while SCC demonstrated disruption of numerous histone modifying enzymes as well as the transcription factor E2F1. In silico screening of candidate therapeutic compounds based on subtype-specific pathway alterations identified HDAC and PI3K inhibitors as potential treatments tailored to lung SCC. Our findings suggest that AC and SCC are distinct disease entities that develop through divergent molecular pathways, and should therefore be treated as such. Furthermore, the gene networks and signaling pathways identified through our analysis provide a rationale for elucidating the mechanisms underlying subtype development and differentiation, and developing subtype specific therapeutic strategies.

1223T

Genetic testing and immunohistochemistry for SDHB in phaeochromocytoma-paraganglioma syndromes: The South Australian experience. *N. Poplawski[1,2,3], A. Duszynski[2], L. Rawlings[4], J. Seymour[4], D. Benn[5], A. Gill[6,7].* 1) Familial Cancer Unit, Women's and Children's Hospital, North Adelaide, South Australia, Australia; 2) Familial Cancer Unit, Genetics and Molecular Pathology Directorate, SA Pathology (WCH site), North Adelaide, South Australia, Australia; 3) University of Adelaide, Adelaide, South Australia, Australia; 4) Genetics and Molecular Pathology Directorate, IMVS (Frome Road Site), Adelaide, South Australia, Australia; 5) Cancer Genetics, Kolling Institute of Medical Research, Royal North Shore Hospital, St Leonards, New South Wales, Australia; 6) Department of Anatomical Pathology, Royal North Shore Hospital, St Leonards, New South Wales, Australia; 7) University of Sydney, Sydney, New South Wales, Australia.

INTRODUCTION: Most phaeochromocytomas and paraganglioma occur sporadically, but some occur as part of a hereditary cancer syndrome caused by mutations in one of a number of genes.

AIM AND METHODS: A retrospective review of germline genetic testing (VHL, RET and succinate dehydrogenase subunit genes SDHB, SDHC and SDHD) and immunohistochemical staining for SDHB in tumour tissue (SDHB-IHC), in patients referred to the South Australian Familial Cancer Unit with an adrenal phaeochromocytoma (PC) and/or paraganglioma (PGL).

RESULTS: Between January 1999 and May 2011, 21 probands were referred to and fully assessed by our service. Three patients presented with phaeochromocytoma and other personal and family features of multiple endocrine neoplasia type 2; all had RET mutations identified. The only patient with familial PC had a VHL mutation identified. Of the 8 with an apparently sporadic unilateral PC, only 1 had a mutation identified; a 30 year old man with an SDHB mutation who developed a head-and-neck PGL 14 years after the PC. SDH mutations were identified in 2 of 4 who presented with an apparently sporadic head-and-neck PGL (1 SDHD and 1 SDHC), 2 of 3 who presented with an apparently sporadic malignant abdominal PGL (both SDHB) and none of 2 who presented with familial head-and-neck PGL.

Tumour tissue was available from 16 probands and SDHB-IHC was abnormal in all probands with an SDH mutation (5/5; 100%), 0/1 with a VHL mutation and 2/10 (20%) with no identified mutation (both presented with familial head-and-neck PGL). Tissue was unavailable for testing in the remaining 4 patients.

CONCLUSION: Our experience supports using SDHB-IHC as a tool to triage genetic testing (see references).

References: Gill *et al*. Hum Pathol 2010;41:805-14; van Nederveen *et al*. Lancet Oncol 2009;10:764-771.

1224T

Integrated epigenetics of human breast cancer subtypes: synoptic investigation of targeted genes, microRNAs and proteins upon demethylation treatment. *R. Radpour[1], Z. Barekati[1], C. Kohler[1], M.M. Schumacher[2], T. Grussenmeyer[3], P. Jenoe[4], J. Bitzer[5], I. Lefkovits[3], F. Staedtler[2], X.Y. Zhong[1].* 1) Laboratory for Gynecological Oncology, Women's Hospital/ Department of Biomedicine, University of Basel, Switzerland; 2) Biomarker Development, Novartis Institutes of BioMedical Research, Novartis Pharma AG, Basel, Switzerland; 3) Department of Biomedicine and Department of cardiac surgery, University hospital Basel, Switzerland; 4) Biozentrum, University of Basel, Switzerland; 5) Department of Obstetrics and Gynecology, Women's Hospital, University of Basel, Switzerland.

The contribution of aberrant DNA methylation in silencing of tumor suppressor genes (TSGs) and microRNAs has been investigated. Since these epigenetic alterations are reversible, it became of interest to determine the early and late effects of the 5-aza-2'-deoxycytidine (DAC) demethylation therapy in breast cancer within different molecular levels. Here we investigate a synoptic model to predict complete DAC treatment effects at the level of epigenome, transcriptome, and proteome for several human breast cancer subtypes. The present study assessed an effective treatment dosage based on the cell viability, cytotoxicity, apoptosis and methylation quantification assays for different breast cancer cell lines. Using the optimal treatment dosage, a highly aggressive and a non-aggressive lines were investigated at various time points using omics approaches such as MALDI-TOF MS, mRNA- and microRNA expression arrays, 2-D gel electrophoresis and LC-MS-MS. Complete molecular profiles of the studied subtypes including the biological interactions and possible early and late systematic stable or transient effects of the methylation inhibition were determined in depth. Beside the activation of several epigenetically suppressed TSGs, we also showed significant dysregulation of some important oncogenes, oncomirs, oncosuppressors miRNAs as well as drug tolerance genes/miRNAs/proteins in breast cancer related pathways. In present study, the results give new therapeutic clues based on chemical modification of the pathological methylation patterns in breast cancer as well as investigation of some new molecular DAC targets and involved pathways. The presented approach might become a useful epigenetic treatment model for other human solid tumors in management of cancer patients.

1225T

polymorphism of TYMS in Mexican breast cancer patients. *A. Ramos[1,2], A. Solorzano[1], LE. Figuera[1], AM. Puebla[3], MP. Gallegos[1].* 1) IMSS, CIBO, Guadalajara, Jalisco, Mexico; 2) Doctorado en Farmacologia, UdeG; 3) Laboratorio de Inmunofarmacologia, CUCEI. UdeG.

Thymidylate synthase (TYMS) catalyzes the reductive methylation of dUMP to form dTMP, which are essential for cell proliferation. Has been show that patients high levels of TYMS are no respond to 5-fluorouracil. The polymorphisms 2R/3R in 5'untranslated region (5'UTR) of TYMS gene contains repeated of a 28 bp sequence. The alleles with two repeat (2R), have low translational efficiency of TYMS mRNA than three (3R) repeats with greater translational efficiency. In the present study we describe to frequency of 2R/3R polymorphism of TYMS gene breast cancer patients and woman health controls from Mexico. In a case-control study of 110 breast cancer patients and 251 controls we observed a frequency of 34.5% (38/110) and 27.8% (70/251) of 3R/3R genotype; 13.5% (152/110) and 16% (40/251) of 2R/2R genotype, and heterozygous genotypes were: 1%(1/110) and 0.8% (2/251) of 1R/2R, 2% (2/151), and 0.8% (2/252) 2R/4R genotype, 1% (1/151) and 1.2% (3/251) of 3R/4R genotype and 48% (53/151) and 53% (134/252) of 2R/3R genotype in cases and controls respectively, without to be statistically different ($p>0.05$). These results suggest that the 2R/3R polymorphism on the TYMS promoter is not associated with Mexican breast cancer patients.

1226T

Rhesus macaque as an animal model for hereditary non-polyposis colorectal cancer. *M. Raveendran[1], P. Gillespie Jr[3], D.W. Brammer[4], D.P. Young[3], J.G. Gelovani[3], J. Rogers[1,2].* 1) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Department of Experimental Diagnostic Imaging, The University of Texas MD Anderson Cancer Center, Houston, TX; 4) University of Houston, Houston, TX.

Rhesus (Macaca mulatta) have been used as an animal model to study several human diseases, e.g., coronary heart disease, osteoporosis, diabetes mellitus. There are only few reports that show the incidence of colon cancer in rhesus, and detailed genetic studies are lacking. Colon cancer is the third most common form of cancer diagnosed in the United States and second leading cause of cancer death. Diagnosing colon cancer at early stage can reduce the incidence of advanced stage tumors and therefore reduce the mortality. Defects in the MLH1 gene account for the majority of cases of hereditary non-polyposis colorectal cancer (HNPCC-Lynch syndrome). In this study we sequenced the full length of the rhesus MLH1 gene, exons 1 to 19, promoters, introns and UTRs in 18 rhesus samples using Sanger sequencing methods. We used PET-CT images and colonoscopy to confirm the colon cancer in our study animals. We identified 124 SNPs and three of them are in coding regions. Two of these coding SNPs are non-synonymous. A total of 47 SNPs were found to be shared in monkeys with known colon cancer (n = 4) and were absent in normal animal (n = 1) and animals of unknown disease status (n=13). We also identified a two base pair deletion in the promoter region of colon cancer monkeys. These SNPs and the deletion will be useful as screening markers prior to the development of “detectable” tumors in rhesus, which in turn can be used as an animal model to study the onset and progression of colon cancer in a suitable animal model.

1227T

Identification of a BRCA2 truncating mutation in a hereditary prostate cancer case with a family history of breast and ovarian cancer through next-generation sequencing. *A.M. Ray[1], K.A. Zuhlke[1], C.M. Robbins[5], W.D Tembe[5], J. Xu[6], S.L. Zheng[6], J.D. Carpten[5], E.M Lange[3], W.B. Isaacs[4], K.A. Cooney[1,2].* 1) Department of Internal Med, Hem/Onc, University of Michigan Medical School, Ann Arbor, MI; 2) Department of Urology, University of Michigan Medical School, Ann Arbor, MI; 3) Department of Genetics, University of North Carolina School of Medicine, Chapel Hill, NC; 4) Department of Urology, The Johns Hopkins University School of Medicine, Baltimore, MD; 5) Translational Genomics Research Institute, Phoenix, AZ; 6) Center for Cancer Genomics and Center for Personalized Medicine Research, Wake Forest University School of Medicine, Winston-Salem, NC.

Prostate cancer (PCa) is the most commonly diagnosed cancer and the second leading cause of cancer deaths among American men. Family history is a known PCa risk factor, with ~10% of all PCa cases assumed to be hereditary. The hereditary breast cancer (BrCa) gene BRCA2 has previously been associated with an increased risk of PCa, especially early-onset PCa. Here we used targeted next-generation sequencing to examine multiple candidate PCa susceptibility genes in probands from 94 hereditary PCa families from the University of Michigan and Johns Hopkins University. A BRCA2 truncating mutation, K3326X, was identified in 1 individual with a family history of breast and ovarian in addition to prostate cancer. Analysis of the family revealed the mutation was also carried by 2 brothers and 1 maternal cousin of the proband with PCa as well as 2 maternal cousins with BrCa and 1 with bladder cancer. Additionally, 1 brother and 1 maternal cousin with PCa, 1 niece with melanoma and 1 unaffected maternal cousin were not carriers. Further screening of 917 Caucasian, early-onset PCa cases and 909 Caucasian, screened controls revealed an additional 18 affected and 17 unaffected carriers. K3326X has been previously associated with increased risk of both lung (OR=1.72) and pancreatic cancer (OR= 4.84) as well as Fanconi Anemia. However, to our knowledge, this is the first report of K3326X in a hereditary PCa family. Further investigation is needed to determine the exact role of K3326X in prostate and other cancers.

1228T

High Prevalence of Germline p53 Mutations Among a Consecutive Series of Unselected Patients with Adrenal Cortical Carcinoma. *VM. Raymond[1], JN. Everett[1], JL. Long[1], T. Else[2], GD. Hammer[2], SB. Gruber[1,3,4].* 1) Department of Internal Medicine, University of Michigan, Ann Arbor, MI; 2) Department of Metabolism, Endocrinology and Diabetes, University of Michigan, Ann Arbor, MI; 3) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 4) Department of Epidemiology, University of Michigan, Ann Arbor, MI.

Introduction: Adrenocortical carcinoma (ACC) is a rare, aggressive tumor which when diagnosed in children prompts an evaluation for Li Fraumeni Syndrome (LFS) due to germline p53 mutations. The Chompret criteria advocate for p53 genetic testing in individuals with ACC regardless of age at diagnosis or family history. The University of Michigan Multidisciplinary Adrenal Cancer Clinic (UMMACC) is a Center for Excellence in ACC diagnosis, treatment and management, a referral center for ACC patients internationally and evaluates greater than 25% of all ACC patients in the United States. To date, no prospective studies quantifying the prevalence of LFS in unselected cases of ACC have been performed. Methods: Patients were recruited through the UMMACC between December 2009-April 2011. All patients with a personal history of ACC were eligible and offered a genetic consultation, p53 genetic testing and participation in an IRB approved Cancer Genetics Registry. Genetic testing was performed at a CLIA certified clinical laboratory via sequencing and gene dosage analysis or MLPA for large deletions, duplications and rearrangements. Results: Ninety-one patients were evaluated in the UMMACC during the study timeframe. Seventy patients underwent genetic consultation (76.9%) and 36 of 70 (51.4%) pursued p53 genetic testing. Primary reason for not pursuing testing was insurance coverage concerns due to out-of-network location of UMMACC (27 of 34). Seven patients declined genetic testing (10%). Three individuals were positive for deleterious missense mutations: p.V272M, diagnosed at 68; p.R181H diagnosed at 52; p.R158H diagnosed at 3. One individual, diagnosed at 47, was positive for a novel variant of uncertain significance (c.G>T 207bp upstream from exon 1)(4 of 36, 11.1%). No positive patients met Classic Li-Fraumeni or Li-Fraumeni-like criteria. The average age at diagnosis in the cohort testing negative for p53 mutations was 44.2. No patients met Classic LFS diagnostic criteria. Three patients met Birch criteria and 2 patients met Eeles criteria. Conclusions: This prospective series of unselected patients with ACC demonstrates that LFS is under recognized within this population. Germline p53 testing in all patients with ACC yields an 11.1% diagnostic yield for LFS. The majority of patients were interested in a genetic consultation (76.9%) and genetic testing (90%). All patients with ACC, regardless of family history should be offered p53 genetic testing.

1229T

DNA repair pathways and lymphoma susceptibility. *J. Rendleman[1], Y. Antitpin[2], B. Reva[2], J. Przybylo[2], A. Dutra-Clarke[2], A. Heguy[2], K. Huberman[2], O. Paltiel[3], D. Ben-Yehuda[3], J. Brown[4], C. Sander[2], R.J. Klein[2], K. Offit[2], T. Kirchhoff[2].* 1) NYU School of Medicine, New York University, New York, NY, USA; 2) Memorial Sloan-Kettering Cancer Center, New York, NY, USA; 3) Hadassah University, Jerusalem, Israel; 4) Dana Farber Cancer Center, Harvard University, Boston, MA, USA.

The inherited factors contributing to the development of lymphoma remain largely unknown. The molecular and genetic evidence points to DNA repair as a candidate molecular network involved in lymphomagenesis. Several studies have attempted to relate DNA repair pathways with lymphoma risk, but the power or smaller scope of selected candidates were usually the major limitations of these prior efforts. In this study we have conducted a case/control association analysis of lymphoma risk based on the large systematic screen of common genetic variation in the complex DNA repair networks. We have applied bioinformatics approaches for a more unbiased selection of candidate genes. Using these strategies, we have selected >450 genes involved in more than 25 DNA repair processes. For the genetic variants, we have focused on tagging single nucleotide polymorphisms (tSNPs), capturing the common variation (MAF>0.05) of 450 selected genes in European population, used primarily in the study. In order to reduce the number of variants in the association screen, all the tSNPs were uncorrelated with r2<0.6, yielding the selection of ~ 1500 SNPs tagging the common variation in these 450 genes. The 1500 SNPs were genotyped in 2 phases in 2500 lymphoma cases and 2500 controls, both of European ancestry using the Sequenom iPLEX methodology. Here we report the results of phase 1 of the study, involving 531 SNPs in 87 DNA repair target genes genotyped in 2500 Lymphoma cases and 2500 controls, both of European ancestry. In phase 1, we have identified several loci associated with the lymphoma risk in pooled or lymphoma subtype-specific analyses. The preliminary results of NHL scan points to a locus on chromosome 11 with 3 independent SNPs showing the significant associations (OR=1.522, 1.451, .7342; p=1.61E-5, 7.9E-5, .0014). NHL associations were also seen on chromosome 15 (OR=1.821, p=.00032), chromosome 8 (OR=.5001, p= .0016), and chromosome 10 (OR=.7045, p=.0023). Significant associations were also confirmed for common and rare haplotypes surrounding these loci, using the imputed data from HapMap Phase 2 and 1000 genomes. While finalizing the second phase of this large scan, we also address the epistatic effects of SNPs and haplotypes in the different components of DNA repair machinery targeted in the study. The further validation of associations identified here will be necessary in larger and independent subsets, possibly as part of International consortia.

1230T

High quality genotyping data from FFPE tumor samples. *B. Riley-Gillis[1], R. Benayed[1], A. Helm[2], K. Gunderson[2], J. Izzo[2], D. Pokholok[2], J. Le[2], C. Fishman[2], J. Garsetti[2], M. Martin[1].* 1) Translational Research Sciences, Roche, Nutley, NJ; 2) Illumina, San Diego, CA.

Formalin-fixed, paraffin-embedded (FFPE) tumor samples are an invaluable resource for cancer research, drug development, and diagnosis. However, utilizing these samples is complicated by the nucleic acid degradation and base modification that occurs during the fixation and storage processes. In this study, we use the Illumina platform to assess the feasibility of genotyping tumor FFPE DNA for targeted and whole genome association studies. Matched fresh frozen (FF) and FFPE ovarian cancer samples were compared on two platforms: custom GoldenGate genotyping and Infinium OmniExpress with FFPE DNA restoration.

The GoldenGate genotyping technology does not involve an initial whole genome amplification step, therefore is not as susceptible to the effects of degraded DNA. At the recommended input DNA concentration of 250ng, concordance between FF and FFPE samples was greater than 99.7%. Over a range of DNA input concentrations, we found concordant genotyping results for FFPE samples down to 50ng of input DNA (concordance with FF >97%).

Illumina's Infinium FFPE DNA Restoration protocol repairs damaged DNA prior to whole genome amplification. The five matched FF and FFPE samples were restored and genotyped on the OmniExpress array, which includes >693,000 genomic markers and coverage of 97% of the cancer genes identified by the Sanger Cancer Genome Project. The restored FFPE samples had call rates between 92% and 97% and concordance between FF and restored FFPE samples was greater than 99.8% for all five samples.

The results indicate that DNA from FFPE tumor samples can be successfully genotyped and yield high quality genotyping data on both custom and genome-wide genotyping platforms. The next step for this project includes testing the FFPE restoration protocol on a custom Infinium iSelect panel containing ~3000 somatic mutations, CNVs and fusion genes. The ability to capture the significant information available in FFPE samples will be critical to deepen the disease understanding through identification of variants in drug targets and pathways driving tumorigenesis and progression. Additionally, baseline assessment of tumors from patients in clinical trials using custom genotyping platforms is necessary to define tumor mutations, stratify patient populations and evaluate or predict therapy response.

1231T

BRCA1 and _BRCA2_ large genomic rearrangement testing in a large cohort of hereditary breast/ovarian cancer patients: prevalence and mutation profiles in risk-stratified patient groups of different ethnicities. *B. Roa, E. Rosenthal, C. Arnell, L.A. Burbidge, W. Geary, J. Schoenberger, J. Trost, R. Wenstrup, T. Judkins.* Myriad Genetic Laboratories, Inc., Salt Lake City, UT, USA.

Large rearrangements in *BRCA1* and *BRCA2* make up a significant proportion of mutations in hereditary breast and ovarian cancer (HBOC). Since August 2006 our laboratory has offered clinical testing for deletions and duplications in *BRCA1* and *BRCA2* using the BRAC*Analysis* Rearrangement Test (BART). Nearly 64,000 patients have received BART testing, revealing a broad spectrum of large rearrangements in both *BRCA1* and *BRCA2*. To characterize the mutation profile of *BRCA1/2* within this referral testing cohort, results from a defined subset of patients were stratified by mutation type (sequencing vs. large rearrangements), prior risk, and patient ethnicity. Among patients tested between July 2007 and April 2011, a subset of 25,535 received BART testing automatically because they met high-risk criteria; 22,925 did not meet high-risk criteria but ordered elective BART at the same time as *BRCA1/2* sequencing. The overall *BRCA1/2* mutation prevalence among high-risk patients was 23.8% compared to 8.2% for the elective group. High-risk patients showed a mutation profile of 90.1% sequencing mutations vs. 9.9% large rearrangements. The corresponding mutation profile in the elective group was 94.1% vs. 5.9%, with significantly fewer large rearrangements in this group versus the high-risk group ($p << 0.001$). Possible explanations for this difference may include bias introduced by the high proportion of large rearrangements found in *BRCA1*, which is more penetrant than *BRCA2*. Results were correlated to eight self-reported ethnic groups to characterize the mutation spectrum. This analysis included only patients who self-reported one ethnicity. In the Western/Northern European group, 9.7% of mutations in high-risk patients were large rearrangements vs. 5.9% in the elective group. Among Latin American/Caribbean patients, large rearrangements accounted for 20.6% of mutations in the high-risk group and 12.8% in the elective group, indicating more large rearrangements in this ethnic group overall ($p << 0.001$, $p = 0.02$). Those reporting African ancestry showed the highest positive rate for sequencing mutations but large rearrangements contributed only 8.3% and 1% of all mutations in the high-risk and elective groups, respectively. Large rearrangements are a significant contributor to the overall spectrum of mutations, and show ethnic-specific variation in their contribution to the *BRCA1* and *BRCA2* mutation profile.

1232T

Elucidation of the role of miR-302 in the induction of TGF-beta signaling pathway in tumor cells. *S. Rohban[1], M.R Rafiee[2], A. Malekzadeh Shafaroudi[2], M. Malakootian[1], N. Ghorbanmehr[1], S.J. Mowla[1].* 1) Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran; 2) Nanomedicine and Tissue Engineering Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

According to the “stem cell origin of cancer” hypothesis, tumors include a small subpopulation of cancer cells which is characterized by stemness properties and is responsible for sustained tumor growth, metastasis and resistance to therapy. Recently, studies have identified the expression of a subset of embryonic stem cell (ESC)-like gene expression signature in invasive, poorly differentiated tumor cells. However, the underlying molecular mechanisms by which these genes contribute to stem cell-like phenotypes shown by many tumors, are not yet fully understood. Here we report the expression of miR-302 family of microRNAs, which are expressed at high levels in ESCs, in the malignant brain tumor tissues and cell lines, combined with transcriptional activity of its cloned promoter in a small subpopulation of tumor cells. Interestingly, by overexpression of miR-302 in these tumor cells, the expression of components of TGF-) signaling pathways, such as TGF) -1, TGF) -2 and TGF) receptor 2, together with several pluripotency markers, was overexpressed in the cells. Moreover, the expression level of miR-141 which can act as translational repressor of TGF-) signaling, was decreased in the transfected cells. Taken together, our data suggest that miR-302 may play a role in cancer stem-like cell behavior, such as self-renewal and maintenance of an undifferentiated state of tumor cells. In addition the effect of miR-302 overexpression on inducing TGF-) pathway, may reflect its probable role in epithelial-to-mesenchymal transition in migrating cancer stem cells. These observations provide important concepts to develop new strategies to target cancer stem cell subpopulation in tumors.

1233T

Large deletions of the APC-gene region in familial adenomatous polyposis patients and the effect on the expression from promoter 1A and 1B. *A. Rohlin[1], Y. Engwall[1], K. Fritzell[2], J. Björk[2], M. Nordling[1].* 1) Department of Molecular and Clinical Genetics, Institute of Biomedicine, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden; 2) The Swedish Polyposis Registry, Department of Medicine, Karolinska Institute, Stockholm, Sweden.

Familial adenomatous polyposis (FAP) is caused by dominant inheritance of germline mutations in the adenomatous polyposis coli (APC) tumour suppressor gene. In classical FAP, mutation carriers develop hundreds to thousands of adenomas in the large bowel and untreated carriers inevitably develop colorectal cancer (CRC) at an early age. A milder form of FAP also exists referred to as attenuated polyposis (AFAP). The APC gene (5q21-q22) encodes several tissue specific transcripts and two promoter regions have been identified 1A and 1B. In this study 54 families with FAP or AFAP were screened for mutations in APC. A deleterious mutation was found in 97% of families with FAP and 26% of families with AFAP. The screening method used to detect mutations in the exons of APC was sequencing. Multiplex Ligation-dependent Probe Amplification (MLPA) was used for detection of larger deletions and duplications. Seven families, among these the largest FAP family in Sweden including 150 individuals of whom 59 are affected, harboring deletions including part or the whole of the APC gene region and/or the promoter region were further investigated. The methods used to characterize the deletions were copy number- and expression analyses using the Affymetrix® Genome-Wide Human SNP Array 6.0 and GeneChip® Human Exon 1.0. Q-PCR (Taqman) was also used for extensive RNA investigations of the APC transcripts using five different probes, one specific for transcripts from promoter 1B, two for promoter 1A and two for the overall expression. Differences in expression from the promoter 1A and 1B were found among the patients given more insight into the regulation of the APC gene in the FAP patients. In families displaying a classical FAP phenotype with no deleterious mutation identified, screening for CNVs in the regulatory regions of APC should follow the initial screening of the coding region as mutations located upstream of promoter 1A could be a possible causative alteration.

1234T

An integrated (epi)genomic approach reveals the role of retinoic acid in cell fate determination. *S. Rossetti, N. Visconti, J. Fischer, N. Sacchi.* Dept Cancer Genetics, Roswell Park Cancer Inst, Buffalo, NY.

The coordinated expression of gene networks in numerous physiological, developmental, and metabolic processes can be ascribed in large part to a superfamily of ligand-inducible transcription factors, the nuclear receptors (NRs). Ligand-binding imparts changes in the NR structure and consequently modulates the recruitment of coactivator/corepressor complexes that are necessary for modifying the chromatin environment at multiple NR direct gene targets. Retinoic acid (RA) a master ligand of prenatal and postnatal developmental processes acts through retinoic acid receptors (RARs). RA can induce or suppress growth in cells with different histotype. Intriguingly, we found that RA can exert a dual action on cell growth within the same cell context. To identify the mechanism at the basis of this dual action, we used an integrated (epi)genomic approach. Unexpectedly, from this analysis it emerged that gene networks and pathways are differentially regulated by RA paradoxically through the very same RA receptor. This work was funded by R01 CA127614 (NS).

1235T

Evaluation of polymorphisms *GSTP1* A313G and *GSTP1* C341T in patients with head and neck squamous cell carcinoma. *A. Russo[1], P.F. Francelin[1], M.A. Palmejani[1], A.L. Galbiatti[1], M.T. Ruiz[1], T.P. Gueroni[1], J.V. Maniglia[2], E.C. Pavarino[1], E.M. Goloni-Bertollo[1].* 1) Genetics and Molecular Biology Research Unit - UPGEM, Faculdade de Medicina São José do Rio Preto - FAMERP , São José do Rio Preto, SP, Brazil; 2) Otorhinolaryngology and Head and Neck Surgery Department of the Faculdade de Medicina São José do Rio Preto - FAMERP, São José do Rio Preto, SP, Brazil.

Introduction: Head and neck cancer is responsible for significant morbidity and mortality, represents the sixth leading cause of cancer death worldwide, and is one of the most prevalent tumor in Brazil. Variations of activation and detoxification of chemical compounds in the xenobiotics metabolism are involved in tumorigenesis, even as polymorphisms in genes of the glutathione S-transferase superfamily, which act in phase II of this metabolic pathway. **Aim:** To investigate the frequency of *GSTP1* polymorphisms A313G (rs1695) and C341T *GSTP1* (rs1138272) in patients with head and neck cancer (case group) and compare with subjects with no history of cancer (control group) to evaluate the association between these polymorphisms and the risk factors and the histopathologic characters of the tumor. **Methods:** Were included 783 individuals, 264 patients and 519 controls. Molecular analysis was performed by PCR-RFLP. For statistical analysis we used the chi-square and multiple logistic regression. **Results:** Results showed that age ⁄ 48 years (OR=12.37; IC 95% 7.89-19.38; p<0.0001), smoking (OR=4.21; IC 95% 2.71-6.55; p<0.0001) and alcoholism (OR=1.65; IC 95% 1.07-2.55; p=0.023) were predictors for the development of cancer of the head and neck, while the *GSTP1* A313G polymorphism was more frequent in control individuals (OR=0.61; IC 95% 0.42-0.90; p=0.013). The clinical pathological parameters were not associated with the presence of the polymorphisms studied. **Conclusion:** In conclusion, the presence of *GSTP1* A313G variant is associated with decreased risk for head and neck cancer and age⁄ 48 years, male gender and tobacco and alcohol consumption are associated with increased risk for developing this disease. **Financial Support:** FAPESP, CNPq and CAPES; support: FAMERP/FUNFARME.

1236T

Study of P53 gene mutations in promoter and exons 2-11 in gastric cancer by PCR-SSCP in a province of Iran. *j. saffari chaleshtori[1], M. Moradi[2], E. Farrokhi[2], M.A. Tabatabaieefar[2], M. Taherzadeh Ghahfarokhi[1], G. Mobini[2], F. Shayesteh[2], F. Azadegan[2], G. Rahimian[2], H. Nazem[3], M. Hashemzadeh Chaleshtori[2].* 1) Payame Noor Univ. Tehran & Cellular and Molecular Research Center Shahrekord Univ. of Med.Sci. Iran. Shahrekord. Islamic Republic of Iran; 2) Cellular and Molecular Research Center Shahrekord Univ. of Med.Sci. Iran. Shahrekord. Islamic Republic of Iran; 3) Payame Noor Univ. Tehran Islamic Republic of Iran.

Background and aim: Gastric cancer is one of the most important human cancers and is the second cause of cancer death worldwide after lung cancer. Genetic factors including oncogens and tumor suppressor genes always contribute to the progression of this cancer. The P53 tumor suppressor gene has a main role in genomic stability and DNA repair. The aim of this study was to determine the P53 gene mutations in gastric cancer specimens in Chaharmahal Va Bakhtiari Province, in southwest Iran. Methods: in this descriptive lab based study, we screened the promoter and exons of P53 gene for mutations in 38 paraffin embedded gastric cancer specimens. DNA was extracted following the standard phenol chloroform protocol. The P53 gene mutations were determined using PCR-SSCP procedure. Results: Our study revealed no P53 gene mutation in promoter and exons 2-11 in the gastric cancer subjects studied. Conclusion: While P53 gene mutations have been reported as the most frequent genetic alterations and are found in about 50% of the human malignancies, no mutation was detected in this study. This might be due to small sample size or mutations on other genetic or epigenetic factors.

1237T

A genome-wide association study of early onset prostate cancer: Increased genetic burden among young cases. *C.A. Salinas[1], E.M. Lange[2,3,4], K.A. Zuhlke[1], A.M. Ray[1], Y. Wang[2], Y. Luo[3], W.B. Isaacs[5], S.L. Zheng[6,7], K.A. Cooney[1,8,9].* 1) Dept. Internal Medicine, University of Michigan Medical School, Ann Arbor, MI; 2) Dept. Genetics, University of North Carolina, Chapel Hill, NC; 3) Dept. Biostatistics, University of North Carolina, Chapel Hill, NC; 4) Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC; 5) Dept. of Urology, Johns Hopkins Medical Institutions, Baltimore, MD; 6) Center for Cancer Genomics, Wake Forest University School of Medicine, Winston-Salem, NC; 7) Center for Genomics and Personalized Medicine Research, Wake Forest University School of Medicine, Winston-Salem, NC; 8) Dept. Urology, University of Michigan Medical School, Ann Arbor, MI; 9) University of Michigan Comprehensive Cancer Center, Ann Arbor, MI.

At least 30 common loci associated with prostate cancer (PC) have been identified by genome-wide association studies (GWAS). Although early age at diagnosis is a recognized marker of genetic susceptibility in several hereditary cancers almost all GWA studies have been conducted in older populations. Men diagnosed with PC at ≤55 years, i.e., early onset PC, may provide increased power to detect novel loci that influence PC incidence. Data from 931 early onset PC cases and 2,985 controls of European descent were analyzed in a first-stage GWAS of PC (n=458,162 single-nucleotide polymorphisms or SNPs). Cases were diagnosed with PC at a mean age of 49.8 years and the majority had low-grade (81% Gleason score ≤7=3+4), localized (81%) disease. Controls were unrelated individuals from Illumina's iControlDB database. Cases were genotyped with the Illumina HumanHap610-Quad Genotyping BeadChip platform. Logistic regression models, adjusted for principal components to account for ancestry, were used to test the association between PC and individual SNPs, assuming a multiplicative genetic model. Overall, no novel SNPs attained genome-wide significance and only rs6983267 ($p=6.07x10^{-9}$) remained significant after correction for multiple tests. Of 23 previously reported PC-associated SNPs, 18 were nominally significantly associated with PC (p<0.05) in our GWAS, with all 23 having a direction of effect consistent with prior findings. We are currently performing genotype imputation and selection of SNPs for Stage 2 genotyping on an independent sample of early onset cases and controls. Compared to older Cancer Genetic Markers of Susceptibility (CGEMS) study cases (>55 years at diagnosis), cases diagnosed with early onset PC had a significantly greater number of risk alleles across 18 previously reported PC-associated SNPs ($p=3.6x10^{-3}$). In a combined analysis of CGEMs and early onset PC cases, the burden of risk alleles carried by each case was significantly related to age at diagnosis, with an average 25.1 (standard deviation=3.2), 24.1 (4.2), 23.8 (3.8), 23.4 (3.2), 23.2 (3.1) and 22.3 (4.7) risk alleles in cases aged 35-44, 45-54, 55-64, 65-74, 75-84 and 85+ years at diagnosis, respectively ($p_{trend}=2.6x10^{-5}$). SNPs identified in older PC cases had stronger associations in men diagnosed with early onset PC. This demonstrates the potential for identifying PC-associated genetic variants by focusing on men with early onset disease.

1238T

Investigation of expression level of MDR1 in Iranian colorectal cancer (CRC) patients. *s. samanian[1], f. mahjoubi[1], b. mahjoubi[2], r. mirzaee[2], r. azizi[2].* 1) National Institute of Genetic Engineering and Biot, tehran, Iran; 2) HAZRAT Rasool Hospital, TehranUniversity of Medical Sciences and Health Care Services.

Purpose: Chemotherapy is one of the most effective treatments for cancer patient but resistance to chemotherapy limits the effectiveness of this useful treatment. Multi drug resistance (MDR) is a phenomenon in which tumor cells become resistant to many structurally and functionally different drugs. Drug resistance can occur at many levels including drug activation, alteration in drug target, DNA repair, apoptosis, drug uptake and increasing drug efflux. ABC transporters super-family is the most important mechanism among all of them. These are trans-membrane proteins that their over expression increase the level of drug afflux by using ATP molecules. Methods used: We investigated the expression level of MDR, one of the ABC transporters' members in 60 Iranian patients diagnosed with colorectal cancer (CRC) employing real time RT-PCR Materials and Methods. Tumor and adjacent normal tissues from 60 Iranian patients with colorectal cancer were assessed for the expression level of MRP1 by Real Time RT-PCR. Result: The project was approved by the local ethical committee of National Institute for Genetic Engineering and Biotechnology (NIGEB) and written informed consent was obtained from all cases. Tissue specimens (tumor and normal tissue adjacent to tumor) were collected from Hazrat Rasool hospital between 2008and September 2010, Histological diagnosis was confirmed for all samples. We found that the expression of MDR1 increased significantly in cancerous regions of these CRC patients compared with their adjacent normal tissues. Furthermore, we demonstrated that the MDR1 expression was dependent on the histological grading. Conclusion: Our results suggest that MDR1likely have an effect on the clinical response to treatment in CRC patients.

1239T

Heat Shock Protein Polymorphism and the Risk of Development of Hepatocellular Carcinoma in Chronic Liver Disease Patients from India. *M.P. Sarma, M. Asim, S. Medhi, P. Kar.* Medicine, Maulana Azad Medical College,University of Delhi, New Delhi, Delhi, India.

INTRODUCTION Genetic variation has been suspected to influence the variable risk of hepatocellular carcinoma (HCC) in which numerous familial HCC clusters has been reported. Functionally significant polymorphisms have been noted in the HSP70-Hom and HSP70-2 genes. HSP70 have been shown to mediate tumorigenesis through inhibition of apoptosis and replicative senescence. AIMS & METHODS The aim of this study was to investigate the potential role of HSP70-Hom and HSP70-2 polymorphisms and the subsequent risk of HCC in chronic liver disease (CLD) patients. Patients diagnosed as HCC with HBV and/or HCV as the main etiology were included in the study. The EASL diagnostic criteria for HCC were followed (Bruix et. al 2001). A total of 75 HCC cases were included in the study of which 35 were positive for HBV and 40 for HCV. An equal number of HBV and HCV related chronic hepatitis cases were also included as controls in which 32 were positive for HBV and 43 were positive for HCV. HSP70-Hom and HSP70-2 polymorphisms were analysed by PCR-RFLP method. The statistical program “EPIINFO” (version 5.0; USD Incorporated 1990, Stone Mountain, Georgia) was used for these analyses. RESULTS HSP70-Hom gene polymorphism was found to be a risk factor for HCC compared to chronic hepatitis irrespective of the etiology of liver disease OR 15.17(3.35-95.35); p= 0.001.On the other hand HSP70-2 gene polymorphism was also found to be a risk factor for HCC compared to chronic hepatitis irrespective of the etiology of liver disease OR 2.36(1.29-4.36); p= 0.002. CONCLUSION: The findings indicate that patients with chronic hepatitis B/ C virus infection who harbor these SNPs represent a high-risk group for HCC and need intensive surveillance for early detection of HCC.

1240T

Single nucleotide polymorphisms in E-cadherin gene confer risk to breast cancer. *V. Satti[1], N. Tipirisetti[1], S. Govatati[2], K. Lakshmi Rao[3], R.R. Digumarti[4], M. Bhanoori[2], M. Deenadayal[5].* 1) Department of Genetics, Osmania University, Hyderabad,; 2) Department of Biochemistry, Osmania University, Hyderabad,; 3) Centre for Cellular and Molecular Biology (CCMB), Hyderabad,; 4) Nizams Institute of Medical Sciences, Hyderabad,; 5) Infertility Institute and Research Centre (IIRC), Secundrabad, INDIA.

Cadherins constitute superfamily of integral membrane glycoprotein's that mediate and regulate Ca2+ dependent homophilic cell-cell adhesion and modulate a wide variety of processes, including cell polarization, migration and cancer metastasis. E-cadherin (CDH1) is an epithelial cellular junction protein expressed in almost all epithelial cells and plays an important role in the maintenance of epithelial development, organization, and cell integrity Reduced CDH1 expression promotes malignant transformation, tumor invasion, and metastasis confirming its role in the suppression of cellular mobility. The present study is an attempt to evaluate the role of CDH1 in the development and progression of breast cancer through SNP analysis. Three SNPs of CDH1 gene viz: -160 C/A, 347 G/GA (located upstream from the transcriptional start site of the CDH1) and +54 C>T transition (located after the stop codon) were analyzed through PCR followed by sequencing. Further the -347G/GA (insertion mutation) SNP was confirmed by RFLP analysis. Blood samples were collected from 101 Breast cancer patients and 250 healthy individuals from department of oncology, NIMS hospital, Hyderabad, India and Infertility Institute and Research Centre (IIRC), Hyderabad, India respectively after ethical committee approval of the hospital and the institute. Elevated risk was observed for -347GA/GA, -160 AA and +54 TT genotypes for development of breast cancer. Further haplotype analysis confirmed that -347GA/-160A/+54C and -347GA/-160A/+54T haplotypes were significantly associated with breast cancer. In conclusion, CDH1 haplotypes may jointly modify the risk of breast cancer development in south Indian women. The lower transcriptional efficiency and m RNA instability associated with these SNP might explain the significant association. The loss of CDH1 protein may also promote tumor initiation through release of b catenin, potentiating the canonical Wnt signaling pathway, or by modulating mitogenic signaling, such as EGF-induced cellular proliferation. To the best of our knowledge, this is the first study from Indian population that reveals the correlation between CDH1 polymorphisms and the risk of developing breast cancer.

1241T

Genomic characterization of bladder cancer initiation and development. *S. Scherer[1, 2], T. Majewski[3], J. Bondaruk[3], D. Muzny[1], J. Drummond[1], L. Trevino[1], J. Niu[1], S. Zhang[3], J. Reid[1], K. Baggerly[4], I. Newsham[1], C. Dinney[5], B. Grossman[5], W. Zhang[3], M. Wang[1], Y-Q. Wu[1], M. Morgan[1], D. Wheeler[1, 2], B. Czerniak[3], R. Gibbs[1, 2].* 1) HGSC, Baylor Col Med, Houston, TX; 2) Mol & Human Gen, Baylor Col Med, Houston, TX; 3) Pathology, UT MD Anderson Cancer Ctr, Houston, TX; 4) Bioinformatics and Comp. Bio., UT MD Anderson Cancer Ctr, Houston, TX; 5) Urology, UT MD Anderson Cancer Ctr, Houston, TX.

We are characterizing the two primary paths of bladder cancer development by combining tumor-normal exome sequencing, RNA-seq and epigenetic analysis in an effort to understand the earliest proliferative events and subsequent progression of the disease. Bladder cancer generally presents as either papillary with relatively high recurrence rates or invasive, and in at least 15% of cases, progresses from the former to the latter. The physiology of resected bladder tissue presents a landscape to employ a unique strategy known as whole organ histologic and genetic mapping. The resected tissue is laid flat and divided into one-centimeter squares over the entire surface. Each square is characterized histologically by a pathologist and then processed to isolate both RNA and DNA. As the tissue blocks range from normal urothelium to carcinoma in situ to invasive transitional cell carcinoma, so too, the underlying mutation profile shifts ahead of these changes from incipient field effects to somatic small scale mutations and finally to larger numbers of mutations and chromosome rearrangements. Previous efforts using SNP mapping at a limited number of loci identified “forerunner genes,” which when mutated, provided proliferative advantage ahead of subsequent mutations in nearby known tumor suppressors. We are now extending these efforts using a combination of NimbleGen whole exome capture and SOLiD sequencing of three tumor-normal pairs on one tissue block per cystectomy. Preliminary analysis revealed mutations present in both the oncogenic RAS-ERK-MAPK signaling pathway and the tumor suppressor p53 regulatory pathway while no variants are observed in Ras, ERK or p53 directly. We are currently expanding these analyses in three ways: we are generating sequencing data on another four tissue blocks per cystectomy, we now have another six whole exome tumor-normal pair datasets generated on the Illumina Hi-seq platform and these results are being augmented by both transcriptome and epigenomic analyses. We believe these results will result in biomarker identification, identification of pathways and therapeutic targets and greater understanding of tumor initiation and development that may be applied to other cancers.

1242T

Proteomic and pathway analyses reveal a network of inflammatory genes associated with differences in skin tumor promotion susceptibility in DBA/2 and C57BL/6 mice. *J. Shen[1], E.L. Abel[1], J.M. Angel[2], P.K. Riggs[3], J. Repass[1], S.C. Hensley[1], L.J. Schroeder[1], A. Temple[1], A. Chau[1], S.A. McClellan[2], K. Lin[1], M.D. Ward[4], O.J. Semmes[4], M.D. Person[2], J. DiGiovanni[2,5].* 1) Department of Molecular Carcinogenesis, Science Park, The University of Texas M.D. Anderson Cancer Center, Smithville, TX 78957; 2) Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX 78712; 3) Department of Animal Science, College of Agriculture and Life Sciences, Texas A&M University, College Station, TX 77834; 4) The Leroy T. Canoles Cancer Research Center, Eastern Virginia Medical School, Norfolk, VA 23507; 5) Department of Nutritional Sciences, College of Natural Sciences, The University of Texas at Austin, Austin, TX 78712.

Genetic susceptibility to two-stage skin carcinogenesis is known to vary significantly among different stocks and strains of mice. In an effort to identify specific protein changes or altered signaling pathways associated with skin tumor promotion susceptibility, a proteomic approach of two-dimensional (2-D) gel electrophoresis and mass spectrometry was used to examine and identify proteins that were differentially expressed in epidermis between promotion-sensitive DBA/2 and promotion-resistant C57BL/6 mice following treatment with the phorbol ester, 12-O-tetradecanoylphorbol-13-acetate. We identified 19 differentially expressed proteins of which 5 were the calcium-binding proteins including annexin A1, parvalbumin (, S100A8, S100A9, and S100A11. The differential expression of two of these calcium-binding proteins, S100A8 and S100A9, was further examined and validated by the following methods: i) one-dimensional (1-D) Western blot analysis; ii) 2-D Western blot analysis; iii) immunohistochemical analysis; and iv) quantitative real-time PCR. Further analyses revealed that S100A8 and S100A9 protein levels were also similarly differentially up-regulated in epidermis of DBA/2 vs C57BL/6 mice following topical treatment with two other skin tumor promoters, okadaic acid and chrysarobin. Pathway analysis of all 19 identified proteins from the present study suggested that these proteins were components of several networks that included inflammation associated proteins known to be involved in skin tumor promotion (e.g. TNF-(, NF2B). Follow-up studies revealed that Tnf(, Nfkb1, Il22, and Il1(mRNAs were highly expressed in epidermis of DBA/2 (>2.5 fold, $p<0.05$) compared with C57BL/6 mice. Taken together, the present data suggest that differential expression of genes involved in inflammatory pathways in epidermis may play a key role in genetic differences in susceptibility to skin tumor promotion in DBA/2 and C57BL/6 mice.

1243T

A Novel Deletion of MEN1 Gene in a Large Family of Multiple Endocrine Neoplasia Type 1 (MEN1) with Aggressive Phenotype. *Y. Shi[1], H. Raef[2], M. Zou[1], E. Baitei[1], R. Al-Rijjal[1], N. Kaya[1], M. Al-Hamed[1], D. Monies[1], N. Abu-Dheim[1], H. Al-Hindi[3], M. Al-Ghamdi[3], B.F. Meyer[1].* 1) Genetics, King Faisal Specialist Hospital and Research Centr, Riyadh, Saudi Arabia; 2) Medicine,King Faisal Specialist Hospital and Research Centr, Riyadh, Saudi Arabia; 3) Pathology, King Faisal Specialist Hospital and Research Centr, Riyadh, Saudi Arabia.

Context: The MEN1 syndrome is associated with parathyroid, pancreatic, and pituitary tumours caused by mutations in the MEN1 gene. In general there is no genotype-phenotype correlation. Objectives: To characterize a large family with MEN1 with aggressive tumour behavior: malignant pancreatic endocrine tumours were present in 5 affected subjects and were the presenting features in 3 subjects. Design: The coding region of MEN1 was sequenced. The gene copy number analysis was performed by multiplex ligation-dependent probe amplification (MLPA) and array comparative genomic hybridization (aCGH).The loss of heterozygosity (LOH) in the tumour tissue was studied by microsatellite analysis. Insulin-like growth factor II (IGF-II) and CDKN1C/ p57KIP2 expression were investigated by immunohistochemistry. Results: Mutation screening by PCR-sequence analysis of patients' peripheral blood DNA did not reveal any mutation in the MEN1 or CDKN1B gene. Gene copy number analysis by multiplex ligation-dependent probe amplification (MLPA) and array comparative genomic hybridization (aCGH) demonstrated a novel monoallelic deletion of 5 kb genomic DNA involving MEN1 promoter, exon 1 and 2. LOH analysis indicated somatic deletion of maternal chromosome 11 including MEN1 locus (11q13) and 11p15 imprinting control regions (ICR). Methylation analysis of ICR demonstrated ICR1 hypermethylation and ICR2 hypomethylation in the tumour specimens. ICR1 and ICR2 control the expression of IGF-2 and CDKN1C/ p57KIP2, respectively. Immunohistochemistry analysis showed that the expression of paternally expressed IGF-2 was up-regulated and the maternally expressed CDKN1C/ p57KIP2 was lost in the pancreatic endocrine tumours. Conclusions: Gene copy number analysis by MLPA should be considered in patients with negative conventional mutation screening. Although large MEN1 deletion causes MEN1, disruption of imprinted CDKN1C/ p57KIP2 and IGF-2 expression may contribute to tumour progression and aggressive phenotype.

1244T

Genetic alterations in FGFR3 and RAS reveal mutual exclusiveness of these genetic events in urinary bladder cancer. A study in Kashmiri population. *M.A. Siddiqi[1], A.A Pandith[1], Z.A Shah[1], N.P Khan[2], M.S Wani[3].* 1) Immunology & Molecular Medicine , Sherl Kashmir Institute of Medical Sciences, Srinagar, Kashmir, India; 2) Clinical Biochemistry, Sherl Kashmir Institute of Medical Sciences, Srinagar, Kashmir, India; 3) Urology, Sherl Kashmir Institute of Medical Sciences, Srinagar, Kashmir, India.

Urothelial cell carcinoma (UCC) is a common malignancy. Worldwide, it is the seventh most prevalent cancer, accounting for 3.2% of all malignancies. The highest incidence is seen in industrialized countries and geographic areas where infection with Schistosoma haematobium is endemic. Men have a higher risk of bladder cancer than women, by a rate ratio of at least 3:1. We conducted a detailed study of the bladder cancer cases registered from 2005 to 2010 in the only tertiary care hospital in Northern India. This study revealed that bladder cancer ranks as the 7th leading cancer and accounts for 5.9% of all prevalent cancers in the Kashmiri population. Thus aim of this study was to analyze the frequency and association of FGFR3 and RAS gene mutations in UCC. We analyzed the mutations of FGFR3 gene and RAS gene family (HRAS, NRAS, and KRAS) to explore the association of FGFR3 & RAS gene alterations as two genetic events in the development of UCC. DNA of tissue specimens of 65 consecutive patients was examined. The DNA preparations were evaluated for the occurrence of FGFR3 and RAS gene mutations by PCR-SCCP and DNA sequencing. Somatic mutations of FGFR3 gene aggregated to 32.30% (21 of 65). The pattern and distribution of FGFR3 mutations were significantly associated with low grade/stage ($p<0.05$). The overall mutations of RAS genes aggregated to 21.5% and showed no association with any clinic-pathological parameters. In total, 53.8% (35 of 65) of the tumours studied had mutation of either a RAS or FGFR3 gene. RAS and FGFR3 mutations were totally mutually exclusive and are thought to reflect activation of the same pathway by either event. Unlike FGFR3 mutation, no obvious relationship of mutation of a RAS gene with tumour grade of stage has been found. FGFR3 and RAS are in the same signal transduction pathway, which might be a possible explanation for the hypothesis of mutual exclusiveness of mutations in these genes in this study. Thus we conclude that FGFR3 and RAS are mutually exclusive genetic events in UCC, suggesting that both provide the same selective advantage most likely activation of MAPK pathway.

1245T

Transcriptome Based Bioinformatic Analysis of a Unique Ovarian Cancer Model. *S.N. Smillie[2], K. Gambaro[2], D. Provencher[4,5], A.-M. Mes-Masson[5,6], P.N. Tonin[1,2,3].* 1) The Research Institute of the McGill University Health Centre, Montreal, Canada; 2) Department of Human Genetics, McGill University, Montreal, Canada; 3) Department of Medicine, McGill University, Montreal, Canada; 4) Division de gynécologie oncologique, Université de Montréal, Montréal, Canada; 5) Centre de Recherche du Centre Hospitalier de l'Université de Montréal / Institut du cancer de Montréal, Montréal, Canada; 6) Department of Medicine, University of Montreal, Montreal, Canada.

Ovarian cancer (OC) is the 5th most deadly female malignancy in Canada with an average 5-year survival rate of 30%. Women are diagnosed at an advanced stage where current therapies are ineffective due to poor screening strategies and a paucity of information regarding the etiology and pathology of the disease. Previously in our laboratory, a unique chromosome transfer technique was applied to OV90, a well-characterized, aggressive OC cell line possessing molecular genetic features commonly observed in high grade serous OC (HGSOC). The derived OV90 hybrids exhibited complete suppression of tumourigenicity and transcriptome analysis revealed that molecular networks characteristics of OC were affected. Current research has expanded the scope of this work to integrate higher density Affymetrix GeneChip expression arrays and a larger sample of independently derived normal ovarian surface epithelial brushings (NOSE) and HGSOC samples. These results have provided support to previous findings from our group (>22% of top candidates recapitulated) and identified additional molecular pathways that are transcriptionally altered both in our cell line model and between NOSE and HGSOC samples. The approach seeks to elucidate the biological mechanisms behind the abrogation of tumourigenicity observed in our model as it relates to HGSOC - the most common type of OC. The complement to this approach has been to investigate genes that exhibit differential expression between NOSE and HGSOC samples, but have stable expression levels in our model. Here we aim to identify pathways underlying the ability to grow indefinitely in culture, a feature retained in our model and one representative of immortalization, an essential phenotype of cancer. Of 153 candidate genes, 43 have been previously identified in cancer, 17 in OC. The integration and utilization of numerous bioinformatics resources and programs has yielded a systematic approach to candidate selection. Top candidates have been identified and steps to validate via qRT-PCR, immunohistochemistry and in vitro assays have been initiated. The characterization of this model aims to address fundamental aspects of OC biology with the hopes of improving our ability to treat and prevent this disease.

1246T

Germline RAD51C mutations in breast and ovarian cancer susceptibility. *F. Soubrier[1], N. Davids[1], A. Fajac[2], C. Colas[1], M. Eyries[1], F. Cornelis[3], A. Cortez[4], R. Rouzier[5], S. Uzan[5], JP. Lefranc[6].* 1) Oncogénétique moléculaire, GH-Pitié-Salpêtrière-APHP, PARIS, France; 2) Histologie Biologie Tumorale, Hôpital TENON-APHP, Paris, France; 3) Anatomo-Pathologie, Hôpital Jean Verdier-APHP, Paris, France; 4) Anatomo-Pathologie, Hôpital Tenon-APHP, Paris, France; 5) Chirurgie Gynécologique, Hôpital Tenon-APHP, Paris, France; 6) Chirurgie Gynécologique, GH-Pitié-Salpêtrière-APHP, Paris, France.

Mutations in the two major susceptibility genes BRCA1 and BRCA2 account for about 16% of familial breast cancers (Van der Groep et al). Genetic factors are mostly unknown for the remaining 84%. Several other genes might explain unelucidated family cases. In the case of breast cancer, deleterious mutations in few genes involved in the Fanconi complex and in the Fanconi-BRCA DNA repair pathway are responsible for Fanconi anemia at the homozygous state and breast cancer susceptibility at the heterozygous state (BRCA2, PALB2, BRIP1). RAD51C plays an important role in the double-strand break repair pathway and, a biallelic missense mutation in the RAD51C gene was found in a Fanconi anemia-like disorder (Somiyajit et al). Subsequently, six monoallelic pathogenic mutations were identified after screening 480 BRCA1/2 negative breast and ovarian cancer pedigrees (Meindl et al). Several reports were unsuccessful to replicate results of the Meindl's study. To investigate whether germline mutations in RAD51C are associated with an increased risk of developing breast and ovarian cancer, we screened, by sanger sequencing of the coding sequence, 92 index cases of breast and ovarian families from French or European origin, and negative for BRCA1/2 mutations. Mutation screening revealed two novel splice mutations flanking exon 7, one donor mutation at position +5 (c.965+5A>G), and one acceptor at position -2 (c.905-2A>C). Either the wild-type exon 7 and its flanking regions, or the two mutated sequences were introduced into the pSPL3 splicing reporter plasmid, and transfected into HeLa cells. The RT-PCR analysis of the transfected cells showed that both mutations induced the loss of exon 7 in the mature transcript, and introduced a frameshift and a codon stop at position 363. This result was confirmed by RT-PCR of the mRNA isolated from blood cells of one index case. One mutated patient presented with an invasive serous ovarian cancer and the other one an invasive endometrioid ovarian carcinoma, and in both cases, several cases of breast cancer were present in the relatives. In our study we found 2 pathogenic mutations among 92 families screened which corresponds to a 2,2 % frequency, a frequency higher than in Meindl et al. Our results confirm that RAD51C is a susceptibility gene for ovarian and breast cancer and that this gene should be screened for mutations in families with multiple breast/ovary cancers.

1247T

Whole Exome Sequencing of Pre- and Post-treatment Glioblastoma Multiforme. *K. Squire[1], A. Lai[2], S.F. Nelson[1].* 1) Human Genetics, UCLA, Los Angeles, CA; 2) Neuro-Oncology, UCLA, Los Angeles, CA.

Glioblastoma mulitforme (GBM) is the most common and aggressive type of primary malignant brain tumor in adults. The standard treatment for GBM is radiotherapy (RT) combined with the alkylating agent temozolomide (TMZ). Despite treatment, GBM remains incurable, with median overall survival under 18 months. Efforts to gain a molecular understanding of GBM have focused on characterization of the untreated tumor. However, RT/TMZ treatment can induce an expanded mutational spectrum potentially involving point mutations, chromosomal aberrations and epigenetic changes. This expanded mutational spectrum within relapsed tumors may contain `driver' aberrations associated with resistance to RT/TMZ. To explore this hypothesis, we are conducting whole-exome sequencing of pre- and post-treatment GBM tumors and matched normal blood samples. In an illustrative sample, copy number analysis based on exome sequencing showed significant amplification of a region containing EGFR on chromosome 7 and loss of chromosome 10 in both tumors, but little difference between the tumors at the chromosomal level. We discovered 1428 and 1779 somatic SNVs/indels in the pre- and post-treatment tumors, respectively, with 1071 shared mutations. Comparing the tumors, we discovered 31 novel non-synonymous, protein-altering SNVs and 5 novel frameshift-causing indels in 31 genes the recurrent tumor, including EGFR, SUFU, and C9orf72. Most novel variants were not detected in the pre-treatment tumor but enrichment of the variants was observed consistent with clonality and a heterozygous state in the recurrent tumor. These findings suggest a selective advantage for the post-treatment haplotypes as heterozygous mutations, although not proven here. SNVs of interest include 3 EGFR variants on the amplified allele. One EGFR variant (R521K) is heterozygous in the blood genome, and both tumors show a gross amplification of the reference allele, typical of DNA amplifications. A second EGFR mutation (R324L) is non-existent in germline, appears in the initial tumor, and is virtually eliminated in the post-treatment tumor, suggesting that the treatment selected against this mutation. In contrast, an EGFR C620Y mutation was also absent in germline, but is strongly present in the initial tumor, and has been highly selected for in the post-treatment tumor. These results begin to highlight the mutational changes between pre- and post-treatment GBM tumors and may inform future prognosis and treatment of GBMs.

1248T

Role of genetic variants of ESR1, ESR2 and PGR in susceptibility to gallbladder cancer. *A. Srivastava[1], K. Sharma[1], S. Misra[2], A. Kumar[3], N. Srivastava[4], B. Mittal[1].* 1) Genetics, Sanjay Gandhi Post Graduate Institute of Medical Science, Lucknow, UP; 2) Surgical Oncology, Chhatrapati Shahuji Maharaj Medical University, Lucknow, UP; 3) Surgical Gastroenterology Sanjay Gandhi Post Graduate Institute of Medical Science, Lucknow, UP; 4) Physiology, Chhatrapati Shahuji Maharaj Medical University, Lucknow, UP.

Rationale: Gallbladder cancer (GBC) shows one of the highest incidence and mortality rates in regions of Northern India. GBC is more common in females, suggesting that sex hormones may play a noteworthy role in the etiology of the disease. The intracellular action of estrogens and progesterone is regulated by the estrogen and progesterone receptors. Therefore, the present study was planned to investigate the associations of common genetic variants in estrogen (ESR) and progesterone (PGR) receptor genes with risk of GBC. Methods: The case control study included subjects who completed an interview and provided blood, which totaled 243 GBC cases, 230 gallstone cases and 220 healthy subjects from North India. The study examined whether polymorphisms of ESR1 IVS1-397T>C (rs2234693), IVS1-351A>G (rs9340799) and Ex4-122C>G (rs1801132), ESR2 -789T>G (rs1271572), Ex6-1082G>A (rs1256049) and variant of PGR Ins/del (rs1042838) are associated with GBC susceptibility. Genotyping was carried out by PCR-RFLP. Statistical analysis was performed by using SPSS ver16. Results: We found that the ESR1 IVS1-397 TT, IVS1-351 GG and ESR2-789 GG genotypes were significantly associated with an increased risk of GBC (Odds Ratio [OR] 2.4, 2.5, 2.0 respectively) and gallstone disease (OR; 2.9 2.0 1.8 respectively) as compared to healthy controls. In contrast, ESR1 Ex4-122C>G and ESR2 Ex6-1082 G>A showed no significant association with GBC and gallstone disease either by allelic or genotypic frequencies. Furthermore, ESR1 T,G,C (OR; 3.0) and T,G,G (OR; 2.0) haplotypes and ESR2 C,G haplotype (OR; 2.2) also showed increased risk for GBC. In PGR, the insertion variant conferred low risk for both GBC (OR; 0.4) and gallstone disease (OR; 0.5). The protective effect of PGR variant (rs1042838) was nullified by ESR1 T,G,C/T,G,G haplotypes in dose dependent manner. Conclusion: Our findings suggest that common variants in hormone-receptor genes ESR1 (rs2234693-rs9340799), ESR2 (rs1271572) and PGR (rs1042838) are associated with risk of gallbladder cancer as well as gallstone disease. Acknowledgements: The study was supported for financial assistance by ICMR, CSIR, DST and DBT, Government of India.

1249T

De novo germline genetic alterations in cancer susceptibility. *Z. Stadler[1], S. Shah[1], B. Yamrom[2], J. Vijai[1], D. Esposito[2], D. Levy[2], J. Kendall[2], K. Sarrel[1], N. Hansen[1], M. Robson[1], N. Kauff[1], D. Feldman[1], G. Bosl[1], L. Norton[1], M. Wigler[2], K. Offit[1].* 1) Memorial Sloan Kettering Cancer Center, New York, NY; 2) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Objective: Genomic structural variations, such as copy number variants (CNVs), contribute significantly to inter-individual genetic variation and have been implicated in susceptibility to neurocognitive disorders. The objective of this study is to determine if de novo CNVs also play a role in cancer susceptibility. Methods: We performed CNV analysis, using 2.1M NimbleGenHD arrays, on a unique ascertainment of DNA samples from “trios”-probands with early-onset cancer and their unaffected biologic parents. Custom algorithms encompassing a series of normalizations including local intensity normalization, lowess normalization and finally archival normalization that adjusts sample hybridizations to a large data set of “standard-standard” hybridizations were used to capture de novo regions. De novo events were characterized and validated with TaqMan copy-number assays for deletions/amplifications. Unaffected siblings of affected probands served as controls for our case-parent trios. Results: In the first 42 testicular germ cell tumor (TGCT) case-parent trios to have undergone analysis, we have identified 5 de novo events affected probands, a rate that exceeds expected background de novo rates for such events. All events occurred outside known regions of common copy-number polymorphisms, and included 3 amplifications and 2 deletions, ranging in size from 23kb to 400kb. Some regions were found to overlap somatic alterations previously described in testicular tumor samples. In one young man with non-seminomatous testicular cancer (NSGCT), two regions, an 80kb amplification on chr3 and a 23kb deletion on chr4 were identified. Another young man with NSGCT was found to harbor a very large deletion, spanning over 400kb, on chr6. Conclusions: Our preliminary data suggests a role for de novo germline deletions and amplifications in cancer susceptibility; however, further validation of results, expansion of the TGCT ascertainment, and assessment in other cancer types is still ongoing. These results mark one of the very first studies focused on the role of de novo mutations as a mechanism of cancer causation and could potentially represent a new paradigm in our understanding of cancer genetics that could have broad applications in terms of cancer risk stratification and cancer prevention.

1250T

Common breast cancer susceptibility loci are associated with triple negative breast cancer. *K.N. Stevens[1], H. Nevanlinna[2], D. Yannoukakos[3], P.A. Fasching[4], D. Eccles[5], J. Chang-Claude[6], P. Miron[7], J.E. Carpenter[8], A.K. Godwin[9], U. Hamann[10], C. Ambrosone[11], R. Winqvist[12], H. Brauch[13], D. Lambrechts[14,15], I. dos Santos Silva[16], J. Peto[16], M.K. Schmidt[17], A. Cox[18], G. Severi[19], E. Sawyer[20], S. Margolin[21], A. Manermaa[22], N.G. Martin[23], G.W. Montgomery[23], P. Pharoah[24], D.F. Easton[25], X. Wang[26], S. Slager[1], C.M. Vachon[1], F.J. Couch[26], Triple Negative Breast Cancer Consortium.* 1) Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA; 2) Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Helsinki, Finland; 3) Molecular Diagnostics Laboratory IRRP, National Centre for Scientific Research “Demokritos”, Athens, Greece; 4) University of California at Los Angeles, David Geffen School of Medicine, Department of Medicine, Division of hematology and Oncology, Los Angeles, CA, USA; 5) University of Southampton, Faculty of Medicine, Southampton University Hospitals NHS Trust, Southampton, UK; 6) Division of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany; 7) Dana Farber Cancer Institute, Boston, MA, USA; 8) Australian Breast Cancer Tissue Bank, University of Sydney at the Westmead Millennium Institute, Westmead, NSW, Australia; 9) Department of Pathology and Laboratory Medicine, Kansas University Medical Center, Lawrence, KS, USA; 10) Molecular Genetics of Breast Cancer, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany; 11) Department of Cancer Prevention and Control, Roswell Park Cancer Institute, Buffalo, NY, USA; 12) Laboratory of Cancer Genetics, Department of Clinical Genetics and Biocenter Oulu, University of Oulu, Oulu University Hospital, Oulu, Finland; 13) Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, and University Tübingen,Germany; 14) Vesalius Research Center, VIB, Leuven, Belgium; 15) Vesalius Research Center, University of Leuven, Leuven, Belgium; 16) Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK; 17) Division of Experimental Therapy and Molecular Pathology and Division of Epidemiology (MKS), Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands; 18) Institute for Cancer Studies, Department of Oncology , Faculty of Medicine, Dentistry & Health, University of Sheffield, UK; 19) Cancer Epidemiology Centre, The Cancer Council Victoria, Melbourne, Australia & Centre for Molecular, Environmental, Genetic, and Analytic Epidemiology, The University of Melbourne, Australia; 20) National Institute for Health Research (NIHR) Comprehensive Biomedical Research Centre, Guy's & St. Thomas' NHS Foundation Trust, London, UK; 21) Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden; 22) Institute of Clinical Medicine, Department of Pathology, University of Eastern Finland and Kuopio University Hospital; Biocenter Kuopio, Kuopio, Finland; 23) QIMR GWAS Collective, Queensland Institute of Medical Research, Brisbane, Australia; 24) Department of Oncology and Department of Public Health and Primary Care University of Cambridge, Cambridge, UK; 25) Department of Genetic Epidemiology, Cancer Research UK Genetic Epidemiology Unit, Strangeways Research Laboratory, Cambridge, UK; 26) Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 First St. SW, Rochester, MN, USA.

Triple negative breast cancers form an aggressive subtype of breast cancer with poor survival. However, little is known about the etiological factors that promote the initiation and development of this form of breast cancer. Commonly inherited breast cancer risk factors identified through genome wide association studies display heterogeneity of effect among breast cancer subtypes defined by estrogen receptor and progesterone receptor status. In this large study of genetic susceptibility to triple negative breast cancer by the Triple Negative Breast Cancer Consortium (TNBCC), 22 common breast cancer susceptibility variants were investigated in 2,980 Caucasian women with triple negative breast cancer and 4,978 healthy controls. Six single nucleotide polymorphisms (SNPs) were highly significantly associated with risk of triple negative breast cancer: rs2046210 (ESR1) (p=4.38 x 10-7), rs12662670 (ESR1) (p=1.13 x 10-4), rs3803662 (TOX3) (p=3.66 x 10-5), rs999737 (RAD51L1) (p=2.96 x 10-4), rs8170 (19p13.11) (p=2.25 x 10-8) and rs8100241 (19p13.11) (p=8.66 x 10-7). These results provide convincing evidence of genetic susceptibility for triple negative breast cancer.

1251T

Effects of phytochemicals on Nickel and Chromium induced DNA damage. *A.R. Patel[1], S.S. Chettiar[2], D.D. Jhala[3], M.V. Rao[3].* 1) Molecular cell Biology, Iladevi Cataract and IOL Research Centre, Ahmedabad, Gujarat, India; 2) Department of Biotechnology, Shree Ramkrishna Institute of Computer Education and Applied Sciences, M.T.B. College Campus, Athwalines, Surat, Gujarat, India; 3) Department of Zoology, School of Sciences, Gujarat University, Ahmedabad, Gujarat, India.

Purpose: Curcumin and Andrographolide are the phytochemicals of medicinal plants Curcuma longa and Andrographis paniculata were investigated to evaluate its ameliorative potential against Nickel (Ni) and/or Chromium (Cr) exerted effects. Ni and Cr compounds are exposed to human population by numerous paths. Hence, this study was undertaken to establish potent herbal antidote against these metal ions. Methodology: In the present study, we report the cytogenetic variations in lymphocyte cultures after exposure to Cr and Ni in the form of potassium dichromate (K2Cr2O7, 1.36 X 10-6M) and nickel chloride (NiCl2, 4.216 X 10-5M) respectively in vitro for 24 and 69 hours exposures and phytochemicals Curcumin (3.87 X 10-7M) and Andrographolide (0.4 x 10-6M) were cosupplemeted. The genotoxic effects of the compounds were analyzed by scoring of genotoxic endpoints like chromosomal aberration, comet assay, and markers of oxidative stress. A known mutagen ethyl methane sulfonate (EMS) was also used as a positive test control. Results: Results revealed that the both curcumin as well as andrographolide significantly reduced chromosomal aberrations and also there was reduction in the comet tail length in these phytochemical supplemented cultures. Cultures supplemented with curcumin showed 92 % where as andrographolide showed 88% amelioration. Exposure dependent effect of the phytochemicals were noticed, results suggested that these phytochemicals showed better effects in the longer (69hrs.) exposure intervals. Conclusion: Curcumin and Andrographolide, medicinal phytochemicals showed protective effect on the DNA damage induced by the Ni and Cr in peripheral blood lymphocyte cultures. Curcumin is more potent ameliorative agent than the andrographolide at this concentration.

1252T

Detecting Chromosomal Inversions Using Chromatid Paints- Use for Synteny Among the Hominidae. *F.A. Ray[1,2], E. Zimmerman[2], M.N. Cornforth[3], J.S Bedford[1,2], E.H. Goodwin[2], S.M. Bailey[1,2].* 1) Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO 80523; 2) KromaTiD Inc., 515 E. Laurel St., Fort Collins, CO 80524; 3) Department of Radiation Oncology, University of Texas Medical Branch, Galveston, TX 77555.

There is no suitable method to routinely detect inversions smaller than a typical metaphase chromosome band (i.e. 10Mb). A chromosomal inversion is an intrachromosomal aberration initiated by misrepair of two double strand breaks and resulting in a segment of DNA within a chromosome being oriented in the opposite direction from the rest of the DNA molecule. We have developed a new methodology to improve inversion detection that involves design of strand specific fluorescent probes that when combined with strand specific removal of the nascent DNA strands (using Chromosomal Orientation- Fluorescence In Situ Hybridization(CO-FISH)) allows hybridization to only one of the remaining two DNA strands within the chromosome. Thus, probes hybridize to an unaltered chromatid producing a fluorescent `chromatid paint' and a chromatid with an inversion produces an easily detectable fluorescence signal switch from one chromatid to the other. For the first chromatid paint, human chromosome 3 was chosen. Hybridization targets, regions of unique DNA, were designed to be located at 1Mb intervals along the length of the four contigs making up chromosome 3. A total of 195 fluorescent probes, along the length of chromosome 3 were developed and tested individually. The probes were then combined and hybridized to cells derived from normal humans. The result in every case has been a fluorescent paint appearing on one chromatid and not the other in both chromosome 3 homologs. To date, no constitutive inversions have been observed in cells from different humans (0/12). The chromatid paint was also hybridized to cells from a patient with one normal chromosome 3 and a known inversion on the other homolog. One homolog produced a clear signal switch at the known breakpoints and the normal homolog did not, clearly demonstrating the ability of chromatid painting to detect inversions. The ability to detect a 1Mb inversion was demonstrated using a chromatid paint with one probe complementary to the rest. It is also important to include inversions as an informative class of evolutionary changes in genome structure. The human chromosome 3 chromatid paint was hybridized to cells from the great apes; chimpanzee, gorilla and orangutan. In each case the chromatid paint hybridized to the corresponding chromosome 3 only. A large inversion observed in orangutan chromosome 3 will be described and the potential of chromatid painting for synteny studies will be further elucidated.

1253T

Implementation of whole genome copy number-SNP arrays in a clinical reference laboratory: expanding the possibilities for molecular karyotyping. *T. Sahoo, R. Owen, L.P. Ross, M.M. Elnaggar, P.H. Kohn, M.H. Haddadin, F.Z. Boyar, L.W. Mahon, B.T. Wang, C.M. Strom, A.L. Anguiano.* Quest Diagnostics Nichols Institute, San Juan Capistrano, CA.

Microarray-based technologies have enhanced the identification of chromosomal abnormalities. The transition from BAC and oligonucleotide-based array comparative genomic hybridization (aCGH) to whole-genome oligonucleotide single-nucleotide polymorphism (oligo-SNP) arrays for postnatal and prenatal constitutional studies is already a reality. Oligo-SNP arrays enable simultaneous high-resolution interrogation of both copy number variations (CNVs) and copy neutral segments of homozygosity (SOH). Our experience using a whole-genome oligo-SNP array to characterize constitutional abnormalities is presented. Since implementation of the Affymetrix SNP Array 6.0 at Quest Diagnostics, >1386 samples submitted for evaluation of constitutional disorders have been analyzed. During the same period, 2642 cases were analyzed with a proprietary, whole-genome BAC aCGH platform. Based on analytical, genomic, and clinical metrics, oligo-SNP revealed clinically significant abnormalities in 178 (13%) cases, findings of unclear clinical significance in 193 (14%), and single or multiple SOH in 48 (3.4%). The clinically significant abnormalities included 168 cases with pure CNVs and 10 with both CNVs and SOH or uniparental disomy. Interpretation of findings of unclear clinical significance was based on multiple criteria including gene content, local genomic architecture, overlap with population variants and a systematic evaluation of similar CNVs identified in our laboratory. Parental analysis in 36 oligo-SNP cases revealed 5 to be de novo and 31 to be inherited from a parent. During the same period, BAC aCGH identified clinically significant abnormalities in 250 cases (9.4%) and findings of unclear clinical significance in 166 (6.3%). Parental BAC aCGH in 39 cases identified 7 to de novo and 32 to be inherited from a parent. Additionally, an average of 12-20 recurrent, small, apparently benign CNVs were identified by oligo-SNP per case. Therefore, implementation of the oligo-SNP platform is improving our ability to detect clinically significant alterations. An important initial outcome has been the identification and characterization of several complex chromosomal rearrangements that would be undetectable with conventional methods or earlier array platforms. These data are of profound value in helping identify novel rearrangements, re-define the nature of known microdeletion-microduplication disorders, and deriving a more explicit genotype-phenotype correlation in many cases.

1254T

Suggestion for post-zygotic translocation; X-chromosome inactivation spred to autosome with a t(X;15)(p21.1;q11.2) translocation. *S. Sakazume[1,2], H. Ohashi[3], Y. Sasaki[4], N. Harada[4], K. Nakanishi[5], H. Sato[5,6], M. Emi[5,6], K. Endoh[7], Y. Kido[2], T. Nagai[2], T. Kubota[7].* 1) Takasaki, Gunma, Japan; 2) Division of Pediatrics, Dokkyo University Koshigaya Hospital, Koshigaya, Japan; 3) Division of Genetics, Saitama Children's Medical Center, Hasuda, Japan; 4) Department of Molecular Genetic Research and Analysis, Advanced Medical Science Research Center, Mitsubishi Chemical Medience Corporation, Tokyo, Japan; 5) DNA Chip Research Inc., Yokohama, Japan; 6) Department of Neurology, Hematology, Metabolism, Endocrinology and Diabetology, Yamagata University School of Medicine, Yamagata, Japan; 7) Department of Epigenetic Medicine, Faculty of Medicine, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Chuo, Japan.

Background: X-chromosome inactivation (XCI) is an essential mechanism in females that compensates for the genome imbalance between females and males. It is known that XCI can spread into an autosome of patients with X;autosome translocations. Methods: The subject was a 5-year-old boy with Prader-Willi syndrome (PWS)-like features including hypotonia, hypogenitalism, and hypo-pigmentation, and developmental delay. G-banding, fluorescent in situ hybridization, BrdU-incorporated replication, human androgen receptor gene locus assay (HUMARA), SNP microarrays, ChIP-on-chip assay, bisulfite sequencing, and real-time RT-PCR were performed. Results: Cytogenetic analyses revealed that the karyotype was 46,XY,der(X)t(X;15)(p21.1;q11.2), -15. In the derivative chromosome, the X and half of the chromosome 15 segments showed late replication. HUMARA showed the two X segments were maternal origin. SNPs confirmed the chromosomes 15 had biparental origin. The DNA methylation level was extremely high in the proximal region from the breakpoint, and the level gradually decreased toward the telomere in the chromosome 15 region. The promoter regions of the imprinted SNRPN and the non-imprinted OCA2 genes were completely and half methylated, respectively. However, no methylation was found in the adjacent imprinted gene UBE3A, which contained a lower density of LINE1 repeats. Conclusions: Our findings suggest that XCI spread into the paternal chromosome 15 leading to the aberrant hyper-methylation of SNRPN and OCA2 and their decreased expression, which contributes to the PWS-like features and hypo-pigmentation. To our knowledge, this is the first report in which the DNA methylation level is demonstrated in an autosome subject to XCI. The methylation status of SNRPN is complete in this patient. Uncommon hypermethylation is suspected in paternal SNRPN in the patient. We assume that, in the derivative chromosome, X region is maternal and 15q is paternal. The derivative chromosome isolation is inevitable step for the verification of post-zygotic translocation.

1255T

Identification of Chromosomal Alterations in Autism Patients. *K. Sasikala[1], V. Balachandar[1,2].* 1) Human Molecular Genetics, Bharathiar University, Coimbatore, India; 2) Thiruvalluvar University, Vellore, india.

Autism is a complex neurobiological disorder that typically lasts throughout a person's lifetime. It is part of a group of disorders known as autism spectrum disorders (ASD). It is often associated with other conditions, such as disorders of the CNS (tuberous sclerosis), developmental delay, attention deficit, epilepsy, and anxiety and mood disorders. At the current time, the exact causes of autism remain elusive, but researchers increasingly believe that both genetics and environment play a role. The prime aim of the present study was to identify the chromosomal aberrations with autism patients in Coimbatore region. In order to investigate the possible cytogenetic damage in autism patients, peripheral blood lymphocyte culture (PBLC) method was carried out on the lymphocytes of 45 autism patient samples and equal number of controls was selected, based on the detailed questionnaire. An autism child has been followed up regularly at frequent intervals of 4-6 weeks both by the paediatrician and child psychologist. Parameters during follow up included enquiry into the parents' assessment of the child as a whole, level of hyperactivity after behavioural and pharmacological intervention. For karyotyping, 100 complete metaphase cells from each subject were evaluated using Trypsin - Giemsa Banding method. In our study chromosomal alterations were frequently observed in chromosomes 2, 3, 8, 16 and X (2q32, 3q25-q27, 16p13, Xp22, and Xq13). In conclusion, in this pilot study, we observed the chromosomal instability in peripheral blood lymphocytes may be a potential biomarker in autism patients. Identification of cytogenetic abnormalities is not only important for providing a cause for the autism in a single individual but is also critical for accurate counselling regarding recurrence risks to parents and family members.

1256T

Molecular cytogenetic characterization of partial deletion Xq and duplication Xp in a patient with premature ovarian failure. *S.H. Shim[2], M.K. Kim[1], S.H. Park[2], J.E. Park[2], Y.H. Cho[3], D.H. Cha[1,2], T.K. Yoon[1].* 1) Department of Obstetrics and Gynecology; 2) Genetics Laboratory, Fertility Center of CHA Gangnam Medical Center, CHA University, Seoul,135-081, Korea,; 3) Department of Medical Genetics, College of Medicine Hanyang University, Seoul, 133-791, Korea.

A 26-year-old female was initially suspected as premature ovarian failure (POF) syndrome because of amenorrhea and high levels of serum FSH and LH, 85.21mIU/ml and 25.05mIU/ml, respectively. Numerical or structural abnormalities of an X chromosome and FMR1 premutation were well known genetic causes of this syndrome. Chromosome analysis with high-resolution GTG banding technique was carried out and her karyotype was apparently 46,XX normal female. FMR1 gene analysis was performed by polymerase chain reaction (PCR) and Southern blotting. The PCR result was normal and one allele in the normal range of (CGG)n repeats was detected. However, Southern blot analysis showed an unusual pattern, no size expansion nor methylated allele detected, which was normal male pattern not female. To figure out this unusual result, multiplex ligation-dependent probe amplification (MLPA) with the subtelomeric region specific probe set (P070) was carried out and the result showed the trisomy Xp and monosomy Xq. This result was confirmed by fluorescent in situ hybridization (FISH) with the TelVysion Xp/Yp and Xq/Yq probes. One of the X chromosomes had two Xp signals on both ends of the X chromosome and no Xq signal. Her family members were also investigated. Her mother is 50 years old and showed no signs of POF syndrome and her 24-year-old younger sister showed normal regular menstruation and normal serum FSH and LH level. Both of them showed apparently normal 46,XX karyotype like the proband. However, FISH analysis showed the mother had a pericentric inversion between Xp22.3 and Xq27 and her younger sister had the same X chromosome abnormality as that of the proband. From these results, the proband's karyotype was 46,X,rec(X)dup(Xp)inv(X)(p22.3q27)mat. To characterize the exact chromosomal regions and genes involved, array based comparative genomic hybridizations (array CGH) using the 135K NimbleGen Whole-Genome Array are applied and the results are pending. Although, the proband's sister does not show any signs and symptoms of POF at this moment, appropriate genetic counseling and clinical follow-up is required.

1257T

A 3.44 MB interstitial duplication of chromosome 3 with no apparent phenotype detected by SNP array. *M. Thangavelu[1], J.H. Tepperberg[2], J. Hume[3], B. Huang[1].* 1) Genzyme Genetics, Orange, CA; 2) LabCorp, NC; 3) Newton-Wellesley Hospital, Newton, MA.

Prenatal cytogenetic studies on amniocytes referred for abnormal sequential screening (1:35 risk Down syndrome and 1:18 risk trisomy 18) and fetal MCA, including heart defect and IUGR on ultrasound, revealed the following karyotype: 47,XY,+mar[10]/46,XY[5]. Whole genome SNP microarray to characterize the marker, revealed an interstitial duplication of a minimum size of 3.443 MB of material from the short arm of chromosome 3 (BP linear position was from 74, 756, 515 to 78, 199, 925 including the gene ROBO2). FISH studies showed that the marker did not contain chromosome 3 material but was derived from the pericentromic region of chromosome 11. Maternal studies showed that the duplication of chromosome 3 material was maternally derived, with insertion of the duplicated material from the short arm on to the long arm of chromosome 3. The pregnancy ended in fetal demise around 20 week gestation. This case illustrates a number of issues to be considered prior to offering and performing microarray; (1) need for parental studies and (2) unexpected findings and (3) the need for appropriate genetic counseling. Irrespective of the size of the duplication/deletion parental follow up studies are important. Unexpected findings in normal individuals may not be entirely benign, because of the potential role of other factors (genetic and non genetic) in the expression of the phenotype. Also, one should be mindful that due to lack of previously documented/reported information satisfactory interpretation may not be feasible in some instances. All these aspects need to be addressed during genetic counseling prior to offering microarray so that the patient is fully aware that the testing may be a lengthy process and may not offer a definitive answer in all cases. When a definitive answer is not available, patients should be prepared to revert to the available phenotype (ultrasound findings) in making a decision. Detailed analysis and documentation of additional cases with copy number variation of relatively large segments with no apparent clinical phenotype will provide valuable information for genetic counseling, as microarray testing gains importance as a powerful tool in genetic diagnosis. Genzyme Genetics and its logo are trademarks of Genzyme Corporation and used by Esoterix Genetic Laboratories, LLC, a wholly-owned subsidiary of LabCorp, under license. Esoterix Genetic Laboratories and LabCorp are operated independently from Genzyme Corporation.

1258T

Cytogenetic study of petrol pump workers occupationally exposed to Benzene. *R.P. Thumbar, P.K. Gadhia.* Department of Biosciences, Veer Narmad South Gujarat University, Surat, Gujarat, India.

Abstract: Petrol has become one of the basic needs of common men. To supply and distribution, there are many industries involved, refinery to petrol pump station. Out of these industries petrol pumps are the only place where petrol fume is directly exposed to maximum numbers of individuals who refill petrol to vehicles. Petrol is not a chemical but mixture of aliphatic hydrocarbons and antiknock agents; aromatic hydrocarbons such as benzene and toluene. Benzene is now proven leukemogen. The concentration of benzene in petrol differs according to mandatory regulation. In developed countries like Europe, Australia and USA, the concentration of benzene in petrol is less than 1%, where as in developing countries like India the concentration of benzene is approximately 3-5%. So it is important to measure the genotoxic risk factor of petrol pump attendants who are serving 8 - 12 hours according to their shift at benzene containing work atmosphere. Methodology: In present study, we have considered smoker (n=25) and non smoker (n=25) petrol pump attendants as a study group where as their respective smokers (n=25) and non smokers (n=25) control individuals, who are not exposed to benzene. Sister chromatid exchange, micronuclei and chromosomal aberration were carried out form blood by peripheral blood lymphocyte culture for genotoxic evaluation. Mitomycin C was used to add in other set of culture vials, as a positive control. Urinary phenol and t,t - muconic acid (HPLC) measured as urinary benzene biomarkers. Result: Micronuclei frequency in smoker petrol pump attendants was seen to be increased ($p< 0.05$) significantly as compared to control. Whereas other cytogenetic parameters such as, SCE and chromosomal aberrations were did not show any significant difference. Urinary phenol and t,t - muconic acid concentration in smokers and non smoker petrol pump workers was found to be significantly increased respectively ($p< 0.05$) and ($p<0.01$) than controls. Conclusion: Increased urinary phenol and t,t - muconic acid in petrol pump attendants ensure the exposure of benzene in work area. Chromosomal endpoints did not show any significant difference in petrol pump attendants than the respective controls, exceptionally micronuclei study showed significant increase in micronuclei frequency of smoker petrol pump attendants than control. Thus, other cytogenetic parameters such as comet assay, FISH etc with cohort study are needed to reach to any concrete conclusion.

1259T

Cytogenetic analysis in material from abortions in couples with recurrent first-trimester miscarriages. *C. Uria Gomez[1, 2], G. Arteaga Ontiveros[1], A. Rodríguez Gómez[1].* 1) Genecon Diagnostico Laboratorio, Toluca Edo. de Mexico, Mexico; 2) Facultad de Medicina UAEM.

The cytogenetic analysis of fetal tissue from spontaneous abortions shows that 50-60% of them were caused by abnormal karyotype. The most frequent chromosomal abnormality is trisomy 16. The aim of this study was to know the frequency of chromosomal aberrations in patients with recurrent miscarriages. Cytogenetic analysis was performed in 100 samples of miscarriage in women with at least 2 first trimester spontaneous abortions. The samples were processed by the explant technique and enzyme digestion using trypsin and collagenase. Cultures were incubated at 37ºC with an atmosphere of 5 % CO_2 and harvested with the usual technique. The chromosomal analysis was performed with GTG banding technique. The cytogenetic results, along with clinical data including gestational age at the time of the miscarriage and maternal age were compiled in a database. The incidence of specific chromosome abnormalities was determined. Cytogenetic results were obtained for 71 cases (71 %), of which 22 (31 %) showed chromosome abnormalities. The most frequent chromosomal aberration was trisomy, detected in 17 cases. The most common autosomal trisomy was that of chromosome 18 (4 cases), followed by trisomy 22 (3 cases) trisomy 13 (2 cases) and trisomy 21 (2 cases). We found other trisomies involving chromosomes 4, 10, 14, 15, 16, and 20, some of them showed mosaicism with a normal cell line. Triploidy was identified in one case, and tetraploidy was detected in one case. A balanced translocation was identified in one case. Trisomy 4 and 10 were detected in earliest gestational age. Of the 100 samples received, 13 of them there was no cell growth and 16 were discarded because they showed bacterial contamination. Our success rate in cell growth, removing the contaminated samples, is 81.6%. This percentage is acceptable according to data reported in literature. Of the 49 samples that were normal, 37 had a karyotype 46,XX and the remaining 12 were 46,XY. The cytogenetic study of abortions is difficult because the study material on occasion is not the most suitable for cell culture, and that may have contamination, maceration or after formalin fixation. Cytogenetic analysis in spontaneous abortions is very important, especially when it has a history of previous losses.

1260T

Fetoplacental discrepancy with normal karyotype in amniotic fluid and two different cell lines in placenta: A case report. *G. Velagaleti[1], K. Higby[2], E. Williamson[1], C. Mendiola[1], V. Ortega[1].* 1) Department of Pathology, University of Texas Health Science Center, San Antonio, TX; 2) Center for Maternal and Fetal Care, San Antonio, TX.

We present a case of fetoplacental discrepancy in a second-trimester fetus with normal karyotype in amniotic fluid and two different Robertsonian translocations in placenta, one balanced and one unbalanced. A 41-year-old woman of Middle-Estern origin, gravida 2, para 1 underwent amniocentesis at 16-weeks gestation due to advanced maternal age. Prenatal aneuvysion testing showed a signal pattern consistent with normal female chromosomes while amniotic fluid showed a normal 46,XX karyotype. Interestingly the karyotype also showed a homozygous inv(9) in all the cells analyzed. Although the inv(9) is considered a polymorphic variant, due to the homozygous nature, parental chromosome studies were suggested. Both parents showed the inv(9) in thier respective karyotypes, thus suggesting the biparental inheritance of the homozygous inv(9) in the fetus. Fetal ultrasound was normal and didnot show any unusual findings. The mother presented to the clinic four weeks later with an intrauterine fetal demise. Although cultures were initiated from fetal, cord and placental tissues, growth was obtained only from placental tissue. Chromosome analysis showed two different cell lines, a balanced (15;21) Roberstonian translocation in 11 cells and an unbalanced (21;21) Robertsonian translocation in 9 cells. The karyotype was interpreted as mos 45,XX,inv(9)(p11q13)x2,der(15;21)(q10;q10)[11]/46,XX,inv(9)(p11q13)x2,+21,der(21;21)(q10;q10)[9]. Mother's history is complicated as she is a carrier for the Cystic Fibrosis mutation (ΔF508) and also for the Factor V Leiden. She is also a carrier for the HbD-Los Angeles (33.9%) and HbQ-India (14.7%) variants. She also had a sibling with term stillbirth. Her husband is normal for both CF and Factor V Leiden. Our case appears to be another example of confined placental mosaicism (CPM) with a normal fetal karyotype. However, we could not confirm the possibility that CPM contributed to the IUFD in our case given the complex medical history of the mother.

1261T

Mosaic isodicentric Y chromosome in a patient with mix gonadal dysgenesis. *Z. Yilmaz[1], B. Yuksel[2], O. Ozer[1], F.I. Sahin[1].* 1) Department of Medical Genetics, Baskent University Faculty of Medicine, Ankara, Turkey; 2) Department of Pediatrics, Cukurova University Faculty of Medicine, Adana, Turkey.

Dicentric chromosomes are the most frequently observed structural abnormalities of Y chromosome. The rearranged chromosome has a breakpoint in the long arm with a loss of the short arm except the proximal part, Thus the patients have SRY gene on the chromosome. Dicentric aberrations are unstable during cell division and can generate various types of cell lines. Most reported patients are chromosomal mosaics, generally including a 45,X cell line. Among the cases, a wide range of variation in phenotype, external genitalia, histology, and chromosomal findings was observed. Here, we report a male patient with perineal hypospadias, unilateral cryptorchidism and a mosaic karyotype 45,X [49]/47,X,idic(Y)(q11.2),+idic(Y)(q11.2)[4]/46,X,idic(Y)(q11.2)[47]. We used fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR) to determine the structure and genetic content of the Y chromosome. FISH analyses revealed double SRY signals on the chromosome suggesting isodicentric Y. Whole chromosome probe hybridized with the derivative chromosome only excluding a translocation. According to PCR results, the patient had an amplified SRY region, whereas a total deletion of AZFabcd regions was detected. Our findings and earlier reports emphasize the importance of the use of a combination of cytogenetic and molecular genetic techniques during characterization of a patient with a derivative Y chromosome before any general conclusions can be reached concerning the relative effects of the Y-chromosome abnormality and mosaicism on sexual differentiation.

1262T

Sex chromosome rearrangement and mosaicism in four patients with short stature and ovarian failure. *C. Yu[1], M. Torchinsky[2], O. Abdul-Rahman[2], R. Morris[3], R. Hines[3].* 1) Dept Pathology; 2) Department of Pediatrics; 3) Department of Obstetrics and Gynecology, University of Mississippi Medical Center, Jackson.

Short stature is one of the main clinical features of Turner syndrome either in the mosaic or non-mosaic form. Phenotypic females with Y chromosomal material have an increased risk for gonadoblastomas. Individuals with X/X rearrangement often have secondary amenorrhea and ovarian failure. The phenotypic features varied depending on the chromosome segment involved in the translocation. We report here four female with either mosaicism of idic(Y) or X/X translocation. Case 1. Patient was referred at 5 years of age because of short stature and suspicious of Turner syndrome. On physical examination patient height was 99.4 cm and weight 14.9 kg. Blood chromosome study revealed a mosaic 45,der(X)t(X;Y)(p22.3;p11.3)[10]/ 46,idem,+idic(Y)(q12)t(X;Y)[36] karyotype. A translocation occurred between the X and Y chromosomes and subsequently the derivative Y formed an isodicentric Y chromosome. FISH study confirmed the X/Y translocation and the idic(Y). Case 2. Patient was referred at 15 years of age because of history of short stature. On physical examination the patient height was 139.4 cm and weight was 44.2 kg. Bone age was read as 11 years. Chromosome analysis revealed a mosaic 45,X[43]/ 46,X,dic(Y)(q11.23)[1]/ 47,X,dic(Y)(q11.23)x2[6] karyotype. FISH study confirmed all three cell lines. Case 3. Patient was referred at 21 years of age because of secondary amenorrhea and ovarian failure. On physical examination patient height was 149.8 cm and weight 67.7 kg. Blood chromosome study reveal a 46,X,der(X)t(X;X)(p22.33;q28) karyotype; the derivative X chromosome had a deletion from Xp22.33 to Xpter and a duplication of Xq28 to Xqter. Chromosome study of her 47-year-old phonotypical normal mother revealed that the mother was the carrier of this derivative X and had a mosaic 45,X[2]/ 46,X,der(X)t(X;X)(p22.33;q28)[27]/ 47,XX,+der(X)t(X;X)[1] karyotype. The X/X translocation was confirmed by FISH. Both case 1 and 2 had idic (Y) and positive for the SRY; the timing of mitotic nondisjunction during early embryogenesis and the level of the concurrent 45,X cell line reportedly account for the female phenotype and the Turner stigmata. In case 3, although the patient and her mother had the same rearranged X, the mother was apparently normal. Nonrandom X chromosome inactivation may play an important role between the patient and her mother.

1263T

Pathogenicity of interstitial deletions 14q investigated by CGH microarrays: When the size of the abnormality does not warrant that it is de novo. *M. Beaulieu Bergeron[1], G. Mathonnet[1], V. Désilet[1], J. Gekas[3], M. Sylvain[4], R. Fetni[2], F. Tihy[1], E. Lemyre[1].* 1) Service de Genetique, CHU Sainte-Justine, Montreal, Canada; 2) Departement de Pathologie, CHU Sainte-Justine, Montreal, Canada; 3) Service de Genetique, CHUQ, Quebec, Canada; 4) Service de Neurologie, CHUQ, Quebec, Canada.

We present four cases of interstitial deletions on the long arm of chromosome 14 that were investigated by CGH microarrays (aCGH). In the first two instances, a deletion of approximately 3 Mb in 14q12 was found in two young girls with microcephaly and central nervous systems abnormalities. Patient 1 also presented with cortical blindness, central hypotonia and peripheral hypertonia, as well as clenched fists and flexed limbs. As for patient 2, she also presented with vocal cord palsy that required intubation at birth as well as tracheostomy, seizures and hypotonia. Previous standard cytogenetic analyses had yielded normal results for this patient. aCGH revealed that these two deletions have in common the loss of FOXG1, a gene known to cause a congenital variant of Rett syndrome when in haploinsufficiency. This correlates with the clinical findings, and further analyses by FISH confirmed the deletion to be de novo in both patients. In the two remaining cases, an interstitial deletion in 14q31 was seen on fetal karyotype. In the first pregnancy, amniocentesis was performed for an increased risk of aneuploidy due to soft markers, whereas increased nuchal translucency on ultrasound prompted amniocentesis in the second pregnancy. In both cases, a similar deletion was suspected on parental karyotype, and later confirmed by FISH. aCGH analyses of both fetuses revealed that the deletions in 14q31.1q31.3 were approximately 7 Mb in size, involve respectively 10 et 7 genes, and have in common the loss of 6 genes: TSHR, GTF2A1, SNORA79, STON2, SEL1L and FLRT2. At almost 4-year-old, the first patient presents with minor facial dysmorphisms and normal psychomotor development. The second patient is yet to be born. Based on the patient's and carrier parents' phenotype as well as literature review, mild facial dysmorphisms and mild anomalies of the cognitive development seem to be associated with these deletions. In conclusion, these four cases of interstitial 14q deletions increase our knowledge regarding the phenotypic features associated with those deletions involving regions 14q12 and 14q31. These cases also illustrate the importance of parental analysis when confirming rearrangements seen by aCGH since rather large deletions can either be inherited or de novo and be associated with a phenotype of mild clinical severity.

1264T

Interstitial 21q22.3 Chromosomal Deletion Associated with Intellectual Disability, Behavioral Abnormalities, and Microcephaly in Siblings. *C. Cottrell[1], S. Kulkarni[1,2,3], M. Vineyard[4], M. Shinawi[4].* 1) Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO; 2) Department of Pediatrics, Washington University School of Medicine, St. Louis, MO; 3) Department of Genetics, Washington University School of Medicine, St. Louis, MO; 4) Department of Pediatrics, Division of Genetics and Genomic Medicine, Washington University School of Medicine, St. Louis, MO.

Background: Chromosome 21q22.3 is a gene rich region containing multiple loci associated with human disease. The phenotypic effects of deletions encompassing the 21q22.3 region have not been previously studied. Methods & Results: We present two male siblings with microcephaly and intellectual disability caused by a microdeletion involving chromosomal region 21q22.3. The older sibling is an 11-year-old male with intractable epilepsy, microcephaly, intellectual disability, and aggressive behavior. The 6-year-old sibling also has intellectual disability, microcephaly and was diagnosed with attention deficit hyperactivity disorder. The siblings exhibit some dysmorphic features including relatively large ears, epicanthal folds, smooth philtrum, and widely spaced teeth. The older sibling also has abnormal skin pigmentation. Microarray analysis using the Affymetrix SNP 6.0 platform revealed an approximately 962kb copy number loss [arr 21q22.3(43,832,384-44,793,940)x1] overlapping a region with a single report of copy number variation. The deletion was inherited from an apparently healthy father except for impaired social behavior. A healthy female sibling was not found to carry the deletion. Conclusions: Our data suggest that deletions encompassing chromosomal region 21q22.3 can be associated with microcephaly and neurobehavioral phenotypes. The deleted region encompasses several genes implicated in human disease, including CSTB and TRPM2. Mutations within the CSTB gene have been associated with the autosomal recessive disease, progressive myoclonic 1 epilepsy (EPM1). The effect of CSTB haploinsufficiency in our patients is unclear; however it may be a contributing factor for the intractable epilepsy observed in the older sibling. The TRPM2 gene product functions as a calcium channel receptor and is highly expressed in the human brain. TRPM2 has been previously implicated in bipolar disorder and its deletion may play a role in the neuropsychiatric phenotypes observed in this the family.

1265T

Deletion 1q24q25: four new cases with recognizable phenotype. *M. de Blois-Boucard[1-3], V. Malan[1-3], O. Raoul[1-3], N. Morichon[1], M. Willems[2], S. Nussbaum[1], A. Munnich[2-3], M. Vekemans[1-3], V. Cormier-Daire[2-3].* 1) Cytogenetics laboratory, Necker Hospital, Assistance Publique, Hopitaux de Paris, France; 2) Department of genetics, Necker Hospital, Paris, France; 3) Universite Paris Descartes, Faculte de Medecine, Paris, France.

Deletion of chromosome 1 are very rare and are usually classified as proximal (1q21q25), intermediate (1q24q32)and terminal (1q32qter). Here, we report on four new cases carrying a deletion 1q24q25. All occurred de novo. Three patients were referred to us because they presented with short stature, small hands and feet, facial dysmorphic features and intellectual disability. Skeleton X-rays revealed mesomelic dysplasia and endocrine workup identified a growth hormone deficiency for two patients. One patient was referred prenatally because of intrauterine growth retardation and short extremities detected on ultrasound survey. Using molecular karyotyping (Array-CGH) we are able to characterize the deletions at the molecular level and to define a 9Mb common deleted region. This region encompasses several genes: PRRX1, SERPINC1, DARS2, presumably responsible for the phenotype observed in our patients. When compared to previously published clinical reports, a recognisable skeletal endophenotype could be delineated. It includes specific clinical and radiological features of the limbs and extremities, namely short stature, shortness of the metacarpus, metatarsus and phalanges. Interestingly, a deletion of the homeobox PRRX1 gene is observed in all described patients. Considering that this gene encodes a protein playing an important role in epithelial-mesenchyme interaction during skeletal organogenesis, one may suggest that the PRRX1 gene is causally related to the skeletal endophenotype. As previously suggested, our data confirm that 1q24q25 deletions are probably responsible for a novel recognizable phenotype with specific skeleton features.

1266T

Chromosome microarray analysis and zebrafish studies identify CCDC165 as a new candidate gene for coloboma. *P. Eydoux[1], B. McGillivray[2], K. Schlade-Bartusiak[1], C. Lyons[3], C. Gregory-Evans[4].* 1) Dept Pathology, Children's & Women's Hosp, UBC, Vancouver, BC, Canada; 2) Dept Medical Genetics, Children's & Women's Hosp, UBC, Vancouver, BC, Canada; 3) Dept Ophtalmology, Children's & Women's Hosp, UBC, Vancouver, BC, Canada; 4) Dept Ophtalmology, Eye Care Center, UBC, Vancouver, BC, Canada.

Coloboma is a rare eye malformation resulting from defective closure of the optic fissure, which may result in poor vision. While most cases of coloboma are sporadic, autosomal dominant, autosomal recessive, and X-linked inheritance patterns have been described. Coloboma is a frequent feature of chromosome abnormalities, such as trisomy 13, partial tetrasomy 22 or Wolff-Hirschhorn syndrome, suggesting that coloboma genes may be copy number sensitive. Molecular mechanisms leading to coloboma remain largely unknown. A female patient was referred at 3 years of age because of a unilateral right-sided infero-nasal iris coloboma. Uncorrected vision with HTOV testing was 20/30 each eye. There was good stereopsis and no strabismus. Fundi were unremarkable. Her milestones and growth were entirely normal. There were no dysmorphic features. An MRI was unremarkable in terms of the orbits, optic nerves, and chiasm. There was a small arachnoid cyst just anterior to the supero-lateral right prefrontal gyrus. Her mother was diagnosed with glaucoma at age 14 and had an otherwise normal ophthalmologic examination. Her father had no visual concerns. He has had recurrent renal calculi and is thought to have bilateral ureteral malformations. Chromosome microarray analysis (CMA) identified a de novo 271.8kb deletion at 18p11.22 (chr18:8,642,237-8,914,059), encompassing the CCDC165 gene, which encodes an unknown protein. Parental FISH studies were normal, showing that this abnormality occurred de novo. We performed *in situ* hybridization in the zebrafish, using a Locked Nucleic Acid (LNA) enhanced exon 5-specific probe from this gene. At 24 hours post-fertilization (hpf) we observed high level expression throughout the developing head, whereas at 48 hpf high level expression was restricted to lens, and a lower level expression in the retina and pharyngeal arch. Knock-down experiments of this gene are pending to further support this gene as causing coloboma. Our results show that CCDC165 is an excellent candidate for the occurrence of coloboma in this patient and illustrates the power of this approach for assessment of pathogenicity of copy number losses in patients with malformations.

1267T

Prenatal detection of mosaicism for del(10)(q11.2) caused by a common chromosomal fragile site FRA10G is associated with a normal phenotype. *J. Liao[1,2], M. Sathanoori[1,4], S.A. Yatsenko[1,3,4], U. Surti[1,2,3].* 1) Pittsburgh Cytogenetics Laboratory, Magee-Womens Hospital of UPMC, Pittsburgh, PA; 2) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 3) Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA; 4) Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Chromosomal fragile sites are specific loci which exhibit structural instability visible as gaps, constrictions or breaks on metaphase chromosomes exposed to certain cell culture conditions or treated with specific chemical agents. To date, over 120 different fragile sites have been identified in the human genome. They are classified as rare or common based on their frequency in the population. Although common fragile sites are currently considered present in all individuals as an intrinsic part of the normal chromosome structure, they have been implicated as breakpoints in the *in vivo* chromosomal rearrangements found in various cancers. In *in vitro* culture conditions, deletions at fragile sites can also occur. However, the possible implication of common fragile sites at prenatal diagnosis is still poorly documented. Here we report 13 prenatal cases with mosaicism for chromosome 10q deletion identified by karyotype analysis of CVS samples. Percentages of mosaicism range from 4% (2 out of 50 cells) to 24% (12 out of 50 cells). These cases share a similar breakpoint at 10q11.2, where a common fragile site, FRA10G is also located. Clinical follow-ups found normal live births without any phenotypic anomalies for all these cases. Therefore, our results indicate that the prenatal mosaicism of del(10)(q11.2) appears to represent an *in vitro* culture event due to FRA10G fragile site in this region and is not likely to cause any clinical consequences, which may ease the anxiety caused by the similar situation at prenatal diagnosis in the future and eliminate the need for a follow-up amniocentesis if the ultrasound is normal. To our knowledge, it is the first report of an association between this common chromosomal fragile site and a prenatal chromosome structural mosaicism.

1268T

Identification of a recombination hotspot sequence at the breakpoint region of the 22q11 deletion using a yeast model. *T. Ohye[1], H. Inagaki[1], H. Kogo[1], M. Tsutsumi[1], B.S. Emanuel[2], H. Kurahashi[1].* 1) Div Molecular Genetics, Fujita Health Univ, Toyoake Aichi, Japan; 2) Div Human Genetics, Children's Hosp Philadelphia.

Chromosomal microdeletions cause a wide spectrum of genomic diseases such as the 22q11 deletion syndrome, the most frequent genomic disorder with a high frequency of *de novo* occurrences. The deletion breakpoints are mainly located within low-copy-repeats (LCRs). These microdeletions are predominantly mediated by unequal non-allelic homologous recombination during meiosis, while some arise via intra-chromosomal recombination between LCRs. In the current study, we established a yeast model system to identify sequences within the 22q11 LCR that induce the deletion. We studied three candidate sequences from within the LCRs in the 22q11 region, a 1.1 kb minisatellite, a 1.4 kb highly AT-rich repeat region (AT-rich-repeat: ATRR) and the ϒ-glutamyltranspeptidase (GGT) gene sequence. Recombination hotspot sequences for other genomic diseases, neurofibromatosis type 1 (NF1) and Sotos syndrome (SoS), were also used as controls. We inserted the *URA3* gene between two copies of *TRP1* genes, one of which carried the candidate sequence at its center. Homologous recombination between the *TRP1* sequences results in an interstitial deletion with the loss of the *URA3* marker, which can be identified in the presence of 5-FOA. As a result, we determined that the deletion frequency for the ATRR was greater than that for the NF1 or the SoS sequences. Other candidates, the microsatellite and GGT, showed a level similar to that of controls. Next, to investigate the mechanism of deletion, we created a POL1-inducible yeast model under the control of a GAL1 promoter. The deletion frequency of the ATRR-harboring yeast with low POL1 expression was significantly higher than that with high expression. Finally, we tested the ability of the candidate sequences to form non-B DNA structures *in vitro*. T7 endonuclease I, that displays cleavage activity for mismatched DNA, cleaved the plasmid with the ATRR. Our results suggest that potential secondary structures of the ATRR in the 22q11 LCR might cause slow or stalled replication inducing a chromosome deletion via intrachromosomal homologous recombination. This might represent a factor that contributes to the high frequency of *de novo* 22q11 microdeletions.

1269T

Subtelomeric microdeletions 19p13.3 are associated with gastrointestinal dysmotility, multiple congenital anomalies and global developmental delay. *S. Peddibhotla[1], S.W. Cheung[1], P. Stankiewicz[1], F. Probst[1], L.L. Harris[1], G.H. Vance[2], G.H. Scharer[3], L.K. Parsley[3], A. Patel[1].* 1) Baylor College of Medicine, Houston, TX; 2) Indiana University School of Medicine, Indianapolis, IN; 3) University of Colorado School of Medicine, Aurora,CO.

Chromosome 19 has the highest gene density, thus even small aberrations can have significant impact on physical and/or cognitive development. Here, we report five cases with a subtelomeric microdeletion in 19p13.3, ranging in size from approximately 400 kb to 1 Mb, which were identified by high-resolution array CGH. The clinical presentation in these patients was complex and included, gastrointestinal (GI) dysmotility with feeding difficulties, reflux esophagitis and delayed gastric emptying, hearing loss, atrial septal defect, unilateral kidney, global developmental delay, and dysmorphic features. The smallest region of deletion overlap contains approximately 40 genes. We are particularly interested in genes responsible for GI dysmotility, cardiac anomalies, and developmental delay. Disturbances in GI motility often result in decreased provision of enteral nutrition. The etiology of GI dysmotility is not well understood, but is believed to be multifactorial. The various contributing factors to GI tract dysmotility are likely impaired function of enteric nerves and smooth muscles of GI tract, systemic inflammatory response and multiple organ dysfunction. We have identified 12 candidate genes in the subtelomeric region of 19p13.3, which can play a significant role in aspects of the clinical phenotypes observed in these patients.

1270T

Narrowing down Congenital Heart Disease (CHD) critical region boundaries on chromosome 4q according to the phonotypes of a patient with 2q34-qter duplication and 4q34.2-qter deletion. *A. Rashidi-Nezhad[1,2], S.M. Akrami[1], N. Parvaneh[3], F. Farzanfar[4], C. Azimi[4], A. Reymond[2].* 1) Department of Medical Genetics, Tehran University of Medical sciences, Tehran, Iran; 2) Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland; 3) Department of Pediatrics, Children's Medical Center, Tehran University of Medical Sciences, Tehran,Iran; 4) Department of Genetics, Cancer Research Center, Cancer Institute of Iran, Tehran University of Medical Science, Tehran, Islamic Rep. of Iran.

Introduction: Deletions of the distal part of 4q have been reported. The severity of the symptoms is dependent on deletion size. It ranges from minor anomalies in individuals with 4q34 deletion to severe abnormalities in patients with 4q31 deletions. In this report, we describe a patient with 2q34-qter duplication and 4q34.2-qter deletion and compare his phenotype with previous publications allowing to further restricting the HSA4 critical region for Congenital Heart Disease (CHD). Patient: - We present a 10 months boy with developmental delay, growth retardation, hearing problem, facial and non-facial minor anomalies. He was born to a 30 years old mother with a history of recurrent fertility problems. - Methods: - Both G-banded metaphase karyotypes and array CGH were used to map the rearrangements in the proband and his mother. - Results: - Proband genotype was as follow: 46,XY , Dup(2)(q34-q35.2) mat, Del(4)(q34-q37.3) mat.arr 2q34q37.3(209,962,499-242,703,145)×3 mat ,4q34.2q35.2(177,717,642-191,220,565)×1 mat. And his mother karyotype was 46,XX,t(2;4)(q34;q34). Discussion: Our patient is deleted for 13.5 Mb on the distal portion of the long arm of HSA4. A region was suggested to harbor a 10 Mb critical region for CHD and PRS at 4q34.1-4q34.3 (Rossi et.al. 2009). Our patient did not present any of these two symptoms allowing us to propose a shorter 3.1 Mb critical region from 4q34.1 to 4q34.2. The proband also harbors a 32.7 Mb duplication of the terminal end of 2q. Notably, none of the major congenital anomalies, such as congenital heart disease or urinary tract malformations found in more proximal duplications, are present suggesting a critical region from band 2q31 to q34.

1271T

Comparison of two different high-resolution genomic arrays, the Affymetrix® Cytogenetics 2.7 versus the Genome-Wide Human SNP 6.0 Array, for diagnosis of CNVs in patients with intellectual disability. *R. Asadollahi[1], B. Oneda[1], S. Azzarello-Burri[1], R. Baldinger[1], A.B. Ekici[2], D. Niedrist[1], A. Reis[2], D. Bartholdi[1], A. Baumer[1], A. Rauch[1].* 1) Institute of Medical Genetics, University of Zurich, Schwerzenbach-Zurich, Zurich, Switzerland; 2) Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen, Germany.

Array-based comparative genomic hybridization (aCGH) has demonstrated to be a valuable tool in the diagnostics of patients with intellectual disability (ID) and multiple congenital abnormalities (MCAs) due to the high-resolution pangenomic coverage. Diagnostic yield of aCGH depends on the number of array markers and signal to noise ratio of the respective array type. Higher number of markers can increase the resolution for detecting small copy number variants (CNVs). However, detecting higher number of small CNVs may decrease the ratio of clinically significant CNVs to polymorphisms and optimized CNV assays may limit SNP genotyping. Affymetrix® Genome-Wide Human SNP Array 6.0 (1.8 M markers) is widely used to perform high-resolution CNV profiling and to obtain SNP genotyping data for homozygosity mapping, linkage analysis and association studies. On the other hand, Affymetrix® Cytogenetics 2.7 Array (2.7 M markers) is technically optimized for CNV detection with even higher resolution and is still capable to detect stretches of homozygosity. Nevertheless, the 2.7 array is not able to genotype single SNPs. To investigate if the 2.7 array optimized for CNV detection indeed reveals more clinically significant CNVs, we compared CNV results of the two array types in 27 patients with ID and/or MCA. Interestingly, we could identify 5 potentially clinically relevant CNVs of small size (2-48 kb, confidence levels 87%-96%) with the 2.7 array which could not be detected with the 6.0 array. 2 out of the 5 aberrations deleted single exons of known disease genes compatible with the phenotype of the patients and were confirmed as de novo with MLPA. Furthermore, 1 large duplication of 34 Mb in mosaic form was detected by the 2.7 array which was absent in the 6.0 array and could explain the patient's phenotype. FISH studies confirmed the presence of an additional marker chromosome. The optimized design of the 2.7 array by removing commonly false positive or highly variable markers and increasing the markers in critical sites has reduced the number of non-significant results considerably (mean: 44.9 in 2.7 array vs. 231 in 6.0 array without any filter) and simplifies the analysis. In our comparison, no relevant aberration detected with the 6.0 array was missed in the 2.7 array. Our results with at least 11% (3/27) of clinically significant small CNVs or mosaicism missed by the 6.0 array demonstrate the higher diagnostic value of the 2.7 array optimized for CNV detection.

1272T

Familial 5q14.3-5q21.1 duplication associated with microcephaly and developmental delay characterized by Array-Based Comparative Genomic Hybridization. *S. Ebrahim[1,2], D. Stockton[3], R. Chikamane[3], M. Hankerd[2], M. Kristofice[2], J. Wojciechowski[2], A.N. Mohamed[1,2].* 1) Department of Pathology, Wayne State University School of Medicine, Detroit, MI; 2) Detroit Medical Center University Laboratories, Cytogenetics; 3) Division of Genetic & Metabolic Disorders, Children's Hospital of Michigan.

We describe a brother and sister from a non-consanguineous family who have a partial duplication of chromosome 5 long arm documented by chromosomal microarray analysis and confirmed by focus high resolution chromosome and or FISH analysis. Since their parents are phenotypically normal and do not have the duplicated 5q in their blood lymphocytes, the most likely explanation is germ-line mosaicism. A seven year old girl with microcephaly, developmental delay and ventricular septal defect (VSD) was found to have a 12.89 Mb duplication involving chromosome 5q14.3q21.1 long arm region which was confirmed by FISH analysis. This duplication contains at least 44 genes including several developmentally important genes. Parental FISH analysis was performed and neither parent was found to carry a duplication of this region in their lymphocytes. The patient has two younger siblings, a six year old brother and a one year old sister, both of whom have microcephaly and developmental delay but no cardiac defect. Microarray analysis revealed that the six year old brother also had the same dup 5q14.3-q21.1 which was confirmed by focus high resolution chromosome analysis. The one year-old sister has not yet been tested. Parental chromosome analysis was performed and neither parent was found to carry rearrangements involving chromosome 5 or a duplication of this region in their lymphocytes. Partial duplication for chromosome 5q is relatively rare, and is associated with a wide range of phenotypes depending on the size and location of the duplicated region. Generally, it includes hypotonia, dysmorphic features, microcephaly, growth, and psychomotor developmental delay. Our cases represent a novel familial duplication syndrome of chromosome 5q with variable expression of features that include developmental delay, dysmorphic features, and cardiac defect. The clinical variability within the family carrying the same 5q duplication is emphasized by the differing associated anomalies in these siblings, who were discordant for congenital heart disease while both had microcephaly and developmental delay. The presence of the dup 5q in the siblings and the normal parental blood chromosome/FISH results is that one of the parents has germ-line mosaicism.

1273T

Pathogenic chromosomal aberrations in the families with Mental Retardation and developmental delay detected by array-CGH. *V. Kucinskas[1], J. Kasnauskiene[1], Z. Ciuladaite[1], E. Preiksaitiene[1], A. Alexandrou[2], G. Koumbaris[2], P. Patsalis[2].* 1) Department of Human and Medical Genetics, Vilnius University, Vilnius, Lithuania; 2) Department of Cytogenetics and Genomics, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus.

The introduction of array CGH into the diagnostic work-up of patients with mental retardation and congenital malformations provides a unique possibility to identify novel microdeletion/ microduplication syndromes, as well as to expand the phenotype, to identify reciprocal products and to elucidate genomic etiology of previously well known conditions and thus allows more accurate and target care and genetic counseling. We report on the results of array-CGH study of 98 karyotypically normal patients with syndromic/ non-syndromic mental retardation/ developmental delay (MR/DD) of unknown origin. The results were confirmed and origin of alterations was revealed by RT-PCR. 15 chromosomal aberrations in 13 patients were considered as pathogenic: 11 de novo (del(2)(q24.2q24.3), del(2)(q27.3), del(4)(q21.22), del(5)(q14.3q14.1), del(5)(q14.3), del(6)(q21), del(16)(q23.1), del(7)(q35q36.1), del(16)(p11.2), dup(5)(q35.3), dup(7)(p22.1)) and 4 inherited (del(16)(p11.2), del(4)(q28.3), del(10)(p14), del(21)(q22.3). The first familial alteration in known pathogenic region was inherited from mother with mild mental retardation, while other three aberrations were inherited from an apparently healthy parent yet have not been reported as polymorphic CNVs in the Database of Genomic Variants. Further molecular analysis is essential to confirm the possibility of incomplete penetrance and variable clinical phenotype in these cases. The efficiency of array-CGH method in our study is 13.3% and demonstrates its successful application in genetic diagnostics of MR/DD. The research was funded by the European Union 7th Framework Programme [FP7/2007-2013] as part of the CHERISH project (www.cherishproject.eu, grant agreement No. 223692).

1274T

Detection of chromosome abnormalities of spontaneous aborted samples using multiple ligation-dependent probe amplification (MLPA). *S.W. Lyu[1], S.R. Sung[2], J.E. Park[2], K.M. Kang[2], M.U. Chin[2], J.W. Kim[1], D.H. Cha[1,2], S.H. Shim[2].* 1) Department of Obstetrics and Gynecology; 2) Genetics Laboratory, Fertility Center of CHA Gangnam Medical Center, CHA University, Seoul, Korea.

Background: About 50% of spontaneously aborted samples shows chromosome abnormalities, mainly numerical abnormalities such as aneuploidies and triploidy. Routine chromosome analyses of aborted samples are performed by tissue culture method. However, this method is labour-intensive and time-consuming and has a significant failure rate (up to 40%) due to poor sample quality. Multiple ligation-dependent probe amplification (MLPA) is a multiplex PCR method detecting abnormal copy numbers of up to 50 different genomic DNA simultaneously. As a molecular cytogenetic method, MLPA can detect various chromosomal imbalances, such as aneuploidies, partial deletions and duplications, depending on the probe set used. Aims: this study aims to evaluate whether the MLPA with subtelomeric probe mix is to be an alternative method of routine chromosome analysis for spontaneous aborted samples. Materials and Methods: Total eighty of abortus samples were examined. The samples were analyzed using both MLPA and routine culture method. Cell culture and conventional chromosome analyses were carried out using standard methods. For MLPA analyses, SALSA70 probe mix which contains probes for subtelomeric regions of all human chromosomes was used. All procedures including multiplex PCR and capillary electrophoresis were performed by manufacturer's protocol. The data were analyzed by the GeneMarker® software and compared with results from routine culture method. Results: Chromosome analyses were performed in 68 cases out of 80. The other 12 (15%) cases were failed to culture. On the other hand, MLPA results were successfully obtained from all 80 cases. The results of sixty four cases were concordant in both MLPA and karyotype results. However, in 4 cases, the results were different from each other due to maternal cell contamination, polyploidy, and mosacism. For 12 culture failed cases, MLPA results showed that seven cases were aneuploidies and the other 5 cases were normal. Conclusion: MLPA is less labour-intensive and more cost-effective than routine karyotype analysis. Moreover, MLPA technique shows a higher success rate and more accuracy than routine karyoptye analysis for abortus samples although MLPA method cannot detect polyploidy (3n or 4n) and balanced translocation.

1275T

Uniparental disomy: Can SNP array data be used for diagnosis? *T. Tucker[1], K. Schlade-Bartusiak[2], P. Eydoux[2], T. Nelson[1], L. Brown[2].* 1) Molecular Genetics Laboratory Dept of Pathology & Lab Medicine Children's & Women's Health Centre of BC; 2) Cytogenetics Laboratory Dept of Pathology & Lab Medicine Children's & Women's Health Centre of BC.

Chromosome microarray analysis (CMA) is recommended as a first tier test for the detection of copy number variants (CNVs) in individuals with developmental delay and autism, replacing disease specific testing using conventional methodologies. In addition to the detection of CNVs, SNP-based CMAs can identify long continuous stretches of homozygosity (LCSH), usually present on multiple chromosomes and associated with consanguinity. When LCSH are restricted to one chromosome, it may reflect UPD; either over the whole chromosome (isoUPD) or segments of the chromosome (heteroUPD). The LCSH in heteroUPD results from recombination during meiosis I (MI). Complete heteroUPD results from a lack of recombination between homologues and on CMA, would have the same appearance as normal biparental inheritance. Several chromosomes have clusters of imprinted genes associated with recognizable syndromes and are therefore sensitive to UPD; developmental delay is a feature of most of these syndromes. Whereas the power of CMA for CNV detection is well documented, very few studies have established the clinical use of CMA for the detection of UPD. To validate the use of Affymetrix 6.0 SNP CMA for the identification of UPD in a clinical laboratory, we retrospectively tested 8 UPD cases identified by conventional polymorphic microsatellite analysis. On CMA, 5 cases showed LCSH limited to a single chromosome, consistent with heteroUPD; the average total LCSH was 29Mb (17Mb-33Mb). Two cases showed complete homozygosity across the entire chromosome, consistent with isoUPD. However, SNP CMA failed to detect one case of UPD; the data showed no LCSH, suggesting a failure of recombination during MI and thus having the appearance of normal biparental inheritance. Recombination during MI is important to ensure proper segregation of the homologous chromosomes, and a failure to recombine at MI may predispose to non-disjunction and, therefore, to UPD. We have demonstrated that UPD will typically be identified by CMA as LSCH. Further, we have confirmed that failure of recombination may result in the inability to detect UPD using CMA. Therefore, a normal SNP CMA result does not exclude the possibility of UPD. Due to the possibility of false negative results, SNP CMA should not be used as a first tier diagnostic test for patients with a clinical diagnosis, or strong clinical suspicion, of a syndrome that may be caused by UPD.

1276T

Mosaic 27.49 Mb 18q terminal deletion with a non-mosaic 461 Kb deletion at the deletion breakpoint. *J. Wang[1], A. Hajianpour[1], B. Huang[2], R. Habibian[1], J. Szymanska[1], H. Zhu[3].* 1) Genzyme Genetics, Monrovia, CA; 2) Genzyme Genetics, Orange, CA; 3) Genzyme Genetics, Westborough, MA.

Chromosome analysis of a 9-day old male with dysmorphic features was reported as normal 46,XY. CGH analysis using Agilent custom 44K oligonucleotide array was performed on the same specimen. A ~38% mosaicism (log2 ratio of -0.304) for a 27.49 Mb terminal deletion of chromosome 18q was detected. Unexpectedly, a log2 ratio of -1 was observed for a 461 Kb sequence at the deletion breakpoint in 18q21.1, indicating a deletion of this region in all cells [arr 18q21.2q21.2(48,585,449-49,046,494)x1,18q21.2q23(49,078,395-76,076,801)x1~2]. A large 18q terminal deletion was subsequently observed cytogenetically in 4 of 50 additional metaphases examined. FISH analyses using the 18q subtelomere probe, BAC clone RP11-49E14 (48,822,719-48,977,691), and BAC clone RP11-45M11 (49,370,974-49,572,264) confirmed the array CGH results: RP11-49E14 (closest to the deletion breakpoint) is deleted in all cells, while RP11-45M11 (slightly distal to RP11-49E14) and the subtelomere probes are deleted only in cells with a visible deletion [ish del(18)(q21.2q21.2)(RP11-49E-,RP11-45M11+,qter+)[80]/del(18)(q21.2q23)(RP11-49E14-,RP11-45M11-,qter-)[4/80]. The much lower level of mosaicism observed cytogenetically as compared to that detected by array analysis can possibly be explained by a decreased viability of cells with the large deletion. Parental blood specimens are being sought in order to determine if the 461 Kb microdeletion is de novo. It is not clear if sequences around the breakpoints of the 461 Kb microdeletion predisposes to further deletion of sequences distally; there are no known segmental duplications that flank this microdeletion. No similar cases are found in the literature. This case again illustrates the power of chromosome microarray analysis in detecting and characterizing chromosome abnormalities. Exclusive of it, the etiology of this patient's abnormal phenotype and the 461 Kb microdeletion in all cells would not have been established. Footnote: Genzyme Genetics and its logo are trademarks of Genzyme Corporation and used by Esoterix Genetic Laboratories, LLC, a wholly-owned subsidiary of LabCorp, under license. Esoterix Genetic Laboratories and LabCorp are operated independently from Genzyme Corporation.

1277T

Duplications of the DGS region on both chromosome 22 homologues in three patients - the co-occurrence on both homologues can involve both inherited and *de novo* events. *W. Bi[1], J. Wiszniewska[1], F.J. Probst[1], B.S. Carter[2], M.D. Williams[3], P. Stankiewicz[1], A. Patel[1], J.R. Lupski[1], S.W. Cheung[1].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Division of Neonatology, Monroe Carell Jr. Children's Hospital at Vanderbilt, Nashville, TN; 3) Division of Medical Genetics and Genomic Medicine, Vanderbilt University School of Medicine, Nashville, TN.

Genomic rearrangements associated with genomic disorders usually occur as heterozygous events involving only one of the two chromosome homologues. Recently, a patient with DiGeorge syndrome (DGS) was shown to inherit a common recurrent deletion from a father who was compound heterozygous for a 22q11.2 deletion and duplication; the father had no phenotype because of genetic compensation for gene dosage of this genomic disorder [Carelle-Calmels, et al.(2009) NEJM 360:1211-6]. We identified four copies of the Velocardiofacial/ DiGeorge syndrome (VCFS/DGS) region in three unrelated patients using chromosomal microarray analysis (CMA) revealed by FISH analyses to be duplications on both chromosome 22 homologues. The first patient is a newborn with cleft palate, mild micrognathia, hypertelorism, and patent ductus arteriosus. She is homozygous for a ~2.5 Mb common 22q11.2 duplication. SNP array analysis confirmed partial tetrasomy of 22q11.2, and did not show evidence of uniparental disomy (UPD) of chromosome 22. This duplication was also identified on one chromosome 22 in the mother, whereas the father was negative for the duplication. Paternity was confirmed by highly polymorphic variants used for identity testing. Thus, one 22q11.2 duplication was inherited from the mother, whereas the duplication in the paternally derived chromosome was presumably generated by a *de novo* nonallelic homologous recombination event during gametogenesis. The second patient also has the common 22q11.2 duplication on both chromosome 22 and the study of etiology for the homozygous duplication is ongoing. The third patient carries a common 22q11.2 duplication on one chromosome 22 homologue and a smaller LCR-flanked duplication of ~1.3 Mb including the *TBX1* gene on the other homologue. The common 22q11.21 duplication was inherited from the father, and the smaller duplication was inherited from the mother. Additional clinically significant copy number changes in the first and the third patient were inherited. Genotype-phenotype correlation showed that the homozygous duplication causes more severe disease; as has been previously reported for homozygous CMT1A duplication > 20 years ago! Our studies demonstrate that both inherited and *de novo* events can result in rearrangements on both chromosome homologues for a given locus and further amplify the role that gene dosage may play in conveying clinically relevant phenotypes.

1278T

The *PRR12* gene is disrupted by a *de novo* balanced t(10;19) chromosome translocation in a girl presenting with psychomotor retardation, aggression and seizures. *C. Córdova-Fletes[1,2], V. Kalscheuer[3], R. Ullman[3], P. Barros-Núñez[4], B. Verduzco-Garza[2], M.G. Domínguez[4], R. Ortiz-López[1,2].* 1) Bioquímica, Universidad Autonoma de Nuevo Leon, Monterrey, Nuevo Leon, Mexico; 2) Unidad de Biología Molecular, Genómica y Secuenciación, Centro de Investigación y Desarrollo en Ciencias de la Salud, Universidad Autónoma de Nuevo León, 64460. Monterrey, Nuevo León, México; 3) Department of Human Molecular Genetics, Max Planck Institute for Molecular Genetics, D-14195 Berlin, Germany,; 4) División de Genética, Centro de Investigación Biomédica de Occidente, CMNO-IMSS, 44340 Guadalajara, México,.

Even though most of the balanced translocations are related to a normal phenotype, the risk to develop a clinical phenotype from a de novo balanced translocation is around 6.1%, and even higher in cases of complex rearrangements. It is suggested that balanced translocations also account for a congenital disorder in about 50% of the cases. This can be due to numerous factors such as the perturbation of dosage-sensitive genes at the breakpoints or any other genes surrounding it, or the not truly balanced or cryptic rearrangements. Frequently, gene disruptions or unbalances from those rearrangements lead to a variety of mental disability forms with or without additional clinical features. In the present case, we thoroughly characterized a de novo balanced translocation present in a girl with seizures, delayed psychomotor development and aggressive behavior. The patient's G-banded karyotype was 46,XX,t(10;19)(q22;q13.1)dn. Further analysis by Genome-Wide SNP arrays 6.0 indicated that the translocation was balanced. Mapping of the breakpoints by array CGH of flow sorted translocation chromosomes showed that both breakpoints disrupted a gene. On chromosome 10 *ZMIZ1* was truncated and on chromosome 19 *PRR12* was disrupted. The function of *PRR12* is presently unknown. Its high expression in brain during human and mouse cerebral cortex development let us suggest that it is the better candidate to account for the patient's clinical phenotype . Conversely, up to date, *ZMIZ1* deficiency has been related to the pathogenesis of inflammatory bowel disease and there is no indication that it plays a role in brain development and cognition. Summarizing, the molecular analyses presented here, revealed for the first time a disruption of the *PRR12* gene, which let us propose that haploinsufficiency of this gene could be responsible for the clinical phenotype present in the patient with the t(10;19) de novo balanced translocaton. This work was partially supported by FOMIX (Convocatoria M0014-2007-2010. Reg. 068251).

1279T

SNP arrays: comparing diagnostic yields for four platforms in children with developmental delay. *G. D'Amours[1,2,5], M. Langlois[3], G. Mathonnet[1], J.L. Michaud[1,2,4,5], M.S. Phillips[3], E. Lemyre[1,2,4,5].* 1) Service de génétique, CHU Sainte-Justine, Montréal, PQ, Canada; 2) Centre de recherche, CHU Sainte-Justine, Montréal, PQ, Canada; 3) Centre de pharmacogénomique, Institut de Cardiologie de Montréal, Montréal, PQ, Canada; 4) Pédiatrie, Université de Montréal, Montréal, PQ, Canada; 5) Faculté de médecine, Université de Montréal, Montréal, PQ, Canada.

Identifying the genetic causes of mental retardation and congenital abnormalities is important, both for the medical care of the patient and for genetic counseling. Recently, array genomic hybridization replaced conventional karyotyping as the first-tier diagnostic test in these patients, as it identifies a chromosomal abnormality in more patients (10-12% vs 3%).

New high-resolution platforms, combining copy number variation (CNV) detection and SNP genotyping, have been developed. In addition to identifying unbalanced chromosome rearrangements, they also allow the detection of loss of heterozygosity (LOH), and by extension, uniparental disomy (UPD).

We tested 21 children with developmental delay, and their parents, on four high-resolution arrays that combine CNV detection and SNP genotyping (Affymetrix and Illumina). We then compared the results with those of the medium-resolution array currently used at CHU Sainte-Justine (NimbleGen), to assess their diagnostic performance.

We identified a pathogenic chromosomal abnormality in three children, and they were successfully detected by each of the studied arrays. No other clear pathogenic change was identified by these high-resolution platforms. However, a number of CNVs that were classified in the final analysis as being of unlikely or uncertain clinical significance considerably burdened the analysis. No UPD for a whole chromosome was detected. Smaller LOH analysis is ongoing, and its usefulness remains to be determined.

Currently, high-resolution SNP arrays identify many CNVs whose clinical significance is presently hard to demonstrate. Therefore, their use does not seem to provide at the present time an additional benefit in the clinical setting. Our observations suggest that LOH and UPD detection using high-resolution SNP arrays appears to be theoretically justifiable, but this remains to be demonstrated.

1280T

Subtelomeric Deletion of Chromosome 10p15: Clinical Findings and Molecular Cytogenetic Characterization. *C. DeScipio[1], L.K. Conlin[2], J. Rosenfeld[3], J. Tepperberg[4], A. Patel[5], M.T. McDonald[6], S. Aradhya[7], D. Ho[7], J. Goldstein[6], M. McGuire[8], L. Medne[2], R. Rupps[9], A. Serrano-Russi[8], E.C. Thorland[10], A. Tsai[11], Y. Hilhorst-Hofstee[12], H. Van Esch[13], D. Clark[2], H. Riethman[14], N.B. Spinner[2,15], I.D. Krantz[2].* 1) Department of Pathology, Johns Hopkins Hospital, Baltimore, MD; 2) Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA; 3) Signature Genomics, Spokane, WA; 4) Laboratory Corporation of Cytogenetics Triangle Park, NC; 5) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 6) Department of Pediatrics, Duke University Medical Center, Durham, NC; 7) Clinical Microarray Services, GeneDx, Gaithersburg, MD; 8) Children's Hospital of Pittsburgh, Pittsburgh, PA; 9) Department of Medical Genetics, University of British Columbia, Vancouver, Canada; 10) Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; 11) Division of Clinical Genetics and Metabolism, Department of Pediatrics, The Children's Hospital, University of Colorado Denver School of Medicine, Aurora, CO; 12) Center for Human and Clinical Genetics, Department of Clinical Genetics Leiden University Medical Center, Leiden, Netherlands; 13) Centre for Human Genetics, University Hospitals Leuven, Leuven, Belgium; 14) The Wistar Institute, Philadelphia, PA; 15) Division of Clinical Labs, The Children's Hospital of Philadelphia and The University of Pennsylvania School of Medicine, Philadelphia, PA.

We describe clinical findings in twenty unrelated individuals with submicroscopic deletions of 10p15.3 characterized by chromosomal microarray (CMA). Interestingly, to our knowledge, only two such cases of isolated, submicroscopic 10p15.3 deletion have been reported. Common features among these individuals include: developmental or intellectual disability (19), craniofacial dysmorphism (9), language/speech delay/disorder (8), seizures (5), brain findings (5) hypotonia (5), short stature (5), GERD (2), constipation (2), spine abnormalities (2) and abnormal nipples (2). Among these twenty individuals, the male to female ratio is 9:10 (one, unknown). Parental CMA studies were performed for six individuals; the 10p15 deletion was de novo in each of the six probands. Two known genes, ZMYND11 (OMIM# 608668) and DIP2C (OMIM# 611380), map within the commonly-deleted 10p15.3 region (UCSC Genome Browser). The ZMYND11 (zinc finger MYND domain-containing protein 11) protein, first identified by its ability to bind the adenovirus E1A protein, localizes to the nucleus and functions as a transcriptional repressor with expression of E1A inhibiting this repression. The DIP2C (disco-interacting protein 2 homolog C) expression has been detected in all adult and fetal tissues and specific adult brain regions examined except lung and pancreas, where expression was low. As little is known about these genes in the 10p deleted region, a direct genotype/phenotype correlation is not clear at this time. We suggest that ZMYND11 and/or DIP2C haploinsufficiency causes clinical features associated with 10p deletions in our patients.

1281T

Cytogenetic analysis of an additional ten cells does not improve the detection of sex chromosome mosaicism. *J.T. Mascarello[1], M. Thangavelu[2].* 1) Genzyme Genetics, Santa Fe, NM; 2) Genzyme Genetics, Orange, CA.

The guidelines for cytogenetic analysis of blood set forth by the American College of Medical Genetics1 states “Cases being studied for possible sex chromosome abnormalities, in which mosaicism is common, should include a minimum of 30 cells counted”. We have examined the value of analyzing an additional 10 cells over and beyond a standard 20-cell analysis in 1081 patients with clinical indications for which a sex chromosome abnormality might be suspected. 996 cases (92%) had a normal karyotype and 85 (8%) had an abnormal karyotype. Of the 85 cases with an abnormal karyotype, 61 had no evidence of mosaicism, 9 had mosaicism involving normal and abnormal cells and 15 had mosaicism involving multiple abnormal cell types. In all mosaic cases, mosaicism was detected in the first 20 cells examined. Moreover, evaluation of more than 20 cells had only minor impact on the estimation of the proportions of the various cell types. These results support the conclusion of Wiktor et al (2009) that “analysis of 20 metaphase cells from a PHA stimulated peripheral blood culture is sufficient to identify sex chromosome abnormalities”. Genzyme Genetics and its logo are trademarks of Genzyme Corporation and used by Esoterix Genetic Laboratories, LLC, a wholly-owned subsidiary of LabCorp, under license. Esoterix Genetic Laboratories and LabCorp are operated independently from Genzyme Corporation.

1282T

21,5 Mb Mosaic Pure Inverted Duplication of Chromosome 1q42.13qter. *M.LM. Morris[1], C.N. Medina[2], E.L. Freitas[3], C. Rosenberg[3], S.F. Oliveira[1], I. Ferrari[1], J.F. Mazzeu[1].* 1) Departamento de Genética e Morfologia, Instituto de Ciências Biológicas, Universidade de Brasilia, Brasilia, DF, Brazil; 2) Secretaria de Estado de Saúde do Distrito Federal, DF, Brazil; 3) Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo, SP, Brazil.

Distal duplications of the long arm of chromosome 1 are rare and have been reported as pure trisomy or as unbalanced translocations and patients demonstrate a wide range of manifestations of variable severity making it difficult to define a "partial trisomy 1q syndrome". In most cases, duplication is the result of an unbalanced translocation with possible imbalance of the other participating chromosome. Cases with pure partial distal trisomy 1q provide an opportunity to better define the partial distal trisomy 1q syndrome. Here we report on a female patient who presented with mildly delayed neuropsychomotor development, facial dysmorphisms and pectus excavatum. She also presented with patent foramen ovale. GTG-banding at 700 bands resolution identified a mosaic inverted duplication defined as 46,XX, dup(1)(q43q44),inv(q43p36.3)[16]/46,XX[14]. Array-CGH and FISH were performed to characterize the duplicated segments, exclude the involvement of other chromosomes and determine the orientation of the duplication. Duplication could be refined to chromosomal region 1q42.13-1q44 to a size of 21,5 Mb (225,696,992-247,164,526 Assembly NCBI36/hg18; 2006). FISH experiments confirmed the inverted orientation of the duplication. The patient presents with a pure 1q42.13qter inverted duplication of 21,5Mb, one of the smallest distal 1q duplications ever described and in mosaic, thus contributing to a better characterization of distal 1q duplication syndrome. However, her clinical signs are consistent to the distal trisomy 1q phenotype thus pointing to a more striking contribution of terminal duplicated segments to the syndrome.

1283T

45,X (40%); 46,X der X (ter rea) (60%) mosaicism. Case report. *M. Pérez Sánchez[1], A. Gonzalez Ramírez[2], A. Enriquez de Luna[3], A. Mora Guijosa[1].* 1) Análisis Clínicos, Hospital Virgen de las Nieves, Granada, Granada, Spain; 2) FIBAO, Hospital Clínico San Cecilio, Granada, spain; 3) Servicio de Ginecología, Hosptal Virgen de las Nieves, Granada, Spain.

End-to-end translocations or terminal rearrangements (ter rea) loading to duplications of nearly the entire X chromosome are rare but well know. Two X chromosome are joined together by either short or either long arms. Theoretically, union of the two chmosome can take place only after each chromosome has been broken. It follows that the ends of both involved arms should be missing. The clinical features rane from full-blown Turner syndrome to simple ovarian dysgenesis without stunted growth or the Turner stigmata. Delections on Xp end are previously been described causing amenorrhea and infertility. On the other hand, Turner mosaicism are present in an important group of Turner syndrome with a wide clinical features ranging from full-blown Turner syndrome to normal phenotype. Here we present a case of a 24 years old woman with that was referred for genetics stuies. The clinical findings were: amenorrhea without treatment response, normal secundary sexual caracteristics, hormonal status at normal levels, and hipoplastic the right ovary and no detection of the left one when magnetic resonance studies were done. No appreciable mental retardation were detected. Chromosome culture and karyotyping were realized by standard thecnics and showed a karyotype of 45,X (40 %); 46,X, der X (ter rea) (60 %). This result was confirmed by FISH in metaphase and interphase with CEPX and CEPY VYSIS probes. To determine the Xp region deleted in the X terminal rearrangement CHG-Array 60 K (Agilent) was done, a Xp terminal delection of 6.58 Mb including 17 genes was detected, with a chromosomic formula of 46,XX,arr cgh Xp22.33-p22.31 (NVBI37:2708827-9294878)x1. Mosaicism was not detected by CGH-Array due to the genomic amount compensation between lost of X chromosome in 45,X cells and the gain in the 46, X, der X (ter rea) cells. Both alterations detected in this patient (mosaicism and X terminal rearrangements) can present (in more or less degree) clinical features of Turner syndrome, but there are no sufficient data to determine previously the phenotypic effect of both alterations in the same patient. In this case the only clinical features detected were amenorrhea and ovaric disfuntion without appreciable mental retardation, that can be in concordance with the delection of Xp end due to the der X (ter rea) chromosome, and no extra effect can be detectable due to the effect of 45, X cells mosaicism.

1284T

Elucidation of inheritance and pathogenicity of CNVs: An approach to a better understanding and clinical utility of SNP arrays. *S. Schwartz[1], C.M. Smith[1], R.D. Burnside[1], I. Gadi[1], V. Jaswaney[1], E. Keitges[2], R. Pasion[1], V.R. Potluri[3], H. Risheg[2], J. Smith[3], J.H. Tepperberg[1], B. Williford[1], P.R. Papenhausen[1].* 1) Lab Corp of America, Research Triangle Park, NC; 2) DynaCare/LabCorp, Seattle, WA; 3) DynaGene/LabCorp, Houston Tx.

Since its introduction, the effectiveness of array analysis to delineate copy number variants not detectable by standard cytogenetic analysis has become apparent and previously undetectable changes are now identifiable. What has become clear is the difficulty in interpreting what these changes actually mean. In order to better understand the significance of the abnormalities detected, we have studied over 5,000 CNVs detected by SNP array. This study has focused on the size, type of abnormality, gene content, and frequency in the general population and inheritance of the CNVs to better understand the clinical significance of the detected abnormalities. Approximately 42% of the CNVs are simple deletions, 30% are simple duplications and 28% are complex abnormalities; 50% of the deletions and 45% of the duplications were greater than 1 Mb. Based on the gene involvement, 33% of the overall changes involved known pathogenic changes, 23% involve susceptibility genes and 44% involve changes with an unknown clinical effect. Parental studies indicate that 33% of the deletions were de novo, whereas only 16% of the duplications were de novo. The vast majority (~70%) of the de novo abnormalities were 1 Mb or greater and the familial cases were less than 1 Mb. 65% of deletions involving suspected pathogenic region but only 31% of duplications were de novo; 2% of duplications and 18% of deletions involve susceptibility regions were de novo. Lastly of those changes involving regions of unknown function, 28% of deletions and 15% of duplications were de novo. These findings provide an intriguing look into the CNVs delineated by array analysis including: (1) Based on size and pathogenicity initially 44% of cases are unknown with respect to phenotypic effect; (2) A larger than expected percent of de novo cases do not involve a reported pathogenic gene. This suggests that the pathogenic gene list needs to be expanded; (3) 34% of de novo cases have genes not previously described suggests that new syndromes can be defined; (4) Deletions are twice as likely as duplications to be de novo; (5) 60 different cases have been identified in our study that have been reported several times, one third of which that are currently not in the literature; (6) Many syndromes are much more variable than originally thought; and (7) This data taken together suggests that 79% of CNVs (and 96% of all samples) can be better defined, assigning a phenotypic range for better counseling.

1285T

A new microdeletion syndrome of 5q31.3 characterized by severe developmental delays, distinctive facial features, and delayed myelination. *K. Shimojima[1], B. Isidor[2], C. Le Caignec[2,3], A. Kondo[4], S. Sakata[4], K. Ohno[4], T. Yamamoto[1].* 1) Inst Integrated Medical Sciences, Tokyo Women's Med Univ, Shinjuku Ward, Japan; 2) Service de Génétique Médicale, Centre Hospitalier Universitaire de Nantes 7, Nantes, France; 3) INSERM, UMR915, l'institut du Thorax, Nantes, France; 4) Division of Child Neurology, Institute of Neurological Sciences, Faculty of Medicine, Tottori University, Yonago, Japan.

Chromosomal deletion including 5q31 is rare and only a few patients have been reported to date. We report here the first two patients with a submicroscopic deletion of 5q31.3 identified by microarray-based comparative genomic hybridization. The common clinical features of both patients were marked hypotonia, feeding difficulty in infancy, severe developmental delay, and epileptic/nonepileptic encephalopathy associated with delayed myelination. Both patients also shared characteristic facial features, including narrow forehead, low-set and dysmorphic ears, bilateral ptosis, anteverted nares, long philtrum, tented upper vermilion, edematous cheeks, and high arched palate. The deleted region contains clustered PCDHs, including PCDHA and PCDHG, which are highly expressed in the brain where they function to guide neurons during brain development, neuronal differentiation, and synaptogenesis. The common deletion also contains Neuregulin 2 (NRG2), a major gene for neurodevelopment. We suggest that 5q31.3 deletion is responsible for severe brain developmental delay and distinctive facial features, and that the common findings in these two patients should be recognized as a new microdeletion syndrome. We need further investigations to determine which genes are really responsible for patients' characteristic features.

1286T

How to recognize a recombinant pericentric inversion at array CGH. *R.F. Suijkerbuijk, L.K. Leegte, T. Dijkhuizen, B. Sikkema-Raddatz, J.B.G.M. Verheij, R.J. Sinke, C.M.A. van Ravenswaaij-Arts.* Genetics, University Medical Centre Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands.

INTRODUCTION: Large pericentric inversions may result in unbalanced offspring due to meiotic recombination within the inserted fragment. Since nowadays array-based analysis is the first diagnostic tool to examine patients with mental retardation and congenital anomalies, one should be aware that recombinant inversions present at array-CGH as a combined gain and loss of the terminal long and short arm, or vice versa, as we will demonstrate with two illustrative cases.

CASE 1 is a 20-year-old man with severe developmental delay and autism. Array-CGH resulted in a gain of 18p11.32 (1.07 Mb) and a loss of 18q21.33q23 (16.8 Mb). The terminal deletion 18q, also known as De Grouchy syndrome, could explain the phenotype in our patient. Parental chromosome studies were offered but denied. Nine months later we received amniotic fluid of the sister-in-law of our proband, because of ultrasound abnormalities. Karyotyping showed an abnormal chromosome 18 with extra material on 18p. Chromosome analysis of the brother of our proband and of their father revealed that both were carrier of an inv(18)(p11.32q21.33). Therefore, the result of the amniocentesis was interpreted as rec(18)dup(18-q)inv(18)(p11.32q21.33)pat and the karyotype of our proband as rec(18)dup-(18p)inv(18)(p11.32q21.33)pat.

CASE 2 is a 14-year-old girl with developmental delay and short stature. Her mother had experienced learning problems and premature ovarian failure. Array-CGH in the daughter resulted in a gain of Xp22.33p21.1 (33.6 Mb) and a loss of Xq27.3q28 (10.4 Mb). Karyotyping of mother and daughter showed that both have the same abnormal X chromosome, most likely the result of an inv(X)(p21.1q27.3) in the maternal grandmother.

DISCUSSION AND CONCLUSION: The concomitant gain and loss of the terminal long and short arm of the same chromosome at array diagnostics should alert to the possibility of a recombinant pericentric inversion. Further karyotyping studies in proband and parents are highly recommended, if necessary combined with metaphase FISH.

1287T

Confirmation testing for CNVs detected by whole genome microarrays: necessary or obsolete. *J. Tepperberg[1], E. Keitges[2], H. Risheg[2], J. Smith[3], V.R. Potluri[3], R. Pasion[1], B. Rush[1], R.D. Burnside[1], J. Jaswaney[1], I.K. Gadi[1], R. Royster[1], S. Moore[1], J. Kesler[1], S. Griffin[1], S. Schwartz[1], P.R. Papenhausen[1].* 1) Dept Cytogenetics, Lab Corp America, Res Triangle Pk, North Carolina; 2) Dept Cytogenetics, DynaCare/LabCorp, Seattle, Washington; 3) Dpet Cytogenetics, DynaGene/LabCorp, Houston, Texas.

Whole genome CMA testing has become the standard diagnostic tool for children with idiopathic MR, developmental delay, autism and congenital anomalies. Confirmation by a second standard method, such as FISH, MLPA, qPCR or chromosomes, if the abnormality was large enough, was recommended for early low resolution targeted arrays. However, new arrays have high density probe coverage that provide considerable redundancy and accuracy. While parental follow-up FISH is necessary to determine origin, recurrence risk and possible mechanism, the need to confirm high resolution arrays is questionable. This is particularly true for SNP arrays which provide an allele call that is not dependent on copy number changes and thus provides a secondary confirmation of genomic aberrations. We reviewed 2439 array positive cases based on size and probe coverage, to determine what percentage of the Affymetrix 6.0 SNP array results were confirmed by targeted BAC or commercial FISH. 2423 of 2439 (99.34%) cases were confirmed by FISH and 3 cases, where FISH confirmation was not appropriate, were confirmed by MLPA. All deletions and duplications > 1 Mb confirmed. Deletions between 500 kb-1 Mb, 100-500 kb and less than 100 kb were confirmed in 98.9%, 99.3%, and 94.0% of the cases, respectively. Duplications between 500 kb-1 Mb, 100-500 kb and less than 100 kb were confirmed in 99.5%, 98.8%, and 94.0% of the cases, respectively. Of the 16 cases that were considered inconclusive by FISH, BAC probe size, alignment of the copy number change (CNC) with respect to the available BACs, structural orientation of some small duplications (inverted vs. direct), absence of a second X chromosome homologue in males for comparison, presence of LCRs and probe coverage within the CNC complicated the analyses. These 16 cases would most likely confirm by other molecular methods. Thus, it appears that the inconclusive results were mainly due to technical problems rather than array reliability. The near 100.0% confirmation emphasizes the high accuracy of microarray testing. It also demonstrates (1) the high clinical concordance between microarray testing and FISH confirmation, (2) that confirmation of high resolution microarray testing for CNCs greater than 1 Mb is not required, (3) that 98.8% of deletions and duplications between 100 kb and 1 Mb confirm, (4) that CNCs below 100kb, particularly duplications, are more difficult to confirm, but would likely confirm by an alternate molecular technology.

1288T

Study on correlation between severe preeclampsia and abnormal expression of long-chain fatty acid oxidative enzyme. *X.L. Sun[1], Z. Yang[1,2], J.L Wang[1].* 1) Department of Obstetrics and G, Peking University Third Hospital, Beijing, China,100191; 2) Corresponding author: Zi Yang, zi_yang@email.com.

Objective: To investigate correlation between severe preeclampsia and abnormal expression of Long-chain 3 hyroxyacyl CoA dehydrogense (LCHAD). Methods: Serum-free trophoblast cells cultured in vitro were divided into four groups, which were stimulated by normal pregnancy serum(NP group), early onset severe preeclampsia serum (E-PE group), late onset severe preeclampsia serum (L-PE group), HELLP syndrome serum (HELLP group) respectively. Expression of mRNA and protein of LCHAD in trophoblast cells were detected by realtime PCR and Western Blot. Results: The relative expressions of mRNA of LCHAD in NP, E-PE, L-PE and HELLP groups were 1.00±0.00, 3.08±0.22,1.62±0.23,3.36±0.18 respectively. The relative expressions of mRNA of LCHAD were significantly reduced in E-PE, L-PE and HELLP groups compared with the NP group(P<0.05). Compared with the L-PE group, gene expressions of LCHAD were significantly decreased in E-PE and HELLP groups (P <0.05)), while no significant difference was found between the E-PE and HELLP group (P> 0.05). The relative expressions of protein of LCHAD in NP, E-PE, L-PE and HELLP groups were 4.94±0.02,2.93±0.13,4.14±0.06,2.80±0.09 respectively. The protein expressions of LCHAD were remarkably reduced in E-PE, L-PE and HELLP groups compared with the NP group (P<0.05). Protein expressions of LCHAD were remarkably decreased in E-PE and HELLP groups compared with the L-PE group (P <0.05)), while no significant difference was found between the E-PE and HELLP group (P> 0.05). Conclusions: These studies demonstrate that long chain fatty acid oxidation was involved in the pathogenesis and development of preeclampsia. The expressions of gene and protein of LCHAD were remarkably affected by early onset severe preeclampsia and HELLP syndrome. The Interaction mechanism and influence between fatty acid oxidation and the development of preeclampsia are worth further explored.(This work was supported by the grant of National Natural Science Foundation of China and Beijing Municipal Natural Science Foundation).

1289T

Study on the expression of LCHAD in trophoblast cells stimulated with different chain length fatty acids. *J.Lue. Wang[1], Z. Yang[1,2], X.L. Sun[1].* 1) Department of Obstetrics and G, Peking University Third Hospital, Beijing, China 100191; 2) Corresponding author: Zi Yang, zi_yang@email.com.

Objective: To investigate the influence among different chain length fatty acids on the expression of long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) in trophoblast cells. Methods: Serum-free trophoblast cells cultured in vitro were divided into five groups, which were stimulated by DMEM/F12 medium without FFA (F-FFA group), short-chain fatty acids (SC-FFA group), medium-chain fatty acids (MC-FFA group), long-chain fatty acids (LC-FFA group), very long-chain fatty acids (VLC-FFA group) individually. Expressions of mRNA and protein of LCHAD in trophoblast cells were detected by realtime PCR and Western Blot. Results: Among F-FFA, SC-FFA, MC-FFA groups, the gene and protein expreesion of LCHAD had no difference (P>0.05).Compared with F-FFA, SC-FFA and MC-FFA groups, the expressions of gene and protein of LCHAD were significantly reduced in LC-FFA group(P<0.05). while the LCHAD gene expression was significantly increased (P<0.05), but there were no difference in protein expression (P>0.05) in VLC-FFA group. Compared with VLC-FFA group, the expressions of gene and protein of LCHAD were significantly reduced in LC-FFA group (P<0.05). Conclusions: This study showed that there were different LCHAD expression in trophoblast cells stimulated with different chain length fatty acids. The Short-chain and medium-chain fatty acids had no effect on LCHAD expression in trophoblast cells. Long-chain and Very-long-chain fatty acids influenced the LCHAD expression in trophoblast cells significantly. (This work was supported by the grant of National Natural Science Foundation of China and Beijing Municipal Natural Science Foundation.).

1290T

Study on the gene and protein expression of p38MAPK and COX-2 in trophoblast cells stimulated by different chain length fatty acids. *Z. Yang, X.L Sun, J.L Wang.* Department of Obstetrics and G, Peking University Third Hospital, Beijing, China,100191.

Objective: To investigate the oxidative stress and inflammation in trophoblast cells stimulated by different chain length fatty acids. Methods: Serum-free trophoblast cells cultured in vitro were divided into five groups which were incubated with DMEM/F12 without FFA (F-FFA), short-chain fatty acids (SC-FFA), medium-chain fatty acids (MC-FFA), long-chain fatty acids (LC-FFA), very long-chain fatty acids (VLC-FFA). Then cells in each group were stimulated by DMEM/F12 medium, NADPH oxidase inhibitor (Apocynin) and p38MAPK inhibitor (SB203580) and were subdivided as each FFA plus-DMEM group, plus-NADPHΘ and plus-p38MAPKΘ groups. Expressions of mRNA and protein of p38MAPK and COX-2 in trophoblast cells were detected by realtime PCR and Western Blot. Results: Compared with other groups, the gene and protein expressions of p38MAPK in LC-FFA + DMEM, VLC-FFA + DMEM groups were increased (P<0.05). Compared with LC-FFA + DMEM group, gene and protein expressions of p38MAPK in LC-FFA + NADPHΘ and LC-FFA + p38MAPKΘ groups were significantly decreased (P<0.05). Compared with VLC-FFA + DMEM group, gene and protein expressions of p38MAPK had no difference in VLC-FFA + NADPHΘ groups(P>0.05), gene expression of p38MAPK in VLC-FFA + p38MAPKΘ group was significantly decreased (P<0.05), but there was no difference in protein expression (P>0.05). Compared with other groups, gene and protein expressions of COX-2 in LC-FFA + DMEM and VLC-FFA + DMEM groups were significantly increased (P<0.05), Compared with LC-FFA + DMEM group, gene and protein expressions of COX-2 in LC-FFA + NADPHΘ and LC-FFA + p38MAPKΘgroups were decreased (P<0.05). Compared with VLC-FFA + DMEM group, gene and protein expressions of COX-2 in VLC-FFA + NADPHΘ and VLC-FFA + p38MAPKΘgroups were all decreased (P<0.05). The correlation analysis showed there were significantly positive correlations between the gene and protein expressions of p38MAPK and COX-2 in LC-FFA group (P<0.05). There were significantly positive correlations in protein expression (P<0.05) but no correlation in the gene expression between p38MAPK and COX-2 in the other groups (P>0.05). Conclusions: The oxidative stress and inflammation may exist in trophoblast cells which were stimulated by Long-chain and Very-long-chain fatty acids. p38MAPK signal transduction pathway may contributed in this process. The interaction mechanism and influence among them are worth further explored. (supported by grant of NNSF of China and Beijing MNSF).

1291T

Biochemical and molecular characterization of the W1327X frequent mutation in Tunisian families with Glycogen storage disease type III (GSD III). *A. MILI[1,2], A. AMARA[2], I. BEN CHARFEDDINE[2], O. MAMAI[2], L. ADALA[2], A. AYADI[3], A. SAAD[2], K. LIMEM[1], M. GRIBAA[2].* 1) Biochemistry Department, Faculty of Medicine, Sousse, Tunisia; 2) Human Cytogenetics, Molecular Genetics and Reproductive Biology Laboratory. Farhat Hached Hospital, Sousse, Tunisia; 3) Paediatric Department, Tahar Sfar Hospital, Mahdia , Tunisia.

BACKGROUND: glycogen storage disease type III (GSD-III) is an inborn error of glycogen metabolism caused by a deficiency of the glycogen debranching enzyme (AGL). Some of the mutations appear to be population specific, whereas others are found in probands from a variety of different ethnic backgrounds. In this study we report a series of patient with homozygous AGL gene nonsense mutation 3980G>A (W1327X). METHODS: sixteen unrelated Tunisian families (from the region of Mahdia in the centerest of Tunisia), including 20GSD type III patients were presented with hepatomegaly and clinically symptoms of hypoglycemia. The routine laboratory investigations showed an elevated serum aspartate aminotransferase, alanine aminotransferase, creatine kinase and triglyceride levels. The blood lactate and uric acid levels were within normal limits. RESULTS: the 20 patients showed a striking elevation of glycogen content in the erythrocytes after several hours fasting and completely loss of debranching enzyme activity in leucocytes. Genotyping study, using four polymorphic microsatellite markers around AGL gene, identified a common haplotype for all patients. Direct sequencing of this gene reveal an homozygous W1327X mutation in exon 31. CONCLUSIONS: W1327X is the most characteristic Mahdia patient's mutation with GSD III. A common haplotype confirms the existence of a founder effect in this population.

1292T

Association Of Adiponectin And Leptin Gene Polymorphisms With Hypertension In Type 2 Diabetic Patients. *O. Khabour[1], S. Wehaibi[1], S. Al-Azzam[2], K. Alzoubi[2].* 1) Medical Laboratory Sciences, Jordan Univercity of Science and Technology, Irbid, Jordan; 2) Clinical Pharmacy, Jordan Univercity of Science and Technology, Irbid, Jordan.

Leptin and adiponectin are adipokines secreted by adipose tissue that play a role in regulating body metabolism and immune response. In this study, association of adiponedtin gene (ADIPQO) polymorphisms (G276T) and leptin gene polymorphisms (-G2548A) with hypertension in type 2 diabetic patients were examined. About 449 type-II diabetic patients from both genders were recruited in the study (199 normotensive and 250 hypertensive). Serum levels of adiponectin and leptin were measured using commercially available ELISA kits. Genetic polymorphisms were genotyped using polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) method. Adiponectin level was significantly higher in normotensive compared to hypertensive subjects (P<0.05). However, there was no difference between the genotypes and alleles frequencies of SNP G276T between the two groups. In addition, G276T SNP did not affect serum adiponectin level in the two groups. Regarding leptin, neither serum level nor SNP G2548A were different between the normotensive and hypertensive groups. However, high G allele of SNP G2548A was associated with increased leptin level (P<0.05). When gender was included in the analysis, similar results were obtained. In conclusion, ADIPQO SNP G2548A and leptin G276T were not associated with hypertension in Jordanian type 2 diabetic patients.

1293T

Deficiency in Mthfr or low dietary choline may lead to adverse reproductive outcomes by modulating ApoAI and inflammatory mediators PPAR-alpha, IFN-gamma or IL-10. *L.G. Mikael, J. Pancer, Q. Wu, R. Rozen.* Human Genetics and Pediatrics, Montreal Children's Hospital, McGill University, Montreal, Quebec, Canada.

Methylenetetrahydrofolate reductase (Mthfr) deficiency, due to a polymorphism at bp 677, is the most common genetic cause of hyperhomocysteinemia (Hhcy). Mthfr synthesizes the major source of carbon units for folate-dependent remethylation of homocysteine (Hcy) to methionine. Choline, through its metabolite betaine, can also function as a methyl donor in folate-independent Hcy remethylation. Both Hhcy and mild MTHFR deficiency have been associated with birth defects, pregnancy complications and other adverse reproductive outcomes. We have previously shown that Hcy reduces synthesis of Apolipoprotein A-I (ApoAI), the major lipoprotein in HDL-cholesterol; ApoAI is regulated by PPAR-(and has anti-inflammatory properties. We had also shown that MTHFR deficiency and low choline diets in mice are associated with Hhcy and increased risk of developmental anomalies. To further address the mechanisms by which Hhcy influences pregnancy outcomes, we fed pregnant mice, with or without a deficiency of MTHFR, either a control diet or a choline-deficient diet (ChDD) and examined ApoAI and other inflammatory mediators (PPAR-(, IFN-. and IL-10). ApoAI mRNA was reduced in livers of Mthfr+/- mice; protein levels were reduced in livers due to Mthfr deficiency as well as choline deficiency; placental ApoAI protein was also reduced, particularly in developmentally delayed embryos. Reduced expression of PPAR-(mRNA and protein was observed in livers of mice fed ChDD and was associated with increased methylation of a CpG dinucleotide in its promoter. Hepatic levels of the pro-inflammatory cytokine IFN-. were increased in Mthfr+/- dams, and placental IFN-. levels were highest in Mthfr+/- dams fed ChDD. Levels of the anti-inflammatory cytokine IL-10 were reduced in livers of mice fed ChDD. PPAR-(levels correlated positively with ApoAI and IL-l0 levels in liver, while ApoAI correlated negatively with IFN-. in maternal and placental tissues. In conclusion, we propose that Hhcy, due to genetic or nutritional disturbances in one-carbon metabolism, reduces expression of Apoa1 and Ppara; the latter effect may involve promoter hypermethylation. Hcy also decreases IL-10 and increases IFN-. levels. This disturbance of the Th1 (IFN-.):Th2 (IL-10) ratio and the increase in inflammatory mediators may contribute to Hcy-induced pregnancy complications.

1294T

Large-scale replication using "Metabochip" array identifies additional genetic loci influencing glycaemic traits. *R.A. Scott[1], V. Lagou[2], E. Wheeler[3], R. Welch[4], R. Mägi[2], J. Luan[1], T.M. Teslovich[4], C. Langenberg[1], I. Prokopenko[2], I. Barroso[3] on behalf of the MAGIC investigators.* 1) MRC Epidemiology Unit, Cambridge, United Kingdom; 2) WTCHG, University of Oxford, Oxford, UK; 3) Wellcome Trust Sanger Institute, Cambridge, UK; 4) Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI, USA.

Background: The Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) previously published the largest meta-analysis of genome-wide association studies (GWAS) for glycaemic traits in non-diabetic individuals, demonstrating 16 genome-wide significant loci for fasting glucose (FG) and 2 for fasting insulin (FI). The "Metabochip" is a custom iSELECT array of 217,697 SNPs, developed to support large-scale follow-up of putative associations for cardio-metabolic traits and fine-mapping of established loci. It contains >65,000 top-ranked, independent SNPs selected for replication of previous stage 1 meta-analyses of metabolic traits, including ~5k/1k selected for FG/FI replication, respectively ($10^{-75}<P_{\text{stage one}}<0.02$). **Methods:** To identify additional loci associated with FG/FI, we meta-analysed FG/FI replication SNPs in Metabochip genotyped studies (24/21 studies with 53,149/42,252 individuals, respectively), non-overlapping MAGIC GWAS discovery studies, and 15/13 new GWAS studies (25,618/23,130 individuals) using fixed effects meta-analysis (including up to 120,845/99,610 individuals in total). **Results:** We identified 11 novel loci for FG ($P<5\times10^{-8}$), 6 of which were in or near genes associated with other metabolic traits: *CENTD2*/*ARAP1* (type 2 diabetes [T2D]), *FOXA2* (T2D), *GRB10* (T2D), *PDX1* (MODY), *PCSK1* (obesity) and *PPP1R3B* (HDL-cholesterol). Five novel loci were located in or near genes encoding transcriptional repressors (14q31, 20q12), tRNA synthetase (14q32), nucleotide phosphodiesterase (10q24), and proinflammatory signalling kinase (9q31). Further, we identified 4 novel loci for FI including variants in or near *FTO* (BMI), *TCF7L2* (T2D, FG, HbA1C), *PPP1R3B* (HDL-cholesterol) and at 4q24 in a gene encoding a myelopoiesis-related protein. Also, 3 loci showed suggestive evidence of association ($P<5\times10^{-7}$) with FI, including *LYPLAL1* (WHR, fat distribution, fatty liver disease) and *ARL15* (adiponectin, T2D). **Conclusions:** Metabochip replication has allowed us to extend the number of genetic loci for FG and FI to 27 and 6, respectively. Identification of FI loci associated with lipids and fat distribution suggests a potential link to hepatic insulin resistance. The overlap of the novel FG/FI loci with established loci for T2D, metabolic and anthropometric traits highlights the complex relationships between these phenotypes. The Metabochip provides an efficient opportunity to widen our knowledge about the genetics of metabolic traits.

1295T

Gene and Pathway-based Analysis of 61 Genetic Variants in the Nicotinic Acetylcholine Receptor Genes and Insulin Resistance in American Indians. *J. Yang[1], SA. Cole[2], K. Haack[2], BV. Howard[3], LG. Best[4], RB. Devereux[5], ET. Lee[1, 6], J. Zhao[1].* 1) Biostatistics & Epidemiology, University of Oklahoma, Oklahoma City, OK; 2) Texas Biomedical Research Institute, San Antonio, TX; 3) MedStar Health Research Institute, Hyattsville, MD; 4) Missouri Breaks Industries Research Inc, Timber Lake, SD; 5) The New York Hospital-Cornell Medical Center, New York; 6) Center for American Indian Health Research, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Background: Cigarette smoking impairs insulin action and induces insulin resistance which is an independent risk factor for cardiovascular disease and type 2 diabetes. Given the strong genetic predisposition for smoking behavior and insulin resistance, and the role of nicotinic acetylcholine receptors in nicotine dependence, we hypothesize that genetic variants in the nicotinic acetylcholine receptor genes are implicated in the relationship between smoking and insulin resistance. Methods: We genotyped 61 tagSNPs in seven nicotinic acetylcholine receptor genes (CHRNA3, CHRNA4, CHRNA5, CHRNA6, CHRNB2, CHRNB3, CHRNB4) in 3,665 American Indians, residing in Oklahoma (OK), Arizona (AZ) and North/ South Dakota (DK), recruited by the Strong Heart Study. Insulin resistance was assessed using the homeostatic model assessment. Cigarette smoking was categorized as current vs. nonsmokers. We first examined the association of each SNP with insulin resistance, adjusting for age, sex, smoking status, body mass index, systolic blood pressure, lipids, physical activity, fibrinogen, and renal function (assessed by estimated glomerular filtration rate). We then performed a gene-based analysis by including the adjusted p-values of all SNPs within a gene using the truncated product method. Pathway-based analysis was done using the p-values of all seven genes based on gene-based analysis. All analyses were stratified by study center (OK, AZ, and DK). Multiple testing was adjusted using the Bonferroni correction. Results: Prevalence of current smokers was highest in participants from DK (65.9%), followed by OK (57.8%) and AZ (50.3%). Insulin resistance level was highest in participants from AZ (6.3±6.6), followed by DK (5.5±8.2) and OK (4.7±6.3). Gene-based analysis showed that five genes were significantly associated with insulin resistance in participants from AZ ($P<0.0001$ for CHRNB2 and CHRNB4) and DK ($P<0.0003$ for CHRNA3, CHRNA4, CHRNA5 and CHRNB4), but not those from OK. Pathway-based analysis indicated that the nicotinic acetylcholine receptor pathway was significantly implicated in insulin resistance among participants from AZ and DK (both $P<0.0001$), but not those from OK ($P=0.18$). Conclusion: The nicotinic acetylcholine receptor gene family may play a role in insulin resistance among American Indians. Targeting this pathway may provide novel therapeutic strategies for the prevention and treatment of insulin resistance and related metabolic disorders.

1296T

MPS BRAZIL NETWORK International Program: Helping to identify MPS patients around the world. *R. Giugliani[1,2,3,4], K. Jesuino[1], A. Brites[1], M. Burin[1], S. Leistner-Segal[1], U. Matte[1,3], M. Wilke[1], A. Federhen[1,4], I.V. Schwartz[1,2,4], MPS Brazil Network members.* 1) Med Gen Service, HCPA, Porto Alegre, RS, Brazil; 2) Dep Genetics, UFRGS, Porto Alegre, RS, Brazil; 3) INAGEMP, Porto Alegre, RS, Brazil; 4) Postgraduate Program in Medicine: Medical Sciences, UFRGS, Porto Alegre, RS, Brazil.

OBJECTIVES: The MPS BRAZIL NETWORK (MBN) was created in 2004, aiming to improve the diagnosis and management of MPS diseases in Brazil. Since then, physicians from many countries have contacted MBN to request support for the investigation of patients with suspected MPS disease. METHODS: The contact with MBN has been mainly performed through the website www.redempsbrasil.ufrgs.br or through the email redempsbrasil@ufrgs.br. Customers could download informative materials and instructions for sample collection and shipment, as well as the educational material on MPS prepared by MBN. Services from several countries sent biological samples, usually dried blood spots, to the MBN headquarters, located at the Medical Genetics Service of Hospital de Clínicas de Porto Alegre, Brazil (MGS-HCPA), where the laboratory tests needed for the diagnosis of MPS were performed. RESULTS: From April 2004 to May 2011, 216 foreign patients with MPS suspicion were investigated. The MPS diagnosis was confirmed in 177/216 (82%) patients. The most frequent type of MPS diagnosed was MPS II, confirmed in 77/177 (42.9%) of the MPS patients, followed by MPS VI (23.1%) and MPS I (15.2%). Most of these patients are from Latin American countries (133/177, or 75.1%). However, there is an increasing referral rate from other continents, with significative numbers coming from Middle East (21%). CONCLUSIONS: The MPS-BRAZIL NETWORK is improving the diagnosis of MPS not only in Brazil, but also in other countries where access to diagnosis is not easily available. These results highlight the importance of not only having the information on MPS widely available, but also providing access to diagnosis of these diseases, especially now that treatment is possible for many of these conditions.

1297T

Simple and rapid testing for Citrin deficiency. *A. Kikuchi[1], O. Sakamoto[1], T. Ohura[2], Y. Matsubara[3], T. Saheki[4], K. Kobayashi[5], S. Kure[1].* 1) Department of Pediatrics, Tohoku University School of Medicine, Sendai, Japan; 2) Division of Pediatrics, Sendai City Hospital, Sendai, Japan; 3) Department of Medical Genetics, Tohoku University School of Medicine, Sendai, Japan; 4) Institute for Health Sciences, Tokushima Bunri University, Tokushima, Japan; 5) Department of Molecular Metabolism and Biochemical Genetics, Kagoshima University Graduate School of Medicine and Dental Sciences, Kagoshima, Japan.

Citrin deficiency is an autosomal recessive disorder caused by mutations of the *SLC25A13*, and has two phenotypes: adult-onset type II citrullinemia (CTLN2) and neonatal hepatitis associated with intrahepatic cholestasis (NICCD). The clinical appearance of these diseases is variable, ranging from almost no symptoms to coma, brain edema, and severe liver failure that requires liver transplantation. Mutation analysis in *SLC25A13* is indispensable because of difficulties in the chemical diagnosis of citrin deficiency. Ten prevalent *SLC25A13* mutations account for 95% of mutant alleles in Japanese patients with CTLN2 or NICCD. The presence of these prevalent mutations is therefore favorable for genetic testing. Development of a simple test for these mutations would be desired. We have established the rapid and simple detection system of these seven mutation regions in *SLC25A13* using real-time PCR followed by melting curve analysis with adjacent hybridization probes (Hybprobe). We have made seven probe sets for the mutation detection. Each of six probe sets detects one mutation site, and another detects four mutation sites, which were clustered within a 21-bp region in exon 17. In the validation of the assay by single-blind manner, 76 test samples were correctly genotyped in the closed-tube assay, within an hour. This system would facilitate the genetic diagnosis of citrin deficiency, especially in East-asian populations. Also, it can be applicable not only to rapid diagnosis in any patients with suspected citrin deficiency, even showing atypical clinical presentation, but also to newborn screening at a much lower cost per sample by more high-throughput real-time PCR system, such as 384 or 1536 well format.

1298T

Molecular Genetics of Glycogen Storage Diseases. *J. Wang, G.L. Wang, F.Y. Li, M.L. Landsverk, W. Zhang, E.S. Schmitt, L.C. Wong.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Glycogen storage diseases (GSDs) are a group of inherited genetic defects of glycogen metabolism that is categorized into types 0-XII, based on the specific enzyme deficiency. Common GSD symptoms include hypoglycemia, hepatomegaly, developmental delay and muscle cramps. The GSD types 0a, I, III, IV, VI, IXa, IXb and X mainly affect liver; types 0b, V, VII and IXd mainly affect muscle. GSD type II affects nearly all organs. GSD type 0 caused by glycogen synthase deficiency. The outcome for untreated GSDs can be devastating. Early and accurate diagnosis, followed by proper treatment can greatly improve quality of life. DNA sequencing is the most effective method to confirm clinical diagnosis of GSD since it is rapid, accurate. The results will greatly facilitate carrier testing, prenatal analysis and genetic counseling. We have developed molecular testing for GSD types 0a, 0b, Ia, Ib, III, IV, V, VI, VII, IXa, IXb, IXc, IXd and X. All coding exons and their flanking regions were PCR amplified and sequenced. A total 305 patients with suspicion of GSD were referred to Medical Genetics Laboratories at the Baylor College of Medicine for sequence analyses of specific GSD genes. Among them, 125 (41%) were for GSD type Ia testing, which is the most common type of GSD. A total of 29 different mutations were detected in the G6PC gene (Type 1a), 6 (21%) of them are novel changes. Another 33 mutations have been detected in the remaining 13 GSD genes. Among them, 20 (61%) are novel changes. We have also identified two homozygous large deletions involving single or multiple exons in the GSD genes. The deletion of exons 3-5 of the G6PC gene has been confirmed by array CGH. The targeted array CGH confirmation of exon 16 deletion of the GBE gene (GSDIV) is pending. The overall positive rate in our patient cohort is 20%. Diagnosis of GSD used to depend on invasive liver or muscle biopsy and biochemical assays. Molecular analysis allows accurate diagnosis when enzymology is uninformative and identifies the pattern of inheritance. If a patient suspected to have GSD has hepatopathy as predominant symptom, analyses of GSD types I, III, IV, VI, IXa, IXb and X may be considered. If myopathy is the major symptom, then, testing of GSD types V, VII and IXd sequentially is suggested. This study underscores the importance of comprehensive molecular analysis to include genes of rare GSD types and targeted array CGH when glycogen storage diseases are suspected.

1299T

PHENOTYPE TO GENOTYPE CORALLATION IN A MILD FORM OF ISOVALERIC ACIDEMIA WITH NOVEL G391V IN THREE PATIENTS FROM A CONSANGINEOUS SAUDI FAMILY. *A.I Al-Aqeel[1,2], D. Colak[3], B. Al-Younes[4], A. Al-Bakheet[4], S. Tulba[4], F. Al-Mutairi[1], M. Al-Amoudi[4], A. Al-Odaib[4], N. Kaya[4].* 1) Department of Pediatrics, Riyadh Military Hospital,Riyadh, Saudi Arabia; 2) Stem Cell Therapy Program, King Faisal Specialist Hospital and Research Centre,Riyadh, Saudi Arabia; 3) Department of Biostatistics and Scientific Computing, King Faisal Specialist Hospital and Research Centre,Riyadh, Saudi Arabia; 4) Department of Genetics, King Faisal Specialist Hospital and Research Centre,Riyadh, Saudi Arabia.

Isovaleric acidemia (IVA) is an autosomal recessive rare disorder caused by a deficiency of isovaleryl-CoA dehydrogenase encoded by IVD gene. Numerous mutations in IVD have been reported in the literature. Here we present first Saudi IVD patients from a consanguineous family with a novel transversion mutation, causing conversion of Glycine to Valine at 391th amino acid on Exon 12 (G391V). The region around the site of the mutation was highly conserved among seventeen different species. Bioinformatics analysis proves the deleterious effects of the mutation. The change is “most likely” to interfere with the function of the IVD protein , with likely effect on its 3-D structure. This demonstrates the necessity of integrating the metabolome with the genome and proteome to bridge ``the genotype-to-phenotype gap”.

1300T

Meta-analyses of genetic association with body mass index in over 92,000 individuals using a gene-centric genotyping array. *Y. Guo[1], M. Lanktree[2], K. Taylor[3], G. Papanicolaou[4], N. Timpson[5], K. North[3], H. Hakonarson[1], L. Lange[6], B. Keating[1] on behalf of the IBC-BMI consortium.* 1) Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104, USA; 2) Department of Biochemistry, University of Western Ontario, London, Ontario, Canada; 3) Department of Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514, USA; 4) Division of Cardiovascular Sciences, National Heart, Lung, and Blood Institute (NHLBI), Bethesda, Maryland 20892, USA; 5) MRC Centre for Causal Analyses in Translational Epidemiology, University of Bristol, Bristol, United Kingdom; 6) Department of Genetics, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514, USA.

While genome wide association (GWA) studies have to date associated common variants across 48 independent loci with body mass index (BMI) at genome-wide significance (P < 5e-8), a gene-centric approach with equivalent or better coverage than GWA arrays can provide additional insight into genetic variants underpinning this trait. Thus to evaluate the association of common and low frequency single nucleotide polymorphisms (SNPs) with BMI, we used the ITMAT-Broad-CARe (IBC) genotyping array comprising up to 53,831 SNPs across ~2,100 cardiovascular-related loci. Participants included up to 78,436 individuals of European descent, 10,160 African Americans, 2,625 Hispanics, and 1,087 East Asians, across a total of 41 studies. Of the 48 established loci, ten have equal or greater coverage on the IBC array than GWA arrays. Eight of these ten loci contained a SNP(s) significantly associated with BMI at array-wide significance (P < 2.4e-6) in individuals of European descent. We describe one novel association for BMI in the *TOMM40-APOE-APOC1* locus at genome wide significance and in a previously reported gene *MC4R* we note a novel low frequency SNP, with minor allele frequency of 0.02, associated with BMI. Additionally, we performed secondary meta-analysis where we conditioned on the most significant SNP in each region in 50,933 individual of European descent and found two loci (*BDNF* and *MC4R*) showing independent signals. We also examined array-wide significant variants in the other three populations. The result indicated that directions of effects for all nine BMI signals in African Americans were consistent with those in European Americans. Finally and importantly, we observed more significant P values in seven of the nine loci in meta-analysis inclusive of the additional multi-ethnic samples, with two extra loci attaining array wide significance: *LGR4*, which is in the proximity but with limited linkage disequilibrium to the *BDNF* gene; and *NTRK2*, a BDNF receptor.

1301T

Genotype-phenotype correlations in Pompe disease. *A. Herzog, R. Hartung, E. Mengel, P. Hermanns, H. Runz, S. Gökce, J. Pohlenz, M. Beck.* Childrens Hospital, University Medical Center Mainz, Mainz, Germany.

Background Pompe disease is an autosomal recessive lysosomal storage disorder caused by a deficiency of the acid alpha-glucosidase. There is a broad spectrum of phenotypes from infantile to late onset forms. All patients show progressive skeletal muscle weakness leading to locomotive and respiratory dysfunctions. Objective: To characterize the genotypes of 37 patients with late onset Pompe disease and correlate them with the respective phenotype. Methods We clinically evaluated the patients and determined the molecular defects in the GAA gene by direct sequencing. Results On a total of 65 alleles 28 different mutations were found. In all patients 2 mutant alleles were identified except 1 pair of siblings. 30 late onset patients were compound heterozygous and 1 patient was homozygous for c.-32-13T>G, which leads to alternative splicing of exon 2 with about 10% of normally spliced products. 4 patients showed 2 other mutations each. First complaints of the c.-32-13T>G positive patients started at a median of 22 (3/34) years, whereas the late onset patients with 2 other mutations all had first symptoms in childhood at a median age of 5 (1.5/12.0) years. A milder affection of the patients with the common splicesite mutation also was seen by means of their mobility graduated by the Walton & Gardner-Medwin scale. 31% of them were able to walk and run freely (grade 0) and 51 % were able to climb stairs without banisters (grade 0-2). This wasn't achieved by any of the 4 patients with other mutations. 2 of them could climb stairs only with banisters (grade 3) and 2 of them weren't able to walk (grade 7 and 8). Furthermore, the c.-32-13T>G compound heterozygous patients had higher forced vital capacities with a median of 72% (50/98). One of the other patients needed an invasive ventilation and wasn't able to perform spirometry. The others had forced vital capacities of 32%, 50% and 56%. Conclusions This study confirms that phenotypic expression of Pompe disease primarily depends on the genotype. The common c.-32-13T>G splicesite mutation is associated with a milder phenotype than other mutations leading to the late onset form of the disease. Yet there is a broad variability in the enzyme activities as well as in disease severity between the patients bearing this mutation. Since this variability couldn't be explained by the mutations on the second allele, it is likely that there are existing secondary disease modifying factors.

1302T

MCAD mutation spectrum in individuals identified through newborn screening. *A. Millson[1], A. Openshaw[2], E. Lyon[1,3].* 1) ARUP Institute for Clinical & Experimental Pathology, ARUP Laboratories, Salt Lake City, UT; 2) ARUP Laboratories, Inc, Salt Lake City, UT; 3) Pathology Department, University of Utah, Salt Lake City, UT.

Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is an autosomal recessive inherited disease with a frequency of 1:6,500 to 1:17,000 in the US population. MCADD limits beta-oxidation of fatty acids in mitochondria, presenting in children and infants following a period of fasting and/or stress. A single point mutation in the ACADM gene, c.985A>G; p.304K>E, is the most common mutation associated with this disorder. 80% of patients are homozygous for c.985A>G and an additional 18% are heterozygous. The carrier frequency in the general population is 1/80. Due to the prevalence, and ease and benefit of clinical management, newborn screening for MCADD using MS/MS has been widely adopted. Confirmatory tests include plasma acylcarnitine profile, plasma carnitine, urine organic acids, and molecular testing. Since ARUP began offering ACADM sequencing, 2/3 of the samples received were for follow-up of abnormal newborn screening, and the remaining 1/3 were for other reasons including symptomatic patients. In a subset of cases we receive additional clinical and laboratory information, such as acylcarnitine profile results. Based on the interpretation of the acylcarnitine profile we evaluated the clinical sensitivity and specificity of our sequencing assay. Assessment of the clinical sensitivity (% of positive test results in patients with disease) found that for cases consistent with MCADD by acylcarnitine profile, slightly less than 50% carried two well-characterized pathogenic mutations, nearly 1/3 had one mutation and one variant of unknown significance (VUS) and in 3.5% no mutations were found. The clinical specificity (% of negative test results in persons without the disease) defined as acylcarnitine profiles not consistent with MCADD, identified individuals having either a single mutation consistent with carrier status or no mutations detected. In cases with acylcarnitine profiles possibly consistent with MCADD the vast majority were carriers, although in one case, one known pathogenic and one mild mutation were detected, possibly consistent with mild disease. Evaluation of the mutation spectrum of unique mutations found 48% were classified as uncertain, 41% pathogenic, 7% suspected pathogenic and 3% pathogenic-suspected mild. Of these, 79% were missense, 15% frameshift, 3% splice site and 3% nonsense. Periodic evaluation of clinical results to assess sensitivity and specificity as well as compilation of detected variants improves patient care.

1303T

The KUVAN® Adult Maternal Paediatric European Registry (KAMPER): Interim results on mutation frequencies of PKU patients. *A.C. Muntau[1], F.K. Trefz[2], A. Bélanger-Quintana[3], J. Alm[4], F.B. Lagler[5], A. Burlina[6], B. Destenaves[7], F. Feillet[8] on behalf of the KAMPER investigators.* 1) Molec Pediatrics, Univ Munich/DVH Childrens Hosp, Munchen, Germany; 2) School of Medicine University of Tuebingen, Reutlingen, Germany; 3) Hospital Ramon y Cajal, Madrid, Spain; 4) Karolinska University Hospital, Stockholm, Sweden; 5) Paracelsus Medical University, Salzburg, Austria; 6) University Hospital, Padova, Italy; 7) Merck Serono S.A. - Geneva, Switzerland, an affiliate of Merck KGaA, Darmstadt, Germany; 8) Hôpital d'enfants Brabois, Vandoeuvre les Nancy, France.

Objectives: KAMPER aims at providing information on the long-term safety of Kuvan treated patients with hyperphenylalaninemia (HPA) due to phenylketonuria (PKU) or BH4 deficiency, over the course of 15 years. Data from approximately 625 patients on growth, neurocognitive outcomes, adherence to diet, long-term sensitivity to Kuvan and pregnancy outcomes are expected. Methods: Observational, multi-centre, drug registry, including a maternal subregistry. Results: At first year interim analysis, four countries contributed a total of 58 patients with phenylalanine hydroxylase (PAH) deficiency and 15 with BH4-deficiency. This report includes results from PAH-deficient patients only. Patients were so far recruited in Germany (n=24), France (n= 19), Spain (n=13) and Italy (n=2). All results are expressed as median (Q1-Q3). The median age of recruited PKU patients was 9.7 years (6.4-14.9). Of these, 53% were male and 47% female. Most PKU patients (93%) were identified by newborn screening. Phenylalanine (Phe) concentration at newborn screening was 483 (371-727) µmol/L. A confirmatory test was performed in 86% of patients, the Phe concentration at this time point was 793 (478-1150) µmol/L. Information on the PAH genotype was available in 34 patients resulting in a total of 27 different genotypes. The majority of the reported genotypes were compound heterozygotes (25/27), while the most frequently encountered one was p.R261Q homozygous (n=4). Further analyses using the BIOPKU database to predict expected response showed that 18 genotypes could be classified as BH4-responders, 1 as slow responder and 3 as non-responders. Five of the reported genotypes have not been previously described in BIOPKU. According to the database, most of the genotypes found in KAMPER patients are associated with mild PKU/mild HPA. Almost all patients (95%) were tested for BH4 responsiveness, with the majority (64%) following a 24-hr loading test. Phe concentrations were reduced by ⁄ 30% in 51 of 55 patients tested. The mean daily Kuvan dose was 15 (10-20) mg/kg/day. Mild/moderate adverse events were reported in 3 PKU patients, which were deemed as not drug related. Conclusions: KAMPER provides a unique opportunity to gather a large collection of long-term follow up data related to BH4-responsive HPA in about 10 European countries. Future analyses will attempt to establish a link between the mutations and the metabolic status of the patients.

1304T

Biotinidase Deficiency Genotype-Enzyme Correlation and a Novel BTD Mutation Database. *M. Procter[1], A. Openshaw[1], D. Crockett[1], B. Wolf[3], R. Mao[1, 2].* 1) ARUP Laboratories, Salt Lake City, UT; 2) Department of Pathology, University of Utah, Salt Lake City, UT; 3) Henry Ford Health System, Detroit, MI.

Background: Biotinidase deficiency (BTD) is an autosomal recessive disorder that results in improper recycling of biotin. BTD affects 1 in 60,000 newborns and falls into two classes: profound/complete (<10% enzyme activity) and partial (10-30% enzyme activity). Over 160 mutations in *BTD* cause biotinidase to be nonfunctional or to be produced at very low levels. Biotinidase deficiency is characterized by skin rashes, hypotonia, developmental delay, alopecia, hearing loss or ataxia. These phenotypes may be mild or only exhibited when the patient is ill or stressed. Clinical diagnosis is dependent on presentation and biotinidase enzyme activity. Confirmation of BTD based on serum biotinidase activity is challenging due to enzyme instability. To identify mutations causing partial and profound BTD, we developed a full gene sequencing assay and a 5-mutation panel for the *BTD* gene. **Methods:** Full *BTD* sequencing and enzyme testing was performed on 113 samples. Eighty were extracted from serum and 33 were extracted from whole blood. Samples were amplified by touchdown PCR, Exo-SAP treated, and sequenced using Big Dye Terminator chemistry. We correlated enzyme activity partial (>2.0 U/L) and profound (<2.0 U/L) BTD with genotypes obtained by *BTD* mutation analysis. **Results:** Of 74 samples with enzyme levels <2.0 U/L, 61 (82%) had two causative mutations. In 32 samples with enzyme levels 2.1-3.5 U/L, we found 12 (38%) with two mutations. The remaining seven samples with enzyme activities >3.5 U/L revealed no samples with two mutations and four with only single mutations. **Discussion:** We were likely to find two causative *BTD* mutations when a sample had a very low biotinidase enzyme activity, however we did not find two mutations in partial or profound groups 100% of the time. We believe this is due to inaccurate enzyme quantification since the enzyme degrades rapidly when not frozen. For this reason the laboratory requests a paired sample from an unrelated donor be included with patient samples to for a sample handling control. In some instances *BTD* mutation analysis testing may be necessary for diagnosis of biotinidase deficiency. In the process of evaluating *BTD* mutations, we saw that information on *BTD* mutations was difficult to find. Consequently, we consolidated published mutations and added observed mutations along with clinical data if available, and made it publicly available in the form of a database(http://www.arup.utah.edu/database/BTD/BTD_welcome.php).

1305T

ABCA1 R230C variant do not contribute to dyslipidemia during gestational diabetes. *E. ZAMARRON-LICONA[1,2], M.C. MARTINEZ-LOPEZ[1], F. DE LA CRUZ-RUIZ[1], J.L. CORTÉS-PEÑALOZA[1], P. GARCÍA-GUERRA[2], R. DIAZ-MARTINEZ[3].* 1) LABORATORIO DE DIAGNOSTICO MOLECULAR, UNIVERSIDAD JUAREZ AUTONOMA DE TABASCO, VILLAHERMOSA, TABASCO, MEXICO; 2) HOSPITAL DE LA MUJER DEL ESTADO DE TABASCO, VILLAHERMOSA, TABASCO, MEXICO; 3) UNIVERSIDAD DE GUADALAJARA, GUADALAJARA, JALISCO, MEXICO.

INTRODUCTION. ABCA1 transporter plays a key role in low-HDL concentration [1]. R230C variant have been found in 10% low-HDL individuals. [2,3]. This variant is a risk allele for low-HDL, T2D in <40 years old and obesity in Mexican population. [4,5]. Gestational diabetes [6-8] is present in women with this risk allele (R230C). Objective: To determine association between R230C variant and dyslipidaemias during gestational diabetes. Methods: 182 obese women in the 24-week gestational age were recruited from the prenatal control service from Tabasco State “Hospital de la Mujer”, average age 28 years old. Glucose screening were useful to classify patients as cases when glucose⁄ 130mg/dl and as controls when glucose ≤130mg/dl. They accepted to participate by signing an informed consent. This project was accepted by both the Hospital and university bioethics committee. Glucose screening, oral glucose tolerance test (OGTT), Lipids profile (Cholesterol Total, CHT; triglycerides, TG; High Density Lipoproteins, HDL) A1c Hb, Insulin and blood type were performed in clinical laboratory of “Hospital de la Mujer”. Genotyping was carried out in the molecular diagnose laboratory at the university by Applied Biosystems' SNP Genotyping Assay for Taqman probe rs9282541 in a EcoTM Real-Time PCR system. Genotype-phenotype correlation and the association strength was determine with directional non-parametric tools for dichotomic ordinal variables, odds ratio (OR) was calculated as well in SPSS software v. 17.0. Results. Metabolic profile in both groups was not significantly different. Dyslipidaemias frequency were as follows: 71% hypercholesterolaemia, 89% hypertriglyceridemia, 11.6% High density lipoproteins lower than 35 mg/dl and 66.7% for mixed Dyslipidaemia (CHT+TG). R230C variant frequency between groups was: 25% control y 21.5% cases. Statistics analysis comparing Genotype-diabetes mellitus phenotype had not significant difference p⁄ .592 y OR=1.158: 0.9557, IC 95%. Association between Dyslipidaemias and genotypes CHT >200 mg/dl p= 0.127, OR =0.858: 1.569; TG >150 mg/dl p⁄ 0.985, OR= 1.003: 0.990 and CHT+TG p⁄ 0.235, OR = 0.896:1.405, IC 95%. Conclusión. In spite of R230C variant appeared in a higher frequency for that described for Mexican population (22.3%), It does not contribute to simple Dyslipidaemias (CHT, low HDL or TG) during gestational diabetes, however It is associated with mixed Dyslipidaemia (CHT+TG).

1306T

Massively parallel sequencing in suspected patients with mitochondrial disorders reveals multiple gene mutations unrelated to respiratory chain subunits or assembly factors. *V. Vasta[1], RP. Saneto[2,4], JL. Merritt II[3,4], S. Hahn[1,3,4].* 1) R&D, Seattle Children's Research Institute, Seattle, WA; 2) Department of Neurology/Division of Pediatric Neurology, University of Washington, Seattle, WA; 3) Department of Pediatrics/Division of Genetic Medicine, University of Washington, Seattle, WA; 4) Seattle Children's Hospital, Seattle, WA.

Purpose: Mitochondrial disorders are characterized by multi-organ involvement and often rapidly progressive clinical course. The variability in clinical presentation and the absence of a reliable diagnostic screening make the diagnosis very challenging. We explored a next-generation sequencing (NGS) test for mitochondrial disorders targeting the exons of 910 known and candidate nuclear genes in 25 patients. Methods: Targeted genes include subunits of the mitochondrial respiratory chain (RCC), assembly, transcription and translation factors, enzymes, carrier proteins and genes causing secondary mitochondrial defects or presenting with similar phenotypes. The exons of the genes were captured by in-solution hybridization followed by sequencing on an Illumina GAIIX. Results: Variants identified in two reference controls were 91.7% concordant with previous Sanger sequencing results. A complex insertion/deletion in *SURF1* gene (c.312_321del10ins2) was identified as a deletion of 8 bases. For analytic sensitivity, 90 variants detected by NGS were sequenced by Sanger and found 98.9% concordant. In intra- and inter-assay we observed an average of 1.3% and 1.8% CV respectively in the identification of coding variants. 98% of the targets were covered by >20 reads of Q⁄ 30. None of 16 patients with various RCC enzyme deficiencies presented mutations in either catalytic units or assembly factors. In 5 patients, significant alterations in *POLG1*, *CPT2*, *FASTKD2*, *SDHD*, *UBE3A*, *VARS2* (tRNA synthase) and *MTO1* (tRNA maturation) were found. Mutations in *PDSS1* were identified in a CoQ10 deficient patient. A previously described pathological mutation in *MFN2* gene was found in 2 patients, but further family studies suggest this mutation may not be clinically significant. Some patients carried mutations in two or more genes. Conclusion: This study underscores our hypothesis that sequencing only nuclear genes for RCC enzyme subunits and assembly factors may not provide the diagnostic answers for suspected patients with mitochondrial disorders. Our findings indicate that some previously described variants may not be pathological. Oligogenic cause for complex clinical presentations could be possible in some patients. Approximately one fourth of enrolled participants carried either known pathogenic mutations or variants of potential clinical significance. Despite the limitation in detecting all the pathogenic variants, our approach underscores considerable clinical benefits.

1307T

Mechanisms of *HLA-DQ* predispositions to type 1 diabetes in European and East Asian populations. *H. Miyadera, K. Tokunaga.* Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Japan.

HLA-DQ provide the strong genetic contributions to susceptibility and resistance to type 1 diabetes (T1D). It has been well established that haplotypes carrying non-Asp57) polymorphism in *DQB1* (*DQA1*03-DQB1*03:02*, *DQA1*05-DQB1*02*) associate strongly to T1D susceptibility in European populations. NOD mice model also encode non-Asp57) (*I-A*^{g7}), and the roles of non-Asp57) in selective peptide binding and TCR contacts have been proposed. However, neither models explain the disease pathway that might be shared with T1D susceptible haplotypes in East Asian population (*DQA1*03-DQB1*03:03/*04:01*), which carry Asp57). In this study we provide a comprehensive profile of HLA-DQ protein stability for all major *HLA-DQ* haplotypes, measured by an newly devised quantitative assay. Our data indicate the implication of HLA-DQ protein stability levels in susceptibility/protection to T1D, and offer a common mechanism that may underlie the association of *HLA-DQ* to T1D.

1308T

Study of mutation spectrum in patients with gaucher disease from India. *C. ANKLESHWARIA[1], M. MISTRI[1], A. BAVDEKAR[2], M. MURANJAN[3], J. SHETH[1].* 1) BIOCHEMICAL AND MOLECULAR GENETICS, FRIGE's INSTITUTE OF HUMAN GENETICS, AHMEDABAD, GUJARAT, India.Tele: ++91-79-26921414, Fax: ++91-79-26921415; 2) K.E.M. HOSPITAL, 489, RASTA PETH, SARDAR MUDLIAR ROAD, PUNE- 411011, INDIA. TELE: 020-66037300; 3) K.E.M. HOSPITAL, DEPARTMENT OF PEDIATRICS, PEDIATRICS RESEARCH LAB, PAREL, MUMBAI-400012, INDIA. TELE: 022-24136051.

Gaucher disease (GD), the most common lysosomal storage disorder, is caused by a deficiency of the lysosomal enzyme glucocerebrosidase (EC 3.2.1.45), leading to the accumulation of glucocerebroside within the tissue macrophages in multiple organs. Atleast 200 mutations in the gene for human glucocerebrosidase have been described till date. However in most, genotype-phenotype studies have focused upon screening for a few common mutations. The variants N370S, L444P, 84GG and IVS2+1G>A account for 90% of the mutant alleles in Ashkenazi Jewish whereas 50%-60% of mutant alleles were detected in non-Jewish individuals with type 1 GD. In the present study, nineteen unrelated individuals who were clinically and biochemically confirmed to have GD were investigated for mutation identification referred from various regions of India. N370S (c.1226G), L444P (c. 1448C), R463C (c. 1504T) mutations were analyzed by restriction fragment length polymorphism (RFLP) PCR. Out of 19 subjects investigated, 10 (52.63%) have shown L444P mutation, 2(10.52%) have shown R463C mutation while N370S mutation was not detected in any of the patients. This is the first report from India demonstrating high prevalence of L444P followed by R463C mutation in GBA gene in patients with Gaucher disease.

1309T

Airway obstruction and surgery in mucopolysaccharidosis type I (MPS I). *P. Arn[1], J.E. Wraith[2], L. Underhill[3].* 1) Division of Genetics, Nemours Children's Clinic, Jacksonville, FL; 2) Genetic Medicine, St. Mary's Hospital, Manchester, United Kingdom; 3) Global Medical Affairs, Genzyme Corporation, Cambridge, MA.

Background: Patients with MPS I typically undergo multiple surgical procedures during their lifetime to address various complications of this multisystemic disease. High rates of perioperative morbidity and mortality are observed among MPS I patients due to the anatomical and functional disruption of airway passages caused by glycosaminoglycan (GAG) accumulation in tissues of the respiratory tract and musculoskeletal system. Airway obstruction may be an indication, as well as a risk factor, for surgical intervention. Therefore, we examined the surgical data reported in the MPS I Registry to determine how often surgeries were performed for an airway-related indication. Methods: The MPS I Registry collects observational data to track the clinical progression and management of MPS I among the global patient population. As of May 2011, 987 patients were enrolled and diagnosed by clinical phenotype as follows: 574 Hurler, 220 Hurler-Scheie, 114 Scheie, and 21 undetermined. Patients with surgical data were included for analysis, and the surgical indication field was searched for terms consistent with airway-related surgery, excluding procedures for nasal polyps and ear tubes. Results were summarized as percentages by procedure type and patient phenotype. Results: Overall, 82% of the 987 Registry patients reported at least one surgical procedure for any indication, with similar percentages seen for Hurler (88%), Hurler-Scheie (85%), and Scheie (85%) patients. Surgeries for airway indications were more common among Hurler (24%) and Hurler-Scheie (22%) than Scheie (3%) patients. A total of 263 airway-related surgeries were reported for 165 patients, ranging from 1 to 6 surgeries per patient. Most procedures were performed to alleviate upper airway obstruction. The 3 most common procedures were adenoidectomy/tonsillectomy (n=152); bronchoscopy (n=40), and tracheostomy (n=38). Of note, tracheostomy procedures were performed in 36 patients (23 Hurler, 12 Hurler-Scheie, 1 Scheie), representing 3.6% of all Registry patients. Conclusions: Airway obstruction requiring surgical intervention is common in MPS I, and life-threatening conditions may arise among attenuated as well as severe phenotypes, as indicated by patients requiring tracheostomy. Since surgeries are performed on MPS I patients for indications other than airway obstruction, surgeons and anesthesiologists should be aware of this high-risk population.

1310T

TRANSCRIPTIONAL REGULATION OF CELLULAR CLEARANCE. *A. Ballabio[1, 2].* 1) Fondazione Telethon, Telethon Inst Gen & Med, Naples, Italy; 2) Baylor College of Medicine, Houston, Texas, USA.

As degradative requirements of the cell may vary depending on tissue type, age and environmental conditions, we postulated the presence of a system allowing the coordination of lysosomal activity. We discovered a gene regulatory network (CLEAR: Coordinated Lysosomal Enhancement And Regulation) that controls lysosomal biogenesis and function. The transcription factor TFEB acts as a modulator of the CLEAR network and it is physiologically activated by lysosomal storage. Upon activation, TFEB translocates into the nucleus, binds to CLEAR target sites in the promoter of lysosomal genes, induces lysosomal biogenesis and increases the ability of the cell to degrade complex molecules, such as glycosaminoglycans (GAGs) (Sardiello et al. Science 2009). More recent studies in our laboratory revealed that TFEB is directly involved in the regulation of two important cellular processes mediated by the lysosome: autophagy (Settembre et al. Science, DOI: 10.1126/science.1204592) and lysosomal exocytosis (Medina et al. submitted). Nuclear localization and activity of TFEB are regulated by serine phosphorylation mediated by the Extracellular Regulated Kinase 2 (ERK2), whose activity is tuned by the levels of extracellular nutrients. Similar to starvation, pharmacological inhibition of ERK2 induces autophagy by activating TFEB. Notably, TFEB-mediated activation of lysosomal exocytosis resulted in the clearance of the pathological storage in mouse models of in four different types of lysosomal storage diseases, and to a striking rescue of the cellular morphological abnormalities. These results reveal a novel mechanism that exerts a global control on the cellular degradation machinery and provide a tool to promote cellular clearance in human disease.

1311T

Higher Paraoxonase gene polymorphism frequency among Brazilian Fabry Disease patients. *A.C. Barris-Oliveira[1], K.B. Müller[2], L.T. Turaça[3], J.B. Pesquero[3], A.M. Martins[2], V. D'Almeida[1].* 1) Psychobiology, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil; 2) Pediatrics, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil; 3) Biophysics, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil.

Paraoxonase (PON1) is an antioxidant enzyme which activity was described to prevent oxidation of lipoproteins by reactive oxygen species formed during oxidative stress, and also by its protective role in atherosclerosis. Several polymorphisms had already been described in the gene of this enzyme and these modifications may cause different enzymatic activities, which are related with unbalanced redox state. Oxidative stress has been considered a putative mechanism for pathophysiologycal changes among some lysosomal storage disorders, like Gaucher disease. Fabry disease (FD) is one of these disorders and is caused by a deficiency of alpha-galactosidase A resulting in globotriaosylceramide storage in lysosomes, and due to this accumulation, is associated with cardiomyopathy, early-onset stroke, progressive renal failure, and other features. The objective of the current study is to investigate PON1 polymorphisms, Gln192Arg (switch of an adenosine for a guanine) and Leu55Met (switch of a thymine for an adenosine) in FD patients. A total of 52 subjects were selected for the study, 26 controls and 26 patients (13 men and 13 women). Both polymorphisms were accessed in blood samples DNA, by PCR-RFLP using the restriction enzymes, *BspPI* for Gln192Arg and, *NlaIII* for Leu55Met. The allele frequencies of the polymorphism Gln192Arg for FD patients and controls were respectively, 0.38 and 0.25. For the polymorphism Leu55Met, allele frequencies for FD patients and controls were, 0.27 and 0.24 respectively. Comparing FD patients vs controls from Gln192Arg polymorphism, a significant difference was observed (Chi-square: 7.95), whereas Leu55Met showed no difference between FD patients and controls. Besides these analyses, another 83 FD subjects were analyzed for the same polymorphisms; however these patients were part of 3 families. The allele frequencies of Gln192Arg and Leu55Met obtained were, respectively, 0.44 and 0.19 for the Family 1 (21 patients), 0.60 and 0.33 for Family 2 (24 patients) and 0.28 and 0.33 for Family 3 (38 patients). The higher frequency of Gln192Arg polymorphism among FD patients highlighted the possibility of a correlation between the PON1 genetic variation and the phenotypes since the disease has a wide range of symptoms which until now could not be explained only by mutations on the GLA gene. Financial support: AFIP, FAPESP, CNPq and IGEIM.

1312T

Skin ultrastructural findings in type 2 Gaucher disease: diagnostic implications. *A. Chan[1], D. Crumrine[1], T. Fergusson[2], O. Goker-Alpan[2], R. Schiffmann[3], W. Hollean[1], P. Elias[1], E. Sidransky[2].* 1) Department of Dermatology, VA Medical Center & Uni, San Francisco, CA; 2) 2Section on Molecular Neurogenetics, Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 3) 3Baylor Research Institute, Dallas, TX.

One of the major challenges in Gaucher disease, the inherited deficiency of lysosomal glucocerebrosidase, has been to distinguish type 2 Gaucher disease from the milder forms in young infants. Type 2 Gaucher disease is the rarest and most severe subtype, marked by rapid, early-onset neurodegeneration. Neither enzyme levels nor genotype reliably predict patient outcome. Making this distinction is essential for genetic counseling and for appropriate therapeutic considerations. The enzyme glucocerebrosidase also plays an essential role in maintaining epidermal permeability function, by regulating the generation of ceramides from glucosylceramides in the stratum corneum of the skin. Previous studies in a small number of patients with type 2 Gaucher disease revealed distinctive epidermal abnormalities, resembling alterations seen in a knock-out murine model of type 2 Gaucher disease. To further assess the diagnostic utility of such evaluations, the current study examined skin ultrastructure in an expanded cohort of twenty children with type 2 and two adults with type 1 Gaucher disease with varying clinical manifestations. Epidermal samples were evaluated by electron microscopy on ruthenium tetroxide post-fixed tissue. When compared to controls and subjects with type 1 Gaucher disease, only samples from patients with type 2 Gaucher disease displayed characteristic electron dense, non-lamellar clefts and immature-lamellar membranes in the stratum corneum. These changes were observed in young neonates as well as older babies, and were present even after enzyme replacement therapy. No abnormalities in the ultrastructure of corneodesmosomes, cornified envelopes, and the corneocyte lipid envelope were observed. In conclusion, the consistent and characteristic appearance of these changes continues to suggest that alterations in epidermal ultrastructure can provide a potential early diagnostic tool to distinguish type 2 Gaucher disease from other subtypes.

1313T

Diffusion Tensor Imaging and Volumetric Studies of the Brain in Canine Mucopolysaccharidosis I. *P.I. Dickson[1], J. Provenzale[2,3], S. Chen[2], I. Nestrasil[4], N.M. Ellinwood[5], S.Q. Le[1], S. Kan[1], S. Banakar[1], H. Boutte[2], E.G. Shapiro[4,6].* 1) Dept Pediatrics, LA BioMed Harbor-UCLA Med Ctr, Torrance, CA, USA; 2) Duke University Department of Radiology, Durham, NC, USA; 3) Emory University Department of Radiology, Oncology and Biomedical Engineering, Atlanta, GA, USA; 4) University of Minnesota Department of Pediatrics, Minneapolis, MN, USA; 5) Iowa State University Department of Animal Science, Ames, IA, USA; 6) University of Minnesota Department of Neurology, Minneapolis, MN, USA.

Standard magnetic resonance brain imaging correlates poorly with clinical outcomes in mucopolysaccharidosis I (MPS I), likely because findings lag behind the neuropathology. Ongoing studies (by E.G.S. and I.N.) of MPS patients reveal reduced fractional anisotropy (FA) of the corpus callosum (CC), disorganized fiber distribution in the centrum semiovale, and altered hippocampal volumes. We performed diffusion tensor imaging (DTI) of white matter tracts and volumetric analysis of the hippocampus in the canine model of MPS I. Four normal (age 18-28 m) and four MPS I dogs (age 24-26 m) underwent T1 and T2-weighted, proton density, and DTI of the brain on a 3T GE MRI scanner using an 8-channel knee coil. A three-dimensional fast spoiled gradient echo (FSPGR) sequence was employed for collecting structural data suitable for volumetric analysis (TR/TE/TI = 8.448/3.628/800 ms, flip angle = 8°, 124 axial slices, 1 mm slice thickness without interslice gap, in-plane resolution of 0.35 mm). The hippocampi were manually traced in BRAINS2 to generate a three-dimensional model for volume measurement. For DTI analysis, regions of interest were placed on FA maps in the peripheral frontal lobe white matter, the genu of the CC, the splenium of the CC, and the corticospinal tracts. Each animal was imaged twice, and values from each scan, right and left side (when applicable) were averaged. FA was 12.3 and 13.1% lower in the genu and splenium, respectively, of the CC in MPS I dogs compared to carrier dogs. This appeared to be due to reduced radial diffusivity ($3\perp$), which was 16.3% lower in genu and 19.7% lower in splenium of MPS I dogs. Myelin basic protein evaluation of the CC is underway. Hippocampal volumes were lower in MPS I dogs, averaging 0.553 ± 0.096 cm^3 for MPS I dogs and 0.662 ± 0.035 cm^3 for carrier dogs. The difference in hippocampal volumes persisted when indexed to body weight, but disappeared when indexed to brain weight, possibly indicating a difference in overall brain volume. Advanced brain imaging of the canine model may increase our understanding of MPS brain disease and lead to better ways to assess neuropathology in MPS patients.

1314T

Mucopolysaccharidosis IVA: A multidisciplinary approach. *P. Harmatz[1], C.J. Hendriksz[2], S. Tomatsu[3], W. Mackenzie[4], G.A. Solanki[2], B. Lee[5].* 1) Dept Gastroenterology, Children's Hosp Oakland, Oakland, CA US; 2) Birmingham Children's Hospital, Steelhouse Lane, Birmingham UK; 3) St Louis University, Edward A Doisy Research Center St Louis MO USA; 4) Alfred I Dupont Hospital for Children, Wilmington DE USA; 5) Dept Moleculr and Human Genetics, Baylor College of Medicine, Houston TX USA.

Mucopolysaccharidosis IVA (MPS IVA, Morquio A syndrome)is an autosomal recessive lysosomal storage disease characterized by excess storage of keratan sulfate leading to skeletal chondrodysplasia. Incidences of MPS IVA range from 1 per 76,000 to 600,000 live births. Patients with severe MPS IVA may experience significantly limited mobility associated with impaired endurance. The pathogenesis of bone dysplasia in MPS IVA is largely unknown and there is a lack of complete understanding of all clinical symptoms. To further understand the symptoms and treatments for MPS IVA, a summit was held with multidisciplinary experts to describe and develop a clinical understanding of MPS IVA, including organ-specific phenotypes of affected patients. Participants represented fields of inherited metabolic disorders, genetics, pediatrics, orthopedics, radiology, cardiology, anesthesiology, surgery (neuro and hand), pulmonology, physiotherapy, basic sciences and laboratory diagnostics. Progression and manifestations of severe and mild forms of MPS IVA were described. Management of patients with MPS IVA, including criteria for various corrective surgical procedures, management of airway compromise and risk of anesthesia, was discussed. Atlanto-axial instability with cervical cord compression and thoraco-lumbar kyphosis indicate severe spinal involvement. Concerns related to administration of anesthesia were discussed. In a survey completed by participants, all reported severe bone dysplasia and dwarfism to be the primary clinical characteristics of MPS IVA. Other reported primary clinical characteristics of MPS IVA were abnormal gait and facial features and cervical cord compression. Mitral valve stenosis and regurgitation appear to be the most prevalent cardiac manifestations and corneal clouding is commonly observed. Cardiac function is typically evaluated by flow echocardiogram and ECG and pulmonary function assessed by pulmonary function tests and sleep studies. Ligament laxity tests are not uniformly performed among participants. Participants agreed that a severity scoring system for MPS IVA is possible to develop (83%) and would be beneficial in practice (67%). MPS IVA is a genetic disease that affects multiple organ systems, hence diagnosis and management require a coordinated, multidisciplinary approach. Disease-related surgery should be performed by surgeons having a special interest in MPS diseases and anesthesiologists should be experienced with MPS patients.

1315T

Intrathecal Hydroxy-Propyl-Beta-Cyclodextrin reverses hearing loss in identical twin girls with Niemann-Pick Type C Disease. *C.A. Hastings, J. Torkildson, R. Raphael.* Pediatric Hematology/Oncology, Children's Hospital & Research Center Oakland, Oakland, CA.

Niemann-Pick Type C (NPC) is a rare, fatal, autosomal recessive neurodegenerative disorder caused by a defect in the NPC1 protein. Biochemically, NPC is characterized by intracellular accumulation of unesterified cholesterol and glycosphingolipids. No medical intervention has received regulatory approval in the USA to treat this disorder. Hydroxyl-propyl-beta-cyclodextrin (HPBCD) is a sugar compound shown to reverse cholesterol trafficking defects in murine and feline NPC models. We administered intravenous (IV) infusions of HPBCD under the FDA's Individual IND exemptions to 5 yr, 2 mos old identical twin, white females. Our patients had lost developmental milestones (speech and communication) and increasingly ataxic, despite treatment with miglustat beginning at 4 yrs 10 mos. We initiated IV HPBCD for 96 hours, followed by twice weekly infusions, escalating dosing if no toxicity. We observed no toxicities (stable hematology and chemistry panels, no SAEs occurred, and AEs were judged to be unrelated to study drug). The parents reported transient improvements in alertness and ataxia. HPBCD does not cross the blood-brain barrier in mice. Based on promising results in mice and cats with direct delivery into the central nervous system (CNS) demonstrating complete reversal or prevention of neurodegeneration in addition to prolongation of life, we amended our protocol to include HPBCD deliver to the CNS. Intrathecal (IT) infusion began at age 6 yrs 9 mos with an initial dose of 175 mg administered every 2 weeks by lumbar puncture. The twins received 6 injections without toxicity (CSF cell counts, protein, myelin basic protein remained normal) and continued to receive weekly administration of IV HPBCD (2500 mg/kg over 8 hours). After FDA review, we have continued every 2 week IT injections at an increased dose of 350 mg. Utilizing a validated NIH NPC Clinical Severity Score, the scores decreased from 36 at baseline to 29 (20% improvement). The most dramatic improvement was in hearing, assessed by brainstem auditory evoked response, which improved from moderate loss (80-95dBHL) at low frequencies and 45-65dBHL at high frequencies to mild loss (45-60dBHL) and normal (15dBHL), respectively, following 6 months of directed CNS therapy. This combination of systemic and central nervous system HPBCD drug delivery is a promising new therapeutic approach for children with NPC.

1316T

Chemical chaperone therapy for) -galactosidase deficiency. *K. Higaki[1], K. Ohno[1], Y. Suzuki[2], E. Nanba[1].* 1) Tottori University, Yonago, Japan; 2) International University of Health and Welfare Graduate School, Otawara, Japan.

) -Galactosidase deficiency is a group of lysosomal lipid storage disorder with autosomal recessive trait, which causes two clinically different diseases, GM1-gangliosidosis and Morquio B disease. It is caused by heterogeneous mutations of the GLB1 gene coding for lysosomal acid) -galactosidase. We have reported the chaperone effect of N-octyl-4-epi-) -valienamine (NOEV) on mutant) -galactosidase proteins. In this study we performed genotype analyses in patients with) -galactosidase deficiency and identified 46 mutation alleles including 9 novel mutations. We then examined the NOEV effect on mutant) -galactosidase proteins using 6 strains of patient-derived skin fibroblasts. We also performed mutagenesis to identify) -galactosidase mutants that are responsive to NOEV and found that 22 out of 94 mutants were responsive. Computational structural analysis revealed the mode of interaction between human) -galactosidase and NOEV. Moreover NOEV reduced GM1 accumulation and corrected impaired lipid trafficking and protein degradation in) -galactosidase deficient cells. These results provided further insights to the NOEV effect as a promising chaperone compound for) -galactosidase deficiency.

1317T

Attenuated phenotype in MPS VI (Maroteaux-Lamy) patients carring the p.R152W mutation. *A. Jurecka[1,2], E. Piotrowska[2], L. Cimbalistiene[3], N. Gusina[4], A. Rozdzynska[5], B. Czartoryska[6], K. Qunap[7,8], G. Wegrzyn[2], A. Tylki-Szymanska[1].* 1) Metabolic Diseases Clinic, The Children's Memorial Health Institute, Warsaw, Poland; 2) Department of Molecular Biology, University of Gdansk, Gdansk, Poland; 3) Center for Medical Genetics, Vilnius University Hospital, Santariskiu Klinikos, Vilnius, Lithuania; 4) Institute for Hereditary Diseases, Centre for Medical Genetic Services, Minsk, Belarus; 5) Anthropometry Laboratory, The Children's Memorial Health Institute, Warsaw, Poland; 6) Department of Genetics, Institute of Psychiatry and Neurology, Warsaw, Poland; 7) Department of Genetics, United Laboratories, Tartu University Hospital, Tartu, Estonia; 8) Department of Pediatrics, University of Tartu, Tartu, Estonia.

Background. Maroteaux-Lamy syndrome or mucopolysaccharidosis type VI (MPS VI) is a rare lysosomal, autosomal recessive storage disorder caused by a deficient activity of N-acetylgalactosamine-4-sulfatase, a lysosomal enzyme involved in the degradation of dermatan sulphate. Approximately 140 ARSB gene mutations have been identified thus far. Due to a large number of mutations, which are often private or novel, genotype-phenotype correlation for most MPS VI patients has been difficult. Aim. The aim of the study was to describe the natural clinical course in patients homozygous for R152W mutation from Central and Eastern Europe. Patients and Methods. From our database of 22 patients with MPS VI, we selected those with the p.R152W mutation in the homozygous (n=7, mean age 25 years, range 17-37 years) and compound heterozygous state (n=9, mean age 18, range 9-33 years). We performed a cross-sectional observational study. Results. Patients homozygous for the p.R152W mutation (n=7, age range 17-37 years) showed a much more attenuated course of the disease characterized by a significantly slower disease progression than patients heterozygous for the mutation (n=9, age range 9-33 years). First signs of the disease, usually slight range of motion limitations and mild skeletal malformations were observed at the median age of 6 years (mean age 8, range 1-21 years). Over 57% of these patients showed first signs of the disease after the age of 5 years, but even as late as 21 years. In these patients height was only slightly decreased and MPS VI features developed later in the course of the disease. Patients heterozygous for the p.R152W mutation developed symptoms earlier in life (mean age at the onset of symptoms was less 2.8 years; range 1 month - 8 years, median 1.29 year) and presented with short stature, significant skeletal malformations and other clinical abnormalities. The mean z-scores values for group of heterozygotes for body height and body weight were deviated from the reverence chart more than the z-scores values for the group of homozygotes. The average age for the homozygous group was 25.43 years while the average age for a group of heterozygotes was 18.1 years, therefore the observed differences are not the result of worsening symptoms with age. Conclusion. The p.R152W mutation in the homozygous state is associated with a relatively attenuated phenotype of mucopolysaccharidosis type VI.

1318T

Risk Factors for Fractures and Avascular Osteonecrosis in Type 1 Gaucher Disease: Analysis from the Gaucher Registry. *A. Khan[1], T. Hangartner[2], N.J. Weinreb[3], J.A. Cole[4], A.R. Gwosdow[4], J.S. Taylor[4], P.K. Mistry[5].* 1) Med Gen & Pediatrics, Alberta ChildHosp/Univ Calgary, Calgary, AB, Canada; 2) Wright State University, Dayton, OH, USA; 3) University Foundation for Lysosomal Storage Disorders, Coral Springs, FL, USA; 4) Genzyme Corporation, Cambridge, MA; 5) Yale University School of Medicine, New Haven, CT.

Background: The most debilitating skeletal complications of type 1 Gaucher disease (GD1) are avascular osteonecrosis (AVN) and fractures. These manifestations of GD1 lead to chronic pain, loss of functional mobility, hospitalization, surgical intervention and diminished health-related quality of life. **Objective:** To determine if disease severity measured by clinical and surrogate biomarkers represent risk factors for AVN or fractures in patients with GD1. **Methods:** A matched case-control analysis using the risk-set method was conducted to identify 4 groups of GD1 patients in the International Collaborative Gaucher Group (ICGG) Gaucher Registry: those with and without AVN, and those with and without fractures. Among cases and controls, we compared hematological, visceral, and organomegaly status, chitotriosidase levels, angiotensin converting enzyme, tartrate resistant acid phosphatase, white blood cell count, and spine DXA and femur DXA Z-scores. **Results:** Anemia and higher chitotriosidase activity differentiated patients with AVN compared to the control group. Patients with AVN were 1.5 times more likely to be anemic compared to the control group (OR= 1.51, 95% CI=1.06-2.15, p<0.02). Patients with AVN had higher levels of chitotriosidase activity preceding their event (OR = 2.23, 95% CI=1.13-4.41, p<0.05) compared to the control group. Patients who suffered fractures were 5.85 times (p<0.0005) more likely to have DXA lumbar spine Z-scores ≤-1 compared to the control group. **Conclusions:** Only anemia and chitotriosidase above the 75th percentile were associated with an increased risk of AVN. Risk of fractures was markedly increased among GD1 patients exhibiting low lumbar spine bone density. These findings show disease control, measured by clinical and surrogate markers, and osteopenia are useful measures to guide clinical therapy in Gaucher disease.

1319T

A mechanistic study of Fabry heart disease using induced pluripotent stem cells. *X. Meng[1], J. Shen[1], N. McNeill[1], B. Eberendu[1], R. Brady[2], R. Schiffmann[1].* 1) Institue of Metabolic Disease, Baylor Research Institute, Dallas, TX; 2) NINDs, National Institutes of Health, Bethesda, MD.

Fabry disease is an X-linked glycosphingolipid disorder caused by an insufficient activity of (-galactosidase A ((-Gal A). Cardiac complications including cardiac hypertrophy, conduction abnormalities and congestive heart failure are the leading causes of the mortality of Fabry patients. However, the pathogenesis of these cardiac abnormalities is largely unknown. The major obstacles in the mechanistic study of Fabry heart disease are the difficulty in obtaining heart biopsy from the patients and the lack of in vitro disease model systems. The induced pluripotent stem cell (iPSC) technology provides unique opportunity to solve these problems because iPSCs can be generated from skin fibroblasts and can be differentiated into cardiomyocytes in vitro. Previously, we have reported the establishment of iPSCs from mouse model of Fabry disease. In this study we generated iPSCs from hemizygous Fabry patients and healthy controls. Fabry patient-iPSCs were differentiated into spontaneously contracting cardiomyocytes in vitro. Fabry patient-iPSCs derived cardiac cells had markedly reduced (-Gal A activity and massive accumulation of globotriaosylceramide, the major substrate of (-Gal A. Using iPSC-derived cardiomyocytes we found altered function of L-type Ca2+ channel in Fabry cardiac cells. Fabry mouse iPSC-derived cardiac cells showed a significant relative resistance to Diltiazem, a specific inhibitor of L-type Ca2+ channel, compared to those derived from wild type (Wt). The beating rates of the cardiomyocytes declined with Diltiazem treatment in a dose-dependent manner in both genotypes, but Fabry cardiomyocytes needed a much higher dose of the drug to obtain equivalent decrease of beating rate compared to Wt controls. We also found significantly increased mRNA level of L-type Ca2+ channel (Cav1.2) in Fabry mouse heart tissues compared to Wt. The relationship between (-Gal A deficiency and abnormal L-type Ca2+ channel was further studied. Treatment of Fabry patient iPSC-derived cardiac cells with recombinant (-Gal A enzyme improved the response of the cells to L-type Ca2+ channel inhibitors (measured as decrement of beating rate). In conclusion, our study showed mouse model- and patients-derived iPSCs are useful tools for study of Fabry heart disease and that abnormal L-type Ca2+ channel activity may play a role in the pathogenesis of Fabry heart disease.

1320T

Postnatal and Prenatal Diagnosis of Lysosomal Storage Diseases in China. *Y. Meng[1], W. Zhang[2], H. Shi[1].* 1) Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, School of Basic Medicine of Peking Union Medical College.Beijing 100005, P R. China; 2) Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College.Beijing 100005, P R. China.

The enzyme assay of LSD in China could be traced back to 1984. Till now, 18 microassay methods for enzyme activity have been established using fluorimetric or colorimetric substrates and 21 kinds of LSD could be diagnosed. Molecular testing for LSDs began in 2000. Since 2000, 830 index patients were diagnosed based on the characteristic clinical manifestations and specific enzyme assay(MPS 317, Gausher Disease 202 , MLD 91, ML 57, others 163.)Prenatal diagnosis had been carried out in 252 pregnancies at risk of LSD in early pregnancy, 55 affected fetuses were detected. In 317 cases of mucopolysaccharidosis (MPS), the most common type is Hunter disease (MPSII, 46.6%). The second is MPS IVA(20.5%) and the 3rd is Hurler disease(MPSI, 18%). Since 2000, we have carried out mutation analysis on Gaucher disease, Fabry disease, MPSI, MPSII, MPSIVA & MPSVI, Niemann-Pick disease, and metachromatic leukodystrophy, etc. Many novel mutations had been detected in Chinese LSD patients. For example, 33 types of mutation have been detected in 137 cases with Gaucher disease. L444P was the most common mutation, accounting for 31.9%, and N188S accounts for 7.4%, F213I accounts for 5.9%, M416V accounts for 5.4% and V375L accounts for 4.4%. 8 mutations were novel mutations in Chinese, e.g., F37V, Y205C, P122L, Y363C, N382K, L383R, L385P and M416V. The treatment of LSD in China, only Gaucher disease patients(130 cases) receive enzyme replacement therapy.

1321T

Evaluation of the pattern of X chromosome inactivation in women with Fabry Disease. *K.B. Müller[1], A.C. Barris-Oliveira[2], E.S. Ramos[3], A.M. Martins[1], V. D'Almeida[2].* 1) Pediatrics, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil; 2) Psychobiology, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil; 3) Genetics, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil.

Background: Fabry Disease (FD) is an X-linked lysosomal storage disorder caused by the deficiency of alpha-galactosidase A ((-galA), which results in the intralysosomal accumulation of globotriaosilceramide (Gb3). Heterozygous female FD patients demonstrate a wide spectrum of symptoms, ranging since asymptomatic until a course as severe as in male hemizygotes, but the cause of this variability is still controversial. X-chromosome inactivation (XCI) is a mechanism of dosage compensation determined randomly (50:50). Skewed XCI, that is a marked deviation in this proportion, is an important phenomenon to be considered on women carriers of X-linked disorders, since some authors believe that this process can explain the manifestation of symptoms, including FD females. The aim of this work was to investigate the pattern of XCI in heterozygous FD women patients. Methods: Eight women with biochemical and molecular diagnosis of FD were evaluated for the pattern of XCI by HUMARA assay. DNA from peripheral blood was extracted using commercial kit. After digestion and nested PCR, alleles were observed in 12% polyacrylamide gel electrophoresis stained with silver nitrate. Results: A remarkable difference in the pattern of alleles was observed in one sample, when bands with and without prior digestion with HpaII were compared. This condition indicates a possible skewed pattern of XCI. The other samples showed no visible differences in the pattern of bands when analyzed by polyacrylamide gel. However it does not exclude the possibility of involvement of skewed XCI, since a more efficient evaluation will be achieved by capillary electrophoresis. Conclusion: Although there is no consensus in the literature regarding the involvement of skewed XCI and the manifestation of symptoms in women with FD, it is clear that this mechanism contributes to the physiopathology of the disease in this group of patients. Further analyses by capillary electrophoresis will add more information to confirm the proportion of skewed XCI. Supported by: FAPESP, CNPq, AFIP and IGEIM.

1322T

Screening for Fabry Disease in Japan. *K. Nakamura, K. Hattori, S. Matsumoto, H. Mitsubuchi, F. Endo.* Dept Pediatrics, Kumamoto Univ Sch Med, Kumamoto, Japan.

Fabry disease is an X-linked disorder of alpha-galactocidase A which causes the accumulation of glycolipids in lysosomes. The incidence of the classical type of the disease is approximately 1 in 40,000 males. Recent studies have revealed the late-onset type of the disease to have a higher frequency than previously known. To determine the disease incidence in Japan, we screened newborns to measure alpha-galactosidase A activity in dried blood spots from Japanese neonates. Enzyme-deficient infants were retested, and infants who were double-screening positive were diagnostically confirmed by enzymatic activity and mutation analyses. Thirty eight neonates had a deficiency in alpha-galactosidase A activities and specific mutations, including 5 neonates with classical mutations identified previously. Based on our newborn screening in Japan, the incidence of alpha-galactosidase A deficiency was 1 in 8,000 male. These results suggest that the late-onset phenotype of Fabry disease is underdiagnosed among both males and females in Japan. The recognition of the existence of these patients suggests the need for both early diagnosis and therapeutic intervention. However, ethical issues need to be taken into consideration in terms of when and whom the screening should be performed.

1323T

Validation of chitotriosidase determination in dried blood spots on filter paper as a tool for screening and monitoring of Gaucher disease patients in Colombia. *N. Pacheco Fernandez, A. Uribe.* Centro de Investigaciones en Bioquímica (CIBI), Departamento de Ciencias Biológicas, Facultad de Ciencias, Universidad de Los Andes, Bogota, Colombia.

Gaucher disease is a lysosomal storage disorder (LSD) caused by a deficiency of the enzyme) -glucocerebrosidase. This deficiency generates an accumulation of glucocerebroside in macrophages and causes bone marrow alterations, hepatomegaly and splenomegaly. Although LSDs are rare diseases, Gaucher disease is quite frequent, however there are no specific biomarkers for detection and monitoring of patients with this disease. Chitotriosidase (CT) is the only biomarker that increases 600 to 1000 times in Gaucher patients. This is why it is widely used in disease screening. The enzymatic determination in dried blood spots on filter paper (DBS) is a tool that facilitates transport and lowers costs of sampling, making it more accessible to population at risk. The aim of this study is to validate the quantification of CT in DBS to facilitate detection and monitoring of Gaucher disease patients in Colombia. Serum and DBS samples of 32 individuals between 8 months and 45 years old were analyzed, 4 of them were Gaucher disease confirmed patients by determination of enzymatic activity of) -glucocerebrosidase and the other 28 were normal controls. Using a fluorometric method with 4-methylumbelliferyl-) -D-N,N',N”-triacetylchitotrioside as substrate, the activity of CT was determined both in serum and DBS. The observed activity values do not overlap between controls (0-52 nmol/ml/hr in serum and 0-88 nmol/ml/hr in DBS) and patients (346-373 nmol/ml/hr and 311-1004 nmol/ml/hr in serum and DBS respectively). Although there is more variation in DBS essay, values between controls and patients are discriminating. This variation can be caused by the presence of cells in filter paper samples, which have been previously eliminated in serum samples. Quantification of CT in DBS works as a cheap and practical tool in detection and monitoring of Gaucher disease patients in Colombia.

1324T

Glycogen clearance by BMN 701 and alglucosidase alfa in a mouse model of Pompe disease. *J. Peng, R. Cahayag, M. Fox, C. O'Neill.* BioMarin Pharmaceutical Inc., Novato, CA.

Pompe disease (GSD Type II) is a rare neuromuscular disorder caused by deficiency of the enzyme acid (-glucosidase, resulting in accumulation of glycogen in muscle tissues. The build-up of glycogen causes progressive muscle weakness, organ failure, and death from cardiac or respiratory failure. A mouse model ($GAA^{tm1Rabn/J}$) of Pompe disease containing a disrupted acid (-glucosidase gene emulates the human disease and has been used to evaluate the potential for efficacy of treatments for glycogen clearance. Alglucosidase alfa, a recombinant human acid (-glucosidase, has been previously demonstrated to reduce glycogen accumulation in the $GAA^{tm1-Rabn/J}$ mice. BMN 701, a novel fusion protein of insulin-like growth factor 2 and acid (-glucosidase (IGF2-GAA), is being developed as an alternative therapy for Pompe disease. The glycogen clearance effects of alglucosidase alfa and BMN 701 were compared directly. $GAA^{tm1Rabn/J}$ mice were treated with repeat intravenous bolus administrations of alglucosidase alfa or BMN 701 weekly for four weeks. One week following the final dose administration, heart (left ventricle), diaphragm, quadriceps, psoas, and soleus muscles were collected and evaluated for glycogen content using an enzymatic assay. Administration of either alglucosidase alfa or BMN 701 resulted in a dose-dependent reduction in glycogen levels; however, the glycogen clearance effect of BMN 701 was generally equivalent to a corresponding 5-fold higher dose of alglucosidase alfa. Only BMN 701 demonstrated the ability to reduce glycogen storage to levels indistinguishable from wild-types in the skeletal muscles examined. We propose that BMN 701 may present an effective new enzyme replacement therapy for Pompe disease, offering improved clinical efficacy compared to current treatment.

1325T

TFEB links autophagy to lysosomal bigenesis. *C. Settembre[1,2,3], C. Di Malta[1], V. Polito[1,2,3], M. Arencibia[4], F. Vetrini[2], S. Erdin[2], D. Medina[1], M. Sardiello[2,3], D. Rubinsztein[4], A. Ballabio[1,2,3].* 1) telethon institute of genetic and medicine; 2) baylor college of medicine; 3) neurological research institute; 4) Cambridge Institute for Medical Research.

Autophagy is a lysosomal degradation pathway with a crucial role in health and disease. Polyubiquitinated proteins, protein aggregates and dysfunctional organelles are degraded, and their components recycled, by autophagy. An important step of the autophagic pathway is the fusion between the autophagosome and the lysosome. Dysfunction of the autophagic-lysosomal system has been associated to the pathophysiology of several inherited diseases, including lysosomal storage disorders and neurodegenerative diseases. We have identified a transcriptional program that controls major steps of the autophagic pathway, including autophagosome formation, autophagosome-lysosome fusion and substrate degradation. The transcription factor EB (TFEB), a master gene for lysosomal biogenesis, coordinates this program by driving expression of autophagy and lysosomal genes. We found that TFEB activity is regulated by ERK2-mediated phosphorylation TFEB. Inhibition of ERK2 signaling results in TFEB nuclear translocation and induction of TFEB activity. Thus the identification of a novel transcriptional dependent mechanism that regulates cellular clearance and of the pharmacological approach to enhance it, opens new therapeutic perspectives for the treatment of storage-related diseases.

1326T

Fabry disease mouse model exhibits cardiac hypertrophy and arrhythmias. *J. Shen, X. Meng, B. Durant, R. Schiffmann.* Institute of Metabolic Disease, Baylor Research Institute, Dallas, TX.

Fabry disease is an X-linked glycosphingolipid disorder caused by a deficiency of (-galactosidase A. Fabry disease exhibits a variety of cardiovascular complications including cardiac hypertrophy and arrhythmias. To date, the mechanism of these manifestations is poorly understood. Due to heterogeneous clinical manifestations of Fabry patients, mouse model will be particularly useful in mechanistic studies. Although mouse model of Fabry disease has been generated, there is little information on their cardiac phenotype. In this study, we characterized Fabry mouse compared to wild-type (WT) controls and identified following cardiac abnormalities. (1) We found age-dependent cardiac hypertrophy in Fabry mice. Heart weight (normalized by body weight) in Fabry mouse was significantly increased compared to WT. The increment of heart weight was seen in 12-month old, but not 5-month old, Fabry mice. The mRNA level of atrial natriuretic peptide, a marker of cardiac hypertrophy, was significantly increased in 12-month old Fabry mouse heart. Activation of Akt signaling is known to be associated with cardiac hypertrophy. We found significantly increased phosphorylated Akt in 19-month old, but not 5-month old, Fabry mouse heart. These observations demonstrated that cardiac hypertrophy occurs in Fabry mice but its progression is relatively slow. To further determine whether young `asymptomatic' Fabry mice have higher susceptibility to inducers of cardiac hypertrophy, 5-month old Fabry and WT mice were infused with) -adrenoceptor agonist isoproterenol using osmotic pumps. Both Fabry and WT mice exhibited significant increase in heart-to-body weight ratio in response to isoproterenol infusion. However, the response in Fabry mice was more significant than WT mice. (2) We found that electrocardiogram (ECG) of Fabry mouse was significantly altered in a sex-dependent manner. Compared to controls, 11-month old male Fabry mice showed lower heart rate, increased heart rate variability, longer PR and ST intervals. However, homozygous female mice of the same age had no ECG abnormalities. In conclusion, we found cardiac hypertrophy and arrhythmias in Fabry mice. Overall, these abnormalities match with Fabry patients' cardiac manifestations, and thus, Fabry mouse would be a good model for studying Fabry heart disease. We are currently trying to elucidate underlying mechanism of Fabry cardiac complications using Fabry mouse and cultured cardiac cells.

1327T

Tissue preferential synergistic effect of saposin A and saposin B on glycosphingolipids degradation in mice. *Y. Sun[1,3], M. Zamzow[1], H. Ran[1], W. Zhang[2], B. Quinn[1], S. Barnes[1], K.D.R. Setchell[2,3], G.A. Grabowski[1,3].* 1) Division of Human Genetics, Cincinnati Children's Hospital Medical Center,; 2) Division of Pathology and Laboratory Medicine, Cincinnati Children's Hospital Medical Center,; 3) Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH 45229-3039.

Saposins (A, B, C and D) are lysosomal proteins derived from the precursor, prosaposin. Saposins have specific and overlapping actions in glycosphingolipid metabolism. Individual saposin A (A-/-) and saposin B (B-/-) deficient mice were created and showed unique phenotypes caused by insufficient degradation of the myelin glycolipids: galactosylceramide/galactosylsphingosine and sulfatide, respectively. To gain insight about the interrelated functions of saposin A and B, combined saposin AB null mice (AB-/-) were created by knock-in point mutations into the saposins A and B domains of the prosaposin locus. Saposins A and B were undetectable in AB-/- mice, whereas prosaposin, saposin C, and saposin D were expressed at WT levels. AB-/- mice developed neuromotor deterioration at ~75 days, and they lost 30% of body weight by end-stage. The life span in AB-/- mice (~100 days) was longer than A-/- (~84 days) and shorter than B-/- (>1 year) mice. Large multinucleated macrophages were found in AB-/- mice livers. The ultrastructure studies showed these macrophage storage inclusions to have a twisted filamentous appearance. Glycosphingolipid analyses by LC/MS and TLC identified substantial increases of lactosylceramide and minor increases of globotriaosylceramide in AB-/- mice liver, but not in the brain. Similar to B-/- mice, activated microglial cells were uniformly distributed over the entire brain of AB-/- mice. Storage material was found in Schwann cells and oligodendrocytes indicating demyelination in AB-/- nervous systems. Sulfatide levels were increased and galactosylceramide was at WT levels in the AB-/- brain. These findings indicate that combined saposins A and B deficiencies attenuated GalCer-) -galactosylceramidase and GM1-) -galactosidase functions in degradation of lactosylceramide mainly in the liver. In the brain, blockage of sulfatide degradation diminished galactosylceramide accumulation. These analyses of AB-/- mice continue to delineate the tissue preferential interactions of saposins in glycosphingolipid metabolism and homeostasis.

1328T

KIR/HLA gene variants in patients with Gaucher disease in Southern Brazil. *F. Vairo[1, 2], P. Portela[3], P. Salim[3], T. Alegra[1,2], C. Netto[1], M.L. Saraiva-Pereira[1], M. Jobim[3], L.F. Jobim[3], I.V. Schwartz[1,2].* 1) Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil; 2) Genetics Department, Universidade Federal do Rio Grande do Sul, Brazil; 3) Immunology Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil.

Background: Gaucher disease (GD) is caused by the reduced activity of a lysosomal enzyme, glucocerebrosidase, which leads to the accumulation of glucocerebroside in the cells and a chronic stimulation of the immune system. Natural Killer (NK) cells play an important role in the immune response, and their activity is impaired in GD. Killer immunoglobulin-like receptors (KIR) regulate the activity of NK cells through an interaction with specific human leukocyte antigen (HLA) class I molecules on target cells. Objectives: To analyze the variability of KIR genes in a Southern Brazilian sample of GD patients, to compare it with controls, and to look for associations with clinical manifestations. Results/Discussion: Using the chi-square test, 31 GD patients (25 mild, 2 moderate, and 4 severe) were analyzed and compared to 200 healthy controls. There was no significative difference in the frequencies of KIR/HLA variants between the groups. No severe patient had the KIRD2S3 variant, but 7 mild and all moderate patients had it. Patients who have the HLA C1 variant appear to have less mono/polyclonal bands in protein electrophoresis. Bone mineral density appeared to correlate with the KIR2DS2 variant since 11/17 patients with osteopenia/osteoporosis have this variant. Conclusion: There are no studies about the relationship about KIR/HLA variants and GD. Despite being a limited sample of GD patients, our data suggest a possible association of KIR/HLA variants and this condition. The KIR/HLA variants must be further studied, for they seem to be a phenotype-modifier factor for GD.

1329T

Diagnostic testing for MPS VI (Maroteaux-Lamy syndrome): Laboratory survey results and recommendations from the MPS VI Diagnostic Summit. *T. Wood[1], O. Bodamer[2], M.G. Burin[3], V. D'Almeida[4], C. Eng[5], M. Fietz[6], R. Giugliani[3,7], C. Hendriksz[8], P. Hwu[9], D. Ketteridge[6], Z. Lukacs[10], N.J. Mendelsohn[11], M. Pasquali[12], A. Schenone[13], K. Schoonderwoerd[14], B. Winchester[15], P. Harmatz[16].* 1) Biochemical Genetics Laboratory at Greenwood Genetic Center, Greenwood, SC, USA; 2) University of Miami Miller School of Medicine, Miami, FL, USA; 3) Medical Genetics Service, Hospital de Clinicas de Porto Alegre, Brazil; 4) Federal University of São Palo, São Palo, Brazil; 5) Baylor College of Medicine, Houston, TX, USA; 6) SA Pathology, Women and Children's Hospital, North Adelaide, SA, Australia; 7) Dep Genetics, UFRGS, Porto Alegre, Brazil; 8) Birmingham Children's Hospital NHS Foundation Trust, Birmingham, UK; 9) National Taiwan University Hospital, Taipei, Taiwan; 10) Hamburg University Medical Center, Hamburg, Germany; 11) Children's Hospitals and Clinics of Minnesota, Minneapolis, MN, USA; 12) Univeristy of Utah School of Medicine, Salt Lake City, UT, USA; 13) Fundación para el Estudio de las Enfermedades Neurometabólicas (FESEN), Buenos Aires, R, Argentina; 14) Erasmus Medical Center, Rotterdam, the Netherlands; 15) UCL Institute of Child Health, London, UK; 16) Children's Hospital Oakland, Oakland, CA, USA.

Maroteaux-Lamy syndrome (Mucopolysaccharidosis VI) is a lysosomal storage disease caused by a deficiency of N-acetylgalactosamine 4-sulfatase (arylsulfatase B). This enzyme is required for the degradation of dermatan sulfate which accumulates in patient's cells and is excreted in large quantities in their urine. Specific therapeutic intervention is available; however, accurate and timely diagnosis is crucial for maximal benefit. To better understand the current laboratory practices for the diagnosis of MPS VI, a survey was completed by 13 diagnostic laboratories from around the globe. The data were reviewed by an expert panel as part of a Diagnostic Summit on MPS VI. Participating laboratories were from Asia, Australia, Europe, and North and South America. The various steps in the diagnosis of MPS VI were assessed including urinary glycosaminoglycan (GAG) testing, enzymatic assays, and molecular diagnostics. For urine-based testing, the methodology for qualitative and quantitative GAG measurement, normal ranges, and common problems were assessed. There was general agreement that dilute urine samples posed a significant problem although specific collection requirements were not made by most institutions. Quantitative testing was generally felt to be insufficient for diagnosis as dermatan sulfate may not always be excreted in large quantities and qualitative testing, looking for the accumulation of dermatan sulfate, was preferred. Diagnosis is usually made by demonstrating a deficiency of arylsulfatase B, typically measured in leukocytes with a smaller number of laboratories using dried blood spots and/or fibroblasts. For leukocyte testing, nitrocatechol was the most common substrate and normal ranges were compared among different laboratories. Proficiency testing is available for both urinary GAG and enzyme studies; however participation is not universal. Molecular testing, is available to clinicians, supports enzyme activity test results, and is essential for carrier testing and subsequent genetic counseling. Overall the expert group recommended caution in the use of urine GAG studies alone to rule out or confirm the diagnosis of MPS VI and felt that arylsulfatase B enzyme testing is superior. Molecular analysis, or at least measurement of another sulphatase enzyme to exclude Multiple Sulphatase Deficiency, was also recommended prior to the initiation of therapy. A diagnostic algorithm for MPS VI including laboratory testing was developed.

1330T

The spectrum of mitochondrial DNA mutations in Iranian LHON patients. *M. Houshmand[1, 2], Z. Rezvani[1], E. Didari[1], A. Arasteh[2], V. Ghodsinejad[2].* 1) Medical Genetic, National Institute of Genetic Engineering & Biotec, Tehran, Iran; 2) Genetic diagnostic Dep., Special Medical Center, Tehran, Iran.

Leber's hereditary optic neuropathy (LHON) is a maternally inherited form of central visual loss that occurs subacutely in young adult men. Experimental support for mitochondrial inheritance was obtained when LHON was first associated conclusively with an inherited mutation in the mitochondrial genome (mtDNA). Beyond this broad agreement about the G11778A, G3460A, and T14484C mutations, it is not yet clear how many other mtDNA mutations may have an etiological or pathogenic role in LHON. These three LHON mutations account for >95% of multigenerational LHON pedigrees of northern European descent. The aim of this study was to define the prevalence of a panel of mtDNA mutations associated with LHON in Iranian patients. PCR-RFLP and sequencing methods were used to investigate these mutations and also new mutation in mitochondrial complex I subunit (ND1-6) of 42 patients. 22 patients (52%) showed one of known mutation which 12 of the patients showed G11778A (55%) (8% female and 92% male), 5 showed G3460A (22.5%) (20% female and 80% male), 5 showed T14484C A (22.5%) (40% female and 60% male). In the rest of patients (20), 5 showed 4216 (25%) (20% female and 80% male), 5 showed 12308 (25%) (All male), 2 showed 10399 (10%) (All female), 2 showed 4942 (10%) (All male), and following mutations was found in the rest of the patients (14318, 14766, 3893, 10042, 10557, 3497, 4654, 13802, 14766, 12012H, 4454, 3269, 14200, 9949, 14021, 14199, 14353). We conclude that: a) Known mutations of LHON just diagnose for 52% of patients, b) even the LHON predominant by male 3:1 but more female were detected in patients with 3460 or 14484 mutations than 11778, c) combined mutations or secondary mutations may play role for pathogenicity of the LHON.

1331T

Widely targeted metabolomics for diagnosing inborn errors of metabolism. *T. Adam[1,2], H. Krätschmerova[1], K. Hron[1], P. Wojtowicz[1], A. Baresova[1], E. Hlidkova[1,2], P. Hornik[3,4], D. Behulova[5], D. Prochazkova[6,7], H. Vinohradska[7], K. Peskova[3,4], K. Adamova[2], S. Stastna[3,4], D. Friedecky[1,2].* 1) Palacky University, Olomouc, Czech Republic; 2) University Hospital, Olomouc, Czech Republic; 3) Charles University, Prague, Czech Republic; 4) University Hospital, Prague, Czech Republic; 5) University Children's Hospital, Bratislava, Slovakia; 6) Masaryk University, Brno, Czech Republic; 7) University Hospital, Brno, Czech Republic.

Objective: Metabolomics becomes an important tool in clinical research and diagnosing human diseases. In this work we focused on diagnosing inborn errors of metabolism (IEMs) in plasma samples using a targeted metabolomic approach. **Methods:** Plasma samples were analysed using the AbsoluteIDQ p 150 Kit (BIOCRATES Life Sciences AG, Austria). The standard flow injection method of the kit comprising two subsequent 20 ul injections (one for positive and one for negative detection mode) was applied for all measurements. All experiments were performed on an QTRAP 5500 tandem mass spectrometer (AB SCIEX, USA) with electrospray ionization. Multiple reaction monitoring detection for 163 metabolites and 27 internal standards was used for quantification. **Results:** We analyzed 50 control samples and 34 samples with amino acids defects (phenylketonuria, maple syrup urine disease, tyrosinemia I, argininemia, homocystinuria, carbamoyl phosphate synthetase deficiency, ornithine transcarbamylase deficiency, non-ketotic hyperglycinemia) and with acylcarnitines defects (methylmalonic acidemia, propionic acidemia, glutaric aciduria I, 3-hydroxy-3-methylglutaric aciduria, isovaleric acidemia, medium-chain acyl-coenzyme A dehydrogenase deficiency and carnitine palmitoyltransferase II deficiency). Control samples were distinguished from patient samples by principle component analysis and hierarchical clustering. **Conclusion:** This study shows that targeted metabolomics can be applied for diagnosing broad IEMs. *This work was supported by grants MSM6198959205 and Internal grant agency of Palacky University grant No. LF_2010_013. Infrastructural part of this project (Institute of Molecular and Translational Medicine) was supported from the Operational programme Research and Development for Innovations (project CZ.1.05/2.1.00/01.0030).*.

1332T

Metabolite Pattern on *in vivo* 1H-Magnetic Resonance Spectroscopy (MRS) of the brain in children with metabolic diseases. *W. Al-Hertani[1], E. Mason[1], T. Tam[1], B. Schmitt[2], S. Blaser[3], H. Branson[3], A. Schulze[1].* 1) Department of Metabolic Genetics, The Hospital of Sick Children and University of Toronto, Toronto, Canada; 2) German Cancer Research Center, Heidelberg, Germany; 3) Department of Diagnostic Imaging, The Hospital of Sick Children, Toronto, Canada.

Introduction: Identifying MRS metabolite pattern that are diagnostic of metabolic diseases is a powerful tool in inborn errors of metabolism (IEM). Methods: We analyzed brain 1H-MRS data from 1843 children from the basal ganglia (BG) and periventricular white matter (PVW). Our study involved measurements from two voxel localizations (BG and PVW) and two echo times (35 ms and 144 ms), and compared data from the 1.5T and 3T Philips Achieva clinical MRI scanners. Point-resolved spectroscopy (PRESS) acquisitions were recorded. Post-processing of MRS data and analysis was performed LC Model software. Retrospective analysis of MR spectra and quantitative information on all detected metabolites was done in children with 21 different metabolic diseases including MMA, Citrullinemia, Arginase deficiency, SSADH deficiency and GA type I. Results: We identified a number of metabolite pattern including low total choline (tCho) in MMA, Arginase deficiency and SSADH deficiency. High Inositol (Ins) was observed in Citrullinemia. Low total creatine (tCr) was seen in Citrullinemia and Arginase deficiency. Conclusions: Our retrospective analysis revealed a number of MRS metabolite pattern in children with various metabolic diseases. Further data exploration and prospective studies will identify novel metabolite patterns that are diagnostic for IEM and elucidate the neurometabolic mechanisms of disease.

1333T

Diagnostic Program for the Detection of Niemann-Pick C disease in Brazil (NPC BRAZIL NETWORK). *F. Timm[1], H. Bock[1], S.G.R. Santos[1], S.S. Mello[1], A. Brites[1], M.G. Burin[1], M.L. Saraiva-Pereira[1,2], R. Giugliani[1,3].* 1) Med Genet Serv, HCPA, Porto Alegre, RS, Brazil; 2) Dep Biochemistry, UFRGS, Porto Alegre, RS, Brazil; 3) Dep Genetics, UFRGS, Porto Alegre, RS, Brazil.

Niemann-Pick disease type C (NPC) is an autosomal recessive condition caused by defects on cholestherol trafikking, which leads to a progressive and usually severe visceral and/or neurologic syndrome. The signs and symptoms overlap with other conditions, and diagnosis is difficult as usually requires a staining test performed on cultured fibroblasts and/or comprehensive molecular studies of the two NPC genes (NPC1 and NPC2). The possibility of treating affected patients with substrate reduction therapy (miglustat) makes more important the correct and timely identification of affected patients. With this aim we set up a comprehensive diagnostic program for NPC in Brazil, which includes: 1) providing information on diagnostic procedures, including a collection and transportation kit (for skin biopsy and blood collection); 2) assay of plasma chitotriosidase; 3) culture of skin fibroblasts and Filipin staining; 4)DNA isolation and molecular analysis of NPC1 and NPC2 gene, depending on results of the Filipin testing. This protocol was performed in 140 patients with suspected NPC, referred from physicians from all Brazilian regions. Diagnosis of NPC was confirmed in 15 cases, being abnormal but not conclusive in further 12 patients. These 27 cases were referred to molecular analysis of both genes to complete investigation (molecular analyses in progress).

1334T

Proteomic Analysis of Induced Pluripotent Stem Cells (iPSC) as a Mechanism to Study Non-Alcoholic Fatty Liver Disease (NAFLD). *S. Brown-Ford[1], A. DeLaForest[2], M. Cayo[2], M. Pellitteri-Hahn[3], M. Zelembaba[3], B. Halligan[3], S. Duncan[2], M. Olivier[1,3].* 1) Medical College of Wisconsin, Department of Physiology, Milwaukee, WI; 2) Medical College of Wisconsin, Department of Cell Biol., Milwaukee, WI; 3) Medical College of Wisconsin, BBC, Milwaukee, WI.

BACKGROUND AND AIM: iPSC provide a unique resource to study liver cell dysfunction in a human cell-based model. It is our goal to study the proteome of iPSC lines created from obese patients with Non-Alcoholic Fatty Liver Disease (NAFLD) and weight-matched controls. NAFLD is commonly attributed to risk factors associated with metabolic syndrome and obesity, but some evidence has pointed to an enhanced genetic susceptibility for the development of the disease. Fatty liver increases mortality due to cirrhosis, carcinoma and liver failure. **METHODS:** In our initial analyses of iPSC, we characterized the proteome of undifferentiated hiPSC and hESC lines using mass spectrometry. Fractions were analyzed on an ion-trap mass spectrometer (LTQ Orbitrap Velos). Two biological replicates for each cell line were analyzed using the SEQUEST algorithm. SEQUEST results from the injected replicates of each sample were pooled and analyzed using VISUALIZE 125-136 software. **RESULTS:** Few proteins were detected at significantly different levels within (1.9-3.7%) the same or between (2.7-5.3%) different cell lines. Our iPSC2a and iPSK3 lines had significantly lower levels of the transcription factor, Lin28A (68% and 48%) compared with our H1ESC line. Y-box binding protein 2 was detected at 83% higher levels in H1ESC than in our iPSK3 line. Analysis of additional cell lines will confirm these initial findings. **FUTURE STUDIES:** We will differentiate control and NAFLD-patient iPSC lines to hepatocyte-like cells to detect significant proteomic and functional differences between these cells after differentiation to gain insights into the underlying cell dysfunction.

1335T

Expression of anti-lipolytic receptors in human adipose tissues and metabolic associations. *L. Chamas, M. Neville, F. Karpe.* University of Oxford, Oxford, United Kingdom.

Background and aims: Anti-lipolytic signals in adipose tissue could be related to insulin sensitivity through the generation of systemic non-esterified fatty acids. Recently uncovered G-coupled proteins receptors GPR109A, GPR109B and GPR81 respond to ligands produced in intermediary metabolism and convey an anti-lipolytic signal in adipose tissue. The endogenous ligand for GPR109A is beta-OH butyrate whereas lactate is the endogenous ligand for GPR81. Little is known about the regulation of these receptors in human adipose tissue. We hypothesize that depot-specific expression of these receptors could help explain tissue anti-lipolytic effects. Materials and methods: Due to the high degree of sequence similarity between *GPR109A* and *GPR109B*, the existing commercially available Taqman gene expression assays were tested for specificity on in-house developed specific templates for the two genes. Both gene assays were found to be non-specific. Following this, specific expression assays were developed and validated. Expression for all three genes in human adipose tissue was determined using paired abdominal and gluteal biopsy samples from 20 healthy male and 20 healthy female subjects. The mRNA expression assays were run on an ABI7900HT system and normalized to *PPIA* and *PGK1*. To assess the relevance of possible feedback loops, the plasma concentrations of the endogenous ligands were measured in plasma. Results: *GPR109A* showed a 1.34 fold higher expression in gluteal tissue compared to abdominal tissue (p=0.012). *GPR109B* showed a 1.45 fold increase in expression in gluteal tissue compared with abdominal tissue (p=0.006). When looking for effects of obesity, abdominal expression of *GPR81* was 1.17 fold lower in obese individuals (p=0.013, n=20) driven by an 1.26 fold reduction in the male group (p= 0.041, n=10). The gluteal expression of *GPR81* was negatively correlated with lactate levels (p=0.026, r=-0.33, n=40) and more so in obese men (p=0.006, r=-0.79, n=10). Conclusion: *GPR109A* and *GPR109B* showed adipose tissue depot specific expression differences. Higher expression of the anti-lipolytic *GPR109A* was seen in the gluteal tissue, known for its lower lipolysis rate. Obesity did not affect the expression of *GPR109A* and *GPR109B* but lowered the expression of *GPR81*. Plasma lactate was negatively associated to *GPR81* gene expression suggesting a ligand-receptor auto-feedback.

1336T

Does the CPT1A p.P479L variant confer risk for unexpected infant death in Nunavut, NWT and Yukon? *S. Collins[1], G. Sinclair[2], G. Osborne[3], A. Corriveau[4], M. Santos[4], B. Hanley[5], F. Bamforth[6], C. Greenberg[7], H. Vallance[2], L. Arbour[1].* 1) Department of Medical Genetics, University of British Columbia, Vancouver, BC Canada; 2) Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC Canada; 3) Health and Social Services, Government of Nunavut, Iqaluit, NU Canada; 4) Department of Health and Social Services, Government of Northwest Territories, Yellowknife, NT Canada; 5) Health and Social Services, Government of Yukon, Whitehorse, YK Canada; 6) Department of Laboratory Medicine and Pathology, University of Alberta Hospital, Edmonton, AB Canada; 7) Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, MB Canada.

Canada's three Northern territories are home to ~100,000 people, of which nearly 50% are of Aboriginal ancestry. Infant mortality rates in the territories increase from west to east, with Nunavut having the highest at 15/1000 live births, a rate 3 times the national average. Nunavut has the largest Inuit population in Canada with ~95% of the 750 infants born each year to Inuit women. Population studies have determined that the c.1436C>T (p.P479L) variant of carnitine palmitoyltransferase 1A (CPT1A) is highly prevalent in Inuit, Alaska Native and coastal BC First Nations and has been associated with increased risk for unexpected infant death in Alaska and BC. The hepatic enzyme CPT1A is a major regulatory point for transport of long chain fatty acids into the mitochondrion for use as energy source during fasting. CPT1A deficiency confers risk for hypoketotic hypoglycaemia, hepatic encephalopathy, seizures, and sudden death. It remains controversial whether the p.P479L variant causes susceptibility to decompensation, in particular during times of fever and intercurrent illness. Risk associated with the p.P479L variant in northern Canada was assessed by comparing the frequency of p.P479L homozygosity of unexpected infant death cases (SIDS, SUDI, and death due to infection) to the estimated population p.P479L homozygosity rates for each territory (64%, 3% and 0% for Nunavut, NWT and Yukon respectively). Ethics approval was obtained from university REBs and local research institutes, with consultation with territorial Aboriginal groups. Of the total 79 unexpected infant death cases identified in all the three territories, 20 of 59 Nunavut cases, 7 of 16 NWT cases and all 4 Yukon cases were available for genotyping. In Nunavut, p.P479L homozygosity was associated with increased risk for unexpected death in (OR 5.15, 95%CI:1.22-46.1). In NWT, there was no statistically significant association between infant mortality and the variant, likely due to small sample size. In Yukon, none of the cases were p.P479L homozygous. Interpretation of risk associated with the variant should be considered with caution as population stratification within Nunavut cannot be ruled out. Potential interactions between the variant and other associated risks, including sleep position, tobacco smoke exposure and breastfeeding practices, are also possible. Prospective studies in Nunavut are planned to better explore risk associated with the variant for infant mortality and morbidity.

1337T

Maternal hyperhomocysteinemia lead to methionine cycle alteration of adult offspring. *V. D'Almeida[1], V.C. Silva[2], E.J. Haseyama[1], M.T.C. Muniz[3].* 1) Psychobiology, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil; 2) Pediatrics, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil; 3) Universidade de Pernambuco, Recife, Pernambuco, Brazil.

Aim: Abnormal fetal antecedents have been associated with increased offspring disease risk through maternal environment, placental changes and epigenetic programming. As alterations in the methionine-homocysteine cycle are described in some disease and could have a role on fetal programming through changes on S-adenosylmethionine (SAM) levels, the aim of this study was to investigate the effects of methionine supplementation, during gestation and lactation, on methionine-homocysteine cycle of offspring. Methods and Results: One month before pregnancy, 13 Swiss female mice were distributed into 2 groups: control (CT=6) group and methionine supplemented (MS=7) group (1% of methionine in water ad libitum). After 20 days, plasma homocysteine levels from MS group were approximately 50% higher than CT group (CT= 4.45 μmol/L, MS= 8.71 μmol/L; p=0.0001). Three months old male offspring (CT=15, MS=11) were euthanized by decapitation. The blood was collected in EDTA tubes for homocysteine, cysteine and total glutathione measurement and the liver was harvested for total glutathione quantification. Our results demonstrated that the treatment with methionine throughout the pregnancy and lactation had no significant effect on offspring plasma homocysteine levels (p=0.3). However, there was an increase on plasma levels of cysteine (CT=424.74 μmol/L, MS=471.83 μmol/L; p=0.04) and glutathione (CT=195.91 μmol/L, MS=302.34 μmol/L; p=0.0003), and hepatic levels of glutathione (CT=8.45 μmol/L, MS=11.58 μmol/L; p=0.0004). Conclusion: Considering our results, we suggest that the organism develops compensatory mechanisms during high methionine intake through increase of cysteine and glutathione formation. These compensatory mechanisms may give priority to the transsulfuration instead of remethylation pathway and prevent possible alterations in gene methylation profile, despite the dams hyperhomocysteinemia. Sources of research support: FAPESP, CNPq and AFIP.

1338T

Erythropoietic Protoporphyrias: Frequency of Mutations in the Ferrochelatase Gene Causing Autosomal Recessive Erythropoietic Protoporphyria and Mutations in the 5'-Aminolevulinate Synthase 2 Gene Causing X-Linked Protoporphyria. *D. Doheny[1], I. Nazarenko[1], M. Balwani[1], L. Liu[1], H. Naik[1], K. Anderson[2], D.M. Bissell[3], J. Bloomer[4], H. Bonkovsky[5], J. Kushner[6], J. Phillips[6], D. Bishop[1], R.J. Desnick[1], Porphyrias Consortium of the Rare Diseases Clinical Research Network.* 1) Dept Gen & Genomic Scienc, Mount Sinai Sch Med, New York, NY; 2) Department of Preventive Medicine and Community Health, University of Texas, Galveston, TX; 3) Department of Medicine, University of California, San Francisco, CA; 4) Department of Medicine, University of Alabama, Birmingham, AL; 5) Department of Medicine, Carolinas Medical Center and HealthCare System, Charlotte, NC; 6) Department of Internal Medicine, University of Utah, Salt Lake City, UT.

Erythropoietic Protoporphyria (EPP), the most common erythropoietic porphyria, is an autosomal recessive disorder, resulting from mutations in the ferrochelatase (FECH) gene that cause a deficient activity of ferrochelatase, the last enzyme in the heme biosynthetic pathway. Recently, an X-linked form of EPP, termed XLP, was described due to deletions in the terminal exon 11 of the erythroid-specific 5'-aminolevulinate synthase 2 (ALAS2) gene at Xp11.21. (Whatley et al, 2008) Both EPP and XLP are characterized clinically by acute photosensitivity and painful skin lesions and, biochemically, by elevated erythrocyte protoporphyrins (PROTO), with XLP distinguishable by having about equal amounts of free-PROTO and zinc-PROTO, whereas in EPP, zinc-PROTO is a minor constituent. To date, 126 FECH mutations have been reported (Human Gene Mutation Database, 2011), as well as the common low-expression allele, IVS3-48T>C. In XLP, three ALAS2 exon 11 deletions have been reported. To determine the frequency of FECH and ALAS2 mutations causing this phenotype, we performed mutation analysis of 97 unrelated patients with biochemical and/or clinical evidence suggestive of EPP, identifying FECH mutations in 79 patients. Among these, three patients had two mutations, 74 had one mutation and the low-expression allele, and two were homozygous for the low-expression allele. Among the FECH mutations, 23 were novel, including three deletions, two splice-site, nine nonsense, nine missense. Among FECH mutation-positive patients, 33 family members were tested, identifying 7 additional affected individuals. Of the 18 cases with no detectable FECH mutation, 10 had ALAS2 lesions, nine with c.1706_1709delAGTG (Whatley et al, 2008) and one c.1734delG (Doheny, 2009). Further testing of 16 XLP family members identified three affected males and eight heterozygous females with variable but generally milder manifestations than affected males. Current efforts are directed to identify large deletions and other variations by dosage analysis and genomic techniques. The findings to date indicate that XLP occurs in about 10% of EPP patients in the US and that ALAS2 mutation analysis should be undertaken in patients with EPP symptoms and no detectable FECH lesion. Finally, there was significant clinical variability in heterozygous XLP females.

1339T

Professional and family attitudes regarding large scale genetic information generated through next generation sequencing in research. *A. Cambon-Thomsen[1], A. Soulier[1], G. Bertier[2], S. Leonard[1], S. Julia[1], GEUVADIS consortium.* 1) Faculte de Medecine, INSERM U 1027 and University of Toulouse, Toulouse, France; 2) CRG, Center for Genomic Regulation Barcelona, Spain.

While genomic science is advancing tremendously, medical, ethical, legal, and social questions are arising regarding genetic information. Ethical aspects of genetic testing related to the use of high throughput techniques pose the problems of status of large scale genetic information regarding privacy and confidentiality, clinically useful information and the duties attached, health related information where no immediate clinical measure exists. Although most of the traditional ethical/legal frames that have developed over years for genetic research and applications continue to apply, some aspects need specific attention for research: The source of samples for sequencing; the type of consent; the scope and duration of studies; the right to withdraw; the concerns for the family; privacy issues (sensitivity of data; data access); return of results. A main feature of this accelerating technology development is a certain blurring between clinical and research contexts. Questions raised by large scale genetic technologies applications are: • Can the same type of regulation apply to targeted tests and to genome-wide sequencing? • Does sequencing require a different level or kind of consent than other genetic tests or medical assessments? • Should whole-genome sequencing method be performed for children or incompetent adults? • How to communicate results when their interpretation remains uncertain and what kind of results should be communicated? • Should participants be informed of incidental findings that unequivocally predict serious disease that can be prevented or ameliorated by early detection? What if the disease cannot be prevented or ameliorated? • How to regulate sequencing services offered directly to consumers? In order to get insights on such issues group discussions and questionnaires were conducted in the context of EU funded projects (GEUVADIS, CAGEKID, ESGI) in the professional contexts (research groups in genetics) and in family situations in 5 countries. Results show a variety of attitudes, that seem influenced by the national context; generally the importance of clear information, understanding of the aims and transparency was underlined. Results also highlight the necessity of addressing such issues at an early stage by collaborative efforts of geneticists, ethicists, social scientists, patient representatives and decision makers.

1340T

The Globalization of Reprogenetic Services: A Thematic Analysis of the Current State of Knowledge. *V. Couture, C. Bouffard.* Division of Genetics, Department of Pediatrics, Université de Sherbrooke, Sherbrooke, Quebec, Canada.

Reprogenetics which can be defined as a combination of in vitro fertilization and medical genetics techniques occupy an ever increasing place within the medical tourism market and, more specifically, in the cross-border reproductive cares movement. Cross-border reprogenetic services offer people who have the means the possibility to obtain abroad services that are not available in their country due to lack of expertise or legal or economic reasons. Within the limit of the knowledge developed on the subject, our objectives are: 1) to summarize the global situation of reprogenetic tourism and 2) initiate ethical reflection on this aspect of cross-border reprogenetic services. Methods: Collection, review and thematic analysis of the literature on cross-border transactions associated with reprogenetics; OVID (Journals@Ovid Full text), EBSCO (MEDLINE, CINHAL, etc.), SCOPUS (Elsevier) and FRANCIS. Results: If more and more articles are related to cross-border reproductive cares (112), the number of publications concerning reprogenetics remains low (61). Two main themes emerge from the literature: 1) the number of users and their destinations; 2) the legal and ethical aspects of those cross-border movements. The main legal subtopics deal with: a) the diversity of national regulations and b) the relevance of regulation which can be overcome by going abroad. The ethical aspects concern: a) the psychological burden, b) the support and remote follow-up, c) the accessibility issues and d) the social disparity. It appears that the variety of regulation of reprogenetic practices and countries where they are available have a major influence on transboundary movements of experts, biological material and users. This situation also raises the question of the limits that national, transnational and international laws and ethics have in their ability to protect users and children-to-be. It also interrogates how to ensure a fair accessibility to these services. Conclusion: Cross-border reprogenetic cares have been little studied as a distinct phenomenon. However, they raise their own ethical, legal, medical and logistical challenges that require us to develop, outside national law, original mechanisms that will ensure the respect of the integrity of the body and the person as well as the autonomy and dignity of users and children-to-be. This objective is even more important now that cross-border reprogenetic services engage populations marked by great wealth disparities.

1341T

Managing incidental findings from genomic testing: A public perspective. *S. Daack-Hirsch[1], A. Spore[1], J.K. Williams[1], M. Driessnack[1], C. Siomon[2].* 1) College of Nursing, University of Iowa, Iowa City, IA; 2) College of Medicine, University of Iowa, Iowa City, IA.

Background: The potential for genomic incidental findings (GIFs) is increasing with the use of comprehensive genotyping associated with whole gene analysis. Although the groundwork has been laid, the voice of the public needs to be incorporated to inform policy-making and establish best practice in the management of GIFs. Therefore, we explored issues and concerns about the management of GIFs in clinical and research testing with members of the public. Methods: For this exploratory descriptive study we conducted seven focus groups representing a broad cross-section of public groups, including elders, young adults, Latinos, African Americans, clergy, parents of children with hearing impairment, and an addiction recovery support group. In addition, seven parents whose children had chromosomal microarray testing were individually interviewed. Data were analyzed using qualitative content analysis. Results: Participants generally viewed incidental findings as unexpected results. Although a result may be unexpected to a patient/participant, it may not necessarily be unexpected from a health care provider/researcher perspective. As well, a result may be viewed as unexpected to one person, but not to another person. Participants generally wanted GIFs disclosed to them, regardless of the severity of the associated disease or the context for the test (clinical or research); although, they did understand that others may not want to know. Participants gave the following reasons for wanting to know about GIFs: to make life style changes; financial and life planning; seek early intervention; to inform other family members; identify causes of illness; and general management of health information. An overall theme of shared responsibility for the management of GIFs emerged in data analysis. This theme reflects that while there is an obligation for the clinician or researcher to disclose the information in a manner that gives the recipient guidance on how, when, and where to follow up on GIFs, the ultimate responsibility to act on the information resides with the recipient. Conclusion: Broad public input is needed in order to understand and incorporation the public's perspective on management of GIFs, and is essential as disclosure guidelines and policies are developed in both the clinical and research setting. Further research/policy-driven efforts are needed to explore this theme of the public's desire for shared responsibility.

1342T

The Appropriate Regulation of Genetic Discrimination at European Level: What can we learn from International Legislative Efforts? *A. de Paor.* Centre for Disability Law & Policy, National University of Ireland, Galway (NUI, Galway), Newcastle Road, Galway, Ireland.

The completion of the Human Genome Project and advancing technology have opened up a new era in genetic exploration. In light of these genetic advances, questions arise as to whether an appropriate framework exists to protect the interests of individuals and also to encourage further advances in the medical and scientific fields. With these genetic developments come new economic opportunities, together with ethical, social and legal considerations. One of the main concerns arising is the potential use of genetic testing to discriminate, especially in employment and insurance, leading to a violation of a myriad of fundamental human rights. Employers and insurance companies are using the results of genetic tests (as a powerful predictive tool) to discriminate based on perceptions of long term health risks and possible future disabilities. In 2008, the United States passed the Genetic Information Non Discrimination Act (GINA). GINA targets genetic discrimination by employers and health insurers. The legislation is revolutionary, in that it is the first pre-emptive anti-discrimination statute in American history. GINA attempts to eliminate this novel brand of discrimination before it becomes widespread. The law was enacted primarily to help ease concerns about discrimination that might discourage individuals from taking genetic tests that could benefit their health. Unlike the United States, there is currently no concrete legal position in this particular area at European Union level. The United Nations Convention on the Rights of Persons with Disabilities prohibits discrimination in a range of fields including employment (Article 25 (e)). Ireland and the EU have signed this Convention and the EU made a historic decision to ratify once a majority of Member States ratify. Once ratified by the EU there will be a responsibility on the European Commission to come forward with legislative proposals as appropriate. Genetic discrimination has already been singled out as a priority area of reform as evidenced, for example in Article 21.1 of the EU Charter of Fundamental Rights which expressly prohibits discrimination based on genetic features. This makes an EU-wide response inevitable as well as preferable to isolated solutions within the Member States. This paper will discuss the need for appropriate regulation of genetic discrimination in light of technological advances and explore what shape such a regulatory model might take at European level.

1343T

Carte blanche ou carte rouge? Willingness of U.S. adults to give broad and limited consent for genetic research. *R. Dvoskin[1], J. Murphy Bollinger[1], J. Scott[2], D. Kaufman[1].* 1) Genetics and Public Policy Center, Berman Institute of Bioethics, Johns Hopkins University, Washington, DC; 2) National Coalition for Health Professional Education in Genetics, Lutherville, MD.

Introduction: Biobanks and large cohort studies increasingly collect samples under broad consent for use in unspecified future studies. Yet there is concern that broad consent approaches will result in more refusals, and study samples that are less representative than those using narrow consent models. To compare the acceptability of two consent models, we surveyed Americans aged 18+ about a proposed national cohort study, randomizing people to view and respond to either broad or narrow hypothetical consent language. **Methods:** We administered an online survey to a random sample of 3,061 U.S. residents, including oversamples of black non-Hispanics (n= 675) and Hispanics (n=708). After being shown a description of the proposed study, participants viewed one of two excerpts from a consent form. Half were told that approved researchers would use their samples and information to study a wide range of diseases; the other half were told they would be asked for permission to use their samples for specific projects. All respondents were asked whether they would agree to share their samples and information with researchers in this manner. **Results:** Slightly more respondents would agree to the broad consent model than to the narrow consent (76% vs. 71%; adjusted p=0.005). Greater willingness to agree to broad consent was observed in nearly every category of gender, age, race and ethnic group, English-language proficiency, and education level. Black non-Hispanics and participants younger than 30 found broad and narrow consent equally acceptable. The strongest independent predictors of willingness to share data and samples, adjusting for the method of consent and other demographics, were trust in the study to protect one's privacy (OR=3.0, p<0.0001), concern that researchers will not follow privacy policies (OR= 0.5, p<0.0001), and having 16+ years of education (OR=1.7, p=0.0001). Men (as compared with women; OR=0.8, p=0.008) and black non-Hispanics (as compared with white non-Hispanics; OR 0.6, p=0.05) were less likely to consent under both models. **Conclusions:** These data suggest that broad consent language is unlikely to reduce initial participation rates compared to a model that allows participants to provide consent for specific studies. Additionally, the similar responses across demographic groups suggest that use of broad consent models is unlikely to influence initial participation rates (and resulting biases) among demographic subgroups.

1344T

Internatonal legislative and factors of influence on Preimplantation Genetic Diagnosis. *L.J. Escobar[1], J. Jimenez[1], E. Quesada[2], L. Hernandez[3], M. Jimenez[4].* 1) Hospital Rafael Mende, Lorca, Murcia, Spain; 2) CS Lorca Sur, Ágrea III de Salud del SMS, Lorca, Spain; 3) Hospital Vírgen del Castillo, Área V de Salud del SMS, Yecla, Spai; 4) Biotools B&M Labs S.A.-España, Spain.

PURPOSE: To analyze the current state of legislation on Preimplantation Genetic Diagnosis (PGD) in Canada, USA and other countries of Europe and Asia as well as the influence of different factors involved in each laws and proposal of their improvement. METHOD: Bibliographic descriptive study of the legislation of 18 countries on PGD. RESULT: PGD is one of the most spectacular advances in the detection of genes-related diseases and opens other possibilities such as the elimination of genetically transmitted diseases or the risk of suffering them, and even the genetic selection of individuals. This has led to a variety of ideological trends and to a heterogeneous international legislative framework on DGP, which has opened an interesting debate in the field of science, bioethics and law. Thus, there are countries where there is a mixed framework of state regulations and recommendations of professional societies (Canada, Australia), while in others there is no specific intervention of the legislature even if the DGP is permitted (US, Japan, UK, Netherlands). In Israel and Islamic countries, specific legislation doesn't exist, but there is a significant permissiveness, influenced by religious factors. In India DGP is forbidden with some exceptions. In Europe there is no common legislation, and the next thing closest to it is the consensus reached by the European Convention on Human Rights and Biomedicine of 1997, in which some countries implement a permissive policy (Spain, France, Denmark, Sweden, Norway), and others prohibit it (Germany, Italy, Switzerland, Austria), which is causing a movement of people within the European Union towards member states with permissive laws. As a rule of thumb, in countries where PGD is accepted, such method is allowed in case of serious genetic disease for therapeutic medical purposes; Sex selection is banned except for sex related diseases. As for the donation of human tissues to others for HAL histocompatibility, there is even less consensus. CONCLUSIONS: There is no common international law on DGP and every national legislative framework is influenced by social and religious criteria. We propose to make way for a multidisciplinary debate in order to reach an agreement on a minimum ethic basis for the development of this technique and of genomics in general. The task will involve finding the ethical limit of satisfaction between society and the protection of genetic identity and the extent of legislative action.

1345T

Public attitudes and interest in newborn genetic testing. *H. Etchegary[1], E. Dicks[2], J. Green[3], K. Hodgkinson[1], D. Pullman[4], P. Parfrey[1].* 1) Clinical Epidemiology, Memorial University, St. John's, Newfoundland, Canada; 2) Clinical Epidemiology, Eastern Health, St. John's, Newfoundland, Canada; 3) Genetics, Memorial University, St. John's, Newfoundland, Canada; 4) Community Health, Memorial University, St. John's, Newfoundland, Canada.

BACKGROUND: As newborn screening (NBS) panels continue to expand, they remain a focus of academic and policy debate. Whether or not testing in the newborn period is supported by parents or the public at large remains largely unknown. To help address this gap, we measured attitudes toward newborn genetic testing in our jurisdiction. OBJECTIVES: To measure interest in newborn genetic testing for several autosomal recessive disorders and reasons for interest. METHODS: A cross-sectional, pen and paper survey was administered to the general public and prospective parents in Eastern Canada between April - December, 2010. FINDINGS: A total of 648 individuals completed surveys. Interest in newborn testing for inherited hearing loss, vision loss and neurological disorders was high (over 80% would have their newborn tested). The attitudes of prospective parents and students were positive, but somewhat less so than members of the general public. Across all disorders, interest in testing was driven by the desire to be prepared for the birth of a child with a genetic disorder. Significantly more people would use the information from testing for fatal neurological disorders in future reproductive decisions than the information generated by newborn testing for inherited hearing or vision loss. CONCLUSIONS: Interest is high in newborn testing for a variety of conditions, including those for which no effective treatment exists. Findings lend support to the expansion of newborn screening panels to include those disorders currently lacking treatment and highlight the value of including the views of diverse stakeholders in screening policies.

1346T

Uses of ancestry in structured association mapping: a critical analysis of recent literature. *S.M. Fullerton[1], J. Yu[2], K.A. Edwards[1], J.S. Taylor[3], K.L. Edwards[4].* 1) Bioethics & Humanities, University of Washington, Seattle, WA; 2) Pediatrics, University of Washington, Seattle, WA; 3) Anthropology, University of Washington, Seattle, WA; 4) Epidemiology, University of Washington, Seattle, WA.

In research, genetic ancestry estimation and related methods to control for confounding due to population stratification are widespread and increasingly regarded as best practice. Yet, as noted in the work of the ASHG Ancestry and Ancestry Testing Taskforce (Royal et al. 2010), ancestry testing also brings with it a host of relevant ethical and social concerns, concerns which have been comparatively neglected in the focus on direct-to-consumer ancestry tests. To better understand and assess the impact of using ancestry estimation to address population stratification, we conducted a content analysis of 58 articles that use Ancestry Informative Markers (AIMs) in structured association mapping. Structured association mapping is a preferred method for addressing confounding in candidate variant studies and is expected to become more common in post-GWAS efforts to map the genes and variants contributing to susceptibility to common complex diseases. Articles were identified by a PubMed keyword search of primary research articles published between 1985 and 2009, and subsequent evaluation to retain only those articles that had employed AIMs for structured association. Articles were then reviewed to identify (1) the relation of study aims to structured association methodology, (2) the nature of AIMs use relative to published recommendations, and (3) the impact of ancestry-based correction on association findings. Overall, most of the studies in this sample provided little-to-no information on their approach to AIMs selection, ancestry estimation, or structured association. Justifications for conducting structured association analysis across the articles were also inconsistent. Whereas several studies conducted a test for stratification or described the population structure of their sample, an equal number of studies provided no explanation for why structured analysis was used. Most significant - given concerns about the ethical and social consequences of ancestry estimation - the majority of articles that corrected for population stratification by adjusting for ancestry proportions found that adjustment had little effect on association results. Taken together, these observations suggest that routine correction for population stratification, especially in the context of candidate gene investigations, may be unnecessary in many cases, and should be weighed carefully in light of attendant ethical and social concerns.

1347T

The Presentation of Risk Information by Personal Genotyping Services. *S.T.K. Garcia[1], S.S. Lee[2], M.K. Cho[2,3].* 1) Department of Genetics, Stanford University, Stanford, CA; 2) Stanford Center for Biomedical Ethics, Stanford University, Stanford, CA; 3) Department of Pediatrics, Division of Medical Genetics, Stanford University, Stanford, CA.

The accurate and effective communication of health risks is an important health policy issue as incorrect perceptions of risk may lead to healthcare under or overutilization. Personal genotyping services reporting the association of SNPs with various diseases and traits are of particular concern as they provide risk assessments of unproven clinical utility and positive predictive value. There is little research to date on users' understanding of their genetic risk information and the limitations of SNP genotyping for risk assessment. This pilot study sought to address this gap by assessing personal genotyping service adherence to the best practices for risk communication in the literature and recommendations by professional organizations to disclose the test purpose and current testing limitations. We collected data from demo accounts of three popular services: 23andMe, Navigenics, and Pathway Genomics, and a non-profit program, the Coriell Personalized Medicine Collaborative. A content analysis was performed on the summary result and disease-specific pages for five conditions reported on by all services (hemochromatosis, lupus, macular degeneration, prostate cancer, and type 2 diabetes). We abstracted data on elements relevant to the communication of risk determined by our review of the risk communication literature and relevant professional organization policy statements (NSGC, ASHG, and ACMG). Primary elements included numerical formatting, use of visual aids, strength of evidence, and disclosure of risk uncertainty. Services followed most of the literature recommendations for the communication of numerical risks including providing risks in a variety of formats and using figures to convey risk information. We found significant gaps in the disclosure of the limitations of SNP-based risk assessment as recommended by professional organizations. Limitations including the uncertainty of algorithms for risk calculation and the reported odds ratios are not universally disclosed and their effect on the precision of the risk assessment is not discussed. Additionally, results formatting does not change significantly based on the risk assessment; in-depth disease information including suggested prevention is disclosed regardless of risk status. The failure to disclose uncertainty in point estimates and the lack of risk-tailored results content may lead users to overestimate the precision and personal relevance of the information provided.

1348T

The ethical and scientific challenges of representativeness in nutrigenetics clinical research. *B. Godard, T. Hurlimann, R. Stenne, Omics-Ethics Research Group.* Department of Social and Preventive Medicine, Bioethics Programmes, University of Montreal, Montreal, Quebec, Canada.

Background*:* Nutrigenetics information is expected to be relevant for treatment, prevention of chronic diseases, as well as health promotion. As such, nutrigenetics may not only benefit patients and *at-specific risk* individuals, but all *healthy* individuals. In terms of global health, both industrialized and emerging countries are facing a growing epidemic of the same chronic diseases on which nutrigenetics research tends to focus. In this context, a selection of participants in nutrigenetics studies that is representative of all populations is crucial. Yet, the selection of participants may be a challenging task that raises both methodological and ethical issues. First, researchers may be torn between the desire to exclude individuals who could bias the study results or decrease its statistical power, on one hand, and, on the other hand, the fear of compromising the validity and usefulness of their findings for a broader and general population (external validity). Second, the definition of what constitutes appropriate representation in clinical research is further complicated by major ethical requirements, in particular by issues of justice and equity. Indeed, if results in nutrigenetics research are valid for some individuals, groups, or communities, and not for others, then questions of justice may arise. This paper aims to examine inclusion of ethnic minorities and populations of all ages worldwide in nutrigenetics clinical research and its scientific and ethical challenges. ***Methods:*** 173 publications were identified through a systematic review of clinical studies in nutrigenetics published between 1998 and 2007. Data such as participants' demographics as well as eligibility criteria were extracted. ***Results:*** There is no consistency in the way participants' origins (ancestry, ethnicity or race) and ages are described in publications. A vast majority of the studies identified was conducted in North America and Europe and focused on “white” participants. Our results show that pregnant women (and fetuses), minors and the elderly (/ 75 years old) remain underrepresented. ***Conclusion:*** Representativeness in nutrigenetics research is a challenging ethical and scientific issue. Yet, if nutrigenetics is to benefit whole populations and be used in public and global health agendas, fair representation, as well as clear and coherent descriptions of participants in publications are needed.

1349T

The Kaiser Permanente Research Program on Genes, Environment, and Health: Re-consent for Sharing Data through dbGaP and Predictors of Response. *J.N. Harris[1], S. Rowell[1], A. Altschuler[1], M. Sadler[1], M. Henderson[1], P. Liljestrand[1], D. Olberg[1], N. Risch[2,1], C. Schaefer[1], C. Somkin[1].* 1) Kaiser Permanente Division of Research 2000 Broadway Oakland, CA 94612; 2) Institute for Human Genetics University of California at San Francisco 500 Parnassus Road San Francisco, CA.

The Kaiser Permanente Research Program on Genes, Environment and Health (RPGEH) received a grant to create a genetic epidemiology resource on adult health and aging, the goal of which is to create genome-wide genotypes and telomere length measurements on 100,000 subjects and to link these data to extensive phenotypic information for research. As part of the data sharing plan, data will be deposited in NIH's Database of Genotypes and Phenotypes (dbGaP). The purpose of this study was to: 1)describe the approach and response to a re-consent campaign aimed at obtaining consent from participants to deposit data into dbGaP; 2)identify sociodemographic and clinical predictors of non-response to the campaign and describe potential barriers to participation. The RPGEH maintains biospecimens and clinical, behavioral, and environmental data on more than 180,000 KP members. When the RPGEH initially consented participants, broad permission was obtained to share de-identified data with qualified researchers outside KP. However, the consent form was developed prior to dbGaP and did not include explicit consent for depositing data into this resource. Based on feedback from multiple stakeholders we decided to use an opt-in approach to obtain consent for data sharing in dbGaP, thus requiring participants to return a new, signed consent form. We analyzed responses from the first 50,000 randomly selected individuals approached for reconsent. We received reconsent from 36,600 participants (73%). Response rates differed by race/ethnicity with higher rates in European-Americans (74%) and lower rates in Asian-Americans (66%), African-Americans (67%) and Latinos (68%). Younger individuals were harder to reach with 55% of people in their 20s vs. 77% of those in their 60s consenting. We received few calls from people concerned with data sharing or dbGaP. Overall, RPGEH's opt-in approach has been successful and can serve as a model for other biorepositories that may need additional consent for data sharing. Racial/ethnic minorities and younger individuals as well as different socioeconomic and clinical subgroups may require additional outreach, and community engagement efforts to improve enrollment. While less than 1000 individuals called in to decline consent, we are unclear why they (as well as the non-responders) declined participation. Additional research is needed to elucidate barriers to reconsent and determine the extent to which sharing data with dbGaP was a deterrent.

1350T

Does a duty of disclosure foster special treatment of genetic research participants? *R.Z. Hayeems[1], F.A. Miller[1], J.P. Bytautas[1], L. Li[2].* 1) Dept. Health Policy, Management, Evaluation, University of Toronto, Toronto, ON, Canada; 2) Dept Biostatistics, University of Toronto, Toronto, ON, Canada.

Introduction: Research ethics guidance stipulates that study participants should not receive enhanced medical care compared to non-participants. Fostering a duty to disclose genetic research results warrants consideration in the context of this guidance. **Methods:** We report on interview and survey data from international autism genetics researchers (clinical and non) regarding research result disclosure and care provision to study participants. Qualitative interviews were analyzed thematically. Survey Likert scale data were dichotomized into agree (agree or strongly agree) vs. not (neutral, disagree, strongly disagree); descriptive statistics were computed for all independent variables. The influence of specific variables on judgments about providing additional care to study participants were explored using unadjusted odds ratios (ORs) with 95% confidence intervals (CIs) as well as multivariate linear models, using $p<.05$ for significance. **Results:** Qualitatively, respondents (n=23) were aware that research participation, and the provision of research results, enables access to expertise and therapeutic resources that are otherwise difficult to obtain. Researchers' views varied regarding whether this should occur. Of 168 survey respondents (RR 44%), 52% agreed that study participants should gain access to additional knowledge and 46% agreed they should gain access to additional services that might not be as readily available to non-participants. Respondents who agreed they should report clinically significant results were twice as likely to agree they should provide additional knowledge [OR=2.04, 95% CI (1.012,4.125)]; those who agreed they should report results of uncertain significance were 7.5x more likely to agree they should provide additional knowledge [OR= 7.54, 95% CI (2.123,26.769)]. **Conclusion:** Providing research results to study participants can address needs that may otherwise be unmet. Qualitatively, we identified ambivalence about whether research should seek to ensure or avoid providing special treatment. Survey data found no consensus about providing study participants with care that may not be as available to non-participants. Feeling obliged to report research results to study participants, however, seems to heighten the disposition to ensure special treatment. This instinct runs contrary to fundamental research ethics guidance; fostering a duty to disclose genetic research results may further fuel this distortion.

1351T

Duty to warn: a case report. *K. Hodgkinson[1], R. Singleton[2], F. Curtis[3], D. Pullman[4].* 1) Medicine, Memorial Univ, St John's, NL, Canada; 2) Pastoral Care, Eastern Health, St. John's, NL, Canada; 3) Provincial Medical Genetics, Eastern Health, St. John's, NL, Canada; 4) Community Health, Memorial Univ, St John's, NL, Canada.

Arrhythmogenic Right Ventricular Cardiomyopathy genetic subtype 5 (ARVD5) caused by a p.S358L change in gene *TMEM43* is common in Newfoundland. The disease is autosomal dominant and lethal with 50% of affected males deceased by 40 years, and 80% by 50 years in the absence of treatment; compared to 5% and 20% respectively of women. Treatment with the implantable cardioverter defibrillator (ICD) increases survival significantly. A recently ascertained family via a 56-year-old female proband (subsequently determined to have the common *TMEM43* mutation)had lost a brother aged 32 to SCD many years ago. This proband was able to build some of her extended family tree but despite great effort, was unable to find their current whereabouts. The distress to this proband was obvious, as the death of her brother remained traumatic and she wanted no other families to suffer. She had the names and approximate years of birth of some of her at-risk family members so an ethics consult was convened to determine if hospital records could be broached to find these individuals. This is a `duty to warn' scenario, where the responsibility for contact is extended from the proband to the care givers. The ethics consultation discussion included health care privacy consultants, bioethicists, informatics experts, hospital lawyers, senior hospital physicians (including public health) and genetic counsellors. It was determined that the privacy consultants would broach the system, and the genetic counsellor would contact the family. This scenario is ongoing. It has lead us to consider the `circle of care' and to what diameter that extends. From the proband to the immediate family and potentially from the hospital to the extended family? Maintaining this analogy, it might be extended to public education and screening in the communities in which this gene is prevalent. Our cardiomyopathy research team has a significant ethics component, one major feature of which will be the determination of the extent of the potential issue of `duty to warn' and population screening for this lethal, yet treatable disease caused by the *TMEM43* mutation.

1352T

A qualitative analysis of European clinical geneticists' views of direct-to-consumer genetic testing. *H.C. Howard[1,2], P. Borry[2,3,4].* 1) Institute of Bio- and Medical Ethics Basel, University of Basel, Missionstrasse 24, CH-4056, Basel, Switzerland; 2) Centre for Biomedical Ethics and Law, Katholieke Universiteit Leuven, Kapucijnenvoer 35 BOX 7001, 3000 Leuven, Belgium; 3) Department of Clinical Genetics, section Community Genetics, VU University Medical Center, EMGO Institute for Health and Care Research, Van der Boechorststraat 7, 1081 BT Amsterdam, the Netherlands; 4) Department of Medical Humanities, VU University Medical Center, EMGO Institute for Health and Care Research, Van der Boechorststraat 7, 1081 BT Amsterdam, the Netherlands.

The offer of genetic testing services directly to consumers has been the subject of debate among health care professionals, researchers and policy makers for the last couple of years. There is a growing body of research regarding consumer views and attitudes on the subject but little is known about how clinical geneticists view direct-to-consumer (DTC) genetic testing. We, therefore, conducted a survey to collect information regarding the awareness, experiences and attitudes of European clinical geneticists about genetic tests and test interpretations sold directly to consumers. European clinical institutes where genetic counseling is offered to patients were contacted and 300 clinical geneticists from 28 countries were invited to participate in the survey. One hundred and thirty-one of the eligible respondents (44%) answered the survey. We previously reported the results of the quantitative aspects of the survey. Herein we discuss the results of a content analysis of the written comments made by the respondents. We focus on comments made regarding four main themes: 1) Their views and direct experiences with patients who have asked about DTC GT; 2) their attitudes about offering genetic testing without face-to-face medical supervision; 3) their views on the legal banning of certain types of genetic tests presently sold DTC; and 4) their attitudes on offering genetic counseling to patients who enquire about DTC genetic testing. These results will contribute valuable information to the debate regarding DTC genetic testing.

1353T

Ethical issues related to high throughput technologies for translation into clinical genetic testing. *S. Julia[1], A. Soulier[2], E. Rial-Sebbag[2], A. Cambon-Thomsen[2], TECHGENE consortium.* 1) Medical genetics, Inserm U 1027 and University Hospital of Toulouse, Toulouse, France; 2) Inserm u 1027 and University of Toulouse, Faculty of Medicine, Toulouse, France.

The goal of the EU funded TECHGENE project (www.techgene.eu) is to incorporate the novel massive parallel sequencing technology in routine diagnostic laboratories for the improved diagnosis of genetically heterogeneous diseases. As high throughput technologies (HTT) may reduce the cost and increase the throughput of genomic sequencing, these technologies are expected to become an essential clinical tool. Part of the project is addressing ethical aspects of HTT. Literature survey, conceptual analysis and discussion within the consortium generated a list of agreed points to consider, and corresponding positions. Complexity of individual results will increase the counselling demand and require much more elaborate and detailed informed consent procedure. Clinicians and laboratory personnel will require training to use the sequence data effectively and appropriate methods will need to be developed to deal with the incidental discovery of pathogenic mutations and variants of uncertain clinical significance. In addition the effective translation of genomic advances into better health care will inevitably require access to and integration of multiple databases, which is likely to raise logistical and ethical considerations. The pace of clinical integration will not be limited by the rate of technology development, but by the ability to accurately interpret the resulting data. HTT has the potential to transform practice of medical genetics and related fields, but the vast amount of personal genomic data produced will increase the responsibility of geneticists to ensure that the information obtained is used in a medically and socially responsible manner. There is a need for a sustained drive to collect relevant evidences about the scientific and clinical validity and utility of HTT before they become implemented and utilized. These elements include: well-functioning health systems; access to health services; an evidence-based infrastructure that evaluates, regulates, sets guidelines, and compares cost-effectiveness of interventions; and an understanding of how local contexts influence relevance, uptake and acceptance of interventions. To ensure fair and reasonable translation of sequencing technologies, the issue of prioritization needs to be addressed explicitly by collaborative efforts of geneticists, ethicists, health economists, patient representatives and decision makers. A translational infrastructure is urgently needed with adaptable governance systems.

1354T

Public views of study design features for biobanks and large-scale genetic cohorts: results of a nationally representative conjoint analysis. *D. Kaufman[1], J. Bollinger[1], J. Bridges[2], C. Buttorff[2], R. Dvoskin[1], J. Scott[3].* 1) Genetics & Public Policy Center, Berman Institute of Bioethics, Johns Hopkins University, Washington, DC; 2) Department of Health Policy & Management, Johns Hopkins University, Baltimore, MD; 3) National Coalition for Health Professional Education in Genetics, Lutherville, MD.

Background: Biobanks and large genetic studies need to minimize participant burden and maximize value of the data, while remaining affordable. To inform future research, we measured the public's preferences for design features for a proposed national cohort study. **Methods:** In April 2011, we surveyed a nationally representative sample of 1,538 US adults. Study design preferences were assessed using conjoint analysis. Participants viewed nine pairs of study designs that varied with respect to six features: study length (5, 10, or 15 years); the return of individual research results, or IRRs (none, some validated, all); whom data are shared with (US academics; US and foreign academics; US academics and industry); annual compensation (none, $50, $100), health data collection (via surveys, medical records, or exams); and possible study requirements (fitness test, diet journal, home visit). For each pair of study designs, respondents chose the study they would prefer. A random utility model was estimated using conditional logistic regression. Participants were also surveyed about other aspects of the proposed study. **Results:** After viewing an explanation of the proposed national study, 54% would definitely (13%) or probably (41%) participate. Annual bonuses were the most important design factor, followed by the return of IRRs and the length of the study (all $p<.0001$). The most attractive design features were a 5-year study length (odds of participating in a 5-year study, compared to the odds of participating across all possible study designs, OR=1.29), annual bonuses of $100 (OR=1.75) and $50 (OR= 1.10), and return of all IRRs (OR=1.32) or a few valid IRRs (OR=1.13). Respondents would also be more likely to join studies that conduct fitness tests and use physical exams to update history. The most repulsive elements were lack of an annual bonus (OR=0.52), no IRRs returned (OR=0.67), a 15-year duration (OR=0.74), sharing data with foreign academics (OR= 0.91), and a required diet journal (0.91). A separate question found that 60% preferred a 12-year study returning a small number of actionable IRRs to a 10-year study providing no results (24%) or a 20-year study returning all results (16%). **Conclusions:** Study duration and rewards for participation were the most important factors to potential participants. The importance of receiving personal health information was underscored by people's preferences for the fitness test and physical exams.

1355T

Public views of sample collection methods for biobanks. *J. L'Heureux[1], J.C. Murray[1], C.M. Simon[2,3].* 1) Department of Pediatrics, Univ Iowa, Iowa City, IA; 2) Program in Bioethics and Humanities, Univ Iowa, Iowa City, IA; 3) Department of Internal Medicine, Univ Iowa, Iowa City, IA.

Background: Biobanks often utilize one of two broad methods of collecting biological specimens: 1) collection of discarded clinical specimens or 2) collection of new specimens specifically for use in the biobank. Little has been reported regarding people's views of these two methods. This study explored public perceptions of these two sample collection methods in the context of a hospital-wide biobank that links both discarded and newly collected biological specimens to electronic medical records for research purposes. Methods: Focus groups and telephone surveys were conducted within the University of Iowa Hospitals and Clinics patient catchment area, to assess relevant public opinions and preferences for a number of biobank features including the two methods of sample collection. Results: Forty-eight individuals participated in 7 focus groups, followed by a survey of 751 randomly selected individuals from counties across Iowa and neighboring states that fall within the UIHC catchment area. Focus group participants were predominantly female (58%) and Caucasian (88%), with an average age of 52.4 years (range: 18-92 yrs). Survey participants were predominantly female (63%) and Caucasian (97%), with an average age of 58.4 years (range: 18-94 yrs). Advantages of utilizing discarded specimens identified by focus group participants were: 1) the convenience of not having to have an extra blood draw or appointment, 2) using samples instead of wasting them (like recycling), 3) cost and time savings for the biobank, and 4) the possibility of increasing accrual rates. Perceived advantages of having a blood draw specifically for biobank purposes were: 1) the ability to get more desirable samples and 2) increased participant awareness of their samples being used for research. Seventy-three percent of survey participants viewed either method as equally workable options, 17% preferred use of discarded specimens, and 6% preferred collection of specimens specifically for the biobank. Conclusion: While collection of specimens specifically for biobanking may deter some potential biobank participants, many find it no less acceptable than the collection of discarded specimens. Limiting inconvenience to potential participants by combining blood draws with already scheduled appointments may help to make this method of sample collection even more acceptable to potential participants.

1356T

Diverse Maternal Perspectives of the Return of Results in Pediatric Genetic Research: Results from Formative Research for the National Children's Study. *K.D. Lakes[1], E. Vaughan[1], A. Lemke[2], M. Jones[1], D. Baker[1], J. Swanson[1].* 1) Pediatrics, University of California, Irvine, Irvine, CA; 2) Center for Genetics Medicine, Northwestern University, Chicago, IL.

As the field of genomics rapidly advances, there is growing interest in applying these technologies to study children's health. Discoveries of associations between genetic variants and disease have increased significantly, but the utility of much of this information for any specific individual remains undetermined. Despite uncertainties, some have argued that parental discretion should be allowed regarding disclosure of individual results when research involves comprehensive genomic testing. Ethical concerns include which results to report and through what methods. Our goal was to identify how concerns, preferences, information needs, cultural values and reasoning strategies impact decisions to receive individual-level findings in order to identify potential disclosure impacts and to develop strategies that improve the conduct of genomic research with children from diverse backgrounds. We report findings from formative research conducted for the National Children's Study. We conducted 7 focus groups in English or Spanish with 53 socioeconomically and ethnically diverse women (28% Hispanic, 49% White/Non-Hispanic, 21% Asian American), and analyzed transcripts of discussions using qualitative thematic methods. Themes emerged that support the need for further research and reconsideration of assumptions that currently guide practices for return of results in genomic research. The analysis revealed that: decisions about receiving results may not be immutable and preferences may change over time; clinical utility may not be the primary determinant of a parent's desire to receive results; nondisclosure may present emotional “harms” not currently considered in bioethics discussions; cultural values play a role in the impact of and decisions about return of results; disclosure of certain findings could affect the parent-child relationship; diverse research populations may have strong expectations for the ethical process of information sharing that go beyond commonly approved protocols; the probability that certain genetic patterns are predictive of disease may not represent participants' decision models; and the timing of the return of results could affect emotional impacts of disclosure. Our results provide insights about diverse expectations, preferences and reasoning strategies for decisions regarding the return of results in pediatric genomic research and identify important gaps in scientific evidence to guide the ethical conduct of such research.

1357T

Returning Research Results: A Deliberative Engagement in Southside Chicago. *A. Lemke[1], C. Halverson[2], L. Friedman Ross[2].* 1) Northwestern University, Chicago, IL; 2) University of Chicago, Chicago, IL.

To promote broad representation and participation in biobanks, it is important that policies are developed in a way that is respectful of diverse communities. In an effort to widen knowledge and inform institutional policies, we conducted a deliberative engagement of individuals from various socioeconomic communities to consider biobank policies regarding return of research results, informed consent, and data sharing. Each group engaged in 4 sessions over 2 days, with each session preceded by an educational presentation. Pre- and post-session surveys were also administered to assess participant attitudes and beliefs. Forty-five individuals participated: two groups consisted of primary caregivers of children who receive pediatric care at a Federally Qualified Health Center (n=22), and two groups consisted of primary caregivers of children who receive pediatric care at a university clinic (n=23). All participants self-identified as African-American and 76% were women. Participants from the FQHC compared to the University clinic were more likely to have ≤ high school education (~36% vs. 9%, $p < .05$). Major themes from the focus group data included: 1) overall interest in individual research results, broad consent, and data sharing; 2) awareness of historical events and lack of education as root causes of fear and distrust; 3) the role of research recruitment and biobank strategies for establishing trust; 4) ensuring accessible benefits from biobank research to all, regardless of ability to pay; and 5) positive experience with the deliberative engagement process. Survey data revealed that approximately 90% of participants expressed interest in receiving individual research results indicating a risk for asthma, and Alzheimer's disease; and roughly 80% were interested in receiving gene findings more common in a racial group and findings with uncertain significance. The degree of interest was the same for learning results about themselves and their children, and did not differ between clinic types or over the 2 days. Level of perceived likelihood of identification from their genetic research data, and potential harm as a result of being identified, decreased over the sessions. In contrast, views regarding protection of privacy of genetic research information remained the same (85% concerned). Our findings revealed community insights that may be important in facilitating relationships and policy discussions between biobank researchers and research participants.

1358T

Exploring the ethics of incidental findings from whole genome studies: understanding what research participants, genomic researchers and genetics professionals want. *A. Middleton[1], M. Parker[2], H. Firth[1], N. Carter[1].* 1) Wellcome Trust Sanger Institute, Cambridge, Cambridgeshire, United Kingdom; 2) Ethox, University of Oxford, Oxford, United Kingdom.

The Deciphering Developmental Disorders (DDD) project will use the latest whole genome technologies to investigate 12,000 children with undiagnosed developmental delay and their parents from every Regional Genetics Service in the UK. The aim of this is to identify new genetic causes for developmental disorders. However, in doing so, clinically significant `incidental findings' will be uncovered. For example, a BRCA1 mutation may be found in a two year old. Such a finding is unlikely to be related to the developmental disorder and yet could still be clinically significant to the child in later life as well as to other family members. Incidental findings could include variants of known and unknown significance, information about life-threatening and serious conditions and information about carrier status. In the DDD research project, incidental findings will not be revealed to participants until more is known about the ethical implications of reporting such results. However, pressure is mounting from policy makers and ethicists to share clinically significant incidental findings, the thinking being that it is unethical to withhold genetic information that could enable the research participant to take preventative or therapeutic action. Whilst there is often sympathy with this position, some genomic researchers are concerned that the time spent searching for, interpreting and reporting incidental findings unrelated to the research aims might jeopardise attaining those aims. As yet there are no published large-scale studies that have gathered empirical data on any of these issues; we aim to address this omission as part of the DDD project. We are ascertaining the views of research participants, genomic researchers, genetic health professionals and laboratory staff. Our questions focus on attitudes towards sharing incidental findings, how such findings could be categorized, what to do with findings of unknown significance, attitudes towards mining specifically for certain types of incidental findings as well as views on how consenting procedures in whole genome studies should be structured. We have designed a social sciences study using a mixed methods approach, utilising both quantitative and qualitative techniques. Throughout 2011 more than 20,000 people will be invited to participate in an online questionnaire and 50 invited for face-to-face interview. We aim to report the first findings from our study at the ICHG conference.

1359T

Informing the return of individual research results in biobanks and large-scale genetic studies: results from a public survey. *J. Murphy Bollinger[1], J. Bridges[2], G. Gallego[2], R. Dvoskin[1], J. Scott[3], D. Kaufman[1].* 1) Genetics and Public Policy Center, Berman Institute of Bioethics, Johns Hopkins University, Washington, DC; 2) Department of Health Policy & Management, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 3) National Coalition for Health Professional Education in Genetics, Lutherville, MD.

Researchers have reported a strong public desire for the return of individual research results (IRRs) to participants in genetic studies. As guidelines for the return of IRR emerge, questions remain as to which results to return and how to deliver them. To understand public preferences and inform researchers considering the return of IRR, we surveyed a representative sample of 3,061 US residents aged 18+. **Methods:** Experiment 1: 1,523 respondents were randomly selected for a discrete choice experiment, to measure the relative importance of eight attributes of a policy to return IRR from a proposed study. In each of 12 survey tasks, the attributes were divided into two mutually exclusive groups and respondents chose the preferred policy. Experiment 2: We measured the influence of the actionability, magnitude of risk, and disclaimers surrounding a given research result on peoples' desire for particular types of IRR. Participants (n=3,061) were randomized to view one of eight sample results and asked their opinion of the information. **Results:** Experiment 1: All eight attributes significantly influenced IRR policy preferences ($p<0.0001$). The most influential features were IRR for treatable diseases (odds ratio of selecting a policy with this feature compared to one without=1.8) and common diseases (OR=1.6), IRR indicating major change in disease risk (OR=1.6), and the provision of detailed reports (OR=1.6). Of lower priority were IRR returned at no cost (OR=1.3), IRR for serious diseases (OR=1.1), IRR confirmed in other studies (OR=1.1), and the option to discuss IRR with study staff (OR=1.1). Experiment 2: Participants who viewed a result conferring a 2x increase in risk were significantly more likely than those who saw a 1% risk increase to find the result “more useful” (29% vs. 24%) and “more worrisome” (17% vs. 10%) than they had expected. More participants would pay for IRR that show 2x risk than results showing a 1% increase (43% vs. 38%). Results shown without disclaimers were seen as “more useful than expected” more often than results with disclaimers (29% vs. 24%). Actionable results were seen as “more useful than expected” more often than inactionable data (OR=1.3). **Conclusions**: People place high value on actionable IRR and ones showing large changes in risk. Since a limited number of research results are likely to meet these criteria, returning a small number of findings, including those for common diseases, may satisfy much of the desire for IRR.

1360T

Consumer genetics in East Asia: public attitudes and policy analysis. *K. Muto, Y. Inoue, T. Arauchi, H. Hong, C. Chang, M. Sato.* Dept Pub Policy, IMS, Univ Tokyo, Tokyo, Japan.

Background: In 2010, DTC (direct-to-consumer) genetic testing for health purposes have been featured again in the US for several warnings by FDA. In contrast, several Shanghai-based companies started to advertise in Japan to sell DTC genetic tests including talent identification, such as memory, concentration, music and drawing. However, regulation of DTC genetic tests for non-health purposes have rarely been discussed before. Is this just fun or entertainment using human DNA? The JSHG didn't agree and published the statement for consumer genetics in 2010 for further discussions on genetic tests non-health purposes. Purpose and methods: To address broad ethical, legal and social implications arising from such tests, we've conducted a questionnaire survey to the Japanese general public to know their recognition and interests towards DTC tests. We've also conducted policy analysis towards consumer genetics and clinical genetic testing in East Asian countries. Results: Advertisement is commonly observed in East Asia though some of the tests have been banned in South Korea. Regarding our survey, 3,606 respondents completed (Respond rate=60.1%). 72.9% of respondents replied that they didn't know DTC genetic tests and 1.3% of them have purchased before. Multi-factorial diseases (53.4%), congenital disorders (36.6%) and aging (32%) for themselves are most attractive tests. Younger generation shows higher interests DTC tests of several purposes, including personality (26.2%), intelligence (26.1%) and physical strength (23.3%). Discussions: We confirmed that DTC genetic tests for non-health purposes are less popular than those for health purposes. Advocacy for younger generation is necessary.

1361T

Translational pathways for prenatal aneuploidy testing using cell-free fetal DNA. *L. Sayres[1], M. Allyse[1], J. King[2], S. Kelly[3], M. Nunes[4], M. Cho[1].* 1) Center for Biomedical Ethics, Stanford University, Stanford, CA; 2) Hastings College of the Law, San Francisco, CA; 3) ESRC Centre for Genomics and Society (Egenis), University of Exeter, Exeter, UK; 4) Pediatrics and Medical Genetics, San Diego Medical Center, Kaiser Permanente, San Diego, CA.

Fetal aneuploidy detection using cell-free fetal DNA (cffDNA) in maternal blood may routinize prenatal testing due to its non-invasiveness, earlier detection time, and low cost. Given recent publication of three independent studies that robustly demonstrate the accuracy of sequencing techniques to detect aneuploidy and the emergence of several companies developing these, it is likely that aneuploidy testing using cffDNA will soon be commercialized. However, translation of this technology into clinical application is not necessarily inevitable and may take divergent pathways in different settings. For instance, similar testing using cffDNA for RhD blood typing, although commercially available, has not realized widespread use in the United States whereas it is farther advanced in many European countries. CffDNA testing for aneuploidy must demonstrate analytic and clinical validity, utility, and cost-effectiveness to achieve effective translation. This technology must also surmount issues of patenting, regulation, the potential for direct-to-consumer testing, competition with existing testing regimes, third-party payment, informed consent, and moral issues around diagnosis or prediction of an expanding list of fetal traits. The priorities and perceptions of stakeholders must be integrated into the development process to ensure successful, ethical translation. Stakeholders in aneuploidy testing using cffDNA include patients and their families, practitioners, researchers, testing companies, insurers, government agencies, and members of the disability rights, pro-life, and pro-choice communities. We present an innovative framework that uses socio-technical mapping to evaluate the potential pathways of cffDNA aneuploidy testing implementation and the values underlying each. This framework seeks to align the values of researchers and technology developers with those of other stakeholders. Due to its accelerating development and commercialization, aneuploidy testing using cffDNA provides a valuable case study for the translation of novel genetic technologies into clinical practice. We anticipate that this framework can also be applied to other emerging genetic technologies and that stakeholder opinions surrounding cffDNA testing will have wider implications for the translation of these technologies from bench to bedside.

1362T

Parental reflections on choice and decisions to accept newborn blood-spot screening. *S.G. Nicholls.* PPR, Lancaster University, Lancaster, Lancashire, United Kingdom.

Introduction: Newborn screening programmes, whilst offered to individuals, resemble traditional public health programmes because (a) they are targeted at large groups of the population and (b) they are offered as preventive interventions to a population considered healthy. For many programmes this potentially presents an ethical tension between the goal of promoting high uptake of supposedly `effective' population oriented programs and the goal of promoting genuinely informed decision-making. Methods: I explore this potential tension through an exploratory sequential mixed methods approach using an initial phase of qualitative interviews with parents (n= 18) and a subsequent postal questionnaire (n=154). Qualitative data was coded using a thematic analysis approach with survey responses analysed using descriptive statistics and structural equation modelling (SEM). Results: Interviews indicated that there may be discord between the stated aims of supporting informed choice and the practice of providing a population screening programme. In particular parents referred to aspects of procedurlisation and information presentation as diminishing the perceived availability of choice. Parents also noted aspects of timing that impacted on their ability to make considered informed decisions. Survey results suggested that ability to make a choice was of less concern than the availability of choice with 80% of parents agreeing to some degree that it had been expected that their child have the heel prick and over 30% indicating that they did not feel they had a choice to decline the screening. Perhaps most disconcerting was that over 10% of parents felt that they had not made an informed choice. SEM revealed the importance of this finding with perceived choice having a significant impact on the perceived quality of decision made and a standardised coefficient almost twice as large as the parents' specific attitudes towards screening itself. Conclusions: These results suggest that parents view choice as an important factor in their decision-making. Despite an explicit mandate to gain consent and facilitate informed choice this may not be the case for some parents. The factors identified within the interviews may be appropriate avenues for development, with the provision of information prenatally being spontaneously offered by parents as a way to facilitate decision-making.

1363T

How does the genetic test report format change physician decision making for at-risk relatives of cancer patients? *S. Plon[1,2,3], B. Parks[1], H.P. Cooper[4], T. Wang[2], S.U. Dhar[2,3], S. Staggs[5], A.D. Weinberg[4], S.G. Hilsenbeck[2,4].* 1) Dept Pediatrics, Texas Children's Cancer Center, Baylor College of Medicine, Houston, TX; 2) Dan L Duncan Cancer Center, Baylor College of Medicine, Houston, TX; 3) Dept Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 4) Dept Medicine, Baylor College of Medicine, Houston, TX; 5) Texas Medical Association, Austin, TX.

Background: DNA sequence-based testing is used for prediction of cancer risk with results described as negative, deleterious mutation or variant of uncertain significance (VUS). We previously demonstrated that non-geneticist physicians chose comprehensive sequencing for healthy relatives in situations where the cancer patient carried a deleterious mutation or VUS. We now determined whether changing the genetic test report format would alter physician decision making. Methods: Practicing physicians in Texas from five specialties completed an online case-based survey which asked for genetic testing and management recommendations for the healthy relatives of cancer patients. The new survey only differed in the test report format which for each variant contained: classification, likelihood of pathogenecity and specific follow-up recommendations. Results: Surveys were obtained from an independent sample of 226 physicians. When the cancer patient carried a deleterious mutation (Class 5 variant), there was little change with a minority of physicians recommending “single site testing” of the relative (26% in new survey versus 20% in prior one, p=0.0525). When the cancer patient carried a VUS (Class 3 variant), physicians were more likely to choose “testing not appropriate” with 33% for the new versus 18% on the prior survey (p=0.007). Per physician, estimated median total testing costs for all cases fell from $10,020 to $8430 (p=0.0003) in the new survey, however, still far exceeding total costs of $950 if national guidelines were followed. Experience with BRCA1/2 testing did not impact testing decisions (p=0.0906). In the current survey there was less intense cancer risk management for at-risk relatives found to carry the VUS but only breast MRI and mastectomy recommendations met statistical significance, e.g. 5% versus 11% previously recommended mastectomy (p=0.0248). Conclusions: Using a defined classification scheme for reporting cancer susceptibility test results has only modest impact on physician decision making. For VUS carriers, physicians were less likely to pursue testing of at-risk relatives and less intense cancer risk management was recommended. Physicians overall continued to choose more comprehensive sequencing compared with national guidelines for at-risk relatives. Improving the care of family members and decreasing costs will require utilization of genetic professionals and better education of physicians. Supported by grant 5R01HG004064.

1364T

OMIM.org, A New and Improved Website for Online Mendelian Inheritance in Man. *J.S. Amberger[1], F.J.-M. Schiettecatte[2], C.A. Bocchini[1], A.F. Scott[1], A. Hamosh[1].* 1) Inst Gen Med, Johns Hopkins Hosp, Baltimore, MD; 2) FS Consulting, LLC, Salem, MA.

The advent of whole genome and exome sequencing has allowed information on gene-phenotype relationships to flourish. Online Mendelian Inheritance in Man (OMIM) brings a long, rich history and unique expertise to the challenge of curating these relationships and classifying newly recognized disorders. A new web interface, omim.org, hosted at UCSC Genome Bioinformatics now provides the research community with optimal views of the OMIM data. Gene-phenotype connections are displayed prominently at the top of both the gene and phenotype entries. Phenotypic series have been created to show the relationship of a particular phenotype across the genome. To enhance the clinical utility of OMIM, ICD and SNOMED codes are available for medical records coding, and phenotype entries have links to GeneTests, Clinical Trials, POSSUM, and Orphanet. Links to many other genetics resources are topically organized within each entry. These resources include genome browsers, protein databases (UniProt and HPRD), gene info (e.g., BioGPS, Gene Cards, PharmGKB), variation databases, model organism databases (e.g., MGI, KOMP, IKMC, ZFIN) and cellular pathway databases. OMIM's Synopsis and Morbid Gene Map is now searchable by genomic coordinates and the clinical synopsis information is available in the full view or new quick view format. OMIM terms have been mapped to SNOMED Clinical Terms, the Unified Medical Language System (UMLS), the Phenotype Ontologies, and the Disease Ontology. These terms are also available via the National Center for Biomedical Ontology (NCBO) BioPortal. Additionally, a thesaurus is an optional supplement to the standard search to allow the retrieval of similar concepts (e.g., cryptorchidism and undescended testes). Researchers interested in data mining can access the OMIM data via an API. OMIM, a data mainstay of the genetics community, continues to provide timely and authoritative accounting of the correlation between mendelian disease and the genes that cause them, and looks forward to working with the research and clinical communities to maximize the usefulness of the database.

1365T

The Human Variome Project - Collection of Variation Worldwide. *R.G.H. Cotton, collaborators of the Human Variome Project.* Human Variome Project, Melbourne, Australia.

The Human Variome Project (*Nat Genet*39(4): 423,2007)(www.humanvariomeproject.org) was initiated in June 2006 (Ring et al.*Pharmacogenomics*7(7): 969-72,2006) drawing attention to the importance of collection of variation and its phenotype and to develop programs to put this into effect. The project builds on work and concepts of the HGVS and others over many years (www.hgvs.org) to focus on all variation associated with disease. The project will include those discovering mutations and their effects and then collecting the data making it instantly available for those who need it to inform clinical decisions,therapy,and research. A high level meeting in Spain (Kaput et al.*Hum Mutat* 30(4): 496-510,2009) developed plans to implement the recommendations of the HVP Melbourne meeting (Cotton et al.*Nat Genet*39(4): 433-6, 2007). The HVP was featured recently in relation to Neurogenetic databases in Science (Cotton et al.*Science* 322(5903): 861-2,2008) and a HVP Neurogenetic Database Initiative is being formed after an HVP Neurogenetics forum in Hawaii October 2009. The Nutrigenomics community is establishing protocols in partnership with HVP. This activity culminated in the third HVP meeting hosted by and under the patronage of UNESCO in Paris(Kohonen-Corish et al.*Hum Mutat*31(12): 1374-81,2010). The HVP and InSiGHT (International Society for Gastrointestinal Hereditary Tumours) (www.insight-group.org) has developed a major pilot study to develop procedures and systems to allow effortless flow of de-identified data for the colon cancer genes from the patient/clinic/diagnostic laboratory via curated locus or gene specific databases to central databases/genome browsers such as NCBI, UCSC and EBI. The system will be easily adaptable to other genes and to multiple laboratories,states and countries worldwide. A country specific collection pilot is underway in Australia and an International Confederation of these countries has been initiated with Malaysia, Korea, China, Australia, Egypt, Belgium, Spain, Greece and Saudi Arabia in the application process. Other pilot studies developed include specific ethical studies related to mutation collection, loading of LSDB content to NCBI databases,funding of curation of LSDBs, a system of Microattribution/reward for mutation submission. Recent commitment by the Chinese Government will allow the rate of the program to be increased (Cyranoski.*Nature* 469(455),2011).

1366T

Human Resource Training in Medical Genetics in Cuba. *P. Lantigua-Cruz, N. Gonzalez-Lucas.* National Center of Medical Genetics, La Habana, La Habana, Cuba.

Developing countries face challenges in incorporating advances in genomics in heath services. In Cuba, these are accessible to the entire population as a social benefit. Information from predictive and genetic testing creates an ethical dilemma when there are insufficient numbers of health personnel with the scientific training to explain the preventive possibilities and risks implied by test results. On the other hand, the risks of occurrence and recurrence of genetic diseases become more clear on the basis of information from clinical diagnosis and the assessment and analysis of genetic susceptibility to environmental risk factors for common diseases, identified by epidemiologic research in communities. The present work describes the design and development of human resources training programs in medical genetics and their effect on prevention of genetic diseases in Cuba, focussing on Community Genetics. Cuba's strength in training human resources for genetics is in a 60-hour medical genetics course in the fourth semester of the undergraduate medicine program and related material in undergraduate nursing and health technology programs. Since 1977, there has been a postgraduate medical program in clinical genetics with several subspecialties. Since 1992, medical residents must also complete a specialty in comprehensive general medicine (MGI). There are also master's programs in medical genetics and genetic counselling, lasting two years and eighteen months, respectively. Since 1977, 149 physicians have graduated as specialists in clinical genetics, 78.5 % of them between 1992 and 2010. A further 835 professionals have earned master's degrees in clinical counselling (61.4 % MGI and 36.3 % baccalaureate nurses). Master's degrees in medical genetics have been taken by 48 professionals (25 physicians with various specialties, including neurology, pediatrics and neonatology) and 23 non-medical professionals involved in genetic research. The cornerstone of the mission for equitable distribution of the benefits of genomic research in medicine is human resource development that ensures, based on academic training, a scientific level capable of dealing with the sensitive information communicated between professionals and clients of genetic services.

1367T

Impact of Web-based Case Conferencing on Cancer Genetics Training for Community-based Clinicians. *K. Blazer[1], C. Christie[2], G. Uman[3], J. Weitzel[1].* 1) Division of Clinical Cancer Genetics, City Hope Comprehensive Cancer Center, Duarte, CA; 2) Graduate School of Education and Information Studies, University of California Los Angeles, Los Angeles, CA; 3) Vital Research, 6380 Wilshire Blvd, Los Angeles, CA.

Introduction: Technology and market forces are driving the demand for cancer risk assessment and preventive care in the community setting, where clinicians are often inadequately prepared to order and interpret predictive genetic tests. City of Hope Cancer Center conducts a three-phase training course in genetic cancer risk assessment (GCRA) for community-based clinicians across the Unites States and internationally, comprised of distance didactics, face-to-face workshops and 12 months of post-course professional development. As designed, the course cannot meet growing demands for GCRA training. Previous action research with key course stakeholders identified face-to-face case-based workshops as a barrier to increasing course access and capacity. This study compared the effectiveness of participation in an established Web-based case conference forum as a more accessible alternative to face-to-face case-based training. Methods: A quasi-experimental design compared pre-post knowledge, case-based skills and professional self-efficacy outcomes from 2009-2010 course cohorts (n=96). The intervention group (n = 52) engaged in Web-based case conferences concurrently with distance didactics; the comparison group (n = 44) participated in the course as originally designed. Results: Both groups and all practice disciplines demonstrated significant pre-to-post increases on all measures. Knowledge increases were higher for the intervention group ($p < .015$); skills and self-efficacy increases were comparable between groups ($p < .33$ and $p < .30$, respectively). Discussion: Findings support the efficacy and utility of Web-based case conferencing to support case-based GCRA training for community-based practitioners. Further studies may inform the development of tools to assess the impact of Web-based case conferencing on long-term practice change and patient outcomes, in alignment with the highest standards of continuing professional development.

1368T

Development and dissemination of a knowledge support service in genetics for primary care providers. *J.C. Carroll[1], R. Grad[2], P. Pluye[2], N. Pimlott[3], J. Allanson[4], J. Permaul[1], B. Wilson[5].* 1) Department of Family Medicine, Mount Sinai Hospital, Univ of Toronto, 60 Murray St, Box 25, Toronto, Ontario, Canada, M5T3L9; 2) McGill University, Department of Family Medicine, 517 Pine Avenue West, Montreal, Qc, Canada, H2W 1S4; 3) Department of Family and Community Medicine, Women's College Hospital University of Toronto, 60 Grosvenor Street Toronto, ON M5S 1B6; 4) Department of Genetics Children's Hospital of Eastern Ontario 401 Smyth Road Ottawa, ON, K1H 8L1; 5) University of Ottawa, Faculty of Medicine, Department of Epidemiology & Community Medicine, 451 Smyth Road (3230E), Ottawa ON K1H 8M5.

Background: Patients look to their family physicians (FPs) for credible information and guidance in making informed choices about genetic testing. FPs express a willingness to play a role in delivering genetics services but are challenged by lack of knowledge and by the rapid pace of genetic discovery. In a recent trial (GenetiKit), we demonstrated the effectiveness of a multi-component knowledge translation strategy on FPs' genetics referral decisions and confidence in core genetics competencies. We now report the findings of a follow-up project, designed to disseminate and evaluate one of the components of GenetiKit, a knowledge support service. These 2-page `Gene Messengers' review genetic tests or disorders recently featured in the media. We have combined them with the Information Assessment Method (IAM), a valid and useable evaluation tool for linking the delivery of information on email with continuing professional development of physicians. Using a theory and four constructs derived from information sciences, IAM assesses the value of clinical information: relevance, cognitive impact, use and expected health benefits associated with that information use. Aims: The project goal was to determine the value of Gene Messengers as a genetics e-learning program for FPs. Methods: An email invitation was sent to 19,000 English-speaking members of the College of Family Physicians of Canada in active practice, soliciting participation in this knowledge translation initiative. Those consenting to participate were asked to complete an on-line demographic questionnaire. They were then sent an email with a Gene Messenger on a different topic every 2 weeks for 6 months. After reading each Gene Messenger, participants could click on a link to complete one IAM questionnaire. Demographic data and frequency responses to the relevance, cognitive impact, information use and expected health benefits of Gene Messengers were collected as well as free-text comments. Results: 1400 of 19,000 (7.4%) FPs contacted are participating in this ongoing project. Conclusion: FP feedback regarding the value of Gene Messengers will be used to redesign them, to optimize this e-learning genetics program, and to assess the potential of this approach for supporting primary care providers, from the point of view of effectiveness and longer term sustainability.

1369T

Genetics Awareness Project (GAP) promotes genetics education and genomic research participation in under-represented racial-ethnic groups in South Florida. *K. Czape, C. Jean, M. Gavier, J. Lee, R. Martinez, L.D. Adams, D. Caldwell, K. Murphy, S. Hahn, K. Walz, M.L. Cuccaro, M.A. Pericak-Vance.* John P. Hussman Institute for Human Genomics, University of Miami School of Medicine.

Underrepresented groups in genomic research, especially Hispanics and African Americans, are unlikely to benefit fully from genomic medicine discoveries. In turn, this may further exacerbate these groups' existing health disparities. To remedy this, it is crucial to maximize research participation of all groups. To address barriers due to limited awareness or misconceptions about genetics and research, a novel community-based culturally sensitive educational program was developed called the Genetics Awareness Project (GAP). GAP aims to increase awareness and knowledge by providing presentations and educational materials about the current and potential impact of genetic information on health, the benefits of family health history, and the importance of genetic research participation among the ethnically diverse. Materials, a website, and presentations were developed in Spanish, English, and Haitian Creole (www.geneticsawareness.org). Since mid-2010, GAP has provided educational materials to over 700 South Floridians in community centers, churches, and fairs. Of these, 595 adults and 120 children participated in 45-minute presentations on genetics, and the benefits of one's family health history and research participation. Pre- and post-presentation surveys were administered during presentations to measure the program's effect on genetics knowledge, attitudes, and research participation. Of 405 audience members who opted to complete the demographic information on the survey, 42.5% self-identified as African American. A total of 38.8% identified as White, 47.4% of whom also indicating Hispanic/Latino ethnicity. Participants were 70.1% female with average age of 65 years. A total of 35.3% had a high school education or less and 43.7% had some form of additional education (e.g., college). Over twenty percent did not specify education, we believe due to low literacy levels of some participants. Preliminary data indicate presentations improved participants' 1) attitudes about genetic research, 2) willingness to participate in genetic research, and 3) likelihood of collecting family health histories. Participants did not indicate the importance of including all ethnic groups in research during the pretest, and this was unchanged by the presentation. Further research may identify the reasons for this and may direct future educational efforts in these communities, ultimately increasing participation in genomic research and improving health equity.

1370T

Biotech 101: An educational outreach program in genetics and biotechnology. *K.M. East[1], A.M. Hott[1], N.P. Callanan[2], N.E. Lamb[1].* 1) Education Outreach, HudsonAlpha Institute for Biotechnology, Huntsville, AL; 2) Genetic Counseling Program, University of North Carolina Greensboro, Greensboro, NC.

Recent advances in research and biotechnology are making genetics and genomics increasingly relevant to the lives and health of the general public. For the public to make informed healthcare and public policy decisions relating to genetic information, there is a need for increased genetic literacy. Biotech 101 is a short-course for the public introducing participants to topics in genetics, genomics, and biotechnology, created at the HudsonAlpha Institute for Biotechnology. This study evaluated the effectiveness of Biotech 101 in increasing the genetic literacy of program participants through pre-and-post surveys. Genetic literacy was measured through increases in self-perceived knowledge for each content area covered through the course and the self-reported impact the course had on various aspects of participants' lives. Three hundred ninety-two individuals attended Biotech 101 during the first three course offerings. Participants reported a significant increase in self-perceived knowledge for each content area ($p<0.01$). Participants also reported the program had high levels of impact on their lives and decision-making, a high likelihood for continued self-learning, and overwhelming satisfaction with course content and logistics. Biotech 101 is an effective mechanism for impacting participants' lives and genetic literacy and serves as a model for other similar programs, adding to the currently limited evidence-base regarding public educational strategies in genetics and biotechnology.

1371T

From genetics to genomics: the impact on national genetics education strategies for healthcare professionals in the UK. *P. Farndon, D. Latham.* NHS National Genetics Education Centre, Birmingham Women's Hosp, Birmingham, West Midlands, United Kingdom.

The UK NHS National Genetics Education and Development Centre has developed a continuum of genetics education for healthcare by undertaking educational needs analyses and practice based surveys (www.geneticseducation.nhs.uk). Pre-registration core learning outcomes are built on in postgraduate training, and workforce competences inform clinical practice; they have been incorporated into medical school curricula, and into nationally agreed requirements for trainee medical specialists, general practitioners, nurses and dietitians. The outcomes were developed by health professionals identifying applications of genetics in their practice and identifying knowledge, skills and attitudes required by non-geneticists to provide care for patients with genetic conditions. The outcomes therefore tend to focus on identifying patients, family history and the genetics of single gene and chromosomal disorders. The Centre's educational resources also emphasise the clinical utility of genetics by using clinical scenarios to set genetic science in context. However, advances in laboratory techniques and the application of therapies developed through genomics need new specific learning outcomes about the applications and practice of genomic medicine. These have been devised but it has proved more difficult to generate resources specifically highlighting clinical applications: it has been necessary to mine primary research papers to generate completely new resources. Educational delivery methods too are changing. Until recently learners would embrace only face to face learning, but the willingness to undertake technology assisted learning is increasing, particularly as day release from clinical duties becomes more difficult. Practitioners particularly want “just in time” information in clinical practice delivered electronically. In developing resource material, it is important to identify the questions which other professionals want answered which may well be different from those a geneticist might suppose they would want to know. Genetics education at a national level needs a long term educational policy, inclusive of learners, educationalists and their institutions which is evidence based, flexible and responsive to changes in workforce structure, provision of clinical services and conceptual and financial commitments to education. Engagement of national policy, regulatory and professional bodies is vital. The UK policy is continually adapting to these changes.

1372T

Teaching Medical Genetics in Nepal: Cultural Implications and Teaching Strategies. *J. Gair[1,2].* 1) Dept Med Gen, University of British Columbia, Victoria, BC, Canada; 2) Island Medical Program, Division of Medical Sciences, UVic, Victoria, BC, Canada.

I teach medical genetics to medical students in Canada. Although there are topics and terms that are very “charged” with meaning in genetics, such as abortion, prenatal diagnosis, genetically modified organisms (GMOs), evolution and eugenics, I have been teaching these topics in the culture in which I was raised. Teaching these same topics in Kathmandu, Nepal will require a totally different approach. As I prepare to leave for Nepal to teach medical genetics in the Patan Academy of Health Sciences School of Medicine (PAHS-SOM), I wonder how different it will be. I want to explore the cultural complications that might arise for me, for the students and for the faculty involved in teaching medical genetics in Nepal - from an anthropological point of view. How will my teaching strategies be affected? My main method will be to document my thoughts, work process and experiences as I prepare my materials for Nepal and as I teach in Nepal and I will compare this to the same things in Canada by taking field notes, blogging and consulting with other professionals. I will create a culturally informed manual of terms, concepts and images for Western faculty teaching in Nepal.

1373T

Testing the Efficacy of Conceptual Change Texts in Undergraduate Students' Understanding of Genetics Concepts. *M. Glassford[1], B. Bowling[1], S. Barnes[1], S. Borgman[1], T. Beery[2], E. Reilly[3], C. Huether[4].* 1) Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY; 2) College of Nursing, University of Cincinnati, Cincinnati, OH; 3) Genetic Counseling Program, University of Cincinnati, Cincinnati, OH; 4) Department of Biological Sciences, University of Cincinnati, Cincinnati, OH.

Even after formal instruction in genetics, many students continue to hold misconceptions concerning various genetics concepts. Conceptual change texts (CCTs) are written exercises that introduce a misconception, refute it with scientific evidence, and then introduce the correct concept. This study involved the development of CCTs meant to challenge three common misconceptions among undergraduate non-science majors enrolled in general biology and genetics courses including: 1) DNA is different in different types of cells within one individual, 2) the terms gene, DNA, and chromosome can be used interchangeably, and 3) all mutations have a negative effect. The goal of this research is to test the efficacy of CCTs as an intervention for use in a relatively large lecture course to address and correct genetics misconceptions. This study uses a two-group experimental design to assess students at two Midwestern universities. Students were randomly assigned into the experimental or control group. Eighty of these students will be randomly selected to participate in the one-year post-course assessment. Students in the experimental group received a CCT on the identified misconception, while students in the control group received a text that discussed a topic from the course, but one that has been found to be widely understood after instruction. Participants were assessed pre-course, at the time of intervention, and post-course. Pilot data were used to revise the assessment questions and to create rubrics to quantitatively characterize the responses. Content analysis of responses at the various time points will identify persistent misconceptions throughout the course. Transforming student answers into quantitative data will allow statistical comparisons and determination of the effectiveness of the intervention. Results and conclusions from the data obtained from 150 students will be presented.

1374T

DNA: Key to Diversity in Animals and People Festival. *M. Godfrey[1], E. Mulkerrin[2].* 1) Univ Nebraska Med Ctr, Omaha, NE; 2) Henry Doorly Zoo, Omaha, NE.

DNA Day has now been recognized since the announcement of the sequencing of the human genome in 2003. Activities in schools and communities have been sponsored by the National Human Genome Research Institute and the American Society of Human Genetics, among others. Here we describe a partnership between an academic medical center, the University of Nebraska Medical Center (UNMC), and a zoo, Omaha's Henry Doorly Zoo to commemorate DNA Day. The zoo hosted the event on their 130 acre grounds. The public's fascination with genetics and genomics was energized by the announcement of the sequencing of the human genome. Here in the American Midwest, daily advertisements regarding genetically engineered crops abound. Yet in other parts of the world fear of “Frankenfoods” have created a stir. In all instances, education is the answer to unfounded fears and inaccurate information. Genetics and education professionals have an obligation to present to the public truthful information in a manner that is interesting and understandable. DNA Day programs provide such a means. Our DNA Day program took advantage of the excellent and well respected educational resources of the Henry Doorly Zoo and UNMC to reach a large number of people in a relatively short time. Two days of programming was scheduled one primarily for visiting school groups and the other for the general public. Activities were scattered throughout the zoo, so that no matter where people wandered, an activity related to DNA Day was nearby. Visitors were provided with a map and schedule of events. The program included a tour of the zoo's Center for Conservation Research, talks by conservation or human geneticists, and a special appearance by a Gregor Mendel impersonator. Hands on activities included: creating DNA bracelets; extracting DNA from bananas; Easter egg genetics; Reebops; Fingerprinting and DNA fingerprinting; legume fitness; horse evolution; and a demonstration of Augmented Reality using the DNA double helix. In all some 14,000 visitors participated in DNA Day activities over the two days of the Festival. It is important that educational institutions partner with organizations with a large a public following to provide novel opportunities for education and outreach.

1375T

Assessment of online health information seeking behavior to inform the development of the National Newborn Screening Clearinghouse. *KM. McWalter, AK. Yu, LE. Hasegawa, S. Scollon, SM. Au.* Genetics Program, Hawaii Dept Health, Honolulu, HI.

With more than four million newborns having newborn screening (NBS) every year, there is a need for centralized, accessible, and accurate information for NBS stakeholders. This is illustrated by the NBS Saves Lives Act of 2008: a mandate to improve the ability to disseminate accurate and appropriate NBS resources by creating the nation's first NBS Clearinghouse, a “one-stop shop for newborn screening information”. The Genetic Alliance aims to fulfill this mandate with the BabysFirstTest.org website. In the late 2000s, the proportion of Americans searching for health information online was 61%, and the subsequent trend shows increased use of the internet to access health information. To aid in the development of the Genetic Alliance's NBS Clearinghouse, the Hawaii Genetics Program assessed the online health information seeking behavior of attendees at the 2011 Hawaii Baby Expo. This is a gathering of diverse consumers interested in prenatal to preschool issues, products, and information. 784 surveys were completed via two types of media - iPads (n=487) and paper (n=297). Participants of Asian (37%), Hawaiian (23%), and Pacific Islander (6%) background were well represented; and 34.6% reported being of two or more ethnicities. Over 96% of participants had previously searched online for health information. Almost 58% spend at least 1-2 hours per day online for personal use and 55% had searched online for health information in the last week (83% in the last month). 58% have a health question at least once a week. When looking for answers, participants most commonly try the internet (49%), followed by asking their doctor (31%) or family/friends (16%). Participants most commonly searched online when worried about being sick (48%) or if curious after seeing a news story (20%). They most often began their searches with search engines (89%). 74% said they were likely or very likely to start a future search for health information on the internet. Results suggest that websites with higher rankings in search engine results may generate more traffic, and that giving specific websites to providers to disseminate to patients may be an effective strategy. News stories about the NBS Clearinghouse may also generate website traffic. Overall, the internet can be a powerful tool for the dissemination of accurate NBS information, particularly if the NBS Clearinghouse website is developed in a way that maximizes visitors.

1376T

Why students don't understand meiosis: An analysis of undergraduate textbooks. *D.L. Newman, C. Catavero, L.K. Wright.* Sch Biological & Medical Sciences, Rochester Institute Technology, Rochester, NY.

Students and professors rely on textbooks to accurately and reliably convey information. However, in order for textbooks to be useful, they must meet students where they are conceptually. We hypthesize that biology textbooks routinely fail in this regard. We surveyed >50 faculty from undergraduate and graduate institutions to determine what they felt students should know about the process of meiosis. In parallel, we examined the explanations of meiosis in 12 textbooks from all major publishers, written for different levels (non-majors, majors introductory and advanced). Previous work has shown that students have significant cognitive deficits in the areas of chromosome structure and ploidy. We found that faculty and textbooks both assume that students understand these concepts better than they really do, especially at higher levels. Since students base their conceptual models on textbook diagrams, and faculty use these materials as their primary means of explaining the concepts in the classroom, we suggest that textbooks need to be redesigned to focus on the fundamentals of chromosome structure and ploidy rather than on the superficial aspects such as phase names of meiosis.

1377T

Cyanide and Phenylthiocarbamide: Correlation with Mid-Phalangeal Hair and Color Blindness in Saint Xavier Community. *T. Tatum Parker, D. Cramarosso, E. Barry.* Biological Sciences, Saint Xavier University, Chciago, IL.

The two goals of this experiment were to 1) compare the findings of Giles, et al (1968) of hydrogen cyanide (HCN) and phenylthiocarbamide (PTC) sensitivity, mid-phalangeal hair and color blindness in Yucatan Mexico with a current population at Saint Xavier University in Chicago, Illinois and 2) find a way to increase genetics education by having students actively participate in an experiment. Our preliminary test had 19 volunteer subjects, 14 female and 5 male. For HCN and PTC sensitivities, modifications were made to the protocols for safety reasons. To limit the exposure to PTC, subjects were provided taste strips of phenylthiourea-phenylthiocarbamide from precision laboratories along with plain paper strips of similar size and texture instead of the cups of liquid PTC used in the previous experiment. The modification for HCN sensitivity was using ground peach pits instead of a HCN solution. Ground peach pits contain amygdalin, which has been shown to have a NOAEL of 10.8 mg/kg bw compared with an acute lethal does of 0.5 - 3.5 mg/kg bw for HCN KCN (Montgomery, 1969; Gosselin et al., 1976; Geitler &Baine, 1983). Subjects were asked to smell three vials, one with liquid extracted from the ground peach pits, one with distilled water and one with vanilla. Color vision was tested using the Ishihara pseudoisochromatic plates, testing for red-green color blindness. For mid-phalangeal hair, all ten fingers of each volunteer were examined for the presence of hair or follicles. Presence or absence of hair for each digit was recorded. Our preliminary results for PTC tasting were similar to those in the previous experiment, with 84% of our subjects able to taste the PTC. However for the other traits, our values were lower. Forty two percent of our subjects could not smell anything in the vial with the distilled water or the vial with the peach pits. Only one female, tested for color blindness and 47% of the participants had mid-phalangeal hair compared to over 75%. Similarly to the previous experiment we found no significant genetic variation among the individuals that were tested. Our next steps will be to increase our sample size and examine single nucleotide polymorphisms associated with the ability to taste PTC. This experiment is an ongoing experiment, to increase genetics education in our majors and non-majors classes by increasing student ownership through their participation in both sample collection and being subjects.

1378T

The latent class analysis to clarify the factors associated with the structure of the public attitude toward the genome research. *Z. Yamagata[1], T. Maeda[2], K. Muto[3], A. Nagai[1], A. Tamakoshi[4], I. Ishiyama[5].* 1) Department of Health Sciences, University of Yamanashi, Yamanashi, Japan; 2) The Institute of Statistical Mathematics, Tokyo, Japan; 3) The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; 4) Aichi Medical University, Aichi, Japan; 5) Teikyo-Gakuen Junior College, Yamanashi, Japan.

We clarified the factors associated with the structure of the public attitude toward the genome research by the latent class analysis. The data was derived from the national wide surveys on the attitude toward the genome research were comprised of 4,000 people (age, 20-69) in 2005 and in 2009, randomly selected from the Japanese general population. The response rate was 54% and 52% respectively. We conducted similar survey on a small scale in 2008. Five clusters (“Group of aggressive promotion”, “Group of passive support”, “Group not making judgment”, “Group making prudent judgment”, “Group not interested in genome”.)were assumed as an explanation model of six variables related to the knowledge of genome and attitudes toward genomic research promotion about three themes; basic genome research, genome research related to agriculture and medicine at the survey. These clusters were associated with academic background, socioeconomic status, donation to their blood for genome research, interest of science, consciousness to take genetic testing, behavior to go to genome lectures. These factors become the basis of genome research outreach programs. We tried to make outreach programs having public understood genome researches based on these results.

1379T

Preference for genetic testing among patients offered participation in the Additional KIF6 Risk Offers Better Adherence to Statins (AKROBATS) trial. *S.L. Charland[1], B.C. Agatep[1], V. Herrera[2], E.J. Stanek[1].* 1) Medco Research Institute, LLC, Medco Health Solutions, Inc., Bethesda, MD; 2) Medco Research Institute, LLC, Medco Health Solutions, Inc., Franklin Lakes, NJ.

Background: Utilization of genetic tests has improved personalized healthcare. However, factors that influence a subject's decision to accept or decline genetic testing are poorly defined. The objective of this study is to characterize the rationale from patients new to statin therapy to either decline or accept KIF6 genetic testing associated with participation in the AKROBATS trial. **Methods:** Subjects were contacted via telephone by study nurses. During this call, subjects were provided information on the study and KIF6 variant testing, and were offered enrollment. Based upon response, patients were queried on reasons to accept or decline study participation. Responses were categorized and collapsed for analysis. Categorical and continuous variables were analyzed using chi-squared and t-tests, respectively (significance at $p<0.05$). **Results:** A total of 773 patients declined participation and 682 subjects agreed to enroll in the AKROBATS study. Enrollees were significantly younger (60±12 vs 64±13) and more likely to be prescribed atorvastatin or lovastatin ($p<0.05$). Reasons for enrollment included: Confident that KIF6 test results would improve my disease outcomes (53.8%), help humanity (17.4%), general interest/knowledge (15%); family history of heart disease (5.9%), confident others were receiving KIF6 test (1.6%), asked to participate (0.9%), and no reason provided (5.4%). Those declining enrollment indicated no reason (43.1%), no perceived benefit of KIF6 testing (19.7%), time constraints (18.4%), not enough knowledge about KIF6 test (8.8%), privacy concerns (6.7%), anxiety associated with results or lifestyle changes (0.9%), and other (2.5%). **Conclusion:** Multiple factors, including age, therapy prescribed, logistical considerations, and health beliefs, may influence the decision to decline or accept genetic testing under trial conditions. Lack of interest, lack of perceived or potential benefit, and time concerns were primary reasons for study participation/KIF6 test declination. In contrast, belief in personal value/utility, altruism, and general interest appeared to drive study participation/KIF6 test acceptance. As genetic testing advances, information such as this is key to fully understanding patients' attitudes towards acceptance and reservations about genetic testing, and may serve as a basis to develop effective patient communication and education strategies to support individualized decision making.

1380T

Efficacy of knowledge of Genetics in the Field of Psychiatry and its impact in patient treatment plan and option. *H. Azimi.* Genetic Informatic, Psychogenome, Nepean, Ontario, Canada.

BACKGROUND: Early detection of genes that predispose individuals to behavioural, psychological and psychiatric illness can have benefits such as: to decrease healthcare cost, increase efficacy of treatment and aid health professionals in advising parents of predisposed newborns of environmental changes that can decrease the like hood of psychiatric manifestations. As the field of Clinical Genetics and metabolic is growing, many psychiatrists are interested to help their patients, from individuals with known psychiatric disorders to their potential children in preventing and predicting diseases. OBJECTIVE: To evaluate the knowledge of genetics in the field of Medical Psychiatry and Mental Health. Method: Design: Standardized Questioner Survey. Setting and Participants: 300 practising Psychiatrist in Canada and USA, 100 from Canada, 100 from USA and 100 international. Information was gathered by phone calls, emails or in person interview. Hypothesis: To identify the gaps in genetics knowledge among practising Psychiatrists. Main Outcome: Number of Bipolar, Schizophrenic, Alcoholic, Autistic, Depressed, and Mentally challenged patients which the Psychiatrist was helping to cure was asked from the Psychiatrist. The outcome was compared with the comparison to knowledge of genetics which the Psychiatrist had about the disease. Results: Mean disorder, prevalence, knowledge and core knowledge of genetics was asked from the Psychiatrist. After six month of follow up with the Physicians, the gaps in knowledge of genetics in Psychiatrist those who trained internationally was higher in the field of genetics compared to Canada and USA trained Psychiatrists. (mean (SD), 0.88(0.22) mM vs. 0.68(0.25) mM in North American Group; P=0.001) Among specialist in Canada (P =0.84). Seven psychiatrists in Canada wore involved in daily genetic literature reading and referred the patients that needed genetic counselling to appropriate centers. Over nine psychiatrists in USA wanted to obtain a fellowship in Psychiatric Genetics and thought of this field to be very interesting and stated “the knowledge genetics needs to be more open to us psychiatrist”. 143 psychiatrists did not want to be involved in any further studying and wanted to just retire, but stated “If we had a Psychiatric genetic round during residency at least for 4-week then maybe we be in different shape”.

1381T

Developing a national competence framework in the UK to support the delivery of non-invasive prenatal diagnosis. *M. Bishop, J. Haydon, D. Latham, P. Farndon.* NHS National Genetics Education Centre, Edgbaston, Birmingham, United Kingdom.

The integration of new genetic technologies into clinical practice impacts not only on clinical genetics but also on the wider health service that may be called upon to offer these tests to their patient groups. One such development is non-invasive prenatal diagnoses (NIPD) where the ability to detect and test fetal cell free DNA and RNA circulating in maternal blood has the capacity to transform prenatal diagnosis for single gene disorders and chromosomal anomalies. For effective integration into standard clinical care health professionals providing the service must be knowledgeable about the test in question. To understand how health professionals in the UK National Health Service (NHS) see NIPD impacting on clinical care and their educational needs to provide this service, the NHS National Genetics Education and Development Centre carried out an explorative study leading to the development of a competence framework. A mix of interviews and focus groups with participants from three clinical areas including fetal medicine (n=7), clinical genetics (n=12) and midwives (n=46) were conducted. Participants were provided with a number of clinical scenarios demonstrating how NIPD could be used in clinical practice to initiate discussion. Interviews and focus groups were audio taped and transcribed, with thematic analysis employed to elicit common views regarding the integration of this technology into clinical practice and the education needs of the workforce who will deliver this service. While all three groups of participants recognised that NIPD will impact on the clinical service they provide, they believed the introduction of this new technology should be viewed as supplementing current roles as opposed to changing practice. As such, the identified educational needs focused more on the procedural issues associated with NIPD, such as understanding the clinical reason for offering the test, knowing the laboratory process and appreciating the implications of test results. In addition to identifying the educational needs, participants stated that any educational package developed needed to reflect the service model for delivering NIPD to ensure education was relevant to the health professional's role. These findings have informed the development of a competence framework which provides the basis for educational packages to support the implementation of NIPD into clinical practice.

1382T

A Comprehensive Analysis of High School Genetics Standards: Are States Failing to Keep Pace with Modern Genetics? *M. Dougherty[1], C. Pleasants[2], L. Solow[3], A. Wong[1], H. Zhang[1].* 1) Education, ASHG, Bethesda, MD; 2) 5 Crystal Springs Rd., Greenville, SC; 3) Wesleyan University, Middletown, CT.

Science education in the United States is driven by the testing and accountability requirements of the No Child Left Behind Act (NCLB). Implementation of NCLB relies heavily on learning outcomes, or “standards,” which are currently developed on a state-by-state basis. Standards, in turn, drive curriculum and instruction, yet they have never been evaluated for scientific accuracy and completeness. We investigated the quality of the standards in genetics for all 50 U.S. states and the District of Columbia and found them to be poor, with more than 85 percent of states receiving overall grades of `inadequate'. Individual genetics concepts are covered poorly as well, with particular weakness in areas related to complex traits—an area that is increasingly important in genomics research. The deficiencies we identified may adversely affect genetics instruction and learning, the preparation of students for college and careers in genetics, and the genetic literacy of the U.S. citizenry.

1383T

Usability Testing in the CFTR2 Web Site. *M.H. Lewis[1], P. Sosnay[2], F. VanGoor[3], H. Yu[3], R. Dorfman[4], J. Rommens[4], M. Corey[4], C. Castellani[5], C.M. Penland[6], R. Karchin[7], G. Cutting[2].* 1) Genetics and Public Policy Center, Berman Insitute of Bioethics, Johns Hopkins University, Washington, DC; 2) Departments of Medicine, Pediatrics, Johns Hopkins University, Baltimore, MD; 3) Vertex Pharmaceuticals, San Diego, CA; 4) Hospital for Sick Children, University of Toronto, Toronto, ON, Canada; 5) Ospedale Civile Maggiore, Verona, Italy; 6) U.S. Cystic Fibrosis Foundation, Bethesda, MD; 7) Department of Biomedical Engineering, Center for Computational Medicine, Johns Hopkins University, Baltimore, MD.

Background: The Clinical and Functional Translation of CFTR (CFTR2) project is an international effort to link 1800+ CFTR mutations with expert-reviewed clinical parameters. A Web site was developed to communicate this information to the Cystic Fibrosis research and patient communities. Since genetic information may be confusing to the lay public, prior to the public launch of the Web site, usability testing was conducted. Usability testing is a technique for ensuring that the intended users of a system can carry out the intended tasks effectively. Usability testing is carried out pre-release so that any significant issues identified can be addressed. Methods: In this qualitative study, multiple iterations of usability testing were conducted. Participants were members of the general public and the Cystic Fibrosis patient community. In each iteration, three participants were each asked to view the Web site, complete a series of tasks, and answer questions based upon the information they obtained. Improvements to the Web site design were implemented after each iteration. Testing was completed in-person and over the telephone. Participants were asked to think out loud as they attempted to complete the assigned tasks. Results: Problems identified were related to content rather than technical issues. One participant noted, “I don't understand what a sweat test is. I need education first.” In some cases, participants answered questions correctly but based upon incorrect interpretation of information. For example, one participant answered correctly that a particular mutation would cause pancreatic insufficiency but was unclear whether pancreatic insufficiency meant that the pancreas was not affected or whether the pancreas did not “sufficiently produce what it is supposed to.” Additional content was added to the Web site to address these issues. During early iterations, participants thought the language was “too textbooky,” and commented, “I don't understand this.” As a result, the Web site was divided into 2 parts, one designed for the general public and another designed for researchers. Conclusions: Usability testing identified problems with a Web site designed to provide information about the genotype-phenotype relationship of specific CFTR mutations. This information was used to improve the Web site content, structure, and design. Usability testing has been a crucial element in the Web site development process.

1384T

Automated generation of diseases classification supported by Orphanet Ontology of Rare Diseases. *F. Dhombres[1,2,4,5,6], S. Aymé[1], A. Rath[1], A. Olry[1], PY. Vandenbussche[2,3,5], J. Charlet[2,5,6,7].* 1) Orphanet, INSERM, Paris, France; 2) UMRS872 Eq.20, INSERM, Paris, France; 3) Mondeca, Paris, France; 4) Service de Gynécologie-Obstétrique et Centre de Diagnostic Prénatal de l'Est Parisien, Hôpital Armand Trousseau, AP-HP, Paris, France; 5) UPMC, Paris Sorbonne Universités, Paris, France; 6) Université Paris Descartes, Paris, France; 7) Assistance Publique Hôpitaux de Paris, Paris, France.

Background. Orphanet is evolving towards an integration of semantic technologies in its editorial workflow and representation of data. This evolution aims to improve edition and maintenance of rare diseases classifications and allow cross referencing with other ontologies. An ontology of rare diseases, including all genetic diseases, has been build from (1) Orphanet Knowledge base stored in a standard relational database and (2) Orphanet core ontology consistent with a meta model of the domain (alpha version available on Bioportal). The full Orphanet nomenclature of phenoma (groups of diseases, disorders, subtypes and clinical signs and symptoms), genes, hierarchies and textual resources were used in this design process.

Objectives. Assessment of an automated generation of Orphanet Classification of Genetic Diseases supported by a semantic editorial workflow.

Methods. Using the rare diseases ontology with its meta-model, a unique query allows automated building of Orphanet Classification of Genetic Diseases. This process involve semantic web technologies (Sparql request on RDF Triplestore). Assessment of the generated classification is performed by a strict automated comparison with the original Orphanet Classification of Genetic Diseases.

Result. An automatically generated classification of 4,997 genetic diseases was built. There is an exact match between the original and the generated classification of genetic diseases.

Discussion. This approach simplifies the editorial process, which is then limited to the maintenance of the ontology itself. The process used for the whole genetic classification is reproducible. Therefore, automated generation of classifications may be extended to produce any other classification based on the semantics defined in the ontology (classification of genetic developmental anomalies during embryogenesis, classification of dominant skeletal diseases, ...). Moreover, once the ongoing gene-disease relationships characterization is achieved, new automatically generated classifications based on the contribution of genes to the etiology of diseases will be conceivable.

1385T

Comparisons of Disease Risk Assessment of Direct Consumer Genetic Services for Japanese Individuals. *T. Kido[1], M. Kawashima[2], S. Nishino[3], G. Sherlock[3], A. Butte[3].* 1) Rikengenesis/JST, Tokyo, Japan; 2) The University of Tokyo, Tokyo, Japan; 3) Stanford University, CA, U.S.

Background: Several companies offer direct-to-consumer (DTC) genetic tests to evaluate individual's disease risks. However, Craig Venter et.al. reported that only 50% or less of the predictions of two DTC companies agreed across five individuals for seven diseases. It is important to examine why such a large discrepancy occurs. In addition, most of the disease risk predictions by DTC companies are based on the GWAS researches in Caucasian population; we need to adapt these predictions to other ethnicities. Methods: First, we compared the risk assessment for each disease used in 23andMe, Navigenics, Pathway Genomics and DeCODEme in detail for one healthy Japanese individual. Then we also compared the risk assessments of 23andMe, Navigenics across three healthy Japanese for 23 diseases, using same methodologies as Craig Venter. Next, we investigated where the discrepancies come from past researches have reported that variance in multigenic risk interpretation can be explained by differences in (1) the average lifetime risk, (2) the SNPs selected for the assessment, and (3) the risk assignment methodologies. We evaluated each of these factors for four DTC companies for Japanese individual, and classified the reasons for their discrepancies. Results: 23andMe, deCODEme, Navigenics, Pathwaygenomics provide 95, 42, 25, and 17 risk assessment reports respectively, and 10 reports were shared for same diseases across the four companies. 23 reports were shared for the same diseases between 23andMe and Navigenics. In total, there were 27 mismatches in predicted risk for three Japanese individuals (approximately 9 mismatches per individual in 23 diseases) between 23andMe's and Navigenics's predictions. For seven diseases, a third or less of the predictions of two companies agreed across three individuals. These mismatches owe to differences in the SNPs used in the calculation, the reference population used, and different risk assignment methodologies. Navigenics does not provide the reports specifically for Asian individuals, while 23andMe provides only three reports (for type2 diabetes, rheumatoid arthritis, and prostate cancer) specifically tailored for Asian individuals. Conclusions: Greater consistency is needed for the risk assessment among different DTC genomic tests. The risk assessments in each ethnicity is important, but only limited data is available. Establishments of reliable risk assessment methods for all ethnicities are required.

1386T

Traditional Bone Setting and Healing: Bane or Blessing. *P. SINGH, PANKAJPREET SINGH.* SPORTS SCIENCES, PUNJABI UNIVERSITY, PATIALA, PUNJAB, India.

Traditional Bone setting (TBS) is a practice of healing bones and related injuries .It contributes largely to the alternative medicine in the Asian continent and especially in India. TBS is easily accessible, cheaper and is believed to give quick results. On the other hand, non union, malunion, traumatic osteomyelitis and limb gangrene are some of the major complications of TBS treatment. In spite of these complications, there is a great demand for TBS, that patients leave orthodox hospitals in favour of treatment by a TBS. The possible reasons for this include cultural beliefs, ignorance, third party advice, and the short supply of trained orthodox man power in rural areas and the fear of amputation at an orthodox hospital. If properly educated about the therapy, radiological diagnosis , duration of healing , complications of mismanagement and rehabilitation exercises , the Traditional bonesetter's can be of immense help in sharing the burden of public health service providers in the developing countries.

1387T

The influence of a positive family history of prostate cancer on the initial screening age, screening interval, and biopsy decisions in a population of PSA screened males. *D. Cross[1], D. Reding[2], G. Aryal[2], K. Sparks[1], C. McCarty[1].* 1) Marshfield Clinic Research Foundation, Marshfield, Wi; 2) Marshfield Clinic, Marshfield, Wi.

Purpose: We examined the PSA screening practices in a multispecialty primary care clinic, including the initial age at screening (with and without a family history of prostate cancer), the interval between PSA screenings, the PSA values and elapsed time between PSA testing and biopsy. Materials and Methods: We used a population based cohort consisting of 4632 men within the Personalized Medicine Research Project with at least one recorded PSA test, 935 individuals underwent a prostate biopsy with 466 diagnosed with prostate cancer. Univariate chi squared analyses were performed to determine if family history was used to determine screening frequency and biopsy decisions in the cohort. Results: Within the population the average age of the cohort was 67.2 years with an average age of initial PSA test of 55.7 years and an average of 6.8 PSA tests with a screening interval of 465 days. Individuals who self reported a family history of prostate cancer were older (73.6 years versus 66.8 years $p<0.01$). The average interval between screenings was not different for individuals with or without a family history of prostate cancer (437 days versus 479 days). However, the time between a PSA test and initial biopsy was significantly different (73.3 days for individuals with a family history of prostate cancer versus 201.5 days for those without a positive family history $p<0.01$.) Individuals who reported a family history of prostate cancer were more likely to have a biopsy (50% versus 20%) $p<0.0001$ and were significantly more likely to have a positive biopsy $p<0.0001$ (81% versus 25%). Discussion: Despite a number of recommendations suggesting men with a family history of prostate cancer begin screening early, we did not observe early or more frequent screening in men with a self reported family history of prostate cancer. This may be because individuals do not know their family history early enough to influence the initial screening date. However, knowledge of a positive family history of cancer does appear to be used by physicians and patients for making biopsy decisions and is associated with a quicker time between PSA testing and biopsy, referral for a prostate biopsy, and a positive biopsy.

1388T

FGF8 mutation screening in patients with VCFS-like phenotype. *I.C. Sgardioli, T.P. Vieira, M. Simioni, L.C. Souza, V.L. Gil-da-Silva-Lopes.* Department of Medical Genetics, Faculty of Medical Sciences University of Campinas, Campinas , São Paulo, Brazil.

INTRODUCTION: Velocardiofacial Syndrome (VCFS) is the most common chromosomal microdeletion syndrome in human, with an incidence of 1/ 4.000 to 1/6.000 births. The main clinical features are congenital heart disorders, typical facial appearance, palatal abnormalities, hypocalcemia, immunodeficiency, learning disabilities and developmental delay. A microdeletion in 22q11.2 is the main cause of the syndrome. However, it is not detected in about 10 to 20% of patients with this clinical suspicious. Some of them have other chromosomal aberrations or TBX1 gene mutations. Based on animal models, VCFS phenotype could also be related to mutations in FGF8 gene. AIM: To investigate the clinical suspicion of SVCF by different laboriatorial techniques and To investigate FGF8 mutations in patients with VCFS suspicion without 22q11 deletion or TBX1 mutations. METHODS: Karyopyte with GTG banding, MLPA (kit P250-A1 DiGeorge - MRC-Holland®), FISH (TUPLE1 probe - Kreatech Diagnostics) and direct sequencing (ABI Prism BigDye Terminator kit). RESULTS: Among 102 individuals, seven had chromosomal aberrations (only one related to 22q11.2 deletion); 31 had deletion in 22q11.2 and 69 had normal karyotypes and no deletion in 22q11. These patients were clinically re-evaluated and those with congenital heart disease were screened for TBX1 and FGF8 gene mutations. To date, among 31 individuals selected, the analysis of TBX1 gene identified just polymorphisms previously known and no sequence changes were found in the 3, 4, 5 and 6 exons of the FGF8 gene. CONCLUSION: This study reinforces the clinical and etiological heterogenity of VCFS spectrum. This is the first report in humans of FGF8 gene investigation in this condition; these results could bring new informations about (the etiology) genetic pathway of the VCFS phenotype. Financial support: FAPESP and CNPq.

1389T

Quantitative and qualitative analysis of DNA from human blood samples stored at different temperature for different time intervals. *S. Goyal[1,3], I. Bhadu[1], M. Rajpurohit[3], A. Raina[2], T.D. Dogra[2], A. Agarwal[1], R.P. Agrawal[1], S. Jain[1], D. Goyal[1].* 1) Diabetes Care & Research Centre, S.P.Medical college, Bikaner, Rajasthan, India; 2) All India Institute of Medical Sciences, New Delhi, India; 3) Tanveer Malawat College of Bioscience, MGS University, Bikaner, Rajasthan, India.

Introduction:-DNA is a genetic material which is used in forensic laboratories like blood, semen, blood stain, bones, semen stain, etc. Mainly white blood cells are used as the major source of DNA due to presence of high quantity of genomic DNA. Quality and quantity traces obtained after isolation of DNA from biological samples, have an important role in forensic science. To investigate qualitative and quantitative estimation of DNA extract from human blood samples analysis better DNA yielding. Method: - All protocols used in this research were standard protocol according to the DNA finger printing lab. Standard protocols were used for collection of blood samples, extraction of DNA, qualitative and quantitative analysis. Result:- Blood was collected from unknown (6) dead bodies and divided into 6 tubes containing 1ml in each tube and kept for different temperature (room temperature(37ºc), cooled at 4ºc and freezed at -20ºc) and for different time periods (2, 4, 6, 9 days). It was observed that though DNA was isolated from same amount of blood in all the samples, a variation was seen, as yield of DNA was obtained at sample freeze at -20ºc i.e. 578.50 ng/µl of DNA, 4ºc and 37ºc(Room Temperature) was 429 ng/µl and 390 ng/µl of genomic DNA respectively. According to qualitification of DNA best quality was observed in 4ºc i.e. 1.692 µg/ml, comparison to another temperature-20ºc and 37ºc i.e. 1.610 µg/ml and 1.645 µg/ml. Discussion: -From all the results it was concluded that quality wise 4ºc was best and -20ºc was best for maximum yield of genomic DNA. For long time storage, the quality of DNA was maintained on 4ºc as it prevents the denaturation of DNA.

1390T

Inauguration of a programme for Disorders of Sex Development (DSDs) in Cameroon (sub-Saharan Africa). *A. Wonkam[1,2], W.Y. Joko[1], F. Mouafo[3], S. Dahoun[4], C. Ngongang[1], C. Lecoultre[5], J. Birreaux[5], B. Paturel[6], P.Y. Mure[6].* 1) Service of Medical Genetics, Gyneco-Obstetric and Pediatric Hospital Yaoundé, Cameroon; 2) Division of Human Genetics, Faculty of health Sciences University of Cape Town, Cape Town , South Africa; 3) Service of Pediatric Surgery, Gyneco-Obstetric and Pediatric Hospital Yaoundé, Cameroon; 4) Service of Genetic Medicine, Geneva University Hospitals, Geneva, Switzerland; 5) Service of Pediatric surgery Geneva University Hospitals, Geneva, Switzerland; 6) Service of Pediatric Surgery, CHU of Lyon, France.

BACKGROUND: Practice of genetic medicine was recently introduced in Cameroon. The increasing number of patients with DSDs reveals the lack of local technical and human resources to face the multiples management challenges. A specific national program for DSDs was launched, with the collaboration of the Ministry of Public Health and a Swiss charity organization. OBJECTIVE: 1-To identify patients with DSDs; 2- to describe the genetic diagnosis; 3- to offer treatment options; 4- to address ethical, legal and social issues. METHODS: 1-Inaugural media campaign to call for patients' participation (16-20th November 2009). 2-Multidisciplinary consultations with local and international professionals (geneticist, endocrinologists, paediatric surgeons, gynaecologist, psychiatrist) 3-Cytogenetic and molecular genetics analyses (locally and abroad) 4-Medical and local surgical management RESULTS: 179 patients were consulted; 122 (66.1 %) were diagnosed with various external and internal genitalia anomalies: 1-Hypospadias (44.3%) 2-46, XX DSD (18%): CAH (8 Cases); 46, XX ovotesticular DSD SRY negative (4 cases); mullerian abnormalities (5 cases); cloacal extrophies (5 cases). 3-Sex chromosomes DSD (12.3%): Turner's syndrome (13 cases), Klinefelter's syndrome (2 cases). 4-46, XY DSD (11.5%): Disorders in androgen synthesis or action (9 cases); 46, XY ovotesticular DSD (2 cases); Kallmann' syndrome (3 cases). 19 patients have been operated. 3 cases needed legal gender reassignment. CONCLUSION: The programme evidences universal need of medical genetics services and provide a model for international collaboration to bridge the technical and human recourses gap in the management of DSDs in a Sub-Saharan Africa country.

1391T

Improving quality of clinical services for patients with genetic susceptibility to breast cancer: An evidence-based approach. *J. Jbilou[1, 2], R. Landry[3], N. Amara[3], J. Simard[4, 5].* 1) Public administratio, Université de Moncton, Moncton, Canada; 2) Department of community health, Université de Sherbrooke, QC, Canada; 3) Department of management, Université Laval, QC, Canada; 4) Department of molecular biology, Université Laval, QC, Canada; 5) Canada Research Chair in Oncogenetics.

Background Female with a BRCA1 or BRCA2 mutation carrier have a lifetime risk of breast cancer of between 50% and 80%. To guide health professionals to decide whether or not personalized medical services are indicated and to ensure that women with genetic susceptibility to breast cancer have access to effective and relevant genetic services, effective strategies are to be put into place to help health professionals to adequately predict breast cancer genetic susceptibility and better communicate it to individuals and their families. Nevertheless, there is a lack of knowledge on stressing factors of this process. Objective Identify key steps of evidence-based genetic services Identify the drivers and challenges that impact on the quality and efficiency of genetic services. Method A qualitative study based on a systematic review of the literature (prior to 2010), using an inductive analysis and content analysis approach. We adopted the perspective of health professionals. We assumed that genetic services are based on a combination of a clinical behaviour and a knowledge transfer process and adopted an integrative framework based on: social cognitive theories and Knowledge value creation framework. Results We retrieved 147 articles that encounter the inclusion criteria. We have identified 5 steps that broadly illustrate degree of variation in the content and process of genetic encounters: assessing risk, informing about risk, confirming risk, announcing risk and supporting women at high or moderate risk. We extracted 327 indicators that have been identified in the literature as confirmed (quantitative articles) or potential (qualitative articles) determinants of genetic services. We abstracted them into 4 major conceptual categories: professional's factors, organizational factors, patient's factors and ethical, legal and social factors. We derived a questionnaire and validate through a Delphi technique with a panel of experts representing all professional categories involved. A second validation of the questionnaire is undergone in a clinical setting. Conclusion Our results are easy to transfer in other clinical setting and our framework may be also used in empirical research of assessing healthcare services delivery and performance. The questionnaire will be administrated in a cross-sectional survey among a sample of Canadian health professionals involved in genetic services for patients with genetic susceptibility to breast cancer.

1392T

A working model for developing countries towards fulfilling the promise of molecular medicine in the rare lethal genetic disorder, Duchenne muscular dystrophy. *B.R. Lakshmi.* MDCRC, Coimbatore, Tamil Nadu, India.

The benefits of genomics and biotechnology are concentrated primarily in the developed countries, while their potential to combat neglected/orphan disorders in the developing countries has been largely untapped. Capacity building in developing countries like India becomes crucial to address disorders like Duchenne muscular Dystrophy. In India, with its population over a billion, the anticipated number of DMD affected patients should be high and there is an urgent need to incorporate genetic approaches into their health care services. The challenges in developing countries involve the limited or even absent regulatory infrastructure and limited capacity to offer diagnostics and treatments and strategies adopted pertains to the country specific needs. The centre's accomplishments in capacity building with time and the establishment of a working model to holistically address DMD are elaborated here. Precise molecular diagnosis enables prevention of the disease through carrier and prenatal diagnosis and also is essential to understand the numbers who could probably benefit from emerging therapeutic strategies like exon skipping and PTC 124. With our systematic and passionate efforts in the past 5 years we have close to 1100 cases of which we have offered precise molecular diagnostics to close to 800 cases as per International guidelines, with strict quality and ethical protocols. Networking of Clinicians has been established, informing them on the benefits of genetic testing and the various services offered at our facility. We are the only Government recognized referral centre for the State, where samples are streamlined through nodal centres involving hospitals, physiotherapy centres and private practitioners across the state and other neighboring southern states of India. A DNA bank is maintained for every patient sample received at the lab. Follow up of genetic diagnosis is done by providing genetic, psychosocial and reproductive counseling, strengthened by carrier analysis offered at the centre. Quarterly parents meeting are held to update them on the recent progresses in the field. Recently we have initiated the Multidisciplinary clinical evaluation as per International guidelines to monitor disease progression and offer appropriate and timely interventions to ensure good quality of life. We believe that the above initiatives have set the platform towards a DMD registry database in the country that contributes to global efforts to combat the disorder.

1393T

The Philippine Birth Defects Surveillance Project: a 3 Year Experience. *C. Padilla[1,2], E. de la Paz[1,2], B. Cavan[3], C. Abarquez[4], R. Sales[1], A. Sur[1], J. Posecion[5], L. Orbillo[6], J. Basilio[6] for the Philippine Birth Defects Surveillance Study Group.* 1) Institute of Human Genetics, NIH Philippines,; 2) Department of Pediatrics, College of Medicine, University of the Philippines Manila; 3) The Children's Genetic Center, Cebu City,; 4) Southern Philippines Medical Center, Davao City; 5) West Visayas State University Medical Center, Iloilo City; 6) Department of Health.

The burden of birth defects is not well-established in the Philippines because of constrained diagnostic capabilities, poor health-related statistics, and the absence of any systematic birth surveillance and registries. Causes are unknown and risk factors and preventive strategies are not yet established. Information on birth defects can be used to facilitate clinical services to affected children and families, improve programming and prevention strategies, and enhance community links. An effective surveillance is needed to allow for the ready use of data for implementation of population-based planning for public health programs. Currently, there is no formal national birth defects surveillance system in the country, and no organized efforts to establish the burden of birth defects. In 2008, the Philippines became part of the March of Dimes Global Network on Maternal and Infant Health. One of the objectives of the project was to establish hospital- and community-based surveillance systems to capture data on adverse birth outcomes. This led to the establishment of the Philippine Birth Defects Surveillance Project, a joint project of the National Institutes of Health Philippines and the Department of Health. To cover all newborns including the home delivered (62% of all births) and to facilitate referral of diagnosed cases among health facilities and communities, the surveillance was implemented in sentinel sites, geographically selected. A sentinel site has at least 1 tertiary private hospital, at least 1 tertiary government hospital, local government hospitals, and communities. From Sept 2008 to March 2011 a total of 82 health facilities and communities from 18 sentinel sites distributed in the 3 major island groups (Luzon, Visayas, Mindanao) have participated. A total of 2949 patients were reported. The top ten birth defects were oral and facial cleft, limb deformities/limb reduction, ankyloglossia, skin tags, multiple congenital anomalies, neural tube defects, Down Syndrome, hydrocephalus, hemangioma, and hypospadia. The biggest challenge of this project is the completeness of the submitted forms and the difficulty in accurate description of the patients. This problem is being solved with the introduction of a telegenetic service. The other challenge is increasing coverage in the community setting, considering that 62 percent are home delivered. The project is now expanding to cover more hospitals and more communities.

1394T

Microbial and genomic studies of A.Indica (Neem) for medicinal purpose. *I. Bhadu[1], S. Goyal[1,2], P.K. Ranga[2], D. Gupta[2], R.P. Agrawal[1], A. Agarwal[1], P.R. Jatkhar[2], V. Agarwal[1], H. Bhadu[1].* 1) Diabetes Care & Research Centre, S.P.Medical college, Bikaner, Rajasthan, India; 2) Tanveer Malawat College of Bioscience, MGS University, Bikaner, Rajasthan, India.

Introduction: - A.Indica (Neem) tree can be labelled as wonder tree for its multipurpose uses in real sense. The word Azadirachta is derived from the Persian azaddhirakht meaning “Noble tree”. Compounds of A.Indica have several types of biological activities like antifungal, antibacterial, hypoglycemic, antitumour, anti-inflammatory etc. Method: -The present study was carried out to check the efficacy of aqueous and ethanolic leaves extracts of A.Indica on different strains of S. aureus Discs (5 mm) soaked and dried in different concentrations viz. 30%, 20 %, 10 % of alcoholic and aqueous solution of leaves were prepared and the antimicrobial efficacy was studied. CTAB method was utilized for quantitative analysis of genomic DNA while qualitative analysis was performed by using gel elcetrophoresis. Result: - A zone of inhibition 12 mm, 10 mm and 9mm in alcoholic and 9mm, 8 mm and 7 mm in aqueous solution were formed by 30 %, 20 % and 10 % for both solutions, respectively. It was observed that A.Indica has a good amount of genomic DNA that is 4.55 µg/ml with high purity was analysed i.e. 2,419 bps. Discussion: - Antimicrobial and genomic analysis of A.Indica was carried out to observe all characteristics of plant parts which is useful in governing the valuable medicinal property for its medicinal and therapeutic purpose.

1395T

What genetic competencies are realistic and achievable for US primary care providers? Qualitative study with key informants. *N. Qureshi[1], K. Szegda[2], J. Kai[1].* 1) University of Nottingham, Nottingham, United Kingdom; 2) Pioneer Valley Asthma Coalition, Baystate High Street Health Center Pediatrics, Springfield, USA.

Purpose: Genetic medicine is increasingly being incorporated into clinical practice, but poses challenges for primary care. Further, this may amplify existing disparities in health service provision for ethnic minority populations in the US and other developed countries. This might be counteracted by improving the genetic competency of primary care providers. In the US, these include family doctors, general internists and paediatricians. The purpose of this study was to critically appraise the skills, attitudes and knowledge of primary care providers currently providing genetic provision for underserved populations. Methods: Key informants were identified through academic residency-training primary care practices and through US family practice conferences (Society of Teachers of Family Medicine and American Academy of Family Physicians). A series of 12 semi-structured interviews were completed with key informants from different settings throughout the US. These interviews were transcribed. The quality of transcription were confirmed by one of the researcher or a second transcriber reviewing transcripts against audio files. Data were thematically coded by two independent researchers and analyzed according to agreed emergent themes. Results: Core attributes were identified which are required for primary care providers to develop genetic competency. These included adapting communication skills, working with families and appreciating disease prevalence in local communities. These attributes could be enhanced by primary care providers' keeping up to date, using standard procedures such as genetic-related checklists produced by American Medical Colleges and presence of genetic literacy in consulting patients. However genetic competency is hindered by lack of time and resources, poor awareness of patients' beliefs, and clinicians displaying unsubstantiated confidence in their own competency. Conclusion: Primary care providers' genetic competency can be improved by incorporating transferable skills and concentrating on genetic conditions with higher prevalence in local areas. This can also be enhanced by improving communties' genetic literacy.

1396T

The distribution of telomere length in buccal cells of healthy volunteers. *Y. Shidoji, S. Yabuta, C. Sakane, M. Masaki.* Graduate School of Human Health, University of Nagasaki, Nagayo, Nagasaki 851-2195, Japan.

Telomere length has been implicated as an indicator of physical aging and other health conditions. In considering widely use of this indicator to various biological fields, it is indispensable to establish a method to measure the telomere length with promptness, handiness and high sensitivity by using a buccal swab that is a low-invasive sample. In order to measure the telomere length of the buccal cells, we decided to apply a monochrome multiplex PCR method that was developed and has recently been improved (Cawthon, 2009). The officerss of Nagayo town (89 men and women with age from 20s to 50s) were voluntarily recruited with a written informed consent, and the buccal cells were harvested by using the swab and the resultant genome DNAs were prepared with Qiagen DNA blood-mini kit. Single-copy gene (A: albumin) was assumed to be an internal standardization and the number of relative copies of telomeres (T: telomere) was calculated as ratio (T/A) of respective threshold cycles (Ct) in monochrome multiplex real-time PCR. As a result, typical amplification curves of both albumin gene and telomere were presented in one tube, and each Ct was obtained separately. T/As of the samples analyzed in the present study were 213 ± 205 (13 twenties), 97 ± 126 (21 thirties), 182 ± 200 (21 forties), and 191 ± 185 (34 fifties), in other words, the telomere length resulted in the shortest in thirties. However, because the sample size was too small, and the variations of the data were also large, the re-examination in the future is certainly required why the thirties showed the shortest telomere length. Alternatively, when the correlation analysis with the result of the food-intake frequency investigation done at the same time was conducted, it was interesting to find a negative correlation between the amount of alcohol drinking and the telomere length in man. However, such a correlation was not seen in heavy drinkers. On the other hand, the telomere length has significantly decreased in women who usually take a massive amount of vitamin C. Any association of the telomere length with whichever blood biochemistry test, food frequency questionaire, daily-life activity investigation, or other single nucleotide polymorphisms will be explored. The correlation of the telomere length of the buccal cell and its length of a peripheral-blood lymphocyte is now under investigation.

1397T

Peculiarities of growth hormone and insulin-like growth factor (IGF-1, IGFBP-3) secretion in genetically determined types of short stature in Uzbek population. *N.Sh. Ibragimova, S.I. Ismailov.* Endocrine diseases, Research Institute of Endocrinology, Tashkent, Uzbekistan.

Stunting is a heterogeneous state accompanied by many genetic diseases. Ethnic Uzbeks often marry to close relatives being a risk factor of giving birth to a child with genetic pathology. Medical sources do not have detailed data on studying a hormonal spectrum and pathogenesis of stunting as well as issues of effective utilization of growth hormone in genetically determined types of stunting (GDTS). Goal. To study secretion of GH and insulin-like growth factor (IGF-1, IGFBP-3) in GDTS in the Uzbek population. Materials and methods: 92 patients with GDTS (11 patients with Russell-Silver, 16 ones with Noonan, 10 with Sekkel, 9 with Prader-Willi, 8 with Cornelius de Lange syndromes, 38 girls with TS) at the age of 7 to 18 were examined. Blood serum was tested for the level of GH, IGF-1, IGFBP-3. Anthropometry (SDS) and past history were studied. The control group included 21 healthy children and adolescents of relevant age. Results: According to the past history, parents of 45.6% of patients examined were married to close relatives. Stunting of a various degree was seen in all patients with GDTS but it was most expressed in patients with Russell-Silver (-5.16±1.18 SDS), Sekkel (-4.18±1.12 SDS) and Cornelius de Lange syndromes (-6.10±1.14 SDS). A reliably low level of GH against the control was found in patients with Cornelius de Lange (0.64±0.05 ng/ml, $p<0.05$), Russell-Silver (0.7±0.04 ng/ml, $p<0.05$), Sekkel syndromes (1.02±0.07 ng/ml, $p<0.05$) on the background of a low level of IGF-1 and IGFBP-3. Patients with Noonan syndrome, TS and Prader-Willi syndromes had a GH level within the low limits except for 12 girls of the pubertal age (14 to 16) with TS who had a low level of GH (0.04±0.05 ng/ml, $p<0.05$), the IGF-1 and IGFBP-3 rates corresponded to the low age limits. Conclusions: The results obtained suggest that patients with GDTS of the Uzbek population had stunting of a various degree but the most expressed stunting was found in patients with Russell-Silver, Sekkel and Cornelius de Lange syndromes due to disturbances in the feed-forward and feedback in the GR- IGF-IGFBP-3 system. Girls with TS of the pubertal age had a low GH level and relative deficiency of IGF-1 and IGFBP-3 due to disturbances in the hypophyseal -ovarian system.

1398T

The NINDS Human Genetic Resource Center: A Resource for the Discovery of Genetic Risk Factors for Neurological Disorders. *C. Tarn[1], M. Self[1], E. Londin[1], E. Janeczko[1], A. Scutti[1], K. Reeve[1], K. Gwinn[2], R. Corriveau[2], M. D'Andrea[1].* 1) Coriell Institute for Medical Research, Camden, NJ; 2) National Institute for Neurological Disorders and Stroke-NIH, Bethesda, MD, USA.

The burden of neurological disorders is a serious public health concern and presents a massive challenge to healthcare systems globally. In most cases, the causes of neurological disorders are multifactorial, involving the interactions of many genetic and environmental factors. However, many neurodegenerative disorders such as Parkinsonism and Amyotrophic Lateral Sclerosis (ALS) still lack reliable disease biomarkers. Many studies aimed at identifying novel mechanisms of disease onset require large numbers of biomaterials from both affected and unaffected individuals. The National Institute of Neurological Disorders and Stroke (NINDS) Human Genetics Resource Center (NINDS Repository) is a public resource established to provide a centralized and open resource of biological samples (DNA and cell lines) and corresponding phenotypic data. Many researchers involved in neurodegenerative disease research have integrated into their study protocols the submission of biological samples and associated clinical data to the Repository for processing, storage, and distribution. Since its inception in 2002, biomaterials from more than 30,000 individuals have been banked, with more than 18,000 samples from individuals with cerebrovascular diseases (5,648), Parkinsonism (4,165), motor neuron diseases (2,255), epilepsy (1,043), Tourette syndrome (436), Huntington's disease (15), neurologically-normal controls (5,019) and unaffected primary blood relatives (1,965) publicly available through the Coriell Cell Repositories' web catalog (ccr.coriell.org). To aid high-throughput gene discovery, the Repository offers disease-focused panels of genomic DNA samples in 96-well plate formats as well as custom plate design services. Additionally, genotype data from over 4,000 NINDS Repository samples are available on dbGaP. More than 50,000 samples have been distributed to researchers by the Repository since inception. Analyses of NINDS Repository samples have been published in more than 200 peer-reviewed scientific articles. Furthermore, the Repository has begun to establish and bank fibroblast cell cultures from individuals with neurodegenerative disorders for purposes of cellular reprogramming to generate induced pluripotent stem cells. By establishing a centralized, publicly accessible resource of biospecimens and associated clinical data, the NINDS Repository facilitates discovery of new genetic and molecular signatures that lead to neurological disorders.

1399T

Family history systems in primary care: a public engagement study. *B. Wilson[1], S.M. Craigie[1], D. Castle[2], H. Etchegary[3], J. Allanson[4], D. Avard[5], J.C. Carroll[6], T. Caulfield[7], P. Chakraborty[4], L. Lemyre[8], J. Little[1], K. Morin[9], F.A. Miller[10], B.K. Potter[1], G.A. Wells[1].* 1) Department of Epidemiology & Community Medicine, University of Ottawa, Ottawa, ON, Canada; 2) ESRC Innogen Centre, University of Edinburgh, Edinburgh, United Kingdom; 3) Faculty of Medicine, Memorial University, St. John's, NL,Canada; 4) Department of Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada; 5) Centre of Genomics and Policy, McGill University, Montreal, QC, Canada; 6) Department of Family & Community Medicine Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada; 7) Health Law Institute, University of Alberta, Edmonton, AB, Canada; 8) School of Psychology, University of Ottawa, Ottawa, ON, Canada; 9) Genome Canada, Ottawa, ON, Canada; 10) Department of Health Policy, Management and Evaluation, University of Toronto, Toronto, ON, Canada.

Background Family history (FH) is associated with the risk of many complex diseases, and there are many tools and systems to store FH information and facilitate its use in primary care. There is also a much literature exploring family perspectives on genetic information, particularly around issues of disclosure and communication. Some of these issues might influence individuals' views on FH information. If so, then a health care provider's request to a patient to gather extensive FH information might raise concerns and potential resistance. In addition, given that collecting this information essentially depends on patient self-report, family relationships and dynamics may have a direct effect on the accuracy of the FH data which make their way into the medical chart. **Aims** We developed this study to complement work on developing evidence-based FH tools for implementation in Canadian health care settings. We wished to identify important issues around the systematic (a) collection and (b) storage and use of FH information in primary care populations. **Methods** This study used a qualitative approach supplemented by a structured survey. Issues salient to the general topic (finding out about FH, and using systems to store this information) were identified through literature review and extensive discussion. These were developed into `information sets' which presented the issues in a logical sequence, using lay language. Within a workshop setting, the information sets were presented incrementally, with pauses for facilitated discussion. Individual responses to two key questions were tracked after each information set, using instant voting technology. These questions were: *Do you think [the province] should invest in setting up family health history systems for family doctors?* and *If your own family doctor asked you to, would you be prepared to find out your family health history information and share it with him or her?* At the end of the workshop, participants completed a structured survey with more extensive question sets on attitudes and demographics. This general approach can be adapted for different participant groups, where different issues may be salient according to life stage or other population attributes. We will present findings from workshops involving two groups: (1) otherwise unselected adults aged 50+, and (2) parents of young children, asked to consider FH systems specifically from a parental context.

1400T

The beta-thalassaemia carrier screening process in Australia- Is it acceptable? *N.E. Cousens[1,2], C.L. Gaff[1,2], S.A. Metcalfe[1,2], M.B. Delatycki[1,2,3].* 1) Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, Victoria, Australia; 2) Department of Paediatrics, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Victoria, Australia; 3) Department of Clinical Genetics, Austin Health, Heidelberg, Victoria, Australia.

Background: There is currently no structured) -thalassaemia carrier screening program in Victoria, Australia. Instead it has been incorporated into routine healthcare practice. Many women are screened through a full blood examination (FBE) at their initial prenatal visit, with a low MCV triggering further) -thalassaemia diagnostic testing. The experiences of and attitudes of women found to be carriers through this ad hoc approach to screening are not known. The views of health professionals also have not been ascertained. Aim: To explore carriers' and health professionals' experiences of and attitudes towards the) -thalassaemia carrier screening process in Victoria. Method: Semi-structured interviews were conducted with 26 female carriers and 10 carrier couples of) -thalassaemia who had been pregnant within the last 12 months as well as 23 health professionals. Interviews were analysed using inductive content analysis. Results: Women were identified as carriers at different times of their lives, either before or during pregnancy, with many being screened incidentally as part of routine antenatal testing or testing to diagnose medical symptoms. Most did not recall being told about thalassaemia before being notified of their carrier status. Most did not make a decision about whether or not to be screened, as they reported being unaware screening was taking place, and therefore did not provide informed consent. Most women were happy for their doctors to decide what tests are conducted, however preferred to have been told that they were being screened for thalassaemia before being tested. Health professionals' views of informed consent varied, with some believing that patients should be informed before they are specifically tested for thalassaemia. Women placed a great importance on the information provided after receiving their test results, believing it to be inadequate with this lack of information leading to misconceptions and confusion. Conclusion: Most women in Victoria are not providing informed consent for thalassaemia screening, and do not recall being told about screening being performed. However there was a greater value placed on awareness of testing and information rather than choice and decision-making by both carriers and health professionals. These results suggest that routine screening as part of clinical care is acceptable, but more emphasis should be placed on awareness of the screening process and post-test education of carriers.

1401T

Establishing a Theoretical Financial Framework for Biobanks for the Purpose of Returning Individual Clinically Significant Genetic Information to Research Participants. *A.M. Peterson[1], M.E. Smith[1, 2], S. Aufox[2, 4], D. Dranove[3].* 1) Clinical and Translational Sciences Institute -Northwestern University, Chicago, IL; 2) The NUgene Project - Center for Genetic Medicine - Northwestern University, Chicago, IL; 3) Kellogg school of management - Northwestern University, Evanston, IL; 4) Center for Genetic Medicine - Northwestern University, Chicago, IL.

Background: Disclosing genetic research results to participants is controversial, particularly when clinical utility or validity has not been established. This debate is founded in ethical, legal, and practical concerns. While ethical and legal concerns have been addressed previously, the practical concerns, including the financial ramifications, have yet to be addressed through scientific analysis. This is especially relevant to large-scale biobanks, which are increasingly accessed for genetic research. The objective of this study is to explore potential avenues where costs may arise throughout the process of returning research results to biobank participants. Methods: Semi-structured interviews were utilized with purposefully selected biobanking professionals. Participants were asked: 1) Their initial impressions surrounding the return process, 2) The methodology they would use to determine clinical significance of a research finding, 3) How they would return results and, 4) What, if any, follow-up would be implemented post-disclosure. Interviewees' responses were analyzed for themes and were used to develop a theoretical model for returning results. The model was assessed for anticipated costs. Results: The seven interviewees had a range of industry experience (0.8-10 years), biobank size (1,400 - 20,000) and consenting processes. Interview data illustrated a lack of consensus on method for returning results. All participants felt their respective IRBs should be consulted and results need to be clinically useful to warrant return. The majority felt there were circumstances where they could consider returning results, would consult advisory groups, utilize multiple attempts and mechanisms for contact, and confirm results in a CLIA lab. Five of 7 would offer genetic counseling for follow-up. The return model illustrates potential costs, ranging from $52.83 - $2,054.65 per participant. Conclusions: Returning research results is not commonly practiced among biobanks. Clearly, biobanking professionals are thinking about the issue. Returning genetic information to research participants could be both time consuming and costly.

1402T

The MiSeq DNA sequencing platform and its application to Public Health. *G.P. Smith[1], W. Meuleman[1], J. Betley[1], J. Becq[1], O. Schultz-Trieglaff[1], L. Murray[1], M. Bauer[1], F. Oaks[2], J. Yeager[2], MiSeq program team.* 1) Illumina Cambridge Ltd, Saffron Walden, United Kingdom; 2) Illumina Inc., Hayward and San Diego, CA. USA.

Next generation sequencing has enabled the routine sequencing of whole human genomes at a scale of many thousands per year. Over the last few years technology advances with the Illumina platform has increased throughput to over 1Tb of high quality data per run, and reduced the costs of sequencing a human genome to less than $10,000. Although these DNA sequencing systems provide an optimised platform for the sequencing of large genomes, there are many projects that require much smaller amounts of data, and faster turnaround times from sample to answer. We have developed a new sequencing system, called MiSeq, that generates over 1Gb of data from 2x150 base reads in just over a single day. Miseq integrates all the steps of the Illumina sequencing process from cluster generation, to paired end sequencing reads, into a fully automated `hands-off' workflow that involves 20 minutes of set up time, and includes the steps of primary and secondary data analysis, including variant calling and de novo assembly. At its core, Miseq runs the same reversible terminator SBS chemistry as the high throughput Hiseq and GAIIx systems, and benefits from the knowledge and understanding that has been acquired in over 1000 peer-reviewed publications. To simplify the upstream library preparation process, we have developed a transposon-based method called Nextera, that generates Illumina-compatible libraries from amplicons or genomic DNA within 1.5 hours and makes possible a complete workflow on MiSeq from sample-to-answer within 8hours, or a single working day, including analysis time. In this study we demonstrate that a single run of the MiSeq instrument is capable of highly accurate and deep sequencing of a range of human pathogens, including E. coli, MRSA and Pseudomonas aeruginosa. In addition, the data is well suited to the de novo assembly of these bacteria, rivalling performance of the higher throughput (and more expensive) machines. The rapid turnaround of the system, coupled with the simple operation of the instrument, makes possible the deployment of Next Generation sequencing in the clinical setting, revealing details of strain identification, antibiotic sensitivity and epidemiology of human pathogens at base-pair resolution. Examples of the integration of the technology into the workflow of MRSA strain characterisation will be provided.

1403T

Candidate gene variants and susceptibility to infection by hepatitis A and E viruses in the US population: analysis using the Third National Health and Nutrition Examination Survey (NHANES III), 1991-1994. *L. Zhang[1], M.H. Chang[2], D.J. Hu[1], E. Teshale[1], N.F. Dowling[2], C.G. Teo[1].* 1) Division of Vrial Hepatitis, Centers for Disease Control and Prevention, Atlanta, GA; 2) Office of Public Health Genomics, Centers for Disease Control and Prevention, Atlanta, GA.

The 2 main causes of enterically-transmitted hepatitis are hepatitis A virus (HAV) or hepatitis E virus (HEV). These viruses are widely prevalent and cause significant public health problems in many countries. Prior to the introduction of hepatitis A vaccine in the US in 1995, HAV periodically caused large, nationwide epidemics, leading to substantial morbidity and economic losses. HEV is not associated with substantial morbidity and mortality in the US, but appears highly prevalent. Human genetic variation is a determinant of viral hepatitis but to date studies of human genetic variation for hepatitis A are few and there are none for hepatitis E. We evaluated associations between 510 single-nucleotide polymorphisms (SNPs) in 200 candidate genes and seropositivity for HAV and HEV among 7159 participants from the second phase (1991-1994) of the Third National Health and Nutrition Examination Survey (NHANES III), a population-based and nationally representative survey of the US. Individuals aged 12 years and older who self-reported as non-Hispanic white (n = 2434), non-Hispanic black (n = 1919), and Mexican American (n = 1919) were included in analysis. Among non-Hispanic whites, non-Hispanic blacks, and Mexican Americans, respectively, the number (weighted frequency) of seropositivity was as follows: 958 (24.9%), 802 (39.2%), and 1540 (71.5%) for HAV; 628 (22.3%), 279 (15.3%), and 449 (21.8%) for HEV; and 304 (32.3%), 156 (21.9%), and 415 (27.3%) for both HAV and HEV. No significant associations between seropositivity for HAV or HEV infection and any of the 510 SNPs were observed among non-Hispanic whites or non-Hispanic blacks. Among Mexican Americans, 3 SNPs located in *ABCB1*, *APOA4*, and *VLDLR* associated with HAV-seropositivity and 1 SNP in GSDML associated with HEV-seropositivity were identified (p-value adjusted for false discovery rate < 0.05). Seropositivity for both HAV and HEV was associated with SNPs in *CDKAL1*, *IL10*, *IL7R*, and *ILF* among non-Hispanic whites, *AFF1* among non-Hispanic blacks, and *TMEM18* among Mexican Americans. **Conclusions**: Susceptibility to infection by HAV and HEV may be genetically associated, and the nature of associations varies across the three racial/ethnic groups. Replication studies involving larger population samples are warranted.

1404T

Evaluation of cystine in urine samples for a diagnostic approach in Colombian cystinuria. *L. Buitrago Alvarado, J. Benavides Sanchez, A. Uribe Ardila.* Centro de Investigaciones en Bioquimica(CIBI), Departamento de Ciencias Biologicas, Facultad de Ciencias, University of the Andes, Bogota, D.C., Colombia.

INTRODUCTION Cystinuria is an autosomal recessive metabolic disorder, with an estimated incidence of 1:7.000 births. It is caused by the defect in intestinal and renal transport of dibasic amino acids cysteine, ornithine, lysine and arginine, causing predisposition for the formation of stones in the urinary system. Early detection improves disease prognosis and prevents further complications, hence the importance of developing screening protocols that do not involve prolonged urine collections, a key objective of this study. METHODOLOGY We performed high-risk screening by qualitative tests (sodium nitroprusside) and semiqualitative (chromatography). Spectrophotometric quantification is performed by a modification of the Brand test for cystine. For the data analysis we used the statistical program SPSS 18, the Kolmogorov-Smirnov normality test, and Ln was used for homogeneity of variance. RESULTS AND ANALYSIS We evaluated samples from 148 individuals, ranging in age from newborns to 63 years, this involves 69 normal controls, 79 patients followed for nephrolithiasis in which five were related to Cystinuria biochemical findings such as positive nitroprusside, chromatographic patterns of cystine, ornithine, and lysine and increased levels of cystine in voided urine samples. We established that the normal value of cystine was 11.9± 11.5 mg/g creatinine. Cystines values of affected individuals were higher than 37.2 mg/g creatinine. Was established as a cutoff 30.3 mg/g creatinine. There is a correlation between the concentration of cystine and creatinine of an individual ($p<0.01$), according to an ANCOVA analysis, and there is a correlation between the concentration of cystine and the age of individuals, men and women ($p<0.02$). These results are highly significant because they suggest age and sex should be taken into account as a parameter for quantification of metabolite creatinuria, and the collection of urine for 24 hours as currently used is not sufficient. CONCLUSION There is a marked difference between affected and healthy individuals in relation to concentrations of cystine in urine. This allows for a preliminary reference value for the Colombian population and improves the differential diagnosis.

1405T

Newborn Screening Education: What information is important to expecting mothers? *S.M. Craigie[1], B.K. Potter[1], B.J. Wilson[1], J. Allanson[2], D. Avard[3], M. Cappelli[4], J.C. Carroll[5], D. Castle[6], P. Chakraborty[7], H. Etcheg-ary[8], J. Grimshaw[9], L. Lemyre[10], J. Little[1], J. Milburn[7], F.A. Miller[11], K. Morin[12], G. Wells[13].* 1) Epidemiology & Community Medicine, University of Ottawa, 451 Smyth Rd. Ottawa, ON K1H 8M5 Canada; 2) Children's Hospital of Eastern Ontario, Department of Genetics, 401 Smyth Road, Ottawa, ON K1H 8L1 Canada; 3) Centre of Genomics and Policy Faculty of Medicine, Dept. of Human Genetics McGill University 740 Dr. Penfield Avenue, Room 5210 Montreal QC; 4) Children's Hospital of Eastern Ontario, Clinical Psy-chology, CHEO Research Institute, 401 Smyth Road, Room R1120 Ottawa, ON K1H 8L1 Canada; 5) Department of Family & Community Medicine, Mount Sinai Hospital, University of Toronto, Granovsky Gluskin Family Medi-cine Centre, 60 Murray St., 4th Floor, Box 25, Toronto, Ontario M5T 3L9 Canada; 6) ESRC Innogen Centre, University of Edinburgh, High School Yards, Edinburgh EH1 1LZ Scotland; 7) Children's Hospital of Eastern Ontario Ontario Newborn Screening Program 401 Smyth Road Ottawa, ON K1H 8L1 Canada; 8) Eastern Health,Clinical Epidemiology, Faculty of Medicine, Memorial University Room H1761, Level 1 Health Sciences Centre 300 Prince Phillip Drive St. John's NL A1B 3V6 Canada; 9) Ottawa Health Research Institute, Clinical Epidemiology Program, 1053 Carling Ave., Ottawa, ON K1Y 4E9 Canada; 10) University of Ottawa, School of Psychol-ogy Institute of Population Health 1 Stewart St, Room 312 Ottawa, ON K1N 6N5 Canada; 11) Department of Health Policy, Management and Evaluation Faculty of Medicine, University of Toronto 155 College Street, 4th Floor Toronto, ON M5T 3M6 Canada; 12) Genome Canada, National GE3LS Program, 150 Metcalfe Street, Suite 2100, Ottawa, ON K2P 1P1 Canada; 13) University of Ottawa Heart Institute H1-1, 40 Ruskin Street Ottawa, ON K1Y 4W7 Canada.

Background: Newborn screening (NBS) in Ontario and many other juris-dictions is delivered as standard care: parents may opt out but it is not explicitly presented as a choice for most. Issues around education and consent have recently come to the fore in the context of debate about expansion of NBS programs and attention to policies related to the storage and secondary use of NBS bloodspots. There is some evidence that parents currently receive limited education about NBS and that what they do receive focuses mainly on the purpose, benefits, and process of screening. Previous work suggests that mothers may prefer more comprehensive information, but NBS programs may be concerned about potential for decreased uptake of the service with provision of additional information, particularly that which emphasizes possible harms. Concerns have also been expressed that pro-viding parents with too much information may compromise understanding or foster anxiety. **Objectives:** The goal of this study is to measure and compare expecting mothers' responses to different educational messages about NBS. By identifying the messages that appear most salient to mothers with respect to their intentions/decision-making about participating in NBS, this work will contribute to ongoing discussions about appropriate educa-tional content and the links between education and parental involvement in decision-making. **Methods and Findings:** We are conducting a self-com-plete survey with pregnant women attending routine 16-20 week ultrasounds at two clinics in Ottawa, ON, Canada. The survey has a factorial design: expecting mothers receive differing messages about newborn screening including those related to the possibility of false positive/negative results; pain from the heel-prick; potential for overdiagnosis of infants not at serious risk of illness; storage and secondary use of bloodspots; and parental choice/consent. Participants respond to questions measuring intention to screen their baby, level of decisional conflict about this decision, and understanding of the messages. Data collection is underway. **Implications:** The findings will have implications for the design and delivery of parental education about NBS in Ontario and other jurisdictions; and will also inform a broader debate about the roles of parents, providers and policy makers in decisions about NBS.

1406T

Haptoglobin phenotypes in Saudi Arabia. *S. Sayeeduddin.* Laboratory Medicine, AlJouf University, Sakaka, AlJouf, Saudi Arabia.

Objective: To find out frequencies of different phenotypes of haptoglobin among Saudi population. Materials and Methods: Eighty one (81) randomly selected people visiting clinics at King Faisal Hospital, Qurayat town, volun-teered to be the subject of this study. Sera collected were analyzed for haptoglobin phenotypes. Haptoglobin phenotypes were determined by verti-cal polyacrylamide gel electrophoresis, and the bands were visualized by staining with benzedine solution (Awadallah and Hamad, 2000). Results: It was observed that maximum number of subjects, 38 (46.9 %) out of 81, belonged to HP 2-2. The HP 2-1 were 29 (35.8 %) and remaining 14 (17.5 %) were of HP 1-1 type. Conclusions: The present work is the preliminary report being reported from the Northern region (Al-Qurayat population in the AlJouf region). Future plans are to extend the study to include the population from other regions of the kingdom and also increase the sam-ple size.

1407T

Access to genetic health care: qualitative study of new universal screening for sickle cell and thalassaemia disorders in England. *J. Kai[1], F. Ulph[2], T. Cullinan[1], N. Qureshi[1].* 1) Division of Primary Care, University of Nottingham, Nottingham, United Kingdom; 2) University of Manchester, UK.

Background: Universal antenatal and newborn population screening for haemoglobin disorders has recently been implemented across the National Health Service in England. This provides an important testing ground for the expansion of genetic health care, and appropriate access to its potential benefits by those diverse minority ethnic communities with highest preva-lence of these disorders in the UK. **Aim:** This study aimed to explore experi-ences of access to new genetic screening for sickle cell and thalassaemia disorders in England. **Methods:** The research was conducted within screen-ing services across 13 hospital provider units and primary care localities in two regions of England with ethnically diverse populations. This involved analysis of service processes and communications to patients; key informant interviews with 19 health professionals and user groups; and substantive qualitative interview study with a purposeful sample of 37 parents with experience of sickle cell or thalassaemia carrier identification following screening, with grounded approach to data analysis; and respondent valida-tion and discussion of findings with parents, health professionals and user groups. **Results:** Users articulated those components of services and out-comes they had valued or would value. However there was wide variation in the quality and accessibility of information for patients. Experience of communication within critical screening interactions with professionals could be poor, with, for example, incomplete exchange or reception of the benefits of screening or diagnostic tests, or the problematic use of family members as interpreters in this context. This compromised access across the screening pathway, generating anxiety and misunderstanding, and lowering opportuni-ties for users to be fully informed. Desire to avoid anxiety or risk during pregnancy could also lead some users to decline services or not pursue information or testing as part of antenatal screening. While service influences and organisation appeared dominant, low community awareness of disor-ders also shaped misperceptions and stigma about screening. **Conclu-sions:** Many factors that may compromise access to haemoglobin disorder screening and the promotion of fully informed choices reflect the way ser-vices are provided rather than patient-specific influences or choice. These factors are likely to be salient to other areas of genetic service development and delivery.

1408T

Familial breast cancer: Risk communication, predictive testing and management in four European countries. *I. Nippert[1], H. Harris[2], C. Julian-Reynier[3], J. Schmidtke[4], C. van Asperen[5], A. Tibben[5,6], D.G. Evans[7,8].* 1) Women's Health Research, Westfälische Wilhelms-Universität, Münster Medical School, Münster, Germany; 2) GenEd Coordinating Centre, Univer-sity of Manchester, Manchester, UK; 3) U912, INSERM, IRD, Aix-Marseille Université, Institut Paoli-Calmettes, Marseille, France; 4) Institute of Human Genetics, Hannover Medical School, Hannover, Germany; 5) Centre for Human and Clinical Genetics, Department of Clinical Genetics, Leiden Uni-versity Medical Centre (LUMC), Leiden, The Netherlands; 6) Department of Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands; 7) Genetic Medicine MAHSC, Central Manchester University Hospitals NHS Founda-tion Trust, St Mary's Hospital, Manchester UK; 8) Genesis Prevention Cen-tre, University Hospital South Manchester, Wythenshawe Hospital, Man-chester, UK.

Background: Genetic testing has its greatest public health value when it identifies individuals who will benefit from specific interventions based upon their risk. This paradigm is the basis for the use of predictive tests, such as BRCA1/2 testing, which has become part of clinical practice for more than a decade. Currently in Western European countries predictive BRCA1/2 testing is offered to women using low, moderate and high risk based upon family history as cut-off levels. Non-genetic health professionals, such as general practitioners (GPs) are seen as gate keepers to manage demand and/or facilitate access for high risk women to adequate services.

Methods: Assess current practice of GP's cancer risk communication and management of familial breast cancer in Germany (DE), France (F), The Netherlands (NL) and the United Kingdom (UK).

Results: 1197 GPs completed a 70 item questionnaire. The majority of GPs report that when an unaffected person presents with cancer concerns they will take a family history (DE 95.7%, F 93.8%, NL 83.7%, UK 80.2%) and provide risk assessment based upon the family history (DE 61.5%, F 67.9%, NL 53.9%, UK 51.7%). However, less than half of the respondents in NL (47.7%) and UK (46.1%) would ask about cancer history of the extended family. Only 44.8% (DE), 26.5% (F) and 43.5% (NL) agreed that inheritance patterns of familial breast cancer should be explained by a genetic specialist whereas 30.1% (DE), 63.6% (F), 49.7% (NL) and 33.8% (UK) state that this should be better done by themselves.

Discussion: There is a critical disconnect between GPs and genetic spe-cialists. The observed tendency in the four countries is that GPs prefer to assess and communicate genetic risks themselves and are unaware that they may not perform adequate risk assessment and risk communication.

1409T

Developing and validating clinical genetics-specific patient reported outcome measures: Are we finally at the end of a long and winding road? *M. McAllister[1], A.M. Wood[2], G. Dunn[3], K. Payne[3], L. Davies[3], S. Shiloh[4], C. Todd[5].* 1) Genetic Medicine, School of Biomedicine, The University of Manchester, Manchester, UK; 2) School of Psychological Sciences, The University of Manchester, Manchester, UK; 3) School of Community-Based Medicine, The University of Manchester, Manchester, UK; 4) Department of Psychology, Univerity of Tel Aviv, Israel; 5) School of Nursing, Midwifery & Social Work, The University of Manchester, Manchester, UK.

An enduring challenge in clinical genetics is how best to measure patient benefits. PROMs offer a possible solution. PROMs are short self-completion questionnaires that can capture aspects of health and health-related quality of life that come directly from the patient. PROMs are used in research to generate evidence of patient benefit from new interventions, for example as the primary outcome in a randomised controlled trial. PROMS are also be used to collect data on patient outcomes in routine clinical practice to enable patient choice and control. US and UK health policy encourages use of PROMs data. However in clinical genetics, there is no consensus about the best PROMs to use. This talk will present findings from a UK programme of research (2003-2011), involving some 450 patients and 130 clinicians, to address the problem of PROMs use in clinical genetics. Mixed-methods were used to identify patient benefits from using clinical genetics: (a) systematic literature review of validated outcome measures used in the field (b) Delphi survey to identify consensus amongst UK clinicians and patients about appropriate outcome domains and (c) qualitative focus groups and interviews. The next stage involved validating two clinical genetics specific PROMs with potential to capture patient benefits: the Genetic Counseling Outcome Scale (GCOS-24) and the Perceived Personal Control (PPC) Scale. The 24-item GCOS-24 was developed to capture a construct, empowerment, summarising patient benefits identified in the qualitative research. Both internal consistency of the GCOS-24 ((=0.87) and test-retest reliability (r = 0.86) are good. Concurrent validity and sensitivity to change over time (Cohens d=0.70) were also demonstrated. The first psychometric validation of the English translation of the Perceived Personal Control scale (PPC-E) was also conducted. The PPC-E was demonstrated to have (a) a one factor structure (b) concurrent validity (c) high internal consistency ((=0.83) (d) sensitivity to change and able to identify moderate changes in PPC (Cohens d=0.40) following clinic attendance. These properties suggest that both the GCOS-24 and the PPC-E are appropriate validated PROMS to (1) evaluate the impact of introducing new interventions in clinical genetics research and (2) collect data on patient outcomes in routine clinical genetics practice. Both PROMs would benefit from further psychometric validation such as testing for known groups and discriminant validity.

1410T

Impact of family history information on accuracy of cardiovascular disease prediction in middle aged women. *Q. Hasanaj[1], BJ. Wilson[1], J. Little[1], Z. Montazeri[1], GJ. Prescott[2] on behalf of CIHR Emerging Team in Genomics in Screening.* 1) Epidemiology, University of Ottawa, Ottawa, ON, Canada; 2) Department of Public Health, University of Aberdeen, Aberdeen, UK.

Background: Positive family history (FH) is associated with risk of many common complex diseases. Most clinical guidelines mention FH as important in assessing disease risks. However, the evidence base to inform the routine use of FH in primary care is largely lacking. The 2009 NIH State-of-the-Science Panel on Family History and Improving Health concluded that, for FH to be established as an evidence-based tool, there is a need to evaluate its predictive ability and prognostic value in combination with traditional risk factors. The limited research published to date suggests that FH might offer statistically significant improvements to disease risk classification, but clinical utility still needs to be adequately evaluated. We report the findings of a study of cardiovascular disease risk prediction in women, in which the impact on clinical decisions of adding FH information to guideline-based risk factor information is provisionally evaluated. **Objectives:** To assess the incremental improvement in individual risk prediction which is gained by adding FH to other clinical information recommended in guidelines, and its potential impact on patient classification and management. **Methods:** This is a secondary cross-sectional analysis of data from the Aberdeen Study of Cardiovascular Health in Women, a retrospective cohort study. The dataset contains information on a range of risk factors as well as FH. Cardiovascular disease outcomes defined according to the Rose Angina Questionnaire and/or Minnesota EKG coding. We used a specific guideline applicable to this British study population (`JBS2') to select variables for univariate and logistic regression analyses. We developed three models: Model 1 included only the covariates in JBS 2; Model 2 added FH to Model 1; and Model 3, which we developed using a fuller range of risk factors available in the dataset, including FH. For each model, the predictive/discriminatory ability was evaluated using ROC curves. **Results:** We will present: the three models, their accompanying ROC curves, and relevant statistics; details of the original classification and reclassification of participants between risk groups resulting from the different models; and comments on the likely effects of reclassification on guideline-based clinical management recommendations, i.e. the changes in specific recommended preventive interventions which follow when participants shift from lower to higher risk strata and vice versa.

1411T

Weighted Metrics for Assessing Quality of Regional Public Health and Clinical Genetics Services. *J. Mulvihill, S. Whitehead, A. Chou, Genetics Systems Assessment Working Group.* Dept Pediatrics & Genetics, OU Medical Ctr, Children's, Oklahoma City, OK.

As health care costs increase, emphasis is being placed on assessing, reporting, monitoring, and improving quality. Using metrics to assure quality has been implemented for management of common chronic diseases, but there is a growing recognition that public health and clinical and laboratory genetics should likewise develop and apply measurements of quality. The objective of this project is to identify and pilot test a set of metrics as a tool for assessing the quality of genetics services within a geographic region of the US. A systematic review of literature (Amer J Med Genet 2009;151C:214-234), prior guidelines, and professional statements yielded 61 measures for further review and selection. A panel drawn from a US pool of experts and stakeholders was assembled and conducted a modified Delphi Technique to rank the measures on a five point Likert scale, according to criteria of relevance, feasibility for evaluation, and strength of scientific evidence. Ratings were computed to generate a score for each measure. Three iterations of the Delphi process and consensus discussion among the panel identified 25 measures for pilot testing. The Departments of Health of Hawaii and of Washington were pilot sites since both have been perceived as “high-performers” in public health and clinical genetics programs. The states' genetics coordinators completed the tool, with input from their databases, local geneticists, genetic counselors, and others. The current metrics tool has five domains (Capacity of Services, Access, Clinical Processes and Quality Improvement, Performance Reporting, and Workforce) with a total of 18 measures. The measures are being weighted for importance in final scoring. After review and clarifications and documentation to guide implementation, the eight states in the Heartland Genetics and Newborn Screening Collaborative (AR, IA, KS, MO, ND, NE, OK, SD) have volunteered to use the Genetics Systems Assessment Tool. The hope is results will facilitate mutual assistance among states and regions and sustain funding to assure the public has access to high quality public health and clinical genetics and genomic services. (US DHHS, Health Services and Resource Administration, Bureau of Maternal and Child Health, Genetics Services Branch, Cooperative Agreement to University of Oklahoma Health Sciences Center, U22MC03962).

1412T

Predictive genetic tests of alcohol intolerance for moderate and responsible drinking; Actions for reducing harmful drinking. *Y. Ohta[1], S. Suzuki[2], F. Kato[1], M. Sami[1], T. Kanda[1], I. Kobayashi[3].* 1) Res Lab for Fundamental Tech, Asahi Breweries, LTD., Ibaraki, Japan; 2) Shonan Kamakura General Hospital, Kanagawa, Japan; 3) Central Hospital, National Cancer Center, Tokyo, Japan.

Health hazards from inappropriate alcohol intakes are increasing year by year(1). To reduce this world-wide public health problems, efforts for eradicate harmful drinking from the society are strongly required. We established a convenient genetic test for the SNP rs671 on Aldehyde Dehydrogenase 2 gene, which uses saliva samples. Additionally, that system does not collect any personal genetic information. With the educational seminars on moderate and responsible drinking and drinking rules, we have provided this DNA test free of charge. (1); World Health Organization, “Global strategy to reduce the harmful use of alcohol”.

1413T

Medical Genetics in Southeast Asia: Status and Current Drivers. *J.K. Thompson[1], M. Laurino[2], D.L. Sternen[3], K. Leppig[4].* 1) Provincial Medical Genetics Program, Children's and Women's Health Centre of British Columbia, Vancouver, BC; 2) Institute for Public Health Genetics, University of Washington, Seattle WA; 3) Division of Genetic Medicine, Seattle Children's Hospital, Seattle, WA; 4) Genetic Services, Group Health Cooperative, Seattle, WA.

There is a broad range of genetic services and testing currently available for the 593 million individuals residing in Southeast Asia (SEA). Some countries have nascent newborn screening while others have a broad spectrum of specialized genetic services. It is foreseeable that the cost of genetic testing will continually decrease with its increasing utilization (e.g., clinical testing, direct to consumer testing, pharmacogenetics, etc.). Thus, we propose possible strategies for infrastructure development of genetic programs in SEA to meet these demands. Through literature review using PubMed, Medline, World Wide Web searches, and personal correspondence, we assessed the current status of newborn screening programs, genetic counseling training programs, and the utilization of clinical genetic technology for diagnoses and carrier screening for each country in SEA. Using the United Nations classification of geographical regions, countries in SEA include: Brunei Darussalam, Cambodia, Indonesia, Lao People's Democratic Republic, Malaysia, Burma (Myanmar), Philippines, Singapore, Thailand, Timor-Leste, and Viet Nam. National newborn screening programs have been implemented in Indonesia, the Philippines, Singapore, and Thailand. Other countries are conducting pilot newborn screening projects and anticipate program implementation in the near future. Clinical genetic services, including diagnostic testing and screening, are available in Indonesia, the Philippines, Singapore, Thailand and Viet Nam, albeit with varying degrees of access, resources, and specialization. The newly implemented genetic counseling program in the Philippines could serve as a potential model for developing an educated work force in SEA that can meet consumer demands for genetic services. Training health professionals to offer genetic counseling services is a cost-effective public health strategy. It provides the opportunity to improve the identification, diagnosis and support of management care of individuals with congenital and genetic conditions. Given the variability of available genetic services in SEA, the development of sustainable and comprehensive genetic services to disseminate accurate genetic information is now of utmost importance. Cultural competency in acknowledging the various cultural and spiritual perceptions on genetic attribution towards disease development and self-sustainability is imperative for success.

1414T

Cystic Fibrosis carrier screening: customer satisfaction study. *L. Ditta, S. Egiziano, C. Salbe, M. Mattiuzzo, L. Tognetto, A. Brugnoli, G. Romagnosi, M. Gion, L. Bartoloni.* Clinical Pathology, ULSS12 Veneziana, Venezia, Italy.

We have been performing pre-conceptional identification of Cystic Fibrosis (CF) heterozygotes since 2001. 13168 subjects were analyzed and 400 carriers found. We refined the distribution of the different CFTR mutations in our population, defining a more sensitive and less expensive mutation panel. To assess the impact of this test on the carriers, we performed more than 100 phone interviews. Even if the couples were followed by gynecologists and family doctors and received an informative leaflet and a written report, the majority of the carriers didn't know what CF was and what a carrier status meant when they received the written report. They asked for information the gynecologist, the family doctor and they searched the internet (three options that often gave wrong information). Talking to a geneticist was the fourth choice, just before asking friends. The majority of the women were prescribed the test during the pregnancy and not before, and there isn't an agreed protocol for testing (both members at the same time or one member first). Only 39% of the carriers decided to have a second level test(DHPLC or sequencing of the entire gene) for their partner, and 12% of them got a positive response too. This five couples were not influenced in their reproductive choices by the positive test, and asked for a CF pre-natal test for all their pregnancies, with no double heterozygote babies to date. The carriers that did not screen the partner with a second level test (61%) did not report the birth of CF babies. Even if the interviewed carriers were happy with the test and suggest to their friends and family to be tested, the results of the questionnaire forced us to change the way we deliver the report and to improve the dissemination of informations among the physicians and the public.

1415T

Recognizing health care needs of orofacial clefts individuals during genetics evaluation in Alagoas, Brazil. *M.I.B. Fontes[1], K.M. Santos[1], J.I. Vieira Filho[1], A.K.M. Andrade[2], F.S. Anjos[1], T.P. Vieira[3], I.C. Sgardioli[3], N.L.V Campos[3], I.L. Monlleó M.D.; PhD.[1], V.L. Gil-da-Silva-Lopes M.D.; PhD.[3].* 1) Department of Pediatrics, State University of Alagoas, Maceió, Alagoas, Brazil; 2) Medical Genetics Sector, Federal University of Alagoas, Maceio, Alagoas, Brazil; 3) Department of Medical Genetics, University of Campinas, Campinas, Sao Paulo, Brazil.

Introduction: Orofacial clefts (OC) occur in approximately 1/600 newborns babies worldwide and are among the most common birth defects. Multidisciplinary, team-based, coordinated and family-centered healthcare is accepted as the state of the art in this field. Public healthcare for patients with orofacial clefts was structured in 1998 in Brazil through 21 units arranged as a network unevenly distributed. Alagoas is a poor state located in the Northeast region of the country in which there is not specialized center for care of patients with OC. Aim: to describe demographic and clinical-genetic characteristics as a subside to structure healthcare for patients with OC in Alagoas. Methods: All patients were systematically seen by two clinical geneticists using a pre-tested protocol from September 2009 to February 2011. Conventional and molecular cytogenetic analyses were performed in selected patients. Fisher Test was used for statistics with p-value<0.05.Results: majority of patients were males, country side residents, with isolated cleft lip with cleft palate. Ages ranged between 0-37 years. Thirty six percent had never undergone surgery while 100% had never attended to genetic evaluation. Isolated cleft was diagnosed in 75%, syndromes in 14.4%, multiple congenital defects in 9.6% cases and additive randomic defects in 1.0%. Recurrence and parental consanguinity were, respectively, 28.7% and 9.7%. Twenty-three individuals without cleft surgery were registered for multidisciplinary treatment. Conclusions: Clinical-genetic characteristics corroborate the literature. Findings revealed high levels of unmet medical needs and provided an evidence base for health care planning. Support: Fapeal, Fapesp, CNPq.

1416T

Novel mouse models for hearing loss associated with proximal 1p36 deletions. *H. Zaveri[1], B.J. Kim[1], O.A. Shchelochkov[2], F.A. Pereira[3], A.K. Groves[1], J.S. Oghalai[4], M. Justice[1], B. Lee[1], D.A. Scott[1].* 1) Molec & Human Gen, Baylor College Med, Houston, TX; 2) Dept of Pediatrics, University of Iowa, Iowa City, IA; 3) Cell & Molecular Biology, Baylor College Med, Houston, TX; 4) Otolaryngology, Stanford School of Medicine, Palo Alto, CA.

Terminal and interstitial deletions of 1p36 are a common cause of cognitive and developmental delay with an incidence of 1 in 5000 newborns. Although 28% of individuals with 1p36 deletions also have sensorineural hearing loss, the gene(s) responsible for this phenotype have yet to be identified. The arginine-glutamic acid dipeptide repeats gene (*RERE*), located in the proximal region of 1p36, encodes a nuclear receptor coregulator that is required for normal embryogenesis, and is highly conserved from *Drosophila* to humans. In zebrafish, reduced expression of the *RERE* homolog *Rerea* leads to inner ear anomalies—fused otoliths and abnormal semicircular canals—and diminished microphonic potentials. We hypothesized that *RERE* plays a similar role in the development of the inner ear in mammals. To test this hypothesis, we used immunohistochemistry to show that RERE is expressed in critical regions of the mouse inner ear including the inner and outer hair cells and the marginal and basal cell layers of the stria vascularis. However, further studies of the function of RERE in the inner ear were hampered by the early lethality seen in *Rere* null mice (*Rere*$^{-/-}$), which die in utero at E9.5 due to cardiac failure. To overcome this, we generated an allelic series of RERE-deficient mice bearing different combinations of a *Rere* null allele and a hypomorphic allele (V193A) we identified in an ENU mutagenesis screen. *Rere*$^{-/\ V193A}$ mice have diminished startle responses to a 108 dB burst at 19.9 kHz emitted from a click box—a common preliminary test for severe hearing loss. Distortion product otoacoustic emission (DPOAE) and auditory brainstem evoked response (ABER) testing at p21 revealed that *Rere*$^{-/\ V193A}$ and *Rere*$^{V193A\ /\ V193A}$ mice have early onset hearing loss. *Rere*$^{-/\ V193A}$ mice also fared poorly in the dowel test which requires normal balance and coordination. Histological analyses and 3D micro-CT reconstructions of the inner ear showed that *Rere*$^{-/\ V193A}$ mice have normal cochlear morphology at p21 but have increased cross-sectional diameter of their semi-circular canals when compared to their wild type littermates. We conclude that RERE is required for normal hearing and vestibular function and that *Rere*$^{-/\ V193A}$ and *Rere*$^{V193A\ /\ V193A}$ mice represent useful models for studying the molecular mechanisms by which RERE-deficiency contributes to the hearing loss in individuals with 1p36 proximal deletions.

1417T

Alternative polyadenylation: gene mechanism to avoid miRNA regulation. *C. Barbacioru[1], F. Tang[3], E. Nordman[1], E. Heard[4], K. Lao[1], A. Surani[2].* 1) Life Technologies, Foster City, CA; 2) Wellcome Trust/Cancer Research UK Gurdon Institute of Cancer and Developmental Biology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QN, UK; 3) BIOPIC, School of Life Sciences, Peking University, Beijing, 100871, China; 4) CNRS UMR3215, INSERM U934, Institut Curie, Paris F-75248, France.

Many protein-coding genes have more than one polyadenylation site, so a gene can code for several mRNAs that differ in their 3' end. The single cell RNA-Seq analysis uses poly(T) primer for reverse transcription, which is biased towards a better coverage of the 3' end of mRNAs and therefore enhances detection of alternative polyadenylation identified as coverage peaks of the 3'UTRs. Here we performed single cell RNA-Seq analysis of single blastomeres of mouse embryos, which revealed significant changes in the transcriptome. We found that 1,718 genes have two transcript isoforms with the same coding region but different lengths of 3'UTR, suggesting that approximately 13% of expressed genes exhibit at least two isoforms with long (distal) and short (proximal) 3'UTR in the same cell at the same time. Prediction of polyadenylation sites are searched for the presence of CPSF binding motif site located at -20 nt upstream of their 3'end and we found 40% of short (proximal) and 70% of long (distal) 3'UTRs, respectively, suggesting that the stop of majority of these 3'UTRs are at the canonical polyadenylation recognition sites. We propose that in individual blastomeres, the majority of transcripts from a gene have a short 3'UTR, which will be translated constantly, whereas for a small number of transcripts from the same gene with long 3'UTR, their translation will be dynamically regulated by microRNAs or RNA-binding proteins. This would allow relatively stable translation of a large set of genes, while permitting subtle adjustment in protein levels. The abundance of transcript isoforms with short 3'UTR is about 5 - 6 fold higher compared with those from the same gene with a long 3'UTR in two-cell embryos. Thus, the impact of miRNA regulation of mRNA translation for these mRNAs with two different lengths of 3'UTRs in early blastomeres is probably negligible. Interestingly, whereas the ratio of long to short 3'UTR is maintained at four- and eight-cell stages, it changes in postimplantation epiblast cells, where mRNAs with the longer 3'UTR isoforms increase from 16% to 33%. This suggests that later in development, transcripts with long 3'UTR might provide greater flexibility for translational regulation, at a time when cells respond to more complex signaling conditions for diverse cell fate decisions.

1418T

Understanding the anomalies in the development of the skull and craniovertebral junction in achondroplasia. *F. Di Rocco[1,2], N. Kaci[1], E. Mugniery[1], C. Benoist-Lasselin[1], N. Litim[1], A. Munnich[1], L. Legeai-Mallet[1].* 1) INSERM U781, Laboratoire de Génétique et Epigénétique des maladies métaboliques, neurosensorielles et du développement, Paris, France; 2) Craniofacial Unit, Pediatric Neurosurgery, Hôpital Necker Enfants Malades, Paris, France.

FGFR3 is a regulator of bone formation. Several pathologies involving this gene in which the skull growth and craniovertebral junction are affected (achondroplasia, Muenke syndrome) have been described. We have used three *fgfr3Y367C/+* mouse models expressing the Y367C mutation ubiquitously (*CMV -fgfr3Y367C/+*), only in the osteoblasts (*Col I-fgfr3Y367C/+*) or only in the chondrocytes (*Col II-fgfr3Y367C/+*) to understand the impact of FGFR3 mutations on the development of the skull and determine the role played by each of these cell types.The three mouse models have been studied at 3 weeks of age using macroscopic, histology, radiology and microCT analysis. The *CMV-fgfr3Y367C/+* mice display severe morphological changes affecting the long bones but also the cranial vault and skull base. The length of the skull was reduced (66%, p<0,001) and the high increased (103%, p<0,05). The size of nasal, frontal, interparietal and occipital bones was significantly altered (55%, 82%, 125%, and 46%, respectively). The posterior fossa was hypoplasic with a small foramen magnum. In all mice, a prognathism was found with an absence of basal synchondrosis. All these features of *CMV-fgfr3Y367C/+* mice closely mimic the human chondrodysplasia. Conversely, *Col I-fgfr3Y367C/+* model results in mild morphological changes. An increase in length of the skull (106%, p<0.01) and alteration in the anterior and occipital foramen angles was found (97%, p<0.05 and 110%, p<0,01, respectively). No anomalies of the calvarial sutures and no prognathism were observed. *Col II-fgfr3Y367C/+* mouse model shows also some severe morphological alterations. Skull length was reduced (75%, p<0.0001), and high increased (103%, p<0.05), with a modification of anterior and posterior angles (76%, p<0.001 and 107%, p<0.05, respectively) and a reduction of the size of the occipital foramen and atlas vertebra. Skull base anomalies were also observed with a complete absence of the synchodrosis and a prognatism. However, no defect in calvaria bones was found. This study allows a better understanding of cranial and craniovertebral abnormalities in achondroplasia. The comparative analysis of our three models confirms the importance of endochondral ossification in the growth of the skull and craniovertebral junction. The differences in phenotypes observed between the three mouse models show that FGFR3 plays a role also in membranous ossification and highlight the impact of encephalic anomalies on calvarial growth.

1419T

Loss of prolyl-3-hydroxylation at position 986 of type I collagen in transgenic mice has a negative effect on bone mass and structure. *I. Grafe[1], D. Baldridge[1], E. Homan[1], T. Bertin[1], Y. Chen[1], M-A. Weis[2], D. Napierala[1], T. Yang[1], B. Dawson[1], C. Lietman[1], E. Munivez[1], S. Chen[1], M. Grover[1], M-M. Jiang[1], D. Eyre[2], B. Lee[1].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Orthopaedics and Sports Medicine, University of Washington, Seattle, Washington.

Purpose Cartilage associated protein (CRTAP) is required for prolyl-3-hydroxylation (3-Hyp) at position 986 of the triple helical domain of fibrillar collagens in vivo. It has been shown that loss of CRTAP results in recessive Osteogenesis Imperfecta (OI), but it is unclear if the concomitant loss of 3-Hyp directly contributes to the bone phenotype. Therefore, this study investigates if loss of 3-Hyp at position 986 of the (1(I) collagen chain has a negative impact on bone tissue through use of a transgenic mouse model. Methods Transgenic mice with overexpression of mutated Col1a1 gene in bone have been generated. Specifically, a point mutation was introduced in the Col1a1 DNA, resulting in a proline to alanine change at position 986. Alanine cannot be 3-hydroxylated and as it is the second most common amino acid in the X-position of the collagen Gly-X-Y triplet, the P986A substitution is predicted not to disrupt folding or stability of the collagen triple helix. The P986A Col1a1 gene is expressed in transgenic mice (P986A-Tg) under control of the osteoblast specific 2.3kb Col1a1 promoter. Similarly, transgenic mice with overexpression of the wildtype col1a1 gene were generated as controls (Control-Tg). Expression levels of Col1a1 mRNA were quantified by RT-PCR and 6 week old female P986A-Tg and Control-Tg mice with similar expression were chosen for further studies. Results In bone of P986A-Tg mice, mass spectroscopy confirmed a 20% expression of the alanine form of total (1(I) collagen chains. As compared to non-transgenic littermates the P986-Tg mice had a 8% reduced weight (p<0.05). MicroCT analysis of vertebral body L4 demonstrated a reduced BV/TV (-11.1%;p<0.05), reduced trabecular number (-9.6%;p<0.05), increased trabecular separation (+14.2%;p<0.05) and a slightly reduced BMD (-8.3%;p= 0.054). No significant differences regarding these parameters were observed in Control-Tg mice compared to non-transgenic littermates. Conclusion Our results indicate that bone specific expression of (1(I) collagen chains lacking 3-Hyp at position 986 affects bone mass and structure in a dominant negative manner. This suggests that 3-Hyp is required for the formation of normal bone and that loss of 3-Hyp, at least in part, may contribute to the OI phenotype related to loss of function of Crtap.

1420T

Consequences of gain-of-function mutations of ALK on central nervous system development. *C. Gordon[1], L. de Pontual[1,2], D. Kettaneh[1], M. Oufadem[1], N. Boddaert[3], M. Lees[4], J. Mollet[5], A. Munnich[1,6], L. Brugière[7], O. Delattre[5], M. Vekemans[1,6], S. Lyonnet[1,6], I. Janoueix-Lerosey[5], J. Amiel[1,6].* 1) Université Paris Descartes, INSERM U-781, Paris, France; 2) Service de Pédiatrie, Hôpital Jean Verdier, AP-HP, Bondy, France; 3) Service de Radiologie Pédiatrique, INSERM U-1000, Hôpital Necker-Enfants Malades, AP-HP, Paris, France; 4) Department of Clinical Genetics, Great-Ormond Street Hospital for Children, London, UK; 5) INSERM U-830, Institut Curie, Paris, France; 6) Département de Génétique, Faculté de Médecine ; AP-HP, Hôpital Necker-Enfants Malades, Paris, France; 7) Service d'Oncologie Pédiatrique, Institut Gustave Roussy, Villejuif, France.

Neuroblastoma (NB) is a frequent embryonal tumour of sympathetic ganglia and adrenals with extremely variable outcome. Recently, somatic amplification and gain-of-function mutations of the anaplastic lymphoma receptor tyrosine kinase (ALK, MIM 105590) gene, either somatic or germline, were identified in a significant proportion of NB cases. We have identified a novel syndromic presentation associating congenital NB with severe encephalopathy and abnormal shape of the brainstem on brain MRI in two unrelated sporadic patients harbouring de novo, germline, heterozygous ALK gene mutations. Both mutations are gain-of-function mutations that have been reported in NB and NB cell lines only. Electroporation of the neural tube of embryonic day 1.5 chicken embryos with a mutant ALK construction results in spreading of neural progenitors beyond the ventricular zone at two days post-electroporation; this phenotype is not observed when wild-type ALK is over-expressed. This supports the hypothesis that activating mutations in ALK disrupt neurogenesis in the central nervous system. These observations further illustrate the role of oncogenes in both tumour predisposition and normal development, and shed light on the pleiotropic and activity-dependent role of ALK in humans. More generally, missing germline mutations relative to the spectrum of somatic mutations reported for a given oncogene may be a reflection of severe effects during embryonic development, and may have to be looked for in patients with extreme phenotypes.

1421T

The Nuclear Receptor Co-Regulator, RERE Regulates the Development of the Cerebellum. *B. Kim[1], H. Zaveri[1], Z. Yu[1], O.A. Shchelochkov[2], M. Justice[1], B. Lee[1], D.A. Scott[1].* 1) Department of Molecular and Human Genetics, Baylor College Med, Houston, TX; 2) Department of Pediatrics, University of Iowa, Iowa, IA.

Cerebellar malformations have been documented in association with over 280 genetic syndromes and account for 3% of all CNS malformations identified in perinatal/neonatal autopsies. Although the cerebellum has been recognized for its importance in motor control and coordination, there is increasing evidence that it also plays an important role in higher cognitive functions. Despite these important roles, little is known about the genes that influence cerebellar development. The arginine-glutamic acid dipeptide repeats gene (*Rere*) is expressed in the developing mouse cerebellum, cerebral cortex, and hippocampus and encodes a nuclear receptor co-regulator that has been shown to interact with several nuclear orphan receptors including NR2F2 (COUP-TFII). Ablation of *Nr2f2* in the mouse cerebellum causes abnormal patterning and cerebellar hypoplasia. This prompted us to hypothesize that RERE interacts with NR2F2 to control cerebellar development. Mice that are homozygous for a *Rere* null allele have open neural tube and structural defects in the developing brain. However, early prenatal lethality precludes their use in studies of cerebellar development, which occurs during the later stages of prenatal period and continues postnatally. To overcome this problem, we created an allelic series of RERE-deficient mice bearing combinations of the previously described *Rere* null allele and a hypomorphic allele (V193A) we identified in an ENU mutagenesis screen. During the prenatal period, the cerebellums of $Rere^{-/V193A}$ mice are hypoplastic and show delayed formation of the principle fissures. Purkinje cell development is also delayed. Although the number of LHX-1 positive Purkinje cell precursors is normal, the expression of calbindin—a marker of mature Purkinje cells is limited to the posterior part of the cerebellum at E18.5 and Purkinje cell dendrite branching in the postnatal period is also attenuated. While the levels of granule cell proliferation and apoptosis are unaffected in $Rere^{-/V193A}$ mice at E18.5, granule cell migration is delayed during the postnatal period. $Rere^{-/V193A}$ mice also fare poorly in the dowel test which requires normal balance and coordination. These results suggest that RERE deficiency causes delayed cerebellar development which, in turn, causes functional deficits in motor coordination. We are presently working to determine if the defects seen in the cerebellum of $Rere^{-/V193A}$ mice are due to interactions between RERE and NR2F2.

1422T

Intellectual disability-associated synaptic protein KIRREL3 interacts with MAP1B and Myr8. *Y.F. Liu[1], S.M. Sowell[1], Y. Luo[1, 3], A.K. Srivastava[1,2].* 1) J.C. Self Research Institute of Human Genetics, Greenwood Genetic Center, Greenwood, SC; 2) Department of Genetics and Biochemistry, Clemson University, Clemson, SC; 3) Present address: Department of Pediatrics, Emory University School of Medicine, Atlanta, GA.

Cell-adhesion molecules of the immunoglobulin (Ig) superfamily play critical roles in brain development, as well as maintaining synaptic plasticity, the dysfunction of which is known to cause cognitive impairment. Recently, we have identified a potential role for KIRREL3, a synaptic molecule of the Ig superfamily, in intellectual disability (ID). We have functionally characterized the human KIRREL3 and determined that the protein is primarily located on the neuronal cell membrane and interacts with the X-linked ID-associated synaptic scaffolding protein CASK through its cytoplasmic domain. Furthermore, its extracellular domain (ECD) contains five Ig-like domains that are post-translationally modified by N-glycosylation and cleaved after expression in neuronal cells. To further elucidate the physiological function of the KIRREL3 ECD, we used yeast two-hybrid screening and found that the domain interacts with MAP1BLC, the microtubule associated protein light chain, and Myr 8, an unconventional myosin protein. The interactions were confirmed by reciprocal co-immunoprecipitation analysis of proteins expressed in transfected HEK293H cells. MAP1B has been previously implicated in synaptogenesis and is involved in the development of the actin-based membrane skeleton. Myr8 is expressed in hippocampal neurons, interacts with Neurexin 1, and was recently found to be deleted in a patient with ID. Together, these findings point to a possibility that the function of KIRREL3 at the synapse is carried out by a cytoplasmic domain-dependent pathway through its interaction with CASK and a pathway through its ECD interactions with other synaptic proteins such as MAP1B and Myr8. The latter function is considered critical as ECD of *syg-1*, a *C. elegans* ortholog of human KIRREL3 that localizes at the site of future synapses and has been shown to be involved in the clustering of synaptic vesicles, is capable of rescuing the terminal phenotype of a syg-1 mutant. Further studies will help define the molecular mechanisms underlying the physiological action of KIRREL3 at the synapse and its role in cognitive function.

1423T

Aristaless brain diseases: a class of pathologies associated with an altered GABAergic homeostasis? *L. Poeta[1,2], D. Drongitis[1], F. Fusco[1], M. Paciolla[1,2], S. Filosa[1], P. Collombat[3], M.B. Lioi[2], M.V. Ursini[1], M.G. Miano[1].* 1) Institute of Genetics and Biophysics, CNR, Naples, Italy; 2) University of Basilicata, Potenza, Italy; 3) INSERM U636 Diabetes Genetics, Nice, France.

ARX is one of the most important X-linked brain disease genes encoding the homeo-Transcription Factor Aristaless with a crucial role in the development of cerebral cortex, thalamus, hippocampus, and striatum. Mutations in humans cause a large spectrum of neurological disorders including lissencephaly, severe epilepsy and mental retardation. Male embryonic Arx null mice shows aberrant migration and differentiation of gamma-aminobutyric acid (GABA)-ergic interneurons, characteristics recapitulating some of the clinical features of lissencephaly and epilepsy in humans. In this report, we present molecular and morphological signatures that appear altered during the transition from neuronal precursors to fully committed Arx deficient GABA cells. In ES and neurosphere cells from wt and Arx-/Y embryonic brains, we analysed specific proliferative and neuronal hallmarks, during in vitro GABAergic maturation. They were investigated in critical transition stages: proliferation, intermediate differentiation and full differentiation. In the absence of Aristaless, we established by real time PCR robust transcriptional changes of a number of critical determinants of proliferating precursor cells and of early neuronal fate. Subsequent analysis performed by immunofluorescence confirms a parallel deregulation of the corresponding proteins. In addition, in full differentiation condition, confocal microscopic study puts in evidence abnormality in neuronal morphology and the high percentage of immature GABAergic neurons. In light of these results, we investigated whether impaired induction of neuronal differentiation was accompanied by generation of cells derived from the other germ layers. Transcript levels of mesoderm/endoderm specific genes revealed abnormal expression during neuronal differentiation. We are currently producing Arx deficient ES cell lines stably transfected with normal Arx to evaluate molecular and morphological rescue effects. In summary, we reveal the existence of changes in critical neuronal markers compatible with an impaired Arx-dependent synaptic function, which impacts on GABA homeostasis. One of the altered markers was suspected to be a direct transcriptional target of Aristaless, even if further work is required to define their functional connection. These findings emphasize the “interneuronopathy” hypothesis and will hopefully lead to the identification of a novel entry point to study ARX physiopathology in epilepsy and mental retardation.

1424T

Candidate Gene Effects on Human Craniofacial Variation in Males and Females. *S.F. Miller[1], D. Defay[1], S. Weinberg[2], C. Kummet[3], J.C. Murray[4], M.L. Marazita[2], G.L. Wehby[5], L.M. Moreno[1].* 1) Department of Orthodontics, University of Iowa, Iowa City, IA; 2) University of Pittsburgh, Pittsburgh, PA; 3) Dows Institute, University of Iowa, Iowa City, IA; 4) Department of Pediatrics, University of Iowa, Iowa City, IA; 5) College of Public Health, University of Iowa, Iowa City, IA.

Purpose: Identifying genetic factors that give rise to human craniofacial variation are of a primary interest within the field of craniofacial biology. This study seeks to further our understanding of this interface between genes and facial morphology. Methods: Thirteen SNPs within candidate genes/ loci for craniofacial development (IRF6, FOXE1, 8q24, and 20q12) and left-to-right asymmetry (LEFTY1, LEFTY2, ISL1, and SNAI1) were analyzed along with facial phenotypes including linear distances, three dimensional geometric morphometric (3D GM) phenotypes via relative warps analysis (RWA) from coordinate data and scores indicating facial shape asymmetry utilizing a multivariate regression model and Spearman correlations. Facial phenotypes were regressed on the genotypes separately for males and females. The facial coordinate data were obtained from 3dMD images of normal adults (62 males and118 females). Results: Suggestive associations ($p<0.05$) were observed between genes and the facial phenotypes. In males, SNPs in IRF6 were associated with nose width and lower face protrusion, SNPs in 8q24 were associated with a principal component (PC) summarizing variation in facial width, SNPs in FOXE1 were associated with philtrum height and a PC summarizing facial width, SNPs in LEFTY1 were associated with nasal base width, maxillary protrusion and overall facial size and finally a SNP in 20q12 was associated with a PC summarizing facial convexity. Interestingly SNPs in 8q24, FOXE1 and LEFTY1 together explained up to 17% of the variation in the PC summarizing facial width ($p=0.01$). In females SNPs in IRF6 were associated with upper facial height and philtrum width, SNPs in 8q24 were associated with a PC summarizing facial convexity, SNPs in FOXE1 were associated with facial height, a SNP in 20q12 explained 20% of the variation in facial shape asymmetry scores ($p=0.03$). Finally LEFT1 SNPs were associated with nasal base width, facial protrusion, cranial base width and overall facial size. Conclusions: These results are among the first to show multiple suggestive correlations between facial phenotypes and candidate genes for left-to-right asymmetry and facial development. This study highlights the importance of identifying genetic factors influencing human craniofacial variation in order to understand abnormal craniofacial development requiring surgical interventions and reducing quality of life.Support:DE016148, CDC5R01DD000295, AAOF 2008 OFDFA.

1425T

The ciliary kinase *Nek8* is required for Polycystin2-mediated signaling. *D.R. Beier[1], D.K. Manning[1], R.G.H.P. van Heesbeen[2,3], M. Sergeev[2,3], I.A Drummond[4], J.V. Shah[2,3].* 1) Genetics Division, Medicine Dept., Brigham & Women's Hospital/Harvard Medical School, Boston, MA; 2) Harvard Medical School, Systems Biology, Boston MA; 3) Renal Division, Medicine Dept., Brigham & Women's Hospital/Harvard Medical School, Boston, MA; 4) Renal Division, Medicine Dept. Massachusetts General Hospital/Harvard Medical School, Boston MA.

The *Nek8* gene encodes a serine/threonine kinase that localizes to the axoneme of primary cilia and is mutated in the juvenile cystic kidneys (*jck*) mouse model of polycystic kidney disease. The *jck* allele harbors a missense mutation that affects an RCC repeat in the C-terminus of NEK8 and full-length mutant protein is expressed in *jck* homozygotes. To elucidate the function of *Nek8* we generated a null allele, and found that *Nek8-/-* embryos exhibit randomization of left-right asymmetry and die shortly after birth. Mutants with abnormal laterality either display situs inversus totalis or right pulmonary isomerism (RPI) coupled with severe structural cardiac anomalies. Whole-mount in situ hybridization of the laterality marker genes *nodal* and *Pitx2* reveal aberrant nodal signaling in *Nek8-/-* embryos. Mutant embryonic nodal cilia appear normal, suggesting there is a defect in signaling rather than an overt cilial structural abnormality. The requirement for *Nek8* in left-right patterning is conserved as knockdown of the zebrafish orthologue causes randomized heart looping. Independent of laterality status, renal function is impaired in all *Nek8-/-* embryos as late-gestation and P0 kidneys have glomerular cysts and proximal tubule abnormalities. Interestingly, PKD-like tubular cysts are not present in mutant kidneys nor can they be induced in renal explant culture cyst formation assays. In addition, *jck/Nek8-* compound heterozygotes develop PKD but cystic disease is much less severe than in *jck* homozygotes, suggesting the *jck* allele is a gain of function allele of *Nek8*. The laterality, cardiac and kidney phenotypes in *Nek8-/-* embryos are strikingly similar to those identified in Pkd2-/- mice. Polycystin2 protein is present and properly localized in *Nek8* mutants but its function is likely perturbed in null embryos and cells, as *Nek8-/-* cells are resistant to triptolide. Together, the data demonstrate a requirement for *Nek8* in proper embryogenesis and suggest the protein is a critical mediator of polycystin2-dependant signaling.

1426T

Exploiting the Mid1 null mouse line to understand cerebellar development. *G. Meroni[1], F. Petrera[1], D. Licastro[1], A. Lancioni[2], R. Ferrentino[3], C. Migliore[1], M. Zanchetta[1].* 1) Functional Genomics, Cluster in Biomedicine (CBM), Trieste, Italy; 2) Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy; 3) Institute of Genetics and Biophysics, CNR, Naples, Italy.

Opitz G/BBB Syndrome (OS) is a genetic disorder characterized by midline developmental defects. Male patients with the X-linked form of OS, caused by loss-of-function mutations in the MID1 gene, show high variability of the clinical signs. MID1 encodes a ubiquitin ligase that controls Phosphatase 2A (PP2A) but its role in the pathogenesis of the disease is still unclear. Recently, we reported the generation and characterization of a mouse line carrying a non-functional ortholog of the human MID1 gene, Mid1. Mid1 null mice show the brain anatomical defect observed in patients, i.e. hypoplasia of the medial cerebellum, the vermis and the presence of motor coordination and procedural learning impairments. The defect is limited to the most anterior lobes of the vermis, the region of the developing cerebellum adjacent to the dorsal midbrain. We reported that lack of Mid1 causes a mis-specification of the midbrain/cerebellar boundary that results in abnormal development of the most anterior cerebellar lobes. To unravel the early events triggered by the lack of Mid1 in the developing cerebellum, we applied a genome-wide approach. We used laser capture microdissection to select the developing posterior dorsal midbrain and cerebellum, i.e. the entire region around the midbrain/cerebellum boundary, from sagittal sections of E12.5 and E13.5 Mid1 null and WT littermate embryos. We extracted RNA from the dissected tissues and perform microarray-based expression profiling. Bioinformatic analysis revealed the presence of several genes differentially expressed between Mid1 null and WT embryos. Gene enrichment analysis of the differentially expressed transcripts shows GO terms statistically significant over-represented in classes defined as `muscle contraction' and `spinal cord neuron specification'. Noteworthy, among the differentially expressed genes in our system the former class is represented by genes coding for proteins of the cytoskeleton and the second by several homeodomain-containing transcription factors. Considering that Mid1 is a microtubular protein involved in cytoskeleton stabilization and that homeodomain containing proteins are important for antero-posterior neural tube determination, these results represent promising bases to understand the origin of the defects observed in Mid1 null mice and in Opitz Syndrome patients.

1427T

Analysis of time-series embryonic diaphragm transcriptomes identifies *Pbx1* as a candidate gene for diaphragmatic defects. *M. Russell[1], M. Longoni[1], J. Wells[2], F. Maalouf[1], A. Tracey[1], K. Ackerman[3], B. Pober[1,4,5], K. Lage[1,6,7,8,9], C. Bult[2], P. Donahoe[1,6,7].* 1) Pediatric Surgical Research Laboratories, Massachusetts General Hospital, Boston, MA 02114 USA; 2) The Jackson Laboratory, Bar Harbor, ME 04609 USA; 3) The University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642 USA; 4) Children's Hospital Boston, Boston, MA 02115 USA; 5) Department of Pediatrics, Harvard Medical School, Boston, MA 02115 USA; 6) Department of Surgery, Harvard Medical School, Boston, MA 02115 USA; 7) Broad Institute, Cambridge, MA 02142; 8) Center for Biological Sequence Analysis, Technical University of Denmark, Lyngby, Denmark; 9) Center for Protein Research, University of Copenhagen, Copenhagen, Denmark.

Congenital Diaphragmatic Hernia (CDH) is a birth defect with significant morbidity and mortality that occurs in 1 in 2,500 live births. Classic genetic studies are in most cases statistically underpowered to identify genes responsible for CDH due to its genetic heterogeneity, polygenic inheritance, and small kindred sizes. To overcome these challenges, time-series whole-genome expression profiles generated from laser capture microdissected embryonic C57BL/6J mouse diaphragms (at E11.5, E12.5, and E16.5) were analyzed. Specifically, gene sets defining biologically relevant pathways (obtained by Gene Set Enrichment Analysis) and temporal expression patterns in the diaphragm (obtained by Short Time-series Expression Miner) were assessed for enrichment of a curated list of “bait” genes known to be associated with diaphragm defects in humans and/or in mutant mice. Genes belonging to the intersecting subset of these two independent algorithms were considered the highest priority CDH candidates. This data filtering approach revealed 31 candidate genes, among which 4 were bait genes, providing positive proof of principle. Each of a dozen candidates tested to date, demonstrate expression in the primordial mouse diaphragm by either immunohistochemistry and/or RT-q-PCR. Among the 28 novel candidates, pre-B cell leukemia transcription factor 1 (*Pbx1*), a known facilitator of muscle differentiation and mesodermal patterning during embryogenesis, was selected to assess further the validity of the enrichment strategy. Ex vivo examination of 3/3 *Pbx1*^{tm1mlc} knock out mouse embryos at E15.5 revealed a range of diaphragmatic defects. Thus, the validity of the computational approach is supported by the discovery of *Pbx1* as a new CDH-associated gene. Sequencing of PBX1 in 150 human subjects with CDH is underway. Finally, we anticipate that our novel expression dataset and the narrowed list of candidates will continue to reveal new CDH-associated genes and provide insight into the developmental biology of this malformation.

1428T

Deletion of *PAX6* entire gene in a patient with aniridia. *E.S.S. França[1], M.S. Guaragna[1], A.T. Maciel-Guerra[2,3], G. Guerra-Júnior[3,4], M.P. de Mello[1].* 1) Centro de Biologia Molecular e Engenharia Genética (CBMEG), Universidade de Campinas (UNICAMP), Campinas, SP, Brasil; 2) Departamento de Genética Médica, Universidade de Campinas (UNICAMP), Campinas, SP, Brasil; 3) 3Grupo Interdisciplinar de Estudos da Determinação e Diferenciação do Sexo (GIEDDS), Universidade de Campinas (UNICAMP), Campinas, SP, Brasil; 4) 4Departamento de Pediatria, Faculdade de Ciências Médicas, Universidade de Campinas (UNICAMP), Campinas, SP, Brasil.

Aniridia is a congenital eye condition characterized by the iris underdevelopment that usually occurs in both eyes. It is inherited as autosomal dominant trait with high penetrance. WAGR syndrome (Wilms'tumor - Aniridia - Genitourinary Anomalies - Mental Retardation Syndrome) is caused by large deletion in 11p13. Normally, this deletion encompasses several genes, including *PAX6* and *WT1*, resulting in both aniridia and increased risk for Wilms' tumor. Heterozygosis for *PAX6* gene mutations are also found in cases of isolated aniridia, suggesting that *PAX6* haploinsufficiency causes this condition. However, not all cases of aniridia are due to mutations. This study was conducted to identify *PAX6* mutations in a Brazilian patient with isolated aniridia. The patient was a girl showing bilateral aniridia, cataract in the left eye and nystagmus. There was no other case in the family. She had no neurological disorders and no other physical problem such as Wilms' tumor, which was monitored periodically by kidney ultrasound scanning. The exons of *PAX6* and *WT1* genes were amplified by polymerase chain reaction and direct sequenced. Copy number variations were investigated by multiplex ligation-dependent probe amplification (MLPA). MLPA was performed with SALSA Kit P219-B1 from MRC Holland (Amsterdam, Netherlands). Molecular analyses for *WT1* and *PAX6* gene did not revealed any mutation in the patient with aniridia. However MLPA demonstrated the heterozygous deletion of every *PAX6* exons. Other probes, including those from *PAX6* 5' and 3' flanking regions and WT1 gene did not show copy number variations. These results discarded deletion and/or mutation on the *WT1* gene. In this study we show the heterozygous deletion of *PAX6* gene in one isolated case of aniridia. This deletion probably occurred de novo, which corresponds to a rare case. Our results indicate that MLPA technique might be included in the molecular diagnosis of *PAX6* gene, mainly to confirm or rule out deleted genes mapping to 11p13 as causes of isolated aniridia or Wilms' tumor. The molecular diagnosis of aniridia in the case described here was only achieved when MLPA technique was used to identify the *PAX6* gene deletion. However, the exact deletion breakpoint was not determined yet. Additional studies such as genotyping with microsatellite markers will be further conducted to delimitate deletion boundaries.

1429T

Knock-down of Zic2 in embryonic stem cells blocks neural differentiation through its effects on miRNA expression. *L. Brown, S. Brown.* University of Vermont Burlington VT.

Heterozygous mutations in the transcription factor ZIC2 result in holoprosencephaly (HPE) in humans and mice, leaving no doubt that ZIC2 plays an important role in early forebrain patterning. Despite this knowledge, there is currently very limited insight into how ZIC2 normally functions in brain development. Our lab has shown that Zic2 is expressed in the preimplantation mouse embryo as well as in embryonic stem (ES) cells and in adult neural stem cells. This observation led us to hypothesize that Zic2 plays a role in regulating early neural differentiation events. To test this hypothesis, we used lentiviral-mediated shRNA to knock down the expression of Zic2 in mouse ES cells by about 90%. ES cells with reduced Zic2 expression ("Zic2-kd-ES") continue to grow with typical ES cell morphology and continue to express markers of pluripotency, such as Oct3/4, Sox2 and Nanog. However, when Zic2-kd-ES cells are allowed to differentiate in culture, they no longer differentiate into neurons or neural precursor cells. Thus, loss of Zic2 appears to block an entire differentiation pathway without affecting known regulators of pluripotency. We used Affymetrix arrays to assess miRNA expression in Zic2-kd-ES cells and show that diminished Zic2 expression major changes in a discreet group of miRNAs. In particular, we show that diminished Zic2 expression results in the 5-60 fold decrease in the expression of the miRNA-302 family. This result is paradoxical, since published data indicate that diminished miRNA-302 expression is associated with loss of expression of standard markers of pluripotency. Current efforts in our lab are aimed at understanding the overall effects of Zic2 on gene expression, as well as understanding how Zic2 regulates miRNA expression. Taken as a whole, our data indicate that Zic2 specifically modulates neural differentiation in pluripotent stem cells and that, by exploring the role of Zic2 in ES cell biology, we will gain insight into the molecular details of neurogenesis. This will be critical for efforts to reprogram adult cells into neural stem cells.

1430T

Asymmetric expression of Claudin-10 is required for correct patterning of the left-right axis. *M.M. Collins[1], A. Simard[2], A.K. Ryan[1,2].* 1) Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Pediatrics, Research Institute of the Montreal Children's Hospital, MUHC, Montreal, Quebec, Canada.

In vertebrates, the asymmetric positioning of internal organs relative to the midline is evolutionarily conserved, and required for normal physiological function. The genetic cascade that defines asymmetric organ positioning initiates during gastrulation, during which several ions including calcium, have been implicated in the initial symmetry breaking event. The mechanisms by which these events are translated into asymmetric gene expression remain unknown. We previously examined the role of the integral tight junction protein, Claudin-1, in patterning the left-right axis in the chick, and showed that overexpression of Claudin-1 on the right side of the embryo randomized the direction of heart looping. To identify other claudins expressed during gastrulation, we performed expression analyses of the claudin family. We identified that 15 claudins exhibit unique and/or overlapping expression patterns during gastrulation. Interestingly, we discovered that Claudin-10 was asymmetrically expressed at Hensen's node, the site where asymmetric gene expression is first observed in the chick embryo and where the initial symmetry breaking events required for left-right patterning occur. Overexpression of Claudin-10 on the left side of the node, or knockdown of endogenous Claudin-10 on the right side of the node, caused a significant increase in abnormal leftward heart looping, indicative of abnormal left-right patterning. We also observed alterations in the expression of the classic left-right morphogenesis gene, Pitx2c, in these embryos. We are currently examining the effects of manipulating Claudin-10 expression on other classic left-right patterning genes, and identifying the functional domains necessary for the role that Claudin-10 plays during left-right patterning. These data suggest that asymmetric expression of Claudin-10 is required for normal left-right patterning, perhaps through regulation of the permeability of the ions that have been proposed to be involved in the initial symmetry-breaking event that occurs at Hensen's node.

1431T

NOTCH signaling pathway and Holoprosencephaly: A transcriptomic approach using chick model. *V. David[1 2], L. Ratié[1], I. Gicquel[1], S. Mercier[1 3], C. Dubourg[1 2], S. Odent[1 3], V. Dupé[1].* 1) Faculté de Médecine, UMR 6061 CNRS/Université Rennes1, Rennes, France; 2) Laboratoire de Génétique Moléculaire, CHU Pontchaillou, Rennes, France; 3) Service de Génétique Clinique, CHU Pontchaillou, Rennes, France.

Holoprosencephaly (HPE) is a congenital malformation of the human brain due to an imperfect division of forebrain during early development. Multiple genetics defects have been identified as involved in this process. It is now currently admitted that HPE is a multihit pathology caused by at least two or more dysfunctional events involving genes from more than one signaling pathway. The SHH and NODAL pathways have been presented to be the main signaling pathways involved in human HPE. To a lesser extend, FGF has also been found to be implicated. Importantly, by CGH array we have recently found several recurrent rearrangements in 6qter in our cohort of HPE patients and identified a component of the NOTCH pathway, DLL1, as a new HPE candidate gene. We described a mutation in *DLL1* in HPE patients and showed that Dll1 was expressed in the chick developing forebrain and notably co-expressed in the anterior neuropore with Fgf8, one of the major genes implicated in ventral forebrain patterning. These results strongly suggest that a disturbance of Notch pathway may well confer a susceptibility to HPE. However, if previous studies have showed the importance of Notch throughout the neurogenic phase of forebrain development, no link with HPE has ever been described. To assess this question, we have implemented an ex ovo chick embryos culture system with a pharmacological inhibitor known to block specifically Notch pathway. Subsequently, the molecular reprogramming of the treated brain has been tested using expression microarray and in situ hybridization approaches. These analyses provide functional data about the NOTCH signaling pathway during HPE appearance as well as throughout normal forebrain development.

1432T

Assisting research into human embryonic and fetal development. *D. Gerrelli[1], S. Suren[1], V. Morrison[1], Y. Cheng[2], L. Overman[2], M. Crosier[2], S. Lisgo[2], S. Lindsay[2], A.J. Copp[1].* 1) Institute of Child Health, University College London, London, United Kingdom; 2) Institute of Genetic Medicine, Newcastle University, Newcastle. United Kingdom.

The Human Developmental Biology Resource (HDBR) is funded by the MRC and Wellcome Trust to provide human embryonic and fetal tissue for gene expression studies related to congenital disease. This research is essential if we are to understand the developmental origin of these conditions, including both malformations and inherited metabolic disorders, and to produce new methods for primary prevention. Use of the HDBR material should also illuminate developmental gene expression underlying aspects of functioning that characterise humans as opposed to lower animals (e.g. higher brain function, language), since such studies are challenging using rodent and other animal models. The HDBR has ethics committee approval for the collection, storage and distribution of human embryonic and fetal material between 4 and 16 weeks of gestation. In addition the HDBR administers a previously collected fetal tissue bank, with specimens available between 8 and 19 weeks of gestation. A significant proportion of the HDBR material is karyotyped and chromosomally normal material is provided for research. Karyotypically abnormal material can also be provided on request. Freshly collected material from the HDBR can be used to generate cell lines (e.g. stem cells), protein, RNA and DNA. cDNA is also available from embryonic tissue for gene expression analysis. In addition, paraffin wax and frozen sections of embryos and early fetuses are available for in situ hybridisation and immunohistochemistry. The HDBR offers an in-house gene expression service (IHGES) using in situ hybridisation and/or immunohistochemistry. The service includes: • Initial testing of in situ cRNA probes or antibodies. A report showing initial results with sense and antisense probes together with positive controls on the human fetal tissue will be provided. • Once good quality results have been generated, a full project can be performed on a number of different developmental stages. High quality images of gene expression patterns suitable for publication will be produced and the HDBR will provide advice on interpretation of results. Recent completed and published IHGES projects have included analysis of gene expression in the limb, eye, liver, kidney, central and peripheral nervous system at a range of human developmental stages. Information on all aspects of the HDBR, can be found on www.HDBR.org or by contacting the centre manager at Hdbr@newcastle.ac.uk or Hdbr@ich.ucl.ac.uk.

1433T

Is TMED2 essential in the chorion for normal interaction between the allantois and the chorion in mice? *W. Hou[1], D. Sarikaya[1,3], L. Jerome-Majewska[1,2].* 1) Department of Human Genetics, McGill University, Montreal, QC, Canada; 2) Montreal Children Hospital Research Institute, Montreal, QC, Canada; 3) Organismic and Evolutionary Biology Department, Harvard University, Cambridge, Massachusetts, USA.

During vesicular transport between the endoplasmic reticulum and the Golgi, members of the transmembrane emp24 domain (TMED) protein family form hetero-oligomeric complexes that facilitate protein cargo secretion and transportation. In our laboratory, we are investigating the function of one member of the TMED protein family, TMED2, in mouse placental labyrinth development. Formation of the mouse labyrinth layer requires proper interactions between two extraembryonic tissues, the allantois and the chorion; and is essential for nutrition, waste, as well as hormone exchange between the fetus and mother. We have shown that Tmed2 is expressed in the allantois and chorion and is required for normal labyrinth layer formation. We hypothesized that TMED2 is essential in the chorion or allantois for normal interaction between the allantois and chorion- a critical step in placental labyrinth layer development. To test this hypothesis, we have generated an ex-vivo allantois and chorion recombination model. We show that the early events of labyrinth layer development: chorioallantoic attachment, fusion of the mesothelium and allantois, and chorionic trophoblast differentiation are recapitulated in this model. We used in situ hybridization and immunohistochemistry to confirm the chorioallantoic attachment event and to monitor development of labyrinth layer in the chimeric explants.We will then use combinations of wild type and Tmed2 null chorion and allantois in these ex-vivo cultures to follow branching morphogenesis in the chorion. Our work will provide insight into the contribution of placental-specific vesicular transport by TMED2 to labyrinth layer morphogenesis. Ultimately we will identify novel mechanisms that may be implicated in the prediction and treatment of placental diseases such as EPL and IUGR.

1434T

Genome wide screening of mesenchymal signalling molecules involved in epithelial differentiation during mice palatogenesis. *K. Kwon, W. Sohn, H. Kim, M. Choi, Z. Ryoo, S. Lee, J. Kim.* IHBR, Kyungpook National University, Daegu, Korea.

In mice palatogenesis, along with the palatal fusion, dorsal and ventral epithelia of palatal shelves are differentiated into the nasal and oral mucosa respectively. Based on the key concept of epithelial-mesenchymal interactions during epithelial morphogenesis, we hypothesized that differentiation of palatal epithelium would be regulated by palatal mesenchymal factors. At E14, prior to palatal fusion, palatal shelves were fronally cryo-sectioned and dorsal and ventral region specific palatal mesenchymal tissues were collected using Laser Micro-Dissection (LMD) method respectively. After the collection of palatal tissue using LMD, total RNAs were harvested and analyzed by the genome wide screening method. We selected the genes which showed the over 2-fold alterations genes then examined RT-qPCR and in situ hybridizations. Tissue specific expression patterns of candidate molecules would suggest that they would play important roles in epithelial differentiation with the region specific manner. Moreover, these region specific expression patterns of mesenchymal factors would be a plausible answer for understanding the pathogenesis of cleft palate.

1435T

Epithelial splicing regulatory proteins 1 and 2 (ESRP1/2) regulate alternative splicing events during mouse embryogenesis. *T. Revil[1], L.A. Jerome-Majewska[1,2].* 1) Dept of Human Genetics, McGill University, Montreal, Quebec , Canada; 2) Department of Pediatrics, Montreal Children's Hospital, Montreal, Quebec, Canada.

The development of a fetus from a single cell requires highly coordinated cell movement and differentiation for proper morphogenesis. We hypothesized that coordinated alternative splicing of mRNAs is a regulatory mechanism via which large groups of genes can change their state and location during embryogenesis. In previously published work, we used a Systems approach to show that a large number of genes are alternatively spliced during embryogenesis. More recently, using the same dataset, we have found that expression levels of two splicing factors, epithelial splicing regulatory proteins 1 and 2, (ESRP1 and 2) are upregulated during differentiation. ESRP1 and 2 are major regulators of epithelial to mesenchymal transition (EMT) in human cancer cells. EMT, the process via which polarized epithelial cells change their states to a mesenchymal cell type, and MET, the reverse process, are the driving forces of morphogenesis. We found that expression of ESRP1 and 2 was increased in the placenta, which represent the more “differentiated” cell types in our dataset when compared to staged match embryos. We also found that alternative splicing of several validated targets of ESRP1 and ESRP2, that were previously shown to be required for embryogenesis, correlated with the expression levels of these genes in our dataset. We have validated our microarray data set and further show that ESRP1 and ESRP2 are expressed throughout embryogenesis. We hypothesize that Esrp1 and 2 are required for the normal morphogenesis of developing embryos and that abnormal alternative splicing of their targets, will result in arrested development as a consequence of abnormal EMT. We are currently performing *in situ* hybridizations, using RNA and LNA probes, to determine the expression of Esrp1 and 2, as well as their putative target exons. Furthermore, we are developing a technique by which to knockdown gene expression of these genes in embryos directly in the uterine horns of mice using morpholinos. Finally, we are creating a conditional knockout mouse for these two genes. These results will allow us to assess the importance of the splicing factors ESRP1 and 2 during mouse embryogenesis.

1436T

Signaling modulations of Rgs19 in palatal EMT process. *W. Sohn[1], Y. Ji[1], H. Kim[1], K. Kwon[1], C. An[1], H. Park[2], H. Jung[3], Z. Ryoo[1], S. Lee[1], J. Kim[1].* 1) IHBR, Kyungpook National University, Daegu, Korea; 2) Forensic Science, Busan Metropolitan Police Agency, Busan, Korea; 3) Yonsei University, Seoul, Korea.

Palatal development is one of the crucial events in craniofacial morphogenesis, according to the significant signaling pathway including the out growth, elevation, and fusion of palatal shelves. In the fusion of palatal shelves, epithelial to mesenchymal transition (EMT) is a fundamental process to achieve the proper morphogenesis of palate. Mechanisms of EMT have been reported as the processes of migration, apoptosis or general EMT through the modulations through various signalling molecules. Rgs19, known as a regulator of G protein signaling (RGS) family through GTPase activity, showed the interesting epithelial expression patterns in various organogeneses including palatal development. To evaluate the precise developmental function of Rgs19 in palatogenesis, we employed the loss of function study using AS-ODN treatments while in vitro palate organ cultivations. Three dimensional reconstructions with the immunostaining of pan-Cytokeratins showed the much decreased number of apoptotic cells in medial edge epithelium (MEE) after the knocking down of Rgs19. These retarded patterns of palatal fusion would be resulted from the altered expression patterns of candidate genes including Axin2, CyclinD1, Lef1, Twist, Snail, Slug and TGFb3. Overall, Rgs19 modulates the palatal EMT process through the apoptotic pathway and signaling interactions among Wnt responsive and EMT related genes.

1437T

Reprogramming Senescent Fibroblasts from Werner Syndrome for Studying Premature Aging. *H. Cheung, X. Liu, O.M. Rennert.* Section on Clinical & Developmental Genomics, NICHD, NIH, Bethesda, MD. USA.

Werner syndrome (WS) is a progeroid-like disease characterized by premature aging features such as loss of hair, osteoporosis, cataracts, myocardial infarction and cancer. These phenotypes become apparent in adolescence. Mutation of the DNA helicase WRN is identified in patients with WS. Deficiency of WRN protein results in genomic instability and telomere erosion, and its clinical manifestations result in phenotypes of accelerated aging. Intriguingly the major affected organs in WS are of mesenchymal origin. Previous studies of WS focused on patient-derived fibroblasts to study the pathology of affected tissues. It remains unresolved as to whether this is reflective of the sequence of events that define the developmental pathology of cells such as cardiomyocytes and osteocytes. Patient specific embryonic stem cells (ESCs) from WS are currently unavailable, making it difficult to study the pathological differentiation in this disease. We have been deriving patient-specific iPSCs (induced pluripotent stem cells) from human skin fibroblasts for studying the pathogenesis of genetic diseases including WS. Unlike normal cells, skin fibroblasts from WS demonstrate slow growth rate, shortened telomere and susceptibility to genotoxic stress. We reprogrammed WS cells to pluripotent state with Yamanaka's transcriptional factors KLF4, OCT4, SOX2, c-MYC. To overcome the accelerated senescence occurring in WS, patient fibroblasts were immortalized with telomerase (hTERT), and/or treated with small molecules targeting the p38/MAPK pathway to revert the aged morphology. The derived iPSCs will be differentiated into mesenchymal stem cells (MSCs) and non-mesenchymal lineages. We will compare cellular senescence and susceptibility to genotoxic stress between WS-iPSC and normal iPSC, as well as their differentiated derivatives. We will establish the consequences of WRN mutation on the ability to reprogram to pluripotency, and their capacity for differentiation into different cell lineages. These studies help establish a cell model for studying human premature aging, using WS as an example.

1438T

Molecular genetic studies of planar polarity gene SCRIBBLE1 in neural tube defects. *K.KH. Fares[1], A.Ra Radouane[1], P.Ma De Marco[2], E.Me Merello[2], V.Ca Capra[2], K.Zo Zoha[1].* 1) biochemistry, university of Montreal, Montreal, Québec, Canada; 2) pathologiy, university of montreal, montreal, québec, Canada.

The neural tube defects (NTDs) including anencephaly and spina bifida, represent a very common birth defect in humans. These anomalies are caused by partial or complete failure of neural tube closure during embryogenesis. Their etiology is quite complex involving environmental and genetic factors and their underlying molecular and cellular pathogenic mechanisms remain poorly understood. Studies in animal models showed an important role of the non-canonical Frizzled (Fz) / Dishevelled (Dvl) signalling pathway in NTDs. This pathway controls planar cell polarity (PCP) in Drosophila and the process of convergent extension (CE) during gastrulation and neurulation in vertebrates. Our study involves the genetic and molecular analysis of the PCP gene SCRIBBLE1 (SCRB1) in human NTDs. The open reading frame and exon-intron junctions of SCRB1 were sequenced in a cohort of 479 NTD patients. We identified 11 novel and rare missense heterozygous mutations in NTDs. Genetic analysis of 7 of these mutations in a cohort of 467 ethnically-matched controls revealed the absence of 3 mutations in all controls analyzed. We are currently finishing the genetic validation of SCRB1 variants in controls and other family members. We will also validate the potential pathogenic effect of these variants by testing their effect on the interaction of SCRB1 with other PCP proteins using the Yeast-two-hybrid system and on CE in a zebrafish model. Our study will help us better understand the role of the PCP pathway, and more specifically of SCRB1, in the complex etiology of the NTDs in humans.

1439T

Dysregulation of DNA damage repair and cell cycle checkpoint control pathways as a mechanism for cleft lip/palate. *G.S. Kobayashi[1], L.A. Cruz[1], D.Y. Sunaga[1], D.F. Bueno[1], S.G. Ferreira[1], M. Aguena[1], L.A. Andrade-Lima[2], C.F. Menck[2], M.R. Passos-Bueno[1].* 1) Human Genome Research Center, Institute for Biosciences, University of São Paulo, Brazil; 2) Institute for Biomedical Sciences, University of São Paulo, Brazil.

Purpose: Non-syndromic cleft lip/palate (NSCL/P) is a multifactorial disease that arises from errors during embryonic development. Although much effort has been put into identifying genetic and environmental factors underlying disease susceptibility, the aetiology of this complex malformation remains obscure. Since a tight regulation of ontogenetic mechanisms is required to ensure appropriate orofacial morphogenesis, our objective was to identify dysregulated pathways that may be accountable for disease manifestation, thus providing more information on the pathogenesis of NSCL/P. **Methods:** We performed a global transcriptome profiling of 7 dental pulp mesenchymal stem cell cultures from NSCL/P patients by comparison to 6 controls, using the Affymetrix platform (HuGene 1.0 ST). Differentially expressed genes (DEGs) were obtained using SAM and RankProd algorithms (MeV TM4 software, $p<0.05$ and $p<0.01$, respectively), followed by validation with qRT-PCR assays (t-test, $p<0.05$). Functional annotation and gene network analysis were performed with Ingenuity Pathways Analysis, while gene clustering, functional pathways/categories, and transcription factor (TF) enrichment procedures were carried out on EXPANDER, using K-means and supervised clustering, KEGG/Gene Ontology and PRIMA, respectively. Finally, TF enrichment was confirmed using the FANTOM 4 database. Quantification of H_2O_2-induced DNA damage was assessed through flow cytometry for anti-. H2AX. **Results/Conclusion:** Among the 228 DEGs obtained by comparing NSCL/P and control cells, we identified a cluster of co-expressed genes that was associated with a general dysregulation of cell cycle checkpoint and DNA repair pathways. We encountered abnormal expression of key molecules that orchestrate these mechanisms, some of which are involved in the aetiology of sporadic forms of cancer, and include *BRCA1*, *BRIP1*, *MSH2*, *RAD51* and *BLM*. TF enrichment analysis on the aforementioned gene cluster pointed *E2F1* as a putative regulator driving the observed expression signature. Moreover, preliminary . H2AX assays confirmed deficiency of DNA repair in NSCL/P cells. Our results suggest that disarrangement of these mechanisms could be related to impairment of embryonic lip and palate morphogenesis, and also provide a link between dysregulation of these pathways and the documented increased occurrence of sporadic cancer in NSCL/P families. Financial Support: CEPID/FAPESP, CNPq, MCT.

1440T

Allele Specific Expression Single cell RNA-Seq Analysis. *K. Lao[1], F. Tang[2], C. Barbacioru[1], E. Nordman[1], S. Bao[2], C. Lee[2], X. Wang[1], B. Tuch[1], E. Heard[3], M. Surani[2].* 1) Molec Cell Biol, Applied Biosystems, part of Life Technology, Foster City, CA; 2) 2Wellcome Trust/Cancer Research UK Gurdon Institute of Cancer and Developmental Biology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QN, UK; 3) 3CNRS UMR3215, INSERM U934, Institut Curie, Paris F-75248, France.

Dissecting the relationship between genotype and phenotype is one of the central goals in biology and medicine. Cell development is driven and controlled by temporal and spatial changes in gene transcription, followed by translation of the resulting messenger RNAs into proteins. Recent advances in high throughput sequencing technology make it possible to obtain information on single cell transcriptomes at high resolution by RNA-Seq analysis, which can be instructive concerning how individual cells respond to signals and other environmental cues at critical stages of cell fate determination, or when they acquire aberrant phenotype. Essentially all cells within an individual organism share a virtually identical genotype, but the individual transcriptomes reflect expression of a subset of genes, which is determined by their epigenetic state, including DNA methylation and histone modifications. If these two copies of a gene are expressed at different levels, the quantities of messenger (mRNA) from individual alleles will differ. This phenomenon is called allele specific gene expression (ASE). ASE is potentially important for development, and during evolution when a new allele emerges from mutation, which might immediately affect development through changes in expression levels in a heterozygous cell. However, the extent of ASE in individual mammalian cells and, especially during early embryonic development has not so far been examined. Here, we took advantage of recent technical and methodological advances concerning single cell RNA-Seq, to analyze allele specific gene expression within individual early mouse blastomeres. We found that around 50% of distinguishable expressed alleles of individual genes showed differential allele specific expression. This shows that ASE is widespread at the earliest stages of mammalian development. ASE is likely to occur as a result of sequence polymorphisms of cis regulatory elements, or this might occur in response to different local chromatin structures mediated by epigenetic modifications.

1441T

Genomic and Genetic Analyses Define Causative Elements of Two Developmental Defects in an Avian Biomedical Model. *E.A. Robb, M.E. Delany*. Animal Science Department, University of California, Davis, Davis, CA.

Advances in genomics can be applied to the study of unique phenotypes in biomedical models to generate new knowledge on pathways involved in human congenital malformations. The chicken has long been a premier research model in the field of vertebrate developmental biology due to the advantages of access and ease of manipulation. The power of the chicken as a model organism has been further improved given the release of its genome sequence and progress in creation of tools and reagents (e.g., SNP arrays, ESTs, gene expression and cDNA databases, microarrays) and availability of unique genetic resources and cell lines. Two single gene mutations in the chicken, *coloboma* (*co*) and *diplopodia-1* (*dp-1*), were studied for the purpose of identifying the element responsible for each developmental syndrome. These mutant models provide an invaluable means of studying craniofacial morphogenesis as well as limb and organ development. In the recessive condition both *co* and *dp-1* produce syndrome phenotypes shared in common with human congenital malformations including cleft palate, facial-tissue defects, polydactyly, and dwarfism. A 60K SNP array study paired with fine-mapping techniques led to the discovery of a specific chromosomal region associated with each syndrome (*co*: 240 kb, 5 genes; *dp-1*: 260 kb, 11 genes). The expression patterns of sixteen genes found within the regions were studied by *in situ* hybridization during early embryogenesis. Examination of the results allowed further prioritization of candidate genes, suggesting that several genes be emphasized in future studies. In addition, next-generation technologies including a targeted-capture array with deep-sequencing were employed to investigate the regions linked to each mutation. Bioinformatic analyses identified unique genetic features (e.g., SNPs, short INDELs, large deletions) associating with each mutation region. These genetic elements are being evaluated for their contribution to the mutant phenotypes using a variety of genetic and genomic techniques. The results of this study will increase our understanding of the mechanisms underlying similar human disorders and will promote insights into the molecular mechanisms of normal development. Ultimately, this new knowledge could lead to screening tools for preventative medicine applications. [USDA NIFA -NRSP8, -NC1170; Fiddyment Endowment].

1442T

Craniofacial abnormalities result from knock down of nonsyndromic clefting gene, crispld2, in zebrafish. *Q. Yuan[1], B.T. Chiquet[1,2], L. Devault[1], M.L. Warman[3], Y. Nakamura[4], E.C. Swindell[1,2], J.T. Hecht[1,2].* 1) Department of Pediatrics, University of Texas Medical School at Houston, Houston, TX; 2) Graduate School of Biological Sciences, University of Texas Health Sciences Center, Houston, Houston, TX; 3) Howard Hughes Medical Institute, Department of Genetics, Boston Children's Hospital, Boston, MA; 4) National Hospital Organization, Clinical Research Center, Murayama Medical Center, Tokyo, Japan.

We have previously found that variation in the CRISPLD2 (cysteine-rich secretory protein LCCL domain containing 2) gene is associated with non-syndromic cleft lip and palate (NSCLP). Crispld2 was first identified in mice and designated Lgl1 (late gestation lung 1). LGL1 plays a role in early branching morphogenesis of the developing lung and kidney and in the epithelial to mesenchymal transition in lung tissue. Defects in the epithelial to mesenchymal transition in craniofacial tissue are thought to underlie nonsyndromic orofacial clefting. Moreover, we found that Crispld2 is expressed in the developing murine craniofacies suggesting that CRISPLD2 may play a role in craniofacial morphogenesis. To define the role of CRISPLD2 in zebrafish craniofacial development, antisense morpholinos (MO) directed against the ATG start site (MO3) and exon/intron boundaries for exons 3 and 4 (MO1 and MO2, respectively) of zcrispld2 were injected into early embryos. We observed a wide range of phenotypic abnormalities including early embryonic death, severe reduction in head size and shortening of the body. All of the resulting offspring showed a range of craniofacial phenotypic abnormalities involving both the jaw and palate with the most severe phenotypes resulting from injection of MO3. Jaw abnormalities included fewer ceratobranchial cartilages (three/four pairs in MO embryos compared to five in uninjected controls) and malformations in Meckel and palatoquadrate cartilages. A variety of palatal malformations included short palate, absent cartilage and abnormal ossification. DLX2, a marker for neural crest cells (NCC), shows abnormal expression in MO injected embryos suggesting that loss of zcrispld2 is specifically affecting these cells. These results show that zcrispld2 is involved in palate and jaw morphogenesis. Moreover, these results demonstrate that genes involved in complex birth defects, such as nonsyndromic cleft lip and palate, can be identified in candidate gene studies and functionally assessed in animal models to define the biological processes that are the basis for these complex birth defects.

1443T

Identification of the Danforth's short tail mouse mutation using next generation sequencing. *C.N. Vlangos[1], A.N. Siuniak[1], D. Robinson[3], A.M. Chinnaiyan[3], J.D. Cavalcoli[4], R.H. Lyons[5], C.E. Keegan[1,2].* 1) Department of Pediatrics, University of Michigan, Ann Arbor, MI; 2) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 3) Center for Translational Pathology, University of Michigan, Ann Arbor, MI; 4) Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI; 5) Biological Chemistry Department, University of Michigan, Ann Arbor, MI.

The Danforth's short tail (Sd^{Sd}) mouse first appeared as a semi-dominant spontaneous mutation in an inbred colony at Stanford University in the 1920s. The phenotype of heterozygous ($Sd^{Sd/+}$) animals includes unilateral kidney agenesis, vertebral anomalies, and a shortened and kinked tail. Homozygous ($Sd^{Sd/Sd}$) mice are more severely affected with bilateral renal agenesis, lack of tail, vertebral anomalies, spina bifida, imperforate anus, and persistence of the cloaca. Homozygous mice are born live but die within 24 hours of birth. The phenotypic characteristics of the *Sd* mouse parallel those of the human malformation syndromes limb-body wall complex, OEIS complex, and VACTERL association. We hypothesize that identification of the *Sd* mutation will provide a model for understanding the etiology behind these human syndromes. Previous work by others genetically mapped the *Sd* mutation to a 1.0cM (1.5Mb) region of mouse chromosome 2qA3. Using bioinformatic analysis of the mouse DNA physical map we identified 9 annotated genes with a total of 86 coding exons spanning the corresponding 1.5Mb critical region. Direct sequencing of the exonic DNA and intron/exon boundaries did not reveal any mutations. Since direct sequencing of the exonic DNA only provided _1% coverage of the *Sd* critical region we performed next generation sequencing (NGS) of the entire 1.5Mb. Prior to NGS we performed an enrichment capture using an Agilent oligo DNA chip designed to cover all unique (non-repeat masked) DNA of the *Sd* critical region. After successful locus specific DNA enrichment, 36bp paired end NGS on an Illumina Genome Analyzer IIx was completed, generating 1.85Gb of sequence. Standard bioinformatics analysis of our NGS data did not reveal any causative mutations. We then turned to interrogation of reads where only a single end of the paired end sequencing mapped correctly to the *Sd* locus. By using this novel technique, we were able to identify the presence of an early transposon (ETn) retroviral like insertion at the *Sd* locus. The ETn insertion was confirmed via Southern analysis, and is not present in additional inbred mouse lines tested. Further, expression studies revealed that the ETn causes mis-expression of the adjacent *Ptf1a* gene. Currently, work is underway to determine the role of *Ptf1a* in proper caudal development in mice and humans.

1444T

Direct or indirect stimulation of an epigenetic change in bladder urothelial cells (UC) in response to uropathogenic *E.coli* (UPEC) infection. *A. Kapila[1,2], C. Tolg[1], B. Weber[1,2], N. Sabha[1], R. Cortese[3], T. Panchal[1], A. Petronis[2,3], K.J. Aitken[1,2], D.J. Bägli[1,2].* 1) Developmental & Stem Cell Bio, Research Institute and Urology Division, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Institute of Medical Science, University of Toronto, Toronto, Ontario, Canada; 3) The Krembil Family Epigenetics Laboratory, Centre for Addiction and Mental Health (CAMH), Toronto, Ontario, Canada.

Introduction: Urinary tract infection (UTI) and UTI recurrence can seriously compromise patients with dilated urinary tract pathologies or other preexisting medical conditions. However the inability to predict which of these patients are at such risk for UTI or its recurrence forces pre-emptive surgical correction of dilating uropathies or widespread indiscriminate antibiotic use. Identification of UPEC-induced DNA methylation marks for UTI recurrence may eventually make it possible to provide a valuable diagnostic tool for refining medical and surgical therapy for such patients, thereby curbing antibiotic use and reducing prophylactic surgery.

Methods: We established an in vitro model of chronic infection using low moi of UPEC and non-UPEC (FimH-) bacteria in the urothelial cell line HTB-9, for 6 days. After the chronic infection, cells were fixed and examined for intracellular bacteria (anti-E.coli antibody) and DNMT1 by confocal microscopy. DNMT1 activity was also assessed in UPEC and non-UPEC inoculated cultures (by Epiquik DNMT activity kit). DNMT1 expression was assessed by real-time PCR. Conditioned media (CM) from UPEC and non- UPEC infected cells was isolated and filtered twice through 0.22 micron filters and was placed on urothelial cell lines for six days and DNMT1 expression was examined by confocal microscopy. Also urothelial cell lines were treated with LPS and examined for DNMT1/3a/3b by confocal microscopy.

Results and Conclusions: Persistent infection of UC with FimH[+] UPEC results in DNMT1 upregulation and decreased cell proliferation. Infection with FimH[-] E.coli had no effect on DNMT1 or UC growth. These results show that FimH adhesin plays an important role in the epigenetic reprogramming of host cell gene expression by E.coli. DNMT1 expression was associated with UC in inoculated cultures; though even neighbouring cells without intracellular UPEC showed an increase in DNMT1 staining. The increase in DNMT1 was replicated in CM from UPEC inoculated cultures, suggesting that the infected UC release paracrine factors which are similarly able to increase DNMT1 expression. Exposure to LPS increased nuclear DNMT 3a and 3b levels but had only minor effects on DNMT1 signifying that the endotoxin may lead to distinct effects from the pathogen itself.

1445T

Evidence of *NFIA* as a dosage-sensitive gene involved in central nervous system development and neurobehavioral functioning. *K. Hovanes[1], M.N. Strecker[1], M. Dasouki[2], E. Youngs[2], D. Superneau[3], S. Hunkapillar[3], P. Miner[4], C. Munn[4], G. Hoganson[4], S. Gunn[1].* 1) CombiMatrix Diagnostics, Irvine, CA; 2) Kansas University medical Center, Kansas City, KS; 3) Our Lady of the Lake Regional Medical Center, Baton Rouge, LA; 4) Rockford Memorial Hospital, Rockford, IL.

We report on four individuals with copy number changes in *NFIA* as the result of non-recurrent microdeletions/duplications at 1p31.3. Six cases of haploinsufficiency (HI) of *NFIA* due to gene disruption or deletion have been reported in association with structural central nervous system (CNS) and urinary tract abnormalities. Previous microdeletions that include *NFIA* have been relatively large: 12Mb (48 genes), 7.9Mb (16 genes), and 2.2Mb (9 genes). To our knowledge, this is the first report of patients with deletions/duplications <1Mb and encompassing fewer than two genes in addition to *NFIA*. All patients were evaluated on a 180K oligonucleotide platform. **Patient 1** is an 8 y/o male with macrocephaly, advanced bone age, attention deficit-hyperactivity disorder (ADHD) and mild dysmorphic features. A non-contrast head CT at 2 years of age demonstrated relative atrophy with a prominent septum pellucidum cavum and prominent ventricles. Chromosomal microarray analysis (CMA) revealed: arr 1p31.3(61,656,200-62,245,026)x1, encompassing *NFIA* (partial), *TM2D1* and *INADL* (partial). **Patient 2** is a 16 y/o female with agenesis of the corpus callosum, developmental delays, cognitive impairment, ADHD and mild dysmorphic features. CMA demonstrated: arr 1p32.1p31.3(60,890,159-61,833,408)x1 mat,2q13(110,186,668-111,127,490)x1. The 1p deletion encompasses only *NFIA*. The 2q deletion includes six genes of uncertain function and one (*NPHP1*) which is associated with autosomal recessive nephronophthisis and Joubert syndrome. **Patient 3** is a 36 y/o female with a Dandy Walker malformation, a history of hydrocephalus requiring shunting, and a history of vesicoureteral reflux. Patient 3 is the mother of Patient 2, and was found to have the same copy number loss as her daughter. **Patient 4** is a 5 y/o male with a history of infantile tremors, autism spectrum disorder, ADHD, anxiety and verbal apraxia. CMA revealed: arr 1p31.3(61,475,400-61,916,101)x3, with a partial gain of *NFIA*. His brain MRI was normal. This report strengthens the evidence suggesting that HI of *NFIA* alone is sufficient to cause CNS malformations. In our patient series, it also was associated with ADHD. Unlike previous reports, only Patient 3 had urinary tract abnormalities. Patient 4, who has a partial gain of *NFIA*, has significant neurobehavioral differences, but no CNS structural abnormalities, suggesting that *NFIA* may be dosage sensitive, but that HI alone is implicated in structural CNS malformations.

1446T

Small RNA deep sequencing reveals co-ordinate expression of microRNAs and argonaute-2 during mammalian embryogenesis. *P.N. Valdmanis, H. Kim, B. Roy-Chaudhuri, Y. Pouliot, M.A. Kay.* Pediatrics, Stanford University, Stanford, CA.

RNA interference occurs by two broad processes: mRNA site-specific cleavage and non-cleavage mRNA degradation and/or translational repression. Mammals have four argonaute genes; however, only argonaute-2 (Ago-2) is able to perform site-specific cleavage, while all four mammalian argonaute proteins (Ago-1 to Ago-4) can carry out non-cleavage-mediated inhibition. The role of Ago-2 in mediating site-specific mRNA cleavage is essential in mammals, in particular during embryonic development, as Ago2 -/- mice suffer severe developmental abnormalities after embryonic day 10.5 (E10.5) and are lethal soon thereafter. To identify the cause of this Ago2-dependent embryonic event, we performed small RNA high throughput sequencing of mouse embryonic fibroblast (MEF) cell lines both before (E10.5) and after (E13.5) this critical time point as well as Ago2 -/- E10.5 MEF cells. MirVana-extracted small RNAs were cloned in a 5'phosphate dependent manner, aligned to linkers and sequenced on an Illumina GAII machine. Reads (17-28bp) were aligned to the mouse genome and to mirBase (release 15) using the Bowtie alignment program. Normalization of microRNA reads revealed a cluster of ~50 microRNAs in a ~800 kilobase pair region on mouse chromosome 12 that are dramatically upregulated in E13.5-derived MEFs relative to E10.5 wildtype and Ago2-/- MEFs. This imprinted region is implicated in uniparental disomy on human chromosome 14. The expression of representative microRNAs in the locus including mir-127 and mir-376 were validated by northern blot analysis. Interestingly, mir-127 is expressed from a transcript antisense to the Rtl1 gene and has been shown to cleave Rtl1 in an Ago-2 dependent manner. Taqman quantitative RT-PCR analysis revealed that the expression of nearby coding and non-coding RNAs including Gtl2, Dlk1 and Rtl1as transcripts are activated after embryonic day 10.5 and are dramatically up-regulated though the remainder of mouse embryo development. Notably, Ago-2 is the only mammalian argonaute that exhibits a similar increase in expression later in embryo development, while Ago-1, Ago-3 and Ago-4 transcripts remain unchanged. This contemporaneous upregulation of these mouse chromosome 12qF1 non-coding RNAs and microRNAs is one that we believe is critically dependent on Ago2 for development.

1447T

Identification and characterisation of endogenous LXR ligands in ventral midbrain development. *S. Theofilopoulos[1], K. Karu[2], S. Kitambi[1], P. Sacchetti[1], K. Sousa[1], J. Sjovall[1], W. Griffiths[3], E. Arenas[1].* 1) Molecular Neurobiology, Medical Biochemistry and Biophysics Department, Karolinska Institute, Stockholm, 17177, Sweden; 2) The School of Pharmacy, 29-39 Brunswick Square, London, WCN 1AX, U.K; 3) Institute of Mass Spectrometry, School of Medicine, Swansea University, Singleton Park, Swansea SA2 8PP, U.K.

The liver X receptors (Lxr(and Lxr)) are ligand-dependent nuclear receptors activated by oxidized derivatives of cholesterol (oxysterols). Our laboratory has recently shown that Lxr(and Lxr) promote ventral midbrain (VM) neurogenesis in vivo and in human embryonic stem cells (Sacchetti et al., Cell Stem Cell 5,409-19, 2009). Our current study focused in identifying and characterizing the endogenous LXR ligands in the embryonic mouse VM. Using LC-MSn and LXR-activation reporter assays, we identified two distinct molecules that are present in the E11.5 mouse VM and are potent activators of LXR(and LXR) . Employing several types of rodent primary cultures, embryonic stem cells, organotypic `open-book'cultures, and zebrafish models, we showed that the two identified LXR ligands have a different mechanism of action and effect in the embryonic VM. We demonstrate that recruitment of specific transcriptional co-activators (such as SRC-1 and ASC-2) and/or transcriptional repressors (such as N-CoR and SMRT) to the transcriptional machinery complex is regulated by the presence of distinct LXR ligands and this leads to different responses in survival versus neurogenesis in the developing VM. We believe that these LXR ligands may lead to improvements in dopaminergic differentiation of human ESCs and their application in drug development and cell replacement therapy for Parkinson's disease.

1448T

Spga-lncRNA3, a novel lncRNA that regulates developmental programs of spermatogonial stem cells. *T. Lee[1,2], W. Chan[2], O. Rennert[2].* 1) School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong, China; 2) Laboratory of Clinical and Developmental Genomics, National Institutes of Child Health and Human Development, National Institutes of Health, Bethesda, MD, U.S.A.

Spermatogonial stem cells (SSCs) are male germ line stem cells that control spermatogenesis by their ability to both self-renew and generate subsequent germ cell types into spermatozoa through rounds of differentiation. Although factor like Glial cell line-Derived Neurotrophic Factor (GDNF) is known to be important in self-renewal of SSCs, the exact mechanisms that govern SSCs differentiation and pluripotency remain largely unknown. To identify the differentiation and self-renwal mechanisms, we performed large-scale transcriptome studies on male germ cells by whole-genome tiling microarrays and sequencing approaches. The findings suggested majority of SSC genome is transcribed and is far more than current gene annotations. We found a huge number of transcript species are known as long non-coding RNAs (lncRNAs). By applying various bioinformatics pipelines, we have identified a number of SSC-specific lncRNA candidates and examined their effects on cellular differentiation using C18-4 SSC in vitro differentiation model. We found a potential candidate, code-named as Spga-lncRNA3, demonstrated significant differentiation inhibition, suggesting it may be important in maintaining stem cell state of SSCs. Our contribution in this study is expected to provide detailed understanding of how lncRNAs regulate cell differentiation and proliferation in spermatogenesis. Dysregulation of these developmental programs is known to associate with gametogenesis defects and disease states such as cancer. The findings are expected to substantially change the concepts in developmental and cancer biology, which could lead to the development of novel therapeutic strategies.

1449T

Notch signaling during chondrogenesis. *S. Chen, J. Tao, M. Jiang, T. Bertin, B. Lee.* Howard Hughes Medical Institute and Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Notch signaling is critical during development directing binary cell fate between progenitors and differentiated cells. Aberrant Notch function causes several human skeletal diseases such as Spondylocostal dysostosis (SCDO) and Alagille syndrome. Recently, cartilage specific gain- or loss-of-function of Notch mouse models have been made in our and other laboratories. Although detailed phenotypic studies of these mice have been conducted, a full knowledge of the mechanisms of the pathway is still lacking. The canonical Notch pathway involves Rbpj as a co-factor to induce target gene expression. However several findings suggest Rbpj-independent mechanisms exist to promote Notch signaling transduction. Our goal is to unravel the molecular mechanisms by which Notch signaling controls cartilage development. We hypothesize that the function of Notch signaling is mediated by both the canonical and non-canonical pathways. To study the gain-of-function of Notch in cartilage, we crossed a Col2a1-cre line with a line having a conditionally activated allele of Notch1 intracellular domain (N1ICD). Resulting mutant mice have shortened limbs, undersized ribs, and virtual absence of the spine and tail. Decreased Alcian blue staining highlights the general defects of chondrogenesis. The decreased expression of Sox9, Col2, Col10 in the mutant suggests the inhibition of chondrocyte differentiation by Notch signaling. On the other hand, complete loss-of-function of Notch (achieved by deleting Presenilin 1/2 in cartilage) causes undersized and underdeveloped skeletal elements. The limbs and spine are about 40% shorter in the mutants at 3 weeks of age. Interestingly, cartilage specific deletion of Rbpj causes only a mild phenotype. The body weight and histology of cartilage appear to be normal at 3 weeks of age. To test whether Notch signal transduction depends on Rbpj, we made mice with deficient Rbpj on the Notch gain-of-function background. These mice have nearly normal limb structure but demonstrate irregular curvature of the spine with a shortened tail, suggestive of incomplete rescue. To completely map the Notch signaling pathway, we are identifying Notch targets by a CHIP-seq approach in the ATDC5 cells, a chondrocyte cell line. We have achieved high specificity and enrichment of the occupancy of the Notch targets using a tagged system. We hope to identify differential targets of canonical vs. noncanonical Notch signaling using this approach.

1450T

WT1 suppresses EZH2 during early nephrogenesis. *M.M. Akpa[1,2], L.L. Chu[1,2], D.M. Iglesias[1,2], P.R. Goodyer[1,2,3].* 1) Human Genetics, McGill University, Montreal, Canada; 2) Montreal Children's Hospital Research Institute, Montreal, Canada; 3) Montreal Children's Hospital, Montreal, Canada.

During development, progenitor cells are induced to form nephron in the embryonic kidney. The exact mechanism involved in this process remains unexplained. The metanephric mesenchyme is known to receive a signal inductive from the ureteric bud, which triggers the differentiation cascade. Wilms tumor suppressor 1 (WT1), a zinc finger transcription factor, is believed to play a key role in this process. Moreover, stem cells retain their pluripotency during embryogenesis by suppressing key differentiation genes through epigenetic modifications, involving Polycomb group protein among others. How this repression is lifted during differentiation is still unclear. We hypothesize that WT1 is crucial to the de-repression of differentiation genes. We further hypothesize that WT1 modulates EZH2, the key catalytic unit of the Polycomb Repressor Complex 2 (PRC2). We showed that WT1 has a direct transcriptional effect on EZH2 through promoter and expression experiments. Using Western immunoblotting, we observed a repression of EZH2 at the protein level in the presence of WT1 compared to the baseline. Using an EZH2 promoter-reporter construct, we also observed a repression of the luciferase activity in the presence of WT1 as compared to the baseline. We confirmed that the effect is mediated through binding of WT1 to a WT1 recognition motif on the EZH2 promoter sequence using Electrophoretic Mobility Shift Assay (EMSA), and that this interaction is specific using an antibody against WT1 that would interfere with the binding ability of WT1 to the motif. Using real-time PCR, we investigated the role of miR-101 and miR-26a in repressing EZH2, mediated through WT1. We showed that WT1 did increase the expression of the two miRNAs and thus also showing an indirect repressive effect of WT1 on EZH2 at the post-transcriptional level. During nephrogenesis, WT1 plays a key role in the differentiation process, achieved in part through the modulation of EZH2's expression, thus lifting the repression exerted on key differentiation genes. The effect of WT1 on EZH2 expression seems to be at the transcriptional level through direct interaction with the promoter region, as well as at the post-transcriptional level indirectly through increased expression of miRNAs. They will bind the 3'UTR and degrade the EZH2 RNA transcript.

1451T

EnSpm-N6_DR DNA transposons shape the repertoire of p53 target genes in zebrafish. *M. Loviglio, L. Micale, C. Fusco, A. Calcagni, B. Augello, G. Cotugno, E.V. D'Addetta, G. Merla.* Medical genetics Unit, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy.

Transposable elements (TEs) represent the largest genomic component of most eukaryotic organisms. In spite of and, to some extent, because of their selfish and parasitic nature, TEs have exerted a strong influence in the structural organization and plasticity of genomes. Recent discoveries support the hypothesis that TEs can directly regulate the expression of nearby genes both transcriptionally and post-transcriptionally. For instance, TEs are known to supply functional elements to their host genomes, as transcription factors binding sites, transcription start sites and enhancer elements. Here we describe the role of zebrafish (Danio rerio) En-SpmN6_DR non-autonomous DNA transposon in shaping the repertoire of a transcription factor, the pleiotropic master regulator p53. Multiple copies of En-SpmN6_DR transposon, which present as single element or clusters, are mapping throughout the genome of zebrafish, the vast majority of which in the upstream or in the introns (less frequently just downstream) of zebrafish coding genes. By luciferase assay, we assessed whether the p53 transcription regulator was able to bind two predicted p53 responsive elements (REs) mapping within the EnSpm-N6_DR transposon, which was found to be proximal (<500 bp) to predicted promoter regions. Notably, we demonstrated that these well conserved p53 binding sites are functionally responsive to Drp53, resulting in the transactivation of several zebrafish genes, including cell cycle regulators and neuron morphogenesis-related factors. Remarkably, a large fraction of orthologs of the genes harboring the EnSpm-N6_DR element in zebrafish are annotated as putative or validated p53 targets in human, and functional annotations evidence a partecipation to diverse neural developmental pathways, such as neurogenesis, synaptic transmission and axonogenesis. Furthermore, predominant expression is found in brain and brain-related structures. Our experimental evidences demonstrate that EnSpm-N6_DR transposon identifies a class of functionally related zebrafish genes, transcriptionally controlled by p53 protein, providing further indication that p53-dependent molecular pathways affect multiple developmental processes, including neuronal maturation and differentiation.

1452T

Modeling Developmental Eye Defects using Zebrafish. *L.A. Schimmenti[1], J. Hatler[1], E. Speltz[1,2], A. Spahn[1], S. Lerach[1].* 1) Dept Pediatrics, Ophthalmology, Genetics, Cell Biology and Development/Inst Human Gen, Univ Minnesota, Minneapolis, MN; 2) Current Address: Yale University, Molecular Biophysics and Biochemistry.

Proper eye development during embryogenesis is critical for normal visual function. Clinically significant ophthalmologic disorders, anophthalmia, microphthalmia and coloboma, result from defective developmental processes and are observed at a rate of 3 per 10,000 live births accounting for approximately ten percent of childhood blindness. The breadth of genetic and cellular mechanisms leading to defective eye development is still not well understood. Dominant mutations in *PAX2*, a member of the paired box transcription factor family, have been identified in patients with renal coloboma syndrome, and *pax2a* mutations in animal models recapitulate this phenotype. The overall goals of this research are to identify and study the network of gene products that function under the control of pax2 and are required for normal eye development. Previous studies in Drosophila identified sparkling (spa) as a pax2 ortholog (Fu & Noll, 1997). Spa mutants have abnormal eye development and reduced expression of cut, a DNA binding protein. These observations led us to hypothesize that cutl1 functions downstream of pax2a during zebrafish eye morphogenesis. The zebrafish embryo is a useful model in which to study eye morphogenesis because its eyes have many similarities with mammalian eyes, particularly in terms of retinal layering. Additionally, eye development in zebrafish is a rapid process with retinal differentiation beginning at ~30 hours post fertilization (hpf) and functional vision occurring at 72 hpf following the formation of photoreceptor outer segments. Moreover, zebrafish embryos are optically clear and develop externally allowing for ease of experimental manipulation and observation. Here, we find that morpholino (MO) antisense oligonucleotide knockdown of the DNA binding protein, cutl1, significantly reduces eye and pupil sizes in zebrafish embryos. Histologically, retinal lamination appears to be partially intact, although some disorganization is observed at higher MO doses. Importantly, the reduced eye size phenotype is likely due to a defect in photoreceptor outer segment (OS) development and/or reduced retinal ganglion cell (RGC) dendrite growth within the inner plexiform layer (IPL). Our findings implicate a novel DNA binding protein, cutl1, in eye development and may represent an avenue by which *PAX2* mutations cause human eye disorders.

1453T

Ofd1 controls dorso-ventral patterning in the brain and plays a developmental stage dependent role in ciliogenesis. *B. Franco[1,4], A. D'Angelo[1], A. De Angelis[1], B. Avallone[2], I. Piscopo[1], M. Studer[3], R. Tammaro[1].* 1) TIGEM, Fondazione Telethon, Naples, ItalyTelethon Institute of Genetics and Medicine, Naples, Italy; 2) Department of Biological Science, Federico II University, Naples, Italy; 3) INSERM U636 University of Nice Sophia Antipolis Nice, France; 4) Medical Genetics Services, Department of Pediatrics, Federico II University, Naples, Italy.

Oral-facial-digital type I syndrome (OFDI) is a human X-linked dominant-male-lethal developmental disorder caused by mutations in the OFD1 transcript. Similar to other inherited disorders associated to ciliary dysfunction OFDI patients display neurological abnormalities. We therefore characterized the neuronal phenotype resulting from Ofd1 inactivation in early phases of mouse embryonic development and at post-natal stages. We determined that Ofd1 plays a crucial role in forebrain development, and in particular in the control of dorso-ventral patterning and early corticogenesis. Interestingly, we observed abnormal activation of the Sonic hedgehog (Shh) and Planar Cell Polarity (PCP) signalling, two major pathways modulating brain development. Ultrastructural studies demonstrated that early Ofd1 inactivation results in the absence of ciliary axonemes despite the presence of mature basal bodies that are correctly orientated and docked. On the contrary, Ofd1 inducible-mediated inactivation at birth does not affect ciliogenesis in the cortex demonstrating for the first time a developmental stage-dependent role for a basal body protein in ciliogenesis. Moreover, we showed a defective cytoskeletal organization in Ofd1 null embryos, most likely due to lack of ciliary axonemes. Thus, the present study identifies Ofd1 as a developmental disease gene that is critical for forebrain development by regulating proper axoneme formation during ciliogenesis in embryonic life, and elucidates the precise step of ciliogenesis in which Ofd1 plays a crucial role in vivo.

1454T

Novel intergenic trans-splicing generating double fusion transcripts in a case of chronic myeloid leukemia in blast phase with the t(7;17) translocation. *S. Hazourli, J. Hébert.* Quebec Leukemia Cell Bank, Maisonneuve-Rosemont Hospital Research Center, Montreal, Quebec, Canada.

Alternative pre-mRNA splicing contributes to protein diversity and plays a crucial role in different cellular mechanisms. Splicing defects have been associated with many human genetic diseases, as well as cancer. We present a novel aberrant intergenic splicing detected in the leukemic cells of a patient with the t(7;17)(p15;q22) chromosomal translocation, associated with chronic myeloid leukemia in blast phase (CML-BP). Using fluorescence in situ hybridization analyses, we studied the breakpoints in the *HOXA* cluster and the RNA-binding protein Musashi 2 gene (*MSI2*), located on chromosomes 7 and 17, respectively. Two different in-frame fusion transcripts, which contain exons 1 to 5 of *MSI2* fused to exon 2 of either the *HOXA9* or the *HOXA11* gene, were identified by RT-PCR and sequencing. The predicted chimeric proteins contain the RNA Recognition Motif domain of MSI2 and the homeodomain of HOXA9 or HOXA11. The presence of fusion transcripts involving two different *HOXA* genes in the same case has already been described in two patients with the t(7;11)(p15;p15)/*NUP98-HOXA* fusion, including one CML-BP case. Only one other case of CML-BP with the t(7;17) has been reported, in which a different translocation breakpoint, fusing exon 9 of *MSI2* to exon 2 of *HOXA9* was detected. A rearrangement of the *MSI2* gene has also been found in nine cases of myeloid malignancies (myelodysplastic syndrome, acute myeloid leukemia and CML-PB) with the t(3;17)(q26;q22). However, *EVI1-MSI2* fusion transcripts have not been detected in these cases. Recently, *MSI2* has been identified as a positive regulator of both mouse and human hematopoietic stem cells. Moreover, *MSI2* overexpression has been associated with advanced CML and with a poor survival in acute myeloid leukemia. This case provides additional evidence that aberrant alternative splicing and the deregulated expression of the genes involved in chromosomal translocations are relevant mechanisms in the pathogenesis of the progression of CML into a more aggressive phenotype.

1455T

Treatment outcome with sequential radiotherapy and chemotherapy based on loss of heterozygosity in both chromosomes 1p and 19 q in anaplastic oligodendroglioma. *HO. Shah[1], A. Yuil[2], A. Pigal[2], M-L. Desormeaux[2], W. Gebre[2], L. Freedman[2].* 1) Dept Pathology, Cytogenetics, Nassau Univ Med Ctr, East Meadow, NY; 2) Dept Pathology, Nassau Univ Med Ctr, East Meadow, NY.

A 46 years old male, who presented to the hospital with symptoms of headaches over the past three years and visual changes during last visit. A CAT scan and MRI of the head were done and both demonstrated a large right frontal mass with significant mass effect. The clinical laboratories were unremarkable. Craniotomy was performed and the specimen of frontal lobe tumor was submitted to Pathology. Anaplastic oligodendrioglioma was diagnosed by the neuropathologist. PCR - based microsatellite analysis that was performed in the paraffin-embedded tissue, revealed a combined loss of chromosome number 1p and 19q, and confirmed the final diagnosis. The patient started treatment with radiotherapy and temozolomide, with good response and no tumor recurrence in the last 6 months. It has been described that patients who have tumors that showed combined 1p and 19q loss in chromoses, had significantly improved outcomes compared with patients who had tumors that lacked 1p/19q loss, hence, clinically testing oligodendroglial tumors for 1p/19q status has become increasingly common, in addition to patient outcomes, 1p/19q status has been associated with tumor location (frontal, parietal, and occipital lobe and bilateral growth). Patients with oligodendrogliomas with the combined loss of 1p and 19q not only have a better response to chemotherapy, they also have a more indolent clinical course and a longer lasting response to radiotherapy. Several studies have found the presence or absence of combined loss of 1p and 19q to be the most important prognostic factor for overall survival in anaplastic oligodendroglial tumors. The median survival duration was 6 -7 years in the presence of the 1p/19q codeletion, and 2-3 years in the absence of the codeletion. The overall picture that emerges from this is that 1p/19q codeleted tumors tend to have slower growth rates and to be more responsive to treatment than tumors without this codeletion. Currently, sequential treatment with radiotherapy and chemotherapy, depending on the expected side effects and treatment efficacy, seems to be the optimal approach.

1456T

Identification of genetics alterations in colorectal cancer patients in Tamil Nadu population, India. *V. Balachandar[1,2], M. Arun[1], P. Manikantan[1], K. Sasikala[1], P. Varsha[3], S.N. Dharwadkar[4], P. Singaravelu[2].* 1) Human Molecular Genetics lab, Bharathiar University, Coimbatore, India; 2) Thiruvalluvar University, Vellore, India; 3) Molecular Biology and Human Genetics, Department of Zoology,Bharathiar University, Coimbatore, India; 4) KLE Medical University, Bangalore,India.

Colorectal cancer (CRC) is the most common inherited cancer syndromes, in which cells in colon or rectum become abnormal and divide without any order forming a tumor. CRC is the second leading cause of malignant mortality in humans in developed countries, accounting for more than 10% of all cancer-related deaths. About 96% of CRC are adenocarcinomas, which evolve from glandular tissue. CRC incidence and mortality rate are 35% higher in men than in women. It is believed that chromosomal anomalies play a major role in tumorigenesis by increasing the rate of chromosome mutations, including deletion and amplification of genes involved in cellular proliferation and/or survival. The main objective of the present study was to identify the chromosomal alternations of CRC patients in Tamil Nadu population, South India. Carcinoembryonic antigen (CEA) has been investigated extensively to test its clinical value in the management of CRC. The aim of CEA monitoring after curative resection of colorectal cancer is to detect recurrent disease at an early and treatable stage. In our study, totally 62 patients were recruited and divided in to 2 groups based on their serum CEA level. In this study chromosomes mainly affected in CRC patients were 1,2, 8, 17, 18 and 22. The major CA was deletions and translocations. The serum CEA level was found to be higher in CRC patients compared to their controls. More number of CA was observed in group II patients who were of higher serum CEA level in comparison to other groups. The present study has been useful in identifying molecular targets for controlling onset of the disease. A good understanding of the genetic changes underlying colorectal carcinogenesis may provide new perspective for prognosis and screening of high risk individuals. Early detection, accurate diagnosis and intensive surveillance are important for best improving a patient's diagnosis and response to therapy.

1457T

CGH+SNP microarrays for copy-neutral aberration detection in cancer research. *P. Costa[1], B. Curry[2], B. Peter[2], P. Anderson[2], N. Sampas[2], S. Giles[1], A. Ashutosh[1], A. Vadapalli[1], A. Ijpma[1], J. Ghosh[1], S. Fulmer-Smentek[1], A. De Witte[1].* 1) Agilent Technologies Genomics, Santa Clara, CA; 2) Agilent Technologies Laboratories, Santa Clara, CA.

Advances in cancer research have greatly benefited from high resolution copy number (CN) measurements provided by oligo array Comparative Genomic Hybridization (aCGH). The addition of single nucleotide polymorphism (SNP) measurements to CGH microarrays enables the detection of copy-neutral loss of heterozygosity (cnLOH) events and allelic imbalances arising from genomic instability during cancer development and progression. Nonetheless, cancer studies face challenges associated with genomes' aneuploidy, polyclonality and mosaicism due to the admixtures of tumor and normal cells. In the quest to decipher tumor complexity, the power and sensitivity of Agilent's CGH+SNP platform has been expanded with new computational methods capable of determining clonal fraction, total CN and allele-specific CN in aneuploid samples. Genomic DNA from hematology-oncology samples, cell lines and HapMap control samples was digested with AluI and RsaI restriction endonucleases to allow for SNP profiling at the enzymes' restriction sites. Experimental samples and a reference HapMap sample of known genotype were differentially labeled and hybridized to the Agilent CGH+SNP array, containing ~120K CGH probes and ~60K SNP probes. The data were analyzed using algorithms with extended capability to determine aneuploid fraction, total and allele-specific CN of the aberrant clone. As expected, significant diversity was found in different chronic lymphocytic leukemia (CLL) tumors. In one CLL sample a small heterozygous deletion and cnLOH were identified on chromosome 13. In another CLL case a trisomy of the entire chromosome 12 was observed, together with a small heterozygous deletion and cnLOH on chromosome 18. An Acute Lymphoblastic Leukemia (ALL) tumor sample was found to harbor an amplification on chromosome 6, as well as two small amplifications on chromosome X. To assess the ability of detecting low level mosaicism, we conducted a mixed sample experiment whereby a sample with a known aberration was mixed at known ratios with a matched sample not containing the aberration. The computed aneuploid fraction, total and allele-specific CN matched the expected values. We have shown that the new algorithms developed for cancer sample analysis determined the genotypes, total copy numbers and clonal fractions in aneuploid samples highly mixed with normal cell populations.

1458T

Analysis of cytogenetic aberration in children with acute lymphoblastic leukemia in relation to other prognostic factors. *E. Maly[1], M. Przyborska[1], K. Derwich[3], O. Spychaka[3], D. Januszkiewicz[1,2,3].* 1) Department of Medical Diagnostics, Poznan, Poland; 2) Institute of Human Genetics Polish Academy of Sciences, Poznań, Strzeszyńska Street 32; 3) Department of Pediatric Oncology, Hematology and Bone Marrow Transplantation of Medical University, Poznań, Szpitalna Street 27/33.

Acute lymphoblastic leukemia (ALL) is the most common malignancy in childhood and accounts for 30% of all cases of cancer. Chromosomal aberrations have a major role in the risk assessment of acute lymphoblastic leukemia. Detection of chromosomal abnormalities is used to determine the risk group, treatment and prognosis of outcome. Karyotypes of 85 ALL patients were analyzed along with immunophenotype and other clinical factors important in assessing the prognosis of ALL. Normal karyotype was encountered in 41 cases (48,2%), translocation t(12;21) - in 12 (14,1%), tetrasomy 21 - in 7 (8,2%), hiperdiploidy - in 5 (5,9%). One case carried translocation t(9;22) (1,2%), two cases- MLL rearrangement (2,3%). Three patients had a complex karyotype (3,5%). Due to lack of metaphases or bad quality of chromosomes karyotype wasn't perform in 14 patients. Girls predominated in common B-ALL, in the rest of subtypes the majority were boys. The highest leukocytosis was observed in the T-ALL subtype, also the L2 morphology was predominated in the T-ALL. Cytogenetic abnormalities with poor prognosis were documented in pro-B ALL and T-ALL. Patients with common B-ALL have had the highest rate of remission on the 15 day of treatment. The highest poor prednisolone response was observed in T-ALL and pro-B ALL patients. Determination of chromosome changes in the karyotype of the patient is an individual and independent prognostic factor in acute lymphoblastic leukemia. Today cytogenetics is a standard in the hematological diagnostics and without it, it is difficult to identify and stratify patients into appropriate treatment groups and to determine the risk of leukemia recurrence.

1459T

Blastic plasmacytoid dendritic cell neoplasm caused by loss of genomic DNA copy numbers in the p18, p16, p27 and RB loci. *N. Oiso[1], Y. Tatsumi[2], T. Arao[3], S. Rai[2], M. Kimura[4], S. Nakamura[5], K. Nishio[3], I. Matsumura[2], A. Kawada[1].* 1) Dept Dermatology, Kinki Univ Faculty Med, Osaka-Sayama, Osaka, Japan; 2) Dept Hematology, Kinki Univ Faculty Med, Osaka-Sayama, Osaka, Japan; 3) Dept Genome Biology, Kinki Univ Faculty Med, Osaka-Sayama, Osaka, Japan; 4) Dept Pathology, Kinki Univ Faculty Med, Osaka-Sayama, Osaka, Japan; 5) Dept Pathology, Nagoya Univ Grad Sch Med, Nagoya, Japan.

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare plasmacytoid dendritic cell precursor-derived aggressive neoplasm with a poor prognosis. Recent studies show that two-thirds of BPDCNs have an abnormal tumor cell karyotype with an average of six to eight abnormalities. We show a 74-year-old Japanese male with BPDCN. The patient's genomic DNA copy numbers were analyzed using array-comparative genomic hybridization (CGH) to identify the chromosomal and genetic features of the malignant cells. The array-CGH analysis revealed the loss of the chromosomes1p31.3-33, 9p/q, 12p13.1-13.2 and 13p/q, and the gain of chromosome 16p/q. These lost regions included the loci of the tumor suppressor genes of CDKN2C/p18, CDKN2A/p16, CDKN1B/p27 and RB1, which are well-known cell-cycle inhibitors. We identified a novel loss of a chromosome 1p31.3-33 region including CDKN2C/p18, which may be involved in the pathogenesis of BPDCN.

1460T

Detection of chromosome alteration and RB1 gene polymorphisms in retinoblastoma patients, India. *P. Varsha[1], V. Balachandar[2,3], S. Mohana Devi[2], K. Sasikala[1,2].* 1) Molecular Biology and Human Ge, Bharathiar University, Coimbatore, India; 2) Human Molecular Genetics Lab, Bharathiar University, Coimbatore, India; 3) Thiruvalluvar University, Vellore, India.

Retinoblastoma is a rare childhood eye cancer initiated by the loss of function of both alleles of the RB1 tumor suppressor gene, mapping to 13q14 chromosomal band. The hereditary form develops at an earlier age than the sporadic one and usually appears as bilateral or multi-centric tumors or both. Approximately 80% of the unilateral cases are a result of a somatic, nonhereditary mutation. The aim of the present study was to assess the risk of retinoblastoma developing in children with chromosomal alterations with mosaic deletions and RB1 gene mutation. Chromosomal alteration and RB1 gene were examined in 42 retinoblastomas (RB) patients (28 with unilateral and 14 with bilateral retinoblastoma) who were treated in Tamil Nadu population, India. We studied 14 patients with bilateral retinoblastoma with or without familial history and 28 patients with unilateral retinoblastoma. Three of the unilateral patients had a positive familial history at diagnosis. All identifiable reported cases of unilateral and bilateral retinoblastoma, which included CA and the RB gene, were analysed in the present study. In the present study, 14 patients, interstitial deletion in chromosome 13 covered region q13~q22. In two URB patient with, a constitutional del 46, XX, del (13) (q14-q21) were observed and four of the patient (2 URB and 2 BRB), a 22- days-old male with BRB, a constitutional 46, XY, 13q14-q22 was transmitted from his asymptomatic mother. In summary, more accurate and reliable genetic testing would provide an important advance in treating patients with retinoblastoma and their families. The molecular techniques used to identify RB gene mutations have improved dramatically in recent years. Conclusive identification of RB1 mutations in retinoblastoma is predicted to improve the clinical management of affected children and relatives.

1461T

Abnormal Signal Patterns Involved in t(12;21) TEL-AML1 in Childhood Acute Lymphoblastic Leukemia Patients. *O. Altiok Clark[1], CF. Sargin Özkaya[2], S. Yakut[2], Z. Cetin[2], A. Kupesiz[3], G. Tezcan[3], V. Hazar[3], G. Luleci[2], S. Berker Karauzum[2].* 1) Department of Medical Genetics, Akdeniz University Medical Faculty, Antalya, Turkey; 2) Department of Medical Biology and Genetics, Akdeniz University Medical Faculty, Antalya, Turkey; 3) Department of Pediatrics, Akdeniz University Medical Faculty, Antalya, Turkey.

The TEL (ETV6)-AML1 (RUNX1) chimeric gene fusion which results from the translocation t(12;21)(p13;q22) is the most common genetic abnormality in childhood B-cell precursor Acute Lymphoblastic Leukemia (ALL). While this translocation is associated with a good prognosis, it is thought that the deletion of the second TEL allele, a gain of the second AML1 allele, and the duplication of the derivative chromosome 21 can each have a negative effect on the clinical outcome. In this study, bone marrow samples from 18 B-cell precursor ALL patients (ages ranging from 6 months to 16 years) were analyzed. Using conventional cytogenetic analysis, it was found that 5 patients had normal karyotypes, 5 patients had numerical and structural abnormalities on chromosome 12 and, no metaphases were observed in eight of the patients. Fluorescence In Situ Hybridization (FISH) was used to determine the translocation between chromosomes 12p13 and 21q22. FISH analysis showed the classical TEL (ETV6)-AML1 (RUNX1) chimeric gene fusion in two cases, whereas double chimeric gene fusion was observed in two other cases, one of which had a wild-type TEL allele deletion while the other had a wild-type AML1 allele deletion. In addition, one t(12;21) positive patient had both a wild-type TEL allele deletion and a wild-type AML1 allele deletion. Four of the patients were found to have the TEL allele deletion without the t(12;21) translocation. Also, the loss of 12p, monosomy 12, and t(6;12)(q16;p12.3) and t(6;9;12)(q13;p22;p13) translocations were observed in these four cases using cytogenetic analysis. In two of patients an extra signal was observed at the AML1 gene locus. One of these two patients had Down Syndrome while the other had a normal karyotype. In the patient with the normal karyotype it is thought that this extra signal was due to amplification of the AML1 gene. During the study, two patients died, one due to an infection which occured during remission and the other because of a relapse. The remaining 16 patients are all still in remission and continuing with their treatment. Our data shows that a deletion of the wild-type 12p13 locus (with or without the t(12;21) translocation) in childhood patients more likely indicates a good prognosis rather than exerting a negative effect on the clinical outcome. Furthermore, the deletion of the TEL allele in patients who do not have the t(12;21) translocation may be used to help identify new ETV6 partner chromosomes.

1462T

Ten years experience of Cytogenetic Investigation in Tunisian Leukemic Patients. *W. AYED[1], O. Kilani[1,2], N. Ben Romdhane[4], S. Ladab[3], L. Torjmane[3], A. Lakhal[3], H. Guermani[1,2], N. Abidli[1,2], F. Talmoudi[1,2], T. Ben Othmen[3], S. Abdelhak[2], A. AMOURI[1,2].* 1) Cytogenetics, Pasteur Institute of Tunis, Tunis, Tunisia; 2) Unité de recherche EMGOOD : Etude des Maladies Orphelines d'Origine Génétique, Institut Pasteur de Tunis, Tunisie; 3) CNGMO of Tunis; 4) La Rabta Hematology.

We analyzed bone marrow and peripheral blood samples of more than 750 Tunisian patients referred from hematology-oncology centers at Tunis. They were either suspected of leukemia at presentation or being monitored for their response to medication. Bone marrow or/and blood cells were cultured, harvested and R-banded according to the standard protocols. Chromosome analysis was performed following ISCN guidelines. The patients were divided into six major groupings as far as the leukemia subtypes were concerned: CML, AML, ALL, MIDS, myeloma and others. Most cases had bone marrow aspiration whereas peripheral blood was utilized only in fraction of cases. The common typical chromosomal abnormalities as well as rare and complex forms were observed. The overall chromosomal abnormality rate obtained was around 30%. The breakdown figures for different categories were roughly as follows: 90% in CML, 25% in AML, 25% in ALL, 30% in MDS and 20% in other types. Compared to published data, the observed chromosomal abnormality rate in the present study is considered average.

1463T

High-resolution confirmation of balanced *RARA* gene rearrangements in acute promyelocytic leukemia by using array CGH. *B.C. Ballif[1], A. Gruver[2], R.R. Tubbs[2], J.R. Cook[2], J.H. Rogers[2], J.R. Batanian[3], A. Furrow[1], L.G. Shaffer[1], R.A. Schultz[1].* 1) Signature Genomic Laboratories, PerkinElmer Inc., Spokane, WA; 2) Clinical Pathology, Cleveland Clinic, Cleveland, OH; 3) Molecular Cytogenetics, SSM Cardinal Glennon Children's Medical Center, St. Louis, MO.

Acute promyelocytic leukemia (APL) is typically characterized cytogenetically by a *PML-RARA* fusion [t(15;17)(q22q12)]. APL cases lacking clear evidence for *PML-RARA* may exhibit alternative chromosomal abnormalities including fusion of *RARA* to other genes (e.g., *ZBTB16*, *NPM*, *NUMA1*, and *STAT5B*), submicroscopic insertions, or complex translocations involving multiple chromosomes. When cryptic, such cases pose a challenge for this critical diagnosis. *ZBTB16* and *STAT5B* fusions are important to recognize as these are resistant to ATRA therapy. Additionally, although it would require a higher resolution test, defining the *PML* breakpoint (bcr1, bcr2 or bcr3) may offer prognostic value. The importance of the genetic lesion in APL has recently been highlighted by the costly use of next generation sequencing technologies to resolve the molecular basis of a cryptic event. As an alternative, we developed a high-resolution microarray-based technology coupled with linear DNA amplification [translocation-CGH (t-CGH)], which can identify balanced translocations and map the breakpoints within ±200 base pairs. We used such a single disease-specific multiplexed assay designed to examine multiple translocations including those aforementioned that are associated with APL to evaluate two cases with a tentative diagnosis of APL. The first case involved a 57-year-old male presenting with classic clinical features and numerous blasts including promyelocytes with Auer rods and high expression of CD13, CD33, and CD117. Although RT-PCR was positive, both karyotype and FISH showed normal results. We performed t-CGH and detected a *PML-RARA* fusion (breakpoints: chr15:72113684-72113889; chr17:35749732-35749887). Both genes showed small copy gains <50 kb presumably resulting from a cryptic fusion. The second patient presented with classic APL symptoms and pathology, with a three-way translocation involving *PML* and *RARA*. The clinician desired additional confirmation with no specimen available for RT-PCR. We performed t-CGH and detected a *PML-RARA* fusion (breakpoints: chr15:72103712-72103789; chr17:35746843-35746876) as well as a 311.6 kb deletion at the breakpoint in *PML*. t-CGH results also provided high-resolution mapping of the *PML* breakpoints (case 1 = bcr1; case 2 = bcr3). Our results illustrate that t-CGH technology applied in a clinical setting provides unambiguous confirmation of APL translocations with additional potentially relevant details not otherwise readily available.

1464T

Evaluation of chromosomal instability in patients with Langerhans cell Histiocytosis. *L. Bobadilla-Morales[1-2], E. Franco-Perez[1], R. Silva-Cruz[1], J.L. De León-Rendón[1], C. Barba-Barba[2], V. Soto-Chavez[2], F. Sánchez-Zubieta[2], A. Corona-Rivera[1-2].* 1) Biol Molecular Genomica, Univ Guadalajara, CUCS, Instituto de Genética Humana ECR, Laboratorio de Citogenetica, Guadalajara, Jalisco, Mexico; 2) Unidad de citogenética, Servicio de Hematología Oncología Pediátrica, División de Pediatría, Hospital Civil “Dr Juan I Menchaca” , Guadalajara.

Introduction: Langerhans cell histiocytosis (LCH) is a clonal proliferative disease of Langerhans cells with local organic or diffuse infiltration of tissues. It has not been possible to establish the physiopathological mechanisms of LCH. Objectives: To evaluate the presence of Chromosomal Instability (CI) in cultured lymphocytes in the presence of mytomicin C (myt C) as a factor for follow-up and response to treatment. Material and methods: We included 8 patients with LCH; lymphocyte cultures were evaluated in the presence of myt C, 100 metaphases per patient, and their respective matched control. We evaluated the presence of CI and performed routine karyotype. Were grouped the patients into 4 stages according to disease course, and in those with localized or multiorgan disease. Results: It was observed statistically significant increase of CI in 78 patients with LCH with respect to the controls. The percentage of aberrations in patients with active and multiorgan disease was the highest observed and the damage more frequently found was chromatid gaps. Conclusions: It was noted the presence of CI in patient`s lymphocytes with LCH respect to the controls, the difference was significant when the disease is multiorganic, and active stage was present, so the CI could be inherent to LCH and the evaluation of CI could reflect the evolution of the disease.

1465T

A simple technique significantly improves the success rate of chromosome analysis from solid tumors. *H. Chaker, D. Turmel, R. Fetni.* Ste Justine, Montreal, Quebec, Canada.

Conventional studies provides valuable information for solid tumors diagnosis and prognostic stratification. However solid tumors cytogenetic is challenging and successful chromosome preparation depends in tumour types, tissue culture and harvesting techniques. We have optimized our laboratory tumors cytogenetic analysis by making a small change in our cytogenetic preparation procedure. We perform in situ culture method for tumor tissue samples and we have combined analysis of metaphases obtained from in situ culture slides and metaphases obtained from harvesting hypotonic solution used for cells swell. Surprisingly the hypotonic solution that is usually discarded contains a considerable number of well spread mitotic cells. A method has been elaborated for obtaining chromosome preparations from different histologic types of human solid tumors and applied to 51 cases. Both quality and quantity of analysed metaphases are significantly improved by this strategy. Use of this technique significantly improves the success rate of chromosome analyses from solid tumors.

1466T

Cytogenetic studies in 84 pediatric neuroepithelial central nervous system tumors in a single institution in Argentina. *M.C. Coccé[1], F. Lubieniecki[2], D. Alderete[3], M.S. Gallego[1].* 1) Genetics Department, Hospital de Pediatría Prof. Dr. Juan P. Garrahan. Buenos Aires, Argentina; 2) Pathology Department, Hospital de Pediatría Prof. Dr. Juan P. Garrahan. Buenos Aires, Argentina; 3) Hemato-Oncology Department, Hospital de Pediatría Prof. Dr. Juan P. Garrahan. Buenos Aires, Argentina.

Primary central nervous system (CNS) neoplasms are the most frequent solid tumor in childhood and include a heterogeneous group showing different histological subtypes, stages of malignancy and varying biological behavior. The pathogenic mechanisms leading to the development and progression of these tumors are still unclear. The aim of the present study was to identify genomic imbalances in samples of pediatric brain tumors and to correlate the findings with the degree of malignancy and clinical outcome. Surgical specimens from 84 institutionally diagnosed neuroepithelial CNS tumors (32 embryonal, 15 astrocytic, 14 ependymal, 14 mixed glioneuronal, 2 choroid plexus and 7 miscellaneous) were referred to our laboratory for cytogenetic studies from September 2005 to January 2011. Of them, 11 were recurrent tumors (2 embryonal, 6 ependymal, 2 mixed glioneuronal and 1 miscellaneous). Combined conventional and molecular cytogenetic techniques, including fluorescence in situ hybridization and comparative genomic hybridization, have been performed. Forty-two primary neuroepithelial CNS tumors (58%) had chromosomal abnormalities (CA): 24 embryonal, 5 astrocytic, 2 ependymal, 6 mixed glioneuronal, 2 choroid plexus and 3 miscellaneous. Nine recurrent tumors (82%) showed CA. The most frequent CA included unbalanced translocations, complex rearrangements and changes of ploidy. Among the medulloblastomas the chromosomes 1, 11 and 17 were the most frequently involved in gains, losses or in structural rearrangements. Most of the WHO grade I astrocytomas and primary WHO grade III ependymomas had normal karyotypes. In contrast, all WHO grade III and IV astrocytomas had complex karyotypes. The highest proportion of CA was found in WHO grade IV and recurrent tumors. We point out the findings of "novel translocation" such as t(1;3)(q21;q21) and t(2;7)(p25;q11.2) in medulloblastoma and t(6;12)(q21;q24.3) in glioblastoma as a sole structural CA. In conclusion, our results demonstrate a significant association between karyotypic complexity and aggressive tumor biology. The finding of novel CA may help to identify specific genes involved in the initiation and progression of these neoplasms. Finally, we would like to emphasize the importance of performing conventional and molecular cytogenetic studies to improve the detection of genomic imbalances and contribute in the understanding of the pathogenesis of these tumors.

1467T

t(X;1) and 5q- from a mediastinal teratoma in a Klinefelter syndrome patient. *A. Corona-Rivera[1,2], C. Barba-Barba[2], M.D. Martinez-Albarran[2], E. Corona-Bobadilla[1], H.J. Pimentel-Gutierrez[1,2], C. Ortega-de-la-Torre[1,2], F. Sanchez-Zubieta[2], L. Bobadilla-Morales[1,1].* 1) Laboratorio de citogenética genotoxicidad y biomonitoreo, Instituto de genética humana "Dr E Corona Rivera", Dpto. de Biología Molecular y Genómica, CUCS, UdeG; 2) Unidad de Citogenética, Servicio de Hematología Oncología Pediátrica, División de Pediatría, Hospital Civil "Dr Juan I Menchaca".

Introduction: Teratomas correspond to 40% of germ cell tumors (GCT), 4.4% of this group are mediastinal type. Chromosome abnormalities found in infants are: 1p-, 6q-, 1p + or 20p +. Interestingly, 10% of the GCT cases behave Klinefelter syndrome (KS). Objectives: To present a previously unreported chromosomal complement observed in a mediastinal teratoma from a patient with Klinefelter syndrome. Clinical report: A 17 years old male. He began his condition at 14 years old with chest pain, dyspnea and cough, ultrasound test reported a mediastinal mass, the biopsy showed mature teratoma, tumor resection was performed, weight 2.350gr, hairy with calcifications. The postoperative pathology report showed: 97% of mature teratoma. 3 cycles of chemotherapy were applied. He completed 2 years and 5 months in control with TAC, Rx, alpha-fetoprotein (AFP) and human chorionic gonadotropin (HCG) values which were normal. At 16 years old he relapsed, presenting high levels of AFP and HCG, bone marrow involvement, then karyotype was done with the following result: 47,XXY[1]/47,XY,+der(X)t(X;1)(q12;q13),del(5)(q23)[1]/48,XY,+der(X)t(X;1)(q12;q13)X2,del(5)(q23)[14], Constitutional alteration was suspected, so XY FISH was performed in oral mucosa to exclude Klinefelter, signals were compatible with XXY complement, After, karyotype was performed in peripheral blood and it was reported chromosome complement of 47,XXY. Conclusions: The presence of mature teratoma in a male patient should always lead to the suspicion of Klinefelter syndrome, as in our case. The discovery of the 5q-may be due to effects of chemotherapy in particular cisplatin, but the finding of the translocation (X;1) found has not been reported previously, and further studies are needed to assess its prognostic utility. Search Klinefeter syndrome is justified in all patients with teratoma and in particular those from the mediastinum.

1468T

Evaluation of MLPA and SISH as two alternative methods for assessing ERBB2 gene amplification status in breast cancer patients. *C. Durajczyk, L. Carson, S. Tennant, P. Batstone, D. Stevenson.* National Health Service, Aberdeen, Scotland, United Kingdom.

The proto-oncogene ERBB2 (HER2/neu) encodes a tyrosine kinase growth factor receptor involved in controlling cell growth, survival and proliferation. ERBB2 amplification in breast cancer is associated with faster relapse times and an unfavourable overall survival. However, the advent of the chemotherapeutic agent Trastuzumab, an anti-ERBB2 monoclonal antibody, has improved the prognosis of these patients, and is now approved as a first-line treatment for ERBB2 positive breast cancer. ERBB2 amplification status is tested in the Aberdeen Cytogenetics laboratory for equivocal 2+ IHC immunohistochemistry referrals. Currently the laboratory employs Vysis HER2/neu fluorescent in situ hybridisation (FISH) as the gold standard test, however other techniques are available as potential alternatives. The aim of this project was to evaluate multiplex ligation-dependent probe amplification (MLPA) and silver enhanced in situ hybridisation (SISH) for use in assessing the ERBB2 amplification status of 22 breast cancer patients. The sensitivity and specificity of these results were compared to FISH. Results showed that SISH was a promising alternative assay, with a high success rate (95.5%) and overall concordance with FISH (95.2%). However, despite the high concordance in determining overall gene amplification status, the equivalent ratios between SISH and FISH varied greatly, particularly in the highly amplified cases. MLPA was the most economical test of the three investigated; however it yielded substandard success (81.8%) and concordance rates (83.3%). Data from each technique will be presented, along with a discussion of the advantages and disadvantages of each assay.

1469T

Detection of telomerase genes (hTERC and hTERT) amplification by FISH in patients with acute myeloid leukemia. *M.M. Eid[1], N.A. Helmy[1], I.M. Omar[2], A.A. Mohamed[2], D. El Sewefy[2], I.M. Fadel[1], R. Helal[1].* 1) Human Cytogenetics, National research center, Cairo, Egypt; 2) Faculty of Medicine - Ain Shams University- Clinical pathology dept.

Acute myeloid leukemia (AML) describes a heterogenous group of hematological disorders. Cytogenetic and molecular assays have allowed patients' follow up aiming for detection of minimal residual disease, prediction of patients' outcome, in addition to providing the rationale for designing novel molecular-targeted therapeutic strategies. Human telomerase reverse transcriptase (hTERT), encoded by the hTERT gene and the telomerase RNA component (hTERC) genes are frequently amplified in human tumors, which may indicate that the hTERT and the hTERC genes may be target for amplification during the transformation of human malignancies including hematological malignancies. This genetic event has implications in diagnosis, prognosis and therapeutics of cancer. To evaluate the hTERC and hTERT genes as a prognostic marker in patients with AML, hTERC and hTERT gene amplification was studied in 20 adult AML patients using a commercial FISH probes (Kreatech) designated to detect the copy numbers of the genes. They were 12 males and 8 females. Their ages ranged from 16 to 67 years. The patients were further divided into two groups; group I (12 patients) containing newly diagnosed AML patients and group II (8 patients) containing patients taken at 28th day of chemotherapy. The hTERC amplification was detected in 19/21 cases (90.5%) The percent of amplification ranged from 2% to 69%. The copy number of the gene ranged from 2-5 copies per interphase cell. For the hTERT gene, the amplification was found in the same percent of the patients. The percent of amplification ranged from 3% to 76%. The copy number of the gene ranged from 2-9 copies per interphase cell. On comparing the de novo group with 28th days chemotherapy group there was a highly statistical significant difference regarding the percent of both genes amplification. The percent of amplification of hTERT gene was found to be higher among patients with poor outcome of the disease than in patients with good outcome. On the contrary the hTERC gene amplification did not exhibit such a correlation. In conclusion, hTERT and hTERC genes amplification were detected in patients with AML; therefore telomerase can be a good cancer marker which may be involved in carcinogenesis of leukemia. Higher amplification was found in de novo cases than cases in remission which emphasize its role in clinical analysis, disease monitoring and detection of minimal residual disease.

1470T

Detection of cytogenetics abnormalities in chronic lymphocytic leukemia using FISH technique and their prognostic impact. *o.m. Eid, M.M Eid, H.F Kayed, W.M Ahmed.* Human Cytogenetics, National research center, Cairo, Dokki, Egypt.

Maha M Eid, Hesham F Kayed, Ola M Eid, Wael Mahmoud Human Cytogenetics Dept., National Research Center Cairo-Egypt Introduction: Chronic lymphocytic leukemia (CLL) is a clonal lymphoproliferative disorder characterized by progressive accumulation of morphologically and immunophenotypically mature lymphocytes. Characterization of genomic aberrations may help to understand the pathogenesis of CLL and may give prognostic information independent from conventional clinical markers for a risk-adapted management of CLL patients. Aim: The aim of the present study is to determine the most common cytogenetics abnormalities between patients with CLL and its prognostic impact. Patients and methods: The present study was carried out on 20 adult patients presented with chronic lymphocytic leukemia. The patients were diagnosed on the basis of standard clinical (lymph node involvement and / or hepatosplenomegaly), hematological and immunophenotypic criteria for diagnosis of B-CLL. All cases were studied at the time of their diagnosis. FISH technique was successfully performed on PB samples using CLL LSI probes for ATM (11q22) / GLI (12q13) and 13q14/ p53 (17p13). Results: For comparative statistical studies, the patients were divided into group I (patients with favorable outcome) and group II (patients with unfavorable outcome). All patients showed one or more cytogenetic abnormality with the prevalent of the p53 in 16 patients out of 20 that perfectly correlated with the poor out come of the patients. This is followed by deletion in the 13q14 and to a lesser extend deletion in ATM gene, but no one has exhibited amplification in the 12q13 locus. Conclusion: p53 deletion as a sole abnormality has a higher prognostic power than the other cytogenetics abnormalities. The cytogenetics study using FISH panel for CLL patients in a complementary fashion to the other clinical and laboratory findings may overcome the pitfalls in the diagnosis also it may assess the assignment of therapeutic protocols for CLL patients according to the results of their cytogenetic analysis at the time of diagnosis.

1471T

Coexistence of t(12;21)(p13;q22)/ETV6-RUNX1 and MLL Rearrangement in a Pediatric Patient with B-Lymphoblastic Leukemia. *M. Hiemenz[1], W. Chen[2], N. Winick[3], C. Tirado[1].* 1) UT Southwestern, Dallas, TX; 2) AmeriPath North Texas, Dallas, TX; 3) Children's Medical Center, Dallas, TX.

The t(12;21)(p13;q22)/*ETV6/RUNX1* is the most common abnormality occurring in approximately 25% of cases of childhood B lymphoblastic leukemia (B-ALL) and is usually associated with a good prognosis. *MLL* aberrations have been described in childhood B-ALL and are considered a poor prognostic marker. Coexistence of *ETV6/RUNX1* translocation with *MLL* (11q23) rearrangements is a rare event as there are only a few reported cases in the literature. Most of these cases harbored an *MLL* deletion. Herein, we report a 3-year-old patient who presented with three days of fever, pallor, and lymphadenopathy. Laboratory examination revealed anemia (hemoglogin 7.7 g/L), thrombocytopenia (platelet count of 60,000×109/L), and a normal white blood cell count (10,700×109/L) but with 41% blasts. Flow cytometry on bone marrow specimen revealed B lymphoblasts [CD10(+), CD13(dim+), CD15(dim +), CD19(+), CD20(-), CD22(+), CD33(dim +), CD34(+), CD38(+), CD45(dim +), CD79a(+), HLA-DR(+), surface Ig(-), TdT(+), and MPO(-)]. Cytogenetic analysis showed a normal male karyotype. Fluorescence in situ hybridization (FISH) studies showed evidence of *ETV6/RUNX1* translocation in 58% of the interphase nuclei examined. One hundred one of those cells showed no normal *ETV6* signal, indicating the loss of the untranslocated *ETV6* allele. There was also a small clonal population (3% of the cells) with an *MLL* rearrangement. The patient was treated with Children's Oncology Group's protocol (AALL0331) for induction chemotherapy. Bone marrow on day 8 showed residual B-ALL (2.6% lymphoblasts by flow cytometry). Bone marrow on day 29 was negative for residual disease. Consolidation chemotherapy was delayed due to low white blood cell counts. The molecular cytogenetic and clinicopathologic features of this case are discussed along with a review of the literature regarding this uncommon subtype of B-ALL. To the best of our knowledge, this is one of the few B-ALL cases with coexistence of *ETV6/RUNX1* translocation [t(12;21)] and a small population with *MLL* rearrangement.

1472T

Juvenile xanthogranuloma with clonal proliferation in the bone marrow. *D. Januszkiewicz[1,2,3], E. Maly[2], M. Przyborska[2], A. Rybczynska[3], B. Konatkowska[3], J. Nowak[1].* 1) Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland; 2) Department of Medical Diagnostics, Poznań, Dobra Street 38a; 3) Department of Pediatric Oncology, Hematology and Bone Marrow Transplantation of Medical University, Poznań, Szpitalna Street 27/33.

Juvenile xanthogranuloma is a common benign tumor in children occurring as a solitary or multiple yellowish-red nodules on skin, but sometimes involving internal organs. Patients with both JXG and NF1 have a 20-32 times higher risk of developing JMML. Triple association between juvenile xanthogranuloma (JXG), juvenile myelomonocytic leukemia (JMML) and neurofibromatosis (NF1) has been also known. The case of an 11-month-old infant boy with a disseminated juvenile xanthogranuloma (JXG) with unusual cytogenetic abnormality in the bone marrow is reported. NF1 and JMML, as well as other leukemias were excluded. Bone marrow and peripheral blood cytogenetic analysis revealed a karyotype with many rearrangements 46,XY,-6,der(12)t(6;12)(p21;p13),del(7)(p13p22),+9. This aberration has been only ones described in the literature in B- acute lymphoblastic leukemia case. On the contrary in our patient a bone marrow immunologic examination revealed a dominant population (61%) of mature T lymphocytes with about 10% of double-positive CD4+/CD8+ cells. Viral infections (CMV, EBV, HPV, HSV, HIV), as a possible T-cell stimulation, were excluded. To the best of our knowledge this is the first described case of systemic juvenile xanthogranuloma with determined karyotype representing unusual clonal chromosomal aberrations.

1473T

***FOXO1-FGFR1* fusion and amplification in a solid variant of alveolar rhabdomyosarcoma.** *J. Liu[1], M. Guzman[1], D. Pezanowski[1], D. Patel[1], J. Hauptman[1], M. Keisling[2], J. Hou[2], P. Papenhausen[3], J. Pascasio[1], H. Punnett[1], G. Halligan[4], J-P. de Chadarévian[1].* 1) Dept Path & Lab Med., St Chris Hosp for Children, Philadelphia, PA. Drexel University College of Medicine, Philadelphia, PA 19129; 2) Department of Pathology and Laboratory Medicine, Hahnneman University Hospital, Philadelphia, PA 19102. Drexel University College of Medicine, Philadelphia, PA 19129; 3) Laboratory Corporation of America, 1912 Alexander Drive, RTP, NC 27709; 4) Department of Pediatrics, Section of Oncology, St. Christopher's Hospital for Children, Philadelphia, PA 19134. Drexel University College of Medicine, Philadelphia, PA 19129.

Rhabdomyosarcoma is the most common pediatric soft tissue malignancy. Two major subtypes, alveolar rhabdomyosarcoma and embryonal rhabdomyosarcoma, constitute 20% and 60% of all cases, respectively. Approximately 80% of alveolar rhabdomyosarcoma carry two signature chromosomal translocations, t(2;13)(q35;q14) resulting in *PAX3-FOXO1* fusion, and t(1;13)(p36;q14) resulting in *PAX7-FOXO1* fusion. Whether the remaining cases are truly negative for gene fusion has been questioned. We are reporting the case of a nine-month old girl with a metastatic neck mass diagnosed histologically as solid variant alveolar rhabdomyosarcoma. Chromosome analysis showed a t(8;13;9)(p11.2;q14;9q32) three-way translocation as the sole clonal aberration. FISH demonstrated a rearrangement at the *FOXO1* locus and an amplification of its centromeric region. Single nucleotide polymorphism based microarray analysis illustrated a co-amplification of the *FOXO1* gene at 13q14 and the *FGFR1* gene at 8p12p11.2, suggesting formation and amplification of a chimerical *FOXO1-FGFR1* gene. This is the first report to identify a novel fusion partner *FGFR1* for the known anchor gene *FOXO1* in alveolar rhabdomyosarcoma.

1474T

Recurrent Cytogenetic Aberrations Predict MYC Breakpoints in Diffuse Large B-cell Lymphoma and Help Define Burkitt Lymphoma versus Diffuse Large B-cell Lymphoma with MYC Rearrangement. *D. Martinez[1], R. Garcia[2], P. Koduru[2], CA. Tirado[2].* 1) University of Texas Health Science Center at San A, San Antonio, USA; 2) Clinical Cytogenetics - UT Southwestern Medical Center, Department of Pathology, Dallas, Tx.

Cryptic MYC rearrangements in lymphomas are not always detected by current cytogenetic methods or Fluorescence In-Situ Hybridization (FISH). It is crucial to properly diagnose diffuse large B-cell lymphoma (DLBCL) from Burkitt lymphoma (BL) with MYC breakpoints since clinical management for each lymphoma differs greatly. In the present study, we are trying (1) to identify recurrent cytogenetic aberrations (RCA) that may predict MYC breakpoints in DLBCL and (2) to generate cytogenetic profiles to differentiate diffuse large B-cell lymphoma (DLBCL) from BL with MYC rearrangements. Two groups of MYC+ and MYC- lymphoma cases were retrieved from the Mitelman database. Karyotypes were assessed to detect associated RCA using bivariate correlations. Results were further analyzed using the Fisher Exact test in order to measure the significance of each association. A $p < .05$ was considered significant. Single hit DLBCL and BL cases were also obtained and analyzed to generate genomic profiles using the same methods. Our results revealed two recurrent associations and a deletion constantly present in our MYC+ group; concurrent gains of chromosomes 11 and 21 (+11/+21), concurrent gain of chromosome 11 and loss of chromosome 15 (+11/-15) and a deletion on the long arm of chromosome 6, del(6)(q15). When comparing DLBCL and BL, we found the following RCA significantly associated with DLBCL: -3/-4, -10/-15, -10/-22, +11/+21, +X, -2, -4, +7, +12, -15, +16, +18, +21, -22, losses of 1p36, 6p13q27 and 17p. In summary, our findings indicate that RCA may be used to predict MYC+ breakpoints that are otherwise not detected by conventional or FISH methods in DLBCL, and may also be used to define BL from DLBCL.

1475T

Proposed FISH panel with diagnostic and prognostic significance for prostate cancer. *N. Mitter[1], J. Belanger[1], J. Blackson[1], A. Singh[2], A. Lindia[2], J. Amberson[3].* 1) Dept Cytogenetics, Dianon Systems (Lab Corp), Shelton, CT; 2) Dept Histology, Dianon Systems (Lab Corp), Shelton, CT; 3) Dept Pathology, Dianon Systems (Lab Corp), Shelton, CT.

Background: Prostate cancer is mostly staged on the basis of Gleason scores on paraffin-embedded needle biopsies. FISH analysis has shown at least four genes to play major role in prostate tumorigenesis - loss of LPL gene (8p22), amplification of MYC gene (8q24.1), loss of PTEN gene (10q23.3)), and a fusion gene created from ERG (21q22.2) and TMPRSS2 (21q22.3) genes. **Statement of Purpose**: To test the prognostic and diagnostic potential of combining these probes to detect different stages of prostate cancer in a blind pilot study on 25 randomly selected patients already analyzed for Gleason scores. **Methods Used**: Four cross sections of paraffin-embedded needle biopsies were cut at 4μm thickness. An additional H&E stained slide was used to identify malignant and benign areas. A study-specific numbering system was used to conduct the study in a blind fashion, with the pathologist's information accessed only after FISH analysis. Standardized and properly validated FISH protocol was used with Abbott probes for MYC, LPL and PTEN genes and Poseidon break-apart probe for TMPRSS-ERG genes. Fifty cells were analyzed for each probe. A case was deemed abnormal if at least five (10%) cells had the same abnormal FISH signal pattern. Loss of LPL and/or PTEN gene(s) is seen in early tumorigenesis and associated with relatively favorable prognosis, while amplification of MYC gene and/or presence of TMPRSS2-ERG fusion is seen in later stages of prostate cancer and associated with a poor prognosis, even if seen in combination with loss of LPL and/or PTEN gene(s). An expected prognosis based on FISH results was then compared to the actual pathologic findings. **Summary of Results**: For 9/25 cases with normal signal pattern for all FISH probes, five had no cancer and four had benign problems. There was a complete correlation between FISH results and Gleason scores for 10/16 cases with prostate adenocarcinomas. Results were inconsistent for the remaining six cases, with three being negative by FISH but positive by Gleason scores for prostate cancer. The other three had abnormal FISH patterns but only benign pathologic abnormalities. The abnormal FISH pattern for these three cases suggests early manifestation of abnormal FISH patterns and possible prognostic significance for later development of prostate cancer. **Conclusion**: A combination of these four FISH probes has a high diagnostic value and prognostic significance for the development of prostate cancer.

1476T

ALK rearrangements in non-small cell lung cancer. *J.J.D. Morrissette, V. Aikawa, J.P. Segal, C. Deshpande.* Dept Pathology, University of Pennsylvania, Philadelphia, PA.

Lung cancer is the leading cause of cancer-related deaths and one of the most common cancers (excepting skin cancer). Non-small cell lung cancer (NSCLC) represents 85% of cases. Identification of the genetic basis of an individual's NSCLC is important for prognosis and treatment, with mutations in KRAS in ~25% of NSCLC, with an increased frequency in smokers. Mutations in EGFR are seen in ~ 20% of NSCLC and are treatable using targeted inhibitors. More recently rearrangements involving the anaplastic lymphoma kinase (ALK) gene, located on 2p23, have been shown to occur in 3% to 5% of NSCLC. These patients are more likely to be younger at presentation, never-smokers, female, and Asian. The most common ALK rearrangement involves the EML4 gene, located 13 Mb away on 2p21, and leads to a novel fusion oncogene, joining the coiled-coil domain of EML4 with the tyrosine kinase domain of ALK, leading to constitutive activation. The rearrangement results from either a small inversion of chromosome 2p23-p21 or an inversion/deletion event leading to the EML4-ALK fusion, with deletion of the reciprocal partner. Importantly, there is a specific inhibitor of ALK, Crizotinib, which has been very effective in reduction of tumor burden. We present our laboratory's experience with ALK analysis of NSCLC. Our validation consisted of 12 triple-negative cases, 6 KRAS positive cases, and 7 EGFR positive cases. We identified ALK rearrangements in 7 validation samples, which included the well-described inversion in 2 cases, the inversion-deletion in 4 cases and 1 case with both the inversion and inversion-deletion. We have subsequently performed ALK FISH on 63 cases, of which there were 3 positive cases, 57 negative cases and 3 cases which had no tumor cells in the submitted specimen. Our 3 positive cases were in a 29 year old Indian female non-smoker, a 46 year old white female non-smoker, and a 65 year old white female with minimal distant smoking history. We have also found an increased number of ALK signals (3~8 copies) in many of the negative samples. Although we have yet to identify a true amplification of ALK, these extra signals appear to represent multiple extra signals of ALK, as opposed to increased copy number of chromosome 2. Identification of this subset of patients is critical for targeted treatment and has altered their clinical course dramatically.

1477T

An unusual myeloid case involving hyperdiploidy. *C. Murray[1], L. Hendry[1], H. Roddie[2], R. Bauld[1], G. Bakirtzis[1], J. Fleming[1], J. Iremonger[1], E. Maher[1].* 1) S. E. Scotland Cytogenetics Department, Western General Hospital, Edinburgh, United Kingdom; 2) Department of Haematology, Western General Hospital, Edinburgh, United Kingdom.

We report a case of a 66 year old male patient who was admitted as an emergency with pyrexia and general malaise, and had a short history of progressive fatigue and shortness of breath. He had no past medical history of note. A full blood count revealed pancytopenia. A bone marrow aspirate demonstrated trilineage dysplasia with an increase in myeloblasts at 8% of total nucleated cells and with 30% ring sideroblasts. Based on these findings a diagnosis of myelodysplasia, WHO subtype RAEB, was made. A specimen of bone marrow was received by our laboratory for cytogenetic analysis. Four clones were identified, including one that showed hyperdiploidy with gains of one extra copy of chromosomes 1, 8, 10, 11, 13, 14 and Y, and two extra copies of chromosome 22. An unidentified marker chromosome was present in some hyperdiploid cells. Whilst hyperdiploidy is well-recognised as an abnormality in lymphoid disorders, it has been rarely reported in myeloid disease. We will present further details of this case, including full cytogenetic results and a literature review.

1478T

GENE EXPRESSION PROFILE OF AURKA AND AURKB IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): CORRELATION WITH CLASSICAL CYTOGENETIC (GTG) AND HEMATOLOGICAL PARAMETERS. *F. Oliveira, AR. Lucena de Araújo, F. Saldanha-Araújo, E. Magalhães Rego, R. Passetto Falcão.* Internal Medicine - Hematology, Faculty of Medicine of Ribeirão Preto, Riberão Preto, São Paulo, Brazil.

Aurora kinase (AURKA/B) overexpression leads to genetic instability and trigger the tumor development. The majority of the studies involving aurora kinase genes are addressed to cancer immunotherapy and no study has comprehensively examined the role of these genes in leukemogenesis. We have evaluated AURKA and AURKB gene expression in CD19+ cells from peripheral blood (PB) of CLL patients, by real time quantitative PCR and correlate the findings with hematological parameters and classical cytogenetics. 62 CLL patients (23 female and 39 male) and 10 age-matched hematologically healthy donors were selected for investigation. AURKA/B gene expression from leukemic samples was calculated as relative quantification to normal CD19+; cells from healthy donors and expressed as 2-ΔΔCt. Metaphase induction in CLL was performed by using the immunostimulatory method that employs the combination of DSP30 and IL-2. All cytogenetic and gene expression data were validated by FISH analysis by using a specific set of probes. According to median value of AURKA/B expression, patients were divided into two groups (≤ 3.4, considered as AURKA+; and > 2.3, considered as AURKB+) and their clinical and biological characteristics were correlated. Higher AURKA/B expression were observed in CLL samples compared with PB normal samples (AURKA [mean value of ΔCt ± SD]: 0.093±0.003 vs 0.071±0.001, p=0.02; AURKB: 0.166±0.01 vs 0.09±0.002, p=0.02). Moreover, AURKA/B+ patients presented a significantly high leukocyte count compared with AURKA/B- patients (AURKA [WBC countx103 ± SD]: 37.8±5.5 vs 68.8±5.8, p=0.0003; AURKB: 40±5.5 vs 66.6±6.2, p= 0.0023, respectively). In addition, Pearson correlation showed that there is a significant association between high expression of AURKA/B and complex karyotype (relative risk: 2.4 [95%CI: 1.46-3.93], p < 0.001). Among the classical cytogenetic profile obtained, normal karyotype was found in 15 patients (24%) and metaphases with abnormal karyotype were seen in 47 subjects (76%). We demonstrated a significant correlation among high expression levels of AURKA/B genes in CLL with chromosomal abnormalities and other hematological parameters. Overexpression of aurora kinase genes have been extensively studied in solid tumors. In CLL this observation may be associated to the genesis of chromosomal abnormalities and possible be used to predict the course of genomic instability in CLL patients. Financial support: FAPESP (Proc. 07/52462-7).

1479T

Frequent cytogenetic alterations in a series of Myelodysplastic Syndrome cases. *S.P. PERDOMO[1,2], L.C. PARDO[1], Y. GUEVARA[1], C. FAJARDO[1], T.I. RONCANCIO[1].* 1) BIOGENETICA DIAGNOSTICA SAS, BOGOTA, Colombia; 2) INSTITUTO DE INVESTIGACION EN NUTRICION GENETICA Y METABOLISMO IINGM. UNIVERSIDAD EL BOSQUE,BOGOTA, COLOMBIA.

During the last decades patients diagnosed with MDS have a better survival rate due to an early diagnosis and management. Cytogenetic analysis are an especial tool in diagnosis, prognosis and classification of MDS, because they are able to detect up to 50% of acquired chromosomal alterations in dysplasic cells from bone marrow. Cytogenetic studies have been focused in some frequent alterations such as del(5q),-7,del(7q),+8,+21,+15 since they represent a valuable tool in clinical evaluation, prognosis and now treatment. In this study we made a retrospective revision of 74 cases diagnosed as MDS in a period of 18 months, using cytogenetic analysis in bone marrow by standard banding procedures and correlated results with biopsy and flow cytometry studies. 59.5% of the cases showed chromosomal alterations, a percentage higher than the reported data in literature. From these cases only 24,3% reported the most frequent and better studied cytogenetic alterations del(5q),-7,del(7q),+8,+21,+15 as the only abnormality. The most frequent alterations are represented by les common abnormalities such as complex karyotypes and balanced translocations, cited below: 46,XX,t(3;5)(p13;p14)[10]/46,sl,der(1)t(1;16),-16,+20[15] 46,XX,t(3;8)(p22;q24.1)[2]/46,XX[28] 46,XY,inv(9)(p21q21),der(1),-7,+mar[12]/46,XY,inv(9)(p21q21)[8] 46,XY,del(1)(p31→pter),+i(1)(q10),-5,-7,13,der(17),18,+r(?),+2mar[16]/45,XY,der(1)t(1;7)(p31:q22),-5,-7,-13,der(17),-18,+r(?),+2mar[14] 46,XX,del(4)(q21→qter)[2]/46,XX[18] 46,XX,i(17)(q10)[20] 50,XY,+1,-5,+8,+9,der(11),+14,+17, -18,-21,t(18;21)(q11.2;p11.2),+r,+mar [18]/46,XY[2] 44,XX,der(2)t(2;11)(p11.2,-p11.2),del(5)(pter->q15),-11,-15,-17,-19,der(20),+2mar[17]/45,sl,+8[3] 45~47,XY,del(1)(P31→pter),+i(1)(q10),del(4)(p15.2),-5,-7,-13,der(17),-18,+r(?),+3mar[6]/46,XY,del(1)(P31→pter),t(1;3)(p13;q21),-4,-5,der(1)t(1;7)(p31;q22),-13,der(17),-18,+r(?),+3mar[12]/46,XY[2] 46,XXt(3;19)(q24;p13)[15]/47,XX,del(3)(q24;q29),+8[10] 47,XX,t(1;3)(p32;q27),+5,i(9)(q10),der(17)(p12)?[12]/46,XX[8] 49,XY,add(3)(q27),-7,-17,-22,-22,+r(?),+6mar[1]/46,XY[29] 46,XY,t(1;11)(p33;p15)[2]/46,XY[23] Our results, show a higher frequency of less common reported and studied cytogenetic alterations in MDS according to scientific reports, with not well known or unknown clinical and/or prognostic value. Follow up studies must be done in order to gain a better understanding of the clinical significance of these abnormalities and reevaluate their frequency in MDS patients.

1480T

Evaluation of AURKB gene amplification status in breast cancer samples. *R.M. Rodrigues-Peres[1], J.K. Heinrich[1], R.G. Paleari[1], J. Vassallo[3], L.O. Sarian[2].* 1) CAISM - Women's Hospital, University of Campinas - UNICAMP, Campinas, SP, Brazil; 2) Department of Obstetrics and Gynecology, Faculty of Medical Sciences, University of Campinas - UNICAMP, Campinas, SP, Brazil; 3) Faculty of Medical Sciences, University of Campinas - UNICAMP, Campinas, SP, Brazil.

Introduction: The Aurora kinase B (AURKB) gene is involved in cell division and mitosis, playing a relevant role in chromosomal segregation and condensation, mitotic checkpoint and cytokinesis. The AURKB protein was shown to be overexpressed in many types of cancer cells, including the increase of a complex formed by Aurkb, Incenp and survivin, which is in turn related to aneuploidy. Thus, AURKB amplification might be associated with other defective genes in cancer such as p53 and BRCA-2. A connection between AURKB amplification and familial breast cancer has also been suggested. Although several authors have described the importance of the AURKB in carcinogenesis, there is still much to understand about its mechanisms in breast cancer cells. Objective: 1) To evaluate gene amplification status of AURKB gene in breast cancer samples; 2) To access genomic instability status of these samples through the correlation of AURKB gene amplification status and ERBB2, C-MYC and CCND1 status; 3) To verify the correlation of AURKB gene with familial breast cancer cases. Materials and Methods: For this study, 48 cases were obtained from mastectomies. A central tumor area and a normal tissue area of the same breast, for each case, were pair-compared. A TMA slide containing these samples was set and used in FISH assays. Homebrew probes were specifically designed for the AURKB gene and applied onto samples with a commercial probe for the chromosome 17 centromere (CEP17, Kreatech), as an internal control. Clinicopathological data were obtained from patients' records. Results: Approximately 23% of the cases presented AURKB amplification and 92% showed genomic instability, i.e., concomitant amplification in other genes. Forty-six percent of the AURKB amplified patients presented positive familiar history. Discussion: Our results corroborate previous findings of AURKB amplification in breast cancer samples. As a high frequency of amplified cases was positive for familial history, further studies should provide new issues regarding the role of this gene in hereditary cancers as well as its association with other genes. The massive correspondence of gene amplification among AURKB, ERBB2, C-MYC and CCND1 genes might suggest an important genetic component involved in the carcinogenesis and tumor development process in the central area of the tumor.

1481T

Strategy using SNP array and interphase FISH for the detection of genetic prognosis factors in neuroblastoma. *M. Roy-Tourangeau[1,2,4], M.-P. Arsenault[1,2,4], C. Nyalendo[1,2,4], S. Cournoyer[1,2,4], P. Teira[3], M. Duval[3,4], H. Sartelet[1,2,4], R. Fetni[1,2,4].* 1) Pathology and Cell Biology, University of Montreal (Quebec), Canada; 2) Pathology; 3) Hemato-oncology; 4) Research Center, CHU Sainte-Justine, Montreal (Quebec), Canada.

Neuroblastoma (NB) is the most common childhood solid tumor and is characterized by its biological and clinical heterogeneity. Some cases have a spontaneous regression, while others are very aggressive, making risk stratification of patients essential. Several genetic markers are currently used to evaluate NB prognosis, such as *MYCN* oncogene amplification, DNA ploidy, 17q gain, 11q23 LOH and 1p36 LOH. Aim of our study is to establish an optimal strategy to detect genetic prognosis factors in NB using Genome Wide analysis, fluorescent *in situ* hybridization (FISH) and DNA index. First, for whole genome screening, we used Affymetrix Genome-Wide Human SNP Array 6.0 platform, enable to detect both single nucleotide polymorphisms (SNP) and copy number variations (CNV). Ploidy was evaluated by flow cytometry and recurrent chromosomal abnormalities were also assessed by a FISH panel, including probes specific for *MYCN*, 17q, 11q23 and 1p36. Among 36 primary NB analyzed, 89 % present one or more genomic abnormalities (by SNP array and/or by FISH), while 4 cases are diploid. Moreover, 22 % are triploid (confirmed by flow cytometry and by SNP); therefore, FISH results were analyzed considering the appropriate ploidy of each sample. The most common CNV/LOH in our cohort are 11q23 deletion (43 %), 17p gain (33%), *MYCN* amplification (17 %) and 1p36 deletion (14 %). When the ploidy status is taking into account for interphase FISH results interpretation, the two techniques show comparable sensitivity. In conclusion, our analysis show that the ploidy changes and highly rearranged genome of NB tumors lead to inaccurate results interpretation for both interphase FISH and SNP microarrays. The integration of DNA index during results analysis is essential for a genetic prognosis factors assessment of NB.

1482T

Chromosomal alterations detected by conventional cytogenetic and FISH in leukemias. *F.J. Sheth[1], M.J. Desai[1], A.P. Patel[2], S.B. Mehta[1], J.J. Sheth[1].* 1) Department of Cytogenetics, FRIGE's Institute of Human Genetics, Ahemadabad, Gujarat, India; 2) Gujarat Research & Medical Institute, Shahibaug, Ahmedabad-380004.

With the increasing global attention to the newly immerging concept of genomic disease management, molecular pathway is gaining a centre stage especially in the management of leukemia and various other cancers. This has become possible due to increasing resolution power and sensitivity offered by various molecular techniques such as FISH. In the present study, 321 individuals were investigated for cytogenetic study due to clinical suspicion of CML [n=175], AML [n= 24], ALL [n= 41], CLL [n=02], MPD [n=24], MM [n=6] and MDS [n=49]. Primary alterations were detected in 158 cases, secondary rearrangements in addition to primary alteration were seen in 10% cases and 163 were found to be cytogenetically normal. Fluorescence *in situ* hybridization [FISH] study has facilitated to recognize not only genomic alteration detected in 33 and 125 cytogenetically normal/abnormal cases respectively. Moreover, dual colour dual fusion specific BAC clones have additionally helped in identifying gene amplification/loss which was missed in 17 cases by routine conventional cytogenetic [CC] analysis. Similarly multiple minor cell lines were identified in one case of AML-M3. Translocations involving more than two chromosomes were observed in 16 cases and of these, two cases showed apparently balanced genome and were responding well to supportive therapy. Together, the combination of CC and FISH analysis incorporates the screening potential of cytogenetics with accuracy of molecular genetics technique, which will lead to better understanding of neoplastic disease, more accurate diagnosis, stratification of patients in to genetic subgroups and eventually improvements in prognostification. This study is supported in parts by DBT, India.

1483T

Acute Promyelocytic Leukemia with a Novel Variant (15;17) Rearrangement. *A. Zaslav[1], M. Bellone[2], B. Kiner-Strachan[3], M. Golightly[4], T. Mercado[1], Y. Hu[2].* 1) Cytogenetics, Dept of Clinical Pathology, Stony Brook Univ Hosp, Stony Brook, NY; 2) Department of Anatomic Pathology, Stony Brook University Medical Center, Stony Brook, NY 11794; 3) Department of Medicine, Bone Marrow Transplant Unit, Stony Brook University Medical Center, Stony Brook, NY 11794; 4) Flow Cytometry, Department of Clinical Pathology, Stony Brook University Medical Center, Stony Brook, NY 11794.

Acute promyelocytic leukemia (APL) is associated with a reciprocal translocation of chromosomes (15;17)(q22;q21), resulting in the fusion of the PML and RARA genes. We describe a patient of 67-year-old with APL carrying a novel variant of (15;17) translocation. The marrow showed typical APL morphology except for absence of Auer-rods. Flow cytometry analysis was consistent with APL. RT-PCR confirmed the PML/RARA transcripts. Standard G-banding analysis on bone marrow cells demonstrated a karyotype of 46,XY,del(17)(q12q21.1),ins(15;17)(q22;q21.2q25.3)[18]/46,XY[2]. Fluorescence in situ hybridization (FISH) using the dual-color, dual-fusion PML(15q22-orange)/RARA(17q21-green) probe also showed a variant abnormal pattern (figure 1): Typical APL patients have 1O (orange):1G (green):2F (fusion) signals. Our patient had 1.5% of cells with the typical abnormal pattern, however, 85% of the cells had 2O:1G:1F. The results demonstrated that the RARA/PML fusion was absent from chromosome 17. PML remained on the der(15) and PML/RARA was inserted into or translocated to the der(15). To our knowledge this abnormality has not been described before. The patient was treated following the Cancer and Leukemia Group B (CALGB) protocol 9710 regimen. His response to ATRA has been slow. Three follow up cytogenetic and FISH analyses demonstrated the absence of the RARA/PML rearrangements in all cells. The patient will be closely monitored. His clinical progress should add to our knowledge of the prognostic significance of this novel variant rearrangement.

1484T

Increased molecular cytogenetic (FISH) abnormality detection rate using plasma cell enriched cell sorting for plasma cell disorders. *S. Zneimer, M. Sasaki.* Cytogenetics, US Labs, Irvine, CA.

The detection of cytogenetic abnormalities in plasma cell disorders (PCD's) are difficult due to the low viability of actively dividing cells in culture. Fluorescence in situ hybridization (FISH) offers a better abnormal detection rate since the cells are analyzed in interphase without the need for actively dividing cells. However, both techniques, when performed on whole bone marrow or peripheral blood samples, do not offer the most reliable means for observing the most common abnormalities seen in PCD's due to the small number of plasma cells in the whole bone marrow or blood specimen. Sorting of plasma cells by various methods have been reported in the literature, and using the autoMACS Pro magnetic cell separator by Milteny Biotech since May 2010, we have been able to increase our abnormal detection rate using FISH analysis of specific recurrent abnormalities. Abnormality rates were compared before and after cell sorting of plasma cells using DNA probes consisting of centromere probes of chromosomes 3, 7, 9 and 11 to detect hyperdiploidy; chromosome 13q14.3 for del(13q) and monosomy 13; IGH/CCND1 for t(11;14); IGH/MAF for t(14;18); FGFR3/IGH for t(4;14); and TP53 gene for del(17p). The abnormality detection rate of 1103 samples using DNA probes of these regions increased from 40.0% before cell sorting to 71.7% after cell sorting and analyzing only plasma cells. The method for plasma cell enrichment had a zero percent failure rate when enough material was received, with a 0.6% insufficient quantity received. These results strongly support the need for cytogenetic labs to use some method for isolating plasma cells to perform FISH analysis for optimum abnormal detection rates of the typical, highly observed abnormalities detected in PCD's for increased sensitivity of abnormal molecular cytogenetic results.

1485T

Gene Expression and SNP Array Analyses of Spindle Cell Lipomas and Conventional Lipomas with 13q14 Deletion. *H. Bartuma.* Department of Clinical Genetics, Lund, Sweden.

Spindle cell lipomas (SCL) are circumscribed, usually subcutaneous tumors that typically occur on the posterior neck, shoulder and back of middle aged men. Cytogenetically, almost all SCL are characterized by deletions of chromosome arm 13q, often in combination with loss of 16q. Deletions of 13q are seen also in approximately 15 % of conventional lipomas. Through single nucleotide polymorphism (SNP) array analyses we identified two minimal deleted regions (MDR) in 13q14 in SCL. In MDR1, four genes were located, including the tumor suppressor gene RB1. MDR1 in SCL overlapped with the MDR detected in conventional lipomas with 13q14 deletion. In MDR2 in SCL there were 34 genes and the two miRNA genes miR-15a and miR-16-1. Global gene expression analysis was used to study the impact of the deletions on genes mapping to the two SCL-associated MDR. Five genes (C13orf1, DHRS12, ATP7B, ALG11, and VPS36) in SCL and one gene C13orf1) in conventional lipomas with 13q-deletions were found to be significantly underexpressed compared to control tissues. Quantitative real-time PCR showed that miR-16-1 was expressed at lower levels in SCL than in the control samples. No mutations were found at sequencing of RB1, miR-15a, and miR-16-1. Our findings further delineate the target region for the 13q deletion in SCL and conventional lipomas and show that the deletions are associated with down-regulated expression of several genes, notably C13orf1, which was the only gene to be significantly down-regulated in both tumor types.

1486T

Aberrant translocations t(3;17)(q22;p13) and t(5;21) (q13;q22) in a case of Ph-positive chronic myeloid leukemia during blastic transformation. *A. Bennour[1], M. Zaier[2], I. Azzouz[1], I. Ouahchi[1], Y. Ben Youssef[2], A. Khelif[2], A. Saad[1], H. Sennana[1].* 1) Cytogenetics Department, CHU Farhat Hached Hospital, Sousse,Tunisia; 2) Department of clinical hematology, CHU farhat Hached Hospital, Sousse, Tunisia.

Secondary chromosome changes are frequently observed during the blastic phase of chronic myeloid leukemia (CML) owing to clonal evolution. We report a patient with Philadelphia (Ph)-positive CML who developed abnormal clones with translocations t(3;17)(q22;p13) and t(5;21)(q13;q22) during the blastic phase. A 44 year old man was diagnosed with CML, he was treated with imatinib during 3 months and a serial chromosome and molecular study was performed over the clinical course of the disease. The patient remained in complete cytogenetic remission after imatinib treatment, when a conventional cytogenetic study performed on the bone marrow was negative for the Ph chromosome. The treatment was discontinued during 11 months, subsequently, the patient developed acute transformation of the disease and cytogenetic examination showed the development of two clones, consisting of the translocations t(3;17)(q22;p13) and t(5;21)(q13;q22) in addition to the standard Ph rearrangement. Whole chromosome paintings with chromosomes 3, 5, 17 and 21 confirmed these translocations. The patient was chemotherapy resistant; he received a second tyrosine kinase inhibitor (dasatinib) for one month. He was then discharged home in a markedly improved clinical condition. Unfortunately, he died. The association of a Ph with abnormalities unusually observed in CML raises the question of whether these aberrant translocations are part of the disease process and casually related to the acute transformation. In our knowledge, t(3;17)(q22;p13) has never been reported previously in the literature, the breakpoint in 17p13 clusters a transcriptional regulator gene PER1 which may play a potential role in the transformation process of the CML. T(5;21)(q13;q22) was rarely described in myelodysplastic syndrome (MDS) and acute non lymphocytic leukemia (ANLL). The gene in 5q13 is yet unknown, and it is therefore uncertain whether this translocation involve a new AML1 (21q22) partner. In conclusion, we find that the combined use of fluorescence in situ hybridization, chromosome painting, and classic cytogenetic analysis allows a better evaluation of the genomic aberration involved in CML blastic transformation, and offers new directions for its further molecular investigations.

1487T

The first case of Philadelphia-negative acute promyelocytic leukemia following imatinib for chronic myeloid leukemia. *J.J.W. WAKIM[1], C.A.T TIRADO[2], W.C CHEN[3].* 1) HEMATOLOGY-ONCOLOGY, UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER, DALLAS, TX; 2) PATHOLOGY, UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER, DALLAS, TX; 3) AMERIPATH NORTH TEXAS, DALLAS, TX.

In chronic myeloid leukemia (CML), chromosomal abnormalities (CA) in Ph-negative cells are scarce and transient, but rarely associated with the development of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). We present the first case of Ph-negative acute promyelocytic leukemia (APL) in a patient in complete cytogenetic response (CCR) following 7 years of treatment with imatinib for CML. A 81 year-old male was diagnosed with t(9;22)(q34;q11.2)/BCR-ABL1 CML in 1999. He received interferon for 9 months then imatinib 400mg daily and achieved CCR. Imatinib was discontinued in November 2007 because of gastrointestinal intolerance. The patient remained in CCR, but in April 2010 presented with APL. FISH showed PML-RARA, but no evidence of BCR-ABL in 1000 interphase nuclei examined which gives the sensitivity of PCR studies. Karyotype revealed: 46,XY,t(15;17)(q24;q21.1)[19]/46,XY[1]. He developed subdural hematomas and died. CA in Ph-negative cells have been observed in 2-21% of CML patients following interferon or imatinib. They are usually transient but 2% of affected patients develop MDS and/or AML. We are the first to report the emergence of t(15;17)/PML-RARA APL in Ph-negative metaphases. Promyelocytic blast crisis of CML was ruled out when FISH showed no evidence of BCR-ABL in 1000 interphase nuclei examined. t(15;17)/PML-RARA was a new event in Ph-negative cells rather than clonal evolution of CML into accelerated phase. Several theories can be advanced to explain this rare event. The first proposition entails a two-step pathogenesis of CML where the Ph-negative stem cell accumulates CA and the Ph chromosome represents a “second hit”. The selective suppression of the Ph-positive clone by imatinib allows the Ph-negative clones expressing the “first hit” to emerge. The second explanation describes a genetically damaged hematopoiesis that produces multiple abnormal clones which remain masked until the eradication of the dominant Ph-positive clone by imatinib. The third proposition implicates imatinib in the appearance of CA since its target, ABL, is involved in DNA repair. The hematopoiesis of a patient with imatinib-induced CCR would be relying on a restricted group of Ph-negative stem cells riddled with genetic instability and prone to the emergence of new CA. In conclusion, CML patients in CCR should be monitored by classical cytogenetics in addition to BM aspirations, as they risk developing additional CA which can rarely be fatal.

1488T

Detection of exonic copy-number changes by high resolution array-CGH of human cancer genes. *H. Chong, A. Elliott, A. Kammesheidt.* Ambry Genetics, Aliso Viejo, CA.

Alterations in genomic copy numbers may lead to changes in gene expression and function and are known to underlay various disease states including congenital anomalies, developmental disorders and numerous cancer syndromes. Array-based comparative genomic hybridization (aCGH) provides a powerful tool to study diseases resulting from genomic copy number variations (CNV). aCGH can identify which chromosomal regions harbor large gains and losses on a genome wide level, with a resolution typically greater than 100Kb on a standard microarray. In the effort to identify smaller CNVs in genes implicated in cancer, we designed and validated an exon focused 180K whole-genome human CGH microarray using the Agilent platform (Santa Clara, CA). The Ambry CancerArrayTM covers the majority of exons in cancer-related genes based on the Sanger Cancer Gene Census at high resolution. The array has coverage of 5024 exons in over 400 cancer-related genes, with an average of 5.1 probes per exon (25,381 probes in 5024 exons) and backbone tiling of about 20 Kb. More than 98% of the exons are covered with at least 3 probes. The array can be used to identify duplications or deletions throughout the genome involving at least one exon. We demonstrate the ability of the custom-designed exon-targeted array to detect clinically relevant intragenic CNVs with subkilobase resolution in patients with various clinical phenotypes. This exon-targeted array enables detection of intragenic copy number changes in situations that would be missed by traditional aCGH and would not be detected by gene-specific DNA sequencing.

1489T

Automating the Dropping Process to Generate Quality Metaphase Spreads in Preparation for Fluorescence In Situ Hybridization (FISH). *B. Kirk[1], A. Avila[1], T. Tisone[1], K. Sundin[2], D. Buller[2], C. Glotzbach[2], C. Kashork[2].* 1) BioDot, Inc., Irvine, CA; 2) Signature Genomic Laboratories, PerkinElmer Inc., Spokane, WA.

Cell `dropping' refers to the process of applying cell samples to a microscope slide in preparation for fluorescence in situ hybridization (FISH). Harvested cells, suspended in a 3:1 (methanol: acetic acid) fix solution, are applied to glass microscope substrate. When applied to the slide, the objective is to have the `dropped' cells spread in such a way that cellular DNA is presented properly for further analysis using FISH. In the case of Interphase FISH, spreading requirements are not as stringent. In the case of Metaphase FISH, however, condensed chromosomes need to be distinct, the proper size, and separated form neighboring metaphase cells. Historically, the dropping process has been done manually in cytogenetic laboratories and while the definition of a quality metaphase is widely accepted, laboratories often differ greatly with respect to the dropping conditions. The process to date is still inherently manual, and is often viewed as an art, rather than a science. Using the BioJet Plus™ dispensing technology from BioDot Inc. (Irvine, CA), a quantitative, non-contact, liquid dispensing system with drop volumes in the range of 10-4,000 nL, we demonstrated the transition to a consistent automated method, thus eliminating the “art” in the process. The BioDot AD1500 configured with the BioJet Plus technology was used to evaluate the automation of the dropping process and produce consistent, quality, metaphase spreads. Control slides were prepared with the standard manual process (protocol supplied by Signature Genomics) and were used to establish quality standards for the BioJet Plus technology. All slides were evaluated using a phase microscope. Experimentally, we have explored the effect of dispense volume (350 nl to 2 ul), surface temperature (30°C to 70°C), surface angle deposition (0° to 60°), surface pre-treatment and local relative humidity (45% to 70%RH) on the quality of metaphase spread preparations. In doing so, an automated protocol was developed that produced metaphase spreads that are sufficient for FISH analysis. In addition to automating the dropping process, drop volumes have been reduced to the point where multiple cell spots can be applied to a single glass slide allowing for high throughput multiplexing. Multiplexing allows for replicate spots of any given sample or multiple samples to be spotted on a single slide.

1490T

Comprehensive Genetic Diagnosis of B-Cell Chronic Lymphocytic Leukemia. *Y.Y. Wen, X.F. Hu, M.M. Li.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

B-cell chronic lymphocytic leukemia (CLL) is the most common leukemia in the United States, predominantly occurring in older males. Some patients with CLL show slow progression of the disease with minimal therapy while others progress aggressively regardless of treatments. Great efforts have been expended on studying the genetic alterations in CLL, leading to the identification of prognostic markers for patients with CLL. Five cytogenetic prognostic categories have been proposed: deletion in the short arm of chromosome 17 is associated with the worst outcome, and deletion of chromosome 13q as the sole aberration appears to have the best outcome. In addition, mutation status of IgVH has also been suggested to correlate with the prognosis of CLL. While many individual prognostic markers have been identified, several open questions remain. First, large-scale study of genes associated with common cytogenetic alternations in CLL was absent. Second, the correlation of cytogenetic aberrations and hypermutation of IgVH is currently unknown. In order to answer these two questions, we performed cytogenetic study, FISH with a panel of probes, IgVH hypermutation study, and chromosome microarray analysis (CMA) using a 400K Cancer-specific CGH/SNP array on ten patients with CLL. Our results demonstrated that CMA not only detected all FISH aberrations, but also helped to define the size and the genes involved in these aberrations. Furthermore, CMA data revealed many previously unknown genomic abnormalities, including copy numbers neutral loss of heterozygosity, which cannot be detected by FISH or cytogenetic study. Based on these results, we suggest that CMA study can replace FISH as a first tier test for CLL. When comparing cytogenetic and FISH results with hypermutation of IgVH, we found that FISH results were not always consistent with IgVH mutation status; however, no conclusion can be drawn due to the small sample size. We believe that cytogenetic, CMA, and IgVH mutation studies provide comprehensive genetic diagnosis and prognosis for CLL. The CGH/SNP array study may uncover the etiology of CLL, and lead to the development of targeted therapies.

1491T

Retrospective microarray analysis in diagnostic acute myeloid leukemia. *C.M. Higgins, D.L. Pickering, J.M. Stevens, W.G. Sanger, B.J. Dave.* Human Genetics Laboratories, Munroe Meyer Institute, University of Nebraska Medical Center, Omaha, NE.

Acute myeloid leukemia (AML) occurs mostly in adults and accounts for 70% of all acute leukemias. Cytogenetics is an important diagnostic and prognostic determinant in AML, however, approximately 30% of cytogenetic studies in diagnostic AML are uninformative due to normal fluorescence in situ hybridization (FISH) studies and/or karyotype. To investigate for the presence of chromosome imbalances not observed by either of the analyses, we examined 10 diagnostic AML cases with normal findings using genomic microarray developed by the Cytogenomic Cancer Microarray Consortium. For validation, we also included a cytogenetically abnormal AML case containing double minutes and ring chromosomes. DNA was extracted from available fresh bone marrow, refrigerated bone marrow, or fixed cell pellets from the cytogenetic cultures. These specimens were hybridized to a customized oligonucleotide array (4x180K) designed to target over 500 selected cancer genes and 130 cancer-associated chromosome regions. To distinguish between disease-associated and other possibly non-pathogenic copy number changes, a select group of controls was utilized. These included cytogenetically normal bone marrows from other hematologic disorders and peripheral blood from clinically normal individuals. Nine of the 10 cases analyzed by microarray did not show any additional clinically relevant genomic imbalances. One case revealed a small copy number gain at 17q25.2 which included the AML specific *SEPT9* (Septin 9) gene. Since this gain is contained within a known copy number variation observed in normal population, the clinical implications of this finding remain uncertain. Microarray revealed a range of 6-20 aberrations, some of which were known benign copy number variants and others were categorized as non-pathogenic based on the findings of our control subset. Additionally, microarray analysis of the cytogenetically abnormal AML case was consistent with the abnormalities defined by conventional cytogenetics and resolved all unidentified abnormalities including the origin of double minutes and ring chromosomes, thus completely characterizing the karyotype. Further investigations utilizing more recent versions of cancer microarrays including a combination of oligonucleotide probes and single nucleotide polymorphism (SNP) content are necessary to determine the benefit of microarray analysis in hematologic disorders.

1492T

Cytogenetic monitoring by use the micronucleus assay (MNs) and the chromosomal aberrations test (CAs) among Tunisian hospital staff exposed to low dose of ionizing radiation (IR). *S. BOURAOUI[1], A. DRIRA[2], H. BEN KHELIFA[1], M. GRIBAA[1], N. BOUALI[1], R. BERGUIGA[1], I. BEN ABDALLAH[1], S. BRAHEM[1], A. BENNOUR[1], O. MAMAI[1], N. MRIZEK[2], F. TABKA[2], H. ELGHEZAL[1], A. SAAD[1].* 1) Department of Cytogenetic and Reproductive Biology ,Farhat Hached University Teaching Hospital, Sousse, Tunisia; 2) Department of occupational and environmental medicine, Farhat Hached University Teaching Hospital, Sousse, Tunisia.

Of all workers exposed worldwide to man-wide sources of radiation, medical personnel represent the group most consistently exposed to low dose of (IR). The high doses of IR are clearly known to produce deleterious consequences in human, including cancer induction. However, the effect of such radiations at lower doses, as in occupational work setting, is less clear. Human cytogenetic biomonitoring studies using somatic cells have been proposed as pertinent tools to assess the possible genotoxic effects of (IR). CAs and MNs have been widely accepted as reliable biomarkers for evaluating damage induced by IR. Our study reports the finding of these genotoxicity tests for Tunisian hospital staff exposed to low dose of IR. Assessment of chromosomal damage was carried out in peripheral lymphocytes of 45 exposed to IR from radiology, radiotherapy, cardiology and nuclear medicine departments of Frahat Hached hospital of Sousse (Tunisia). We used 34 controls from administrative department of the same hospital matched for gender, age, smoking and life style. Heparized venous blood samples were collected and were processed for different cytogenetic methodologies used in this study. MNs frequency was evaluated after cytokinesis block by use the cytochalasine B. Mitomycin C was used for detection of CAs. The frequency of binucleated micronucleated cells was significantly higher ($p < 0.05$) in the exposed groups (15.8‰ ± 4.66) when compared with controls (6.91‰ ± 4.44). The highest frequency of MN was seen in exposed from nuclear medicine department. MN frequency was significantly positively correlated with time of exposure to IR (Spearman's test, $\delta = 0.046$, $p < 0.05$). CAs frequency was significantly higher ($p<0.05$) in the exposed groups (4.85 % ± 2.35) when compared with controls (1.85 % ± 1.84). Our results points to the necessity to develop procedure that allow the occupationally exposed hospital staff to be biomonitored and protected from IR.

325F

eQTL analysis to identify candidate loci influencing cellular response to simvastatin exposure. *L.M. Mangravite[1], B.E. Engelhardt[2], M.W. Medina[3], J.D. Smith[4], M.J. Rieder[4], D.A. Nickerson[4], M. Stephens[5], R.M. Krauss[3].* 1) Sage Bionetworks, Seattle, WA; 2) Department of Computer Science, University of Chicago, Chicago, IL; 3) Children's Hospital Oakland Research Institute, Oakland, CA; 4) Department of Genome Sciences, University of Washington, Seattle, WA; 5) Department of Statistics and Department of Human Genetics, University of Chicago, Chicago, IL.

Statins are prescribed to lower cardiovascular disease (CVD) risk by reducing plasma LDL. This response is variable and, although it has been hypothesized to have a genetic component, identification of genotypes associated with plasma LDL change has met with limited success. We have, however, demonstrated associations of candidate gene loci with cellular response traits in statin-treated lymphoblastoid cell lines (LCL). We sought to extend these analyses by using a genome-wide approach to identify novel loci for statin response. Specifically, we aimed to identify cis-eQTLs, classifying each as interacting or not interacting with statin treatment, and to test for relationships of these loci with change in cell surface expression of LDL receptor (LDLR), the protein that mediates increased LDL plasma clearance in response to statins. Methods: Gene expression profiles and surface LDLR protein were measured following simvastatin or control buffer exposure in 480 LCLs. Measured genotypes were imputed to 7.8 million SNPs and tested for associations with treated and untreated expression profiles. Gene by treatment effects, or differential cis-eQTLs, were identified through the application of a novel multivariate Bayesian regression method designed to explicitly test for five possible models of association with treated or untreated gene expression. Summary: Simvastatin exposure significantly altered expression of 5509 probed transcripts (±1.09±0.08 fold change, 54.0% response rate, q<0.0001) and increased surface LDLR expression (2.22±5.16 fold change, P<0.0001). Univariate analyses identified 2145 and 2032 cis-eQTLs within the untreated and treated datasets respectively (log10BF > 8.0). For 24 genes associated with treatment-stable cis-eQTLs, expression was correlated with change in LDLR (q=0.10). Few differential cis-eQTL were observed, although the multivariate approach was more effective than standard methods for differential eQTL detection. However, statin-mediated changes in expression of genes at these loci were not strongly correlated with change in LDLR. These findings suggest that genetic regulation of LDLR, and hence plasma LDL, manifests through variation in underlying physiology rather than through statin-specific effects. In contrast, treatment-dependent differential cis-eQTLs may influence variation in statin response of non-cholesterol pathways, possibly contributing to the known pleiotropic effects of statins that influence CVD risk.

326F

CRP predicting locus at 6q22 modulates risk of ventricular fibrillation in acute myocardial infarction. *R. Pazoki[1], M.W.T. Tanck[1], D. Dalal[2], J.S.S.G de Jong[3], A.A.M. Wilde[3], C.R. Bezzina[3].* 1) Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center, Amsterdam, the Netherlands; 2) of Cardiology, Johns Hopkins University, Baltimore, Maryland, USA; 3) Heart Failure Research Center, Department of Clinical and Experimental Cardiology, Academic Medical Center, Amsterdam, the Netherlands.

Introduction: Sudden cardiac death is a leading mode of death in adults in the Western world and is largely caused by ventricular fibrillation (VF) during acute myocardial ischemia-infarction (MI). A genetic component in risk of VF in the setting of MI is recognized. However, the underlying genetic factors remain largely unknown. Plasma concentration of C-reactive protein (CRP) is a predictor of sudden cardiac death. In recent genome-wide association studies (GWAS), several single nucleotide polymorphisms (SNPs) have been associated with CRP levels. In this study we investigated the effect of CRP-predicting SNPs in modulation of risk of VF in acute MI.

Methods: Patients studied were from the Arrhythmia Genetics in the NEtherlands Study (AGNES), consisting of patients with a first acute MI, with those suffering VF classified as cases (n=515) and those not suffering VF classified as controls (n=457). Twenty independent SNPs (r^2<0.75) previously found to be associated with CRP levels at genome-wide significant p-values ($P<5\times10^{-08}$) in previous GWAS were identified. Genotypes were obtained by direct genotyping (Illumina 610) or were imputed using HapMap. A Bonferroni-corrected threshold of P<0.002 was used to define statistical significance in testing of individual SNPs. We also generated a genetic risk score based on the number of CRP-increasing alleles. Logistic regression modeling was used to test for association with VF.

Results: SNP rs6901250 in GPRC6A was strongly associated with VF in acute MI (OR:1.39;95%CI:1.14,1.69;$P=9.6\times10^{-04}$), with the CRP-increasing allele being associated with increased risk for VF. Analysis of SNPs in a 50 Kb region around rs6901250 identified a stronger signal at SNP rs633420 ($P=9.7\times10^{-05}$) which was in high linkage disequilibrium (r^2=0.73) with rs6901250. The CRP genetic risk score generated based on the number of CRP-increasing alleles was associated with risk of VF after MI (OR: 1.07; 95%CI: 1.01,1.12; P=0.01).

Conclusions: CRP-predicting loci in aggregate and rs6901250 individually are associated with risk of VF in MI. Our study highlights the role of inflammatory loci on risk of VF after acute MI.

327F

A prescription for personalized wellness: Identification of genomic signatures of VO2max responsiveness among individuals undergoing combined aerobic and resistance training. *E. Rampersaud[1,4,5], L. Nathanson[1,4], T. Musto[2], A. Mendez[3,4], R.L. Belton[1,4], K. Meshbane[1,4], A. Dressen[1,4], N. Hudson[1,4], M. Cuccaro[1,4,5], J.M. Vance[1,4,5], D. Seo[1,4], M.A. Pericak-Vance[1,4,5], P.J. Goldschmidt-Clermont[1,4].* 1) John P. Hussman Inst Human Genomic, Univ Miami, Miami, FL; 2) Department of Kinesiology and Sports Science, University of Miami, Miami, FL, USA; 3) Diabetes Research Institute, University of Miami, Miami, FL, USA; 4) Miller School of Medicine, University of Miami, Miami, FL, USA; 5) Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami, Miami, FL, USA.

Exercise is a proven method for reducing cardiovascular disease (CVD) risk but not all individuals experience equal cardiovascular benefits from exercise and some may be at risk for injury. Thus, while exercise prescription remains a primary clinical prevention tool, a one-size-fits-all approach is not universally effective. Understanding the genomic causes of variation in exercise responsiveness, will enable “personalized health” programs for exercise to be individually tailored. We performed gene expression analysis on 101 individuals from the Genetics, Exercise And Research (GEAR) study to identify a genetic profile for fitness response. Participants completed a 12 week combined aerobic and resistance training program, 3 days/ week, for 45 to 60 minutes at 60% to 80% of their age and gender predicted maximum heart rate capacity. Baseline and post-training measures were collected on VO2max and other fitness variables. Total RNA was analyzed using the Affymetrix GeneChip Human Gene ST array. Analyses were performed separately in men and women. “High responders” had a ⁄ 15% improvement in VO2max and “low responders” had <15% improvement. A set of 16 genes (fold changes of⁄ 1.5;FDR≤5%) were differentially expressed between high and low male responders. We also identified 437 genes whose baseline expression levels differed significantly among men who were subsequently classified as “high responders” compared to those who were classified as “low responders” (FDR<5%). Gene set analysis showed enrichment in oxidative phosphorylation and cardiac muscle contraction pathways (FDR<0.01). Differentially expressed genes among “high” compared to “low” responders in both pre (n=53) and post-menopausal women (25) mapped to inflammatory and immune gene networks. Comparison of our results with those from two independent exercise intervention studies revealed overlap in inflammatory and muscle contraction pathways. In summary, we identified genes that both predict and differentiate VO2max responsiveness to exercise and which map to similar biological pathways as demonstrated by two independent studies. Our findings support the application of genomic approaches for identifying the molecular signature of exercise-induced fitness.

328F

Genotyping of Heat Shock protein-70 Polymorphisms in Hypertrophic Cardiomyopathy. *A. Rangaraju[1], M.L Satyanarayana[1], N. Calambur[2], P. Nallari[1].* 1) Department of Genetics, Osmania University, Hyderabad, Andhra Pradesh, India; 2) Cardiologist, CARE Hospitals, Hyderabad, Andhra Pradesh, India.

Statement of Purpose: Hypertrophic Cardiomyopathy (HCM) is a disease of the heart muscle characterized by hypertrophy of the left/right ventricle with a predominant involvemnent of the interventricluar septum. It has a varied clinical course ranging from being asymptomatic to heart failure to even sudden cardiac death. Of late, the role of Heat shock proteins has been implicated in diseases like coronary artery disease, schizophrenia, Parkinson's disease, High altitude illness etc. Heat shock proteins (HSP) are a group of proteins expressed when the cells are exposed to elevated temperature, stress stimuli including oxidative free radicals and toxic metal ions and act as molecular chaperones, under both stressed and normal conditions, to assist polypeptide chain folding and assembly into its secondary and higher-order structures, apart from playing a role in the repair of damaged proteins and ubiqutination. Recently, three heat-shock protein 70 (HSP70) genes have been mapped within the major histocompatibility complex (MHC) class III region of chromosome 6: **HSP70-1, HSP70-2 and HSP70- Hom***Italic Text*. In the present study, the analysis of heat shock protein 70 gene polymorphisms: +190G/C-(*HSP 70-1*), +1267A/G - (*HSP 70-2*) was carried out to understand its implication in HCM. Methodology: Blood samples from 100 healthy subjects and 100 HCM cases were collected from Osmania and CARE Hospitals respectively.The genotyping of *Hsp 70* gene for the polymorphisms was carried out by PCR based RFLP analysis. Results: The `CC' genotype of +190G/C polymorphism in *Hsp 70-1* gene was found to be 23% in HCM and 7% in controls with the Odds Ratio being 3.8157 (CI:1.4423 - 10.094) and `GG' of +1267A/G in *Hsp 70-2* gene was found to be 36% in HCM and 16% in controls with Odds Ratio estimate being 3.5357 (CI;1.5826 - 7.899). +1267A/G polymorphism is in the coding region of the gene, the GG genotype may play a role in the increased transcriptional activity after receiving a stimulus. The +190G/C polymorphism is a silent polymorphism which lies 26 bp upstream of the 5'UTR region which may act synergistically in relation to other polymorphisms present in the vicinity.

329F

Genetic heterozygosity in Cardiomyopathy. *M.L. Satyanarayana[1], R. Advithi[1], S. Deepa[2], C. Narsimhan[3], N. Pratibha[1].* 1) Department of Genetics, Osmania University, Hyderabad, Andhra Pradesh, India; 2) Centre for Cellular & Molecular Biology, Hubsiguda, Hyderabad, Andhra Pradesh, India; 3) Care Hospital, Banjarahills, Hyderabad, Andhra Pradesh, India.

Cardiomyopathy is the disease of heart muscle which is caused by abnormalities in cardiac wall thickness, chamber size, contraction and relaxation. HCM is a primary cardiac disease characterized by an increase in the thickness (hypertrophy) of left ventricle walls in the absence of secondary causes, while Dilated cardiomyopathy (DCM) is associated with dilation and impaired contraction of the left or both ventricles. Hypertrophic cardiomyopathy (HCM) is known to be manifested by mutations in 12 sarcomeric genes and dilated cardiomyopathy (DCM) by sarcomeric as well as cytoskeletal gene mutations. It is well established that mutations in sarcomeric and cytoskeletal genes account to both HCM and DCM phenotypes. Hence the present study is envisaged to screen for the genetic variations of sarcomeric genes viz., beta-myosin heavy chain gene () -MYH7), Troponin T2 (TNNT2), Troponin I3 (TNNT3), Alpha -Tropomyosin (TPM1), Alpha- Actin (ACTC), Myosin Regulatory Light Chain (MYL2), Myosin Essential Light Chain (MYL3) by SSCP & sequencing in 179 controls, 309 patients (HCM & DCM). Less than 10% of the mutations accounted for the cardiomyopathy but surprisingly 40-50% of the cases harbored genetic compounds in these mutations, which may play a role in the etiopathogenesity of the condition. This can be explained on the basis of dose effect of the mutant protein, or environmental factors wherein the HCM and DCM phenotype affecting both right and left ventricles, leading to heart failure. Further the synonymous SNP and genetic compounds may effect in a dominant negative fashion and insilico analysis may highlight the significance of these variations/ mutations in cardiomyopathy which will be discussed.

330F

A novel haplotype within CRP gene augments the risk of coronary heart disease in Indian Asians. *P.P. Singh[1], M. Singh[1], H.S. Nagpal[2], T.P. Kaur[3], M.D. Napoli[4].* 1) Molecular Genetics Laboratory, Department of Human Biology, Punjabi University, Patiala, Punjab, India; 2) Nova Heart Institute and Research Centre, Bathinda, India; 3) Amrit Poly Clinic, Ferozepur, India; 4) Neurological Section, SMDN - Center for Cardiovascular Medicine and Cerebrovascular Disease Prevention,Sulmona(AQ), Italy.

Background —Blood concentrations of C-reactive protein (CRP) have been associated with coronary heart disease (CHD) risk in epidemiological studies. However, doubts remain whether this association is causal or reflects residual confounding. The influence of CRP gene polymorphisms on elevated CRP levels as genetic mediators of the CRP-CHD link, need to be explored in Indian Asians, where this disease is rising rampantly. Methods and Results —The present study investigated three pertinent CRP single nucleotide polymorphisms (SNPs): -717A>G (rs2794521), +1059 G>C (rs1800947) and +1444C>T(rs1130864) in 180 angiographically diagnosed coronary heart disease cases and 175 control subjects of Punjab, India. Rare allele frequencies (G, C and T) of -717A>G +1059G>C and +1444C>T are found to be 11.4%, 11.7%, 17.8% and 16.9%, 10.0%, 19.7% in cases and controls respectively. Disease association analysis reveals that AGT (in order of -717A>G, +1059G>C and +1444C>T) is a susceptible haplotype for risk of CHD (OR 2.4, 95%CI: 1.23-4.84, P=0.006) which exacerbates after adjusting for the confounding effects of age, sex, BMI, SBP, DBP, lipid lowering drugs, aspirin use, arterial hypertension (OR 2.5, 95%CI: 1.27-4.99, P=0.004).The possession of each unit of this haplotype increases the risk of CHD by a factor of 2.41±0.439 () ±SE) in its recessive mode. Conclusions —In the present study, a novel susceptible haplotype within CRP gene (AGT) is found to be associated with CHD risk and higher CRP levels in Indian Asians.

331F

Association of sequence variants in the USF1, ROS1 and ABCA genes with glycohemoglobin levels in the ClinSeq Study. *H. Sung[1], M. Krishnan[1], D. Ng[2], S. Gonsalves[2], P. Cruz[3], J. Mullikin[3,4], L. Biesecker[2], A. Wilson[1], NISC Comparative Sequencing Prgram.* 1) Genometric Section, NIH/NHGRI/IDRB, Baltimore, MD; 2) Genetic Disease Research Branch, National Human Genome Research Institute, NIH, Bethesda, MD; 3) Comparative Genomics Unit, Genome Technology Branch, National Human Genome Research Institute, NIH, Bethesda, MD; 4) National Institutes of Health Intramural Sequencing Center (NISC), National Human Genome Research Branch, Bethesda, MD.

ClinSeq is a large scale medical sequencing study designed to investigate associations of rare sequence variants to traits related to the risk of developing coronary heart disease (CHD). In this study, the samples are comprised of non-smoking patients, ages 45 to 65 with normal to severe coronary artery calcification. Forty-three phenotypes related to CHD, including glycohemoglobin level, were measured at the NIH Clinical Research Center in Bethesda, MD. Sanger-based sequencing of selected coding regions across 250 genes was performed at the NIH Intramural Sequencing Center. Each genotype was converted to missing if its call was ambiguous, if the Polyphred score was less than 99 or if either forward or reverse reads were missing. After removing sequence variants with a location-specific calling rate of less than 60% and individuals with a genotyping rate of less than 60%, a total of 8,837 sequence variants (SVs) were analyzed in 436 Caucasians. Of 8,837 SVs, 5,035 SVs were not observed in either dbSNP or the 1000 Genomes databases and were considered to be novel SVs. Tests of association of glycohemoglobin level (adjusted for age, sex and abdominal circumference) and each sequence variant were performed with simple univariate linear regression assuming additive allelic effects using PLINK v1.07. Thirty-four SVs, of which 27 were novel, with estimated minor allele frequencies of less than 0.003, were associated with both untransformed and transformed glycohemoglobin level at a significance level of less than 1e-08. In most cases the minor allele of these significant variants occurred only once in the entire sample. These findings will be investigated further by collapsing rare variants by position and by functional domain. The most significant SVs (p-values less than 1e-16) were all novel and included variants in the intronic regions of USF1 (chromosome 1 at 159,276,835 bp), ROS1 (chromosome 6 at 117,749,568 bp) and ABCA1 (chromosome 9 at 106,587,796 bp). USF1 is associated with antilipolytic insulin sensitivity and metabolic risk factors for cardiovascular disease, ROS1 is associated with myocardial infarct and ABCA1 is associated with atherosclerosis, CHD and cholesterol. These significant findings suggest the importance of the USF1, ROS1 and ABCA1 genes in determining glycohemoglobin levels and provide insight into the underlying genetic mechanisms of coronary heart disease in the ClinSeq study.

332F

Analysis of Gene-by-Smoking Interactions in Families with Early-Onset Coronary Artery Disease (CAD) in the GENECARD Study. *C.K. Ward-Caviness[1, 2], C. Blach[2], C. Haynes[2], E. Dowdy[3], S.H. Shah[2, 3], S.G. Gregory[2, 3], W.E. Kraus[2, 3], E.R. Hauser[2, 3].* 1) Computational Biology and Bioinformatics, Duke University, Durham, NC; 2) Center for Human Genetics, Duke Univeristy Medical Center, Durham, NC; 3) Department of Medicine, Duke University Medical Center, Durham, NC.

We explored the genetics underlying cigarette smoke-CAD associations using the Genetics of Early-Onset Coronary Artery Disease (GENECARD) cohort. We have previously shown linkage of CAD to a region on chromosome 3q21, and association studies in independent case-control cohorts, CATHGEN and Intermountain Heart Catheterization Study, revealed significant associations between SNPs in this region and CAD, driven by a CAD-smoking interaction (Horne et al. Ann Hum Genet 2009). To validate and expand on these results we explored the interaction between 431 SNPs on chromosome 3 and smoking using Association in the Presence of Linkage-Ordered Subset Analysis (APL-OSA) and Genotypic Distribution Analysis (GDA). APL-OSA used a permutation procedure to test for increased association of a SNP with CAD in a subset of families with greater smoking (mean pack-years) versus the whole dataset. SNPs significant for CAD association in APL-OSA were then tested for association with smoking using GDA; GDA is a test for biologically plausible models, e.g. additive, dominant, or over-dominant, performed by comparing the distributions of mean pack-years across the genotypes, choosing one member per family. The posterior distribution of mean-pack years is calculated for each genotype and 10,000 independent draws from each posterior are used to determine if the SNP is classified as one of the three aforementioned models. A Kruskal-Wallis test was used to test the significance of the classification. Using APL-OSA we found 16 significant SNPs, permutation p-value < 0.05. Via GDA, 11 of these 16 significant SNPs followed a biologically plausible model for mean-pack years, three with a suggestive Kruskal-Wallis p-value, $p < 0.10$. RS6766899 was the most significant result in both APL-OSA ($p = 0.008$) and GDA ($p = 0.065$). This SNP is located in rho-GTPase activating protein 32 (ARGHAP32) and further examination of the results revealed 2 more rho-GTPase pathway genes with significant SNPs, kalirin (KALRN) (RS4234218 APL-OSA p = 0.018, GDA p = 0.16) and myosin light chain kinase (MYLK) (RS11707609 APL-OSA p = 0.034, GDA p = 0.071). Our APL-OSA results further validated SNPs on chromosome 3 as being associated with CAD in smokers, and the GDA results reveal that at least some of the SNP-pack-years interactions followed a biologically plausible model for smoking (pack-years). This study gives new information for possible biological mechanisms for susceptibility to smoking in CAD.

333F

Mouse models provide insights into the mechanisms underling abnormal cardiac development associated with recurrent 8p23.1 deletions. *M. Wat[1], M. Garcia[2], Y. Chen[1, 4], Z. Yu[1], A. Hernandez[1], R.J. Schwartz[3], B. Lee[1, 4], M. Dickinson[2], D. Scott[1].* 1) Molec & Human Gen, Baylor College Med, Houston, TX; 2) Molecular Physiology and Biophysics, Baylor College Med, Houston, TX; 3) Biology and Biochemistry, University of Houston, TX; 4) Howard Hughes Medical Institute.

Cardiovascular anomalies are a highly penetrant phenotype associated with recurrent 8p23.1 microdeletions. Although haploinsufficiency of *GATA4* clearly contributes to the development of these anomalies, the spectrum of heart defects seen in 8p23.1 microdeleiton patients is more severe than those seen in patients with isolated *GATA4* mutations. This led us to hypothesize that haploinsuficiency of *SOX7*—a transcription factor encoding gene known to regulate *GATA4* expression—also contributes to the development of cardiovascular anomalies in patients with 8p23.1 deletions. To test this hypothesis, we created constitutive and conditional *Sox7* knockout mice. We found that $Sox7^{-/-}$ animals were embryonic lethal around E10.5 with dilated pericardial sacs and failure of yolk sac vascular remodeling suggestive of cardiac failure. Live imaging of wild type and $Sox7^{-/-}$ embryos that expressed green fluorescence protein in their erythrocytes revealed that decreased vascular flow begins between E8.5 and E9.5 in the mutant. Using whole-mount hybridization and immunohistochemistry, we found that *Sox7* expression at this stage of development is limited to the endothelial cells in the developing vasculature and the endocardial cells of the heart. Immunostaining of stage matched embryos for PECAM1—an endothelial cell marker—subsequently revealed evidence of abnormal angiogenesis/vascular remodeling with the intersomitic vessel of $Sox7^{-/-}$ embryos appearing disordered and supernumerary. We found increased prenatal lethality of $Sox7^{+/-}$;$Gata4^{+/-}$ double heterozygotes but only on a pure C57BL6 background. These results allow us to conclude that SOX7 is required for normal endothelial cell-dependent development and demonstrate for the first time that *Sox7* and *Gata4* interact genetically in-vivo. Since the development of the endocardium, an endothelial derived structure, is critical for proper formation of the cardiac valves and the septa of the four-chambered heart, we are currently working to determine if $Sox7^{-/Flox}$;$Tie2^{Cre}$ and $Sox7^{+/-}$;$Gata4^{+/-}$ mice have complex cardiac anomalies similar to those seen in individuals with 8p23.1 deletions.

334F

Fine-mapping of lipid loci in multi-ethnic populations detects known functional variants. *Y. Wu[1], S. Buyske[2], T. Assimes[3], L.S. Adair[4], C. Ballantyne[5], C. Carty[6], I. Cheng[7], D. Duggan[8], L. Dumitrescu[9], C.B. Eaton[10], A.B. Feranil[11], L.A. Hindorff[12], T.C. Matise[13], S. Mitchell[9], Y. Lin[6], T.A. Manolio[12], U. Peters[6], J.G. Robinson[14], F. Schumacher[15], A. Young[6], C. Kooperberg[6], D. Crawford[9], K.L. Mohlke[1], K.E. North[16].* 1) Department of Genetics, University of North Carolina, Chapel Hill, NC; 2) Department of Statistics and Biostatistics, Rutgers University, Piscataway, NJ; 3) Department of Medicine, Stanford University School of Medicine, Stanford, CA; 4) Department of Nutrition, University of North Carolina, Chapel Hill, NC; 5) Baylor College of Medicine, Houston, TX; 6) Public Health Sciences, Fred Hutchinson Cancer Research Institute, Seattle, WA; 7) University of Hawaii Cancer Center, Honolulu, HI; 8) Translational Genomic Science Institute, Phoenix, AZ; 9) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 10) Departments of Family Medicine and Epidemiology, Alpert Medical School of Brown University, Providence, RI; 11) Office of Population Studies Foundation, University of San Carlos, Cebu, Philippines; 12) Office of Population Genomics, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 13) Department of Genetics, Rutgers University, Piscataway, NJ; 14) University of Iowa, Iowa City, IA; 15) Keck School of Medicine, University of Southern California, Los Angeles, CA; 16) Department of Epidemiology and Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, NC.

Genome-wide association studies in European ancestry populations have identified many loci associated with lipids, however, the causal variants at most loci remain unknown. Fine-mapping across populations with diverse linkage disequilibrium (LD) patterns provides an opportunity to localize causal variants. We analyzed high-density SNPs on Metabochip for their association with triglycerides (TG), HDL-cholesterol (HDL-C) and LDL-cholesterol (LDL-C) in 5,884 African Americans in the Population Architecture using Genomics and Epidemiology (PAGE) consortium [drawn from the Atherosclerosis Risk in Communities Study (ARIC), the Multiethnic Cohort Study (MEC) and the Women's Health Initiative (WHI)], and 1,719 Filipinos from the Cebu Longitudinal Health and Nutrition Survey (CLHNS). We examined 6 loci with $P<10^{-5}$ in PAGE or CLHNS and for which variants have been previously associated with function. We evaluated whether these potentially causal variants would show the lowest *P* value of association among Metabochip fine-mapping SNPs. At *SORT1* in PAGE, potentially causal rs12740374 showed notably stronger association with LDL-C ($P=6.1\times10^{-11}$) than nearby SNPs ($P>1.4\times10^{-8}$), including the GWA index rs629301 ($P=2.5\times10^{-7}$). The findings suggested that the more limited LD in PAGE compared to CEU helped pinpoint the potentially causal variant. At *CETP* in PAGE, potentially causal rs17231520 ($P=2.2\times10^{-35}$) showed the strongest HDL-C association; this SNP is monomorphic in CLHNS and CEU. The *CETP* SNP with the lowest *P* value in CLHNS, rs7499892 ($P=4.5\times10^{-9}$), has unclear function. At *LIPC*, as the best Metabochip representative of potentially causal promoter variants, rs1077834 showed the strongest HDL-C association in PAGE ($P=2.7\times10^{-5}$), although CLHNS revealed an even stronger SNP (rs6494007, $P=6.0\times10^{-10}$). At *APOA5* for TG, different potentially causal SNPs exhibited the strongest association: PAGE, rs3135506 (S19W), $P=3.3\times10^{-12}$; and CLHNS, rs651821 (-3A>G), $P=3.8\times10^{-12}$. The strongest TG SNP at *GCKR* in PAGE ($P=3.5\times10^{-6}$) and CLHNS ($P=7.2\times10^{-6}$) was rs1260326 (L446P); and at *APOE*, the reported causal LDL-C SNP rs429358 is not represented well on Metabochip. Thus, at loci with strong signals, if the potentially causal SNPs were genotyped, they often showed the strongest trait association. These results suggest that for loci at which potentially causal SNPs remain unknown, the strongest evidence of association may reveal good candidates for functional study.

335F

Common and rare variants contribute to extreme LDL levels: findings from the NHLBI Exome Sequencing Project. *C. Xue[1], Y. Hu[2], C. Bizon[3], E. Lange[3], C. Willer[4], L. Lange[3] on behalf of the NHLBI Exome Sequencing Project.* 1) Bioinformatics, University of Michigan, Ann Arbor, MI; 2) Department of Biostatistics, University of Michigan, Ann Arbor, MI; 3) Department of Genetics, University of North Carolina, Chapel Hill, NC; 4) Department of Internal Medicine, University of Michigan, Ann Arbor, MI.

Plasma low-density lipoprotein cholesterol (LDL-C) is a major, modifiable risk factor for atherosclerotic cardiovascular disease. Genome-wide association scans have identified many common variants with individually small effects on LDL-C levels. Studies of Mendelian families with severe hypercholesterolemia have identified rare variants in four genes - LDLR, APOB, PCSK9, and ARH. We hypothesized that individuals with LDL-C levels ascertained from the extremes of the population distribution may harbor rare variants, potentially private or previously unknown, with a large effect on the phenotype. We ascertained 412 individuals from the extremes of the LDL-C distribution from 4 population-based cohort studies in the U.S.: ARIC, Jackson Heart Study, Framingham Heart Study and the Cardiovascular Health Study. We selected 196 African-American (AA) individuals (103 with LDL-C in the upper 1% of the distribution after adjusting for age, sex and status of lipid-lowering therapy and 93 with LDL-C in the lower 1%), and 216 European-American (EA) individuals, (108 from the upper 1% and 108 from the lower 1%). The mean LDL-C level for individuals in the high LDL group was 283 mg/dl for EA individuals and 242 mg/dl for AA individuals from the upper tail and from the lower tail; 46 mg/dl in EA individuals and 49 mg/dl in AA individuals. For the 412 individuals, we have performed targeted exome capture, followed by massively parallel sequencing on Illumina GA IIx at the University of Washington. We have generated an average of 102 million mapped reads per sample, with 95% of bases reaching a recalibrated quality score of Q20 or greater. 65% of the reads mapped to the exonic target region with mean depth of 83x. Initial analyses revealed the critical importance of QC, especially the impact of variant filters. To date, analysis of common variants with MAF> 1% under an additive genetic model revealed genome-wide significant association with APOE variants. We also assessed the impact of nonsynonymous variants with frequency below 1% in each gene using the CMC test: for unfiltered variants, the most significant association was observed for rare variants in LDLR (p=1x10-4). Of these LDLR variants, 9 were previously known to cause familial hypercholesterolemia and 7 are newly identified in the CMC test. We also noticed that 12 of the 16 LDLR variants are singletons. These rare variant findings offer insight on the design of future sequencing studies and follow-up strategies.

336F

Implication of the Pro12Ala , G972R polymorphisms of the PPAR- . 2 and IRS1 gene in type 2 diabetes and Coronary Artery Disease. *S. Vats, K. Matharoo, V. Sambyal, AJS. Bhanwer.* Human Genetics, G.N.D.U, Amritsar, INDIA 143005.

Coronary artery disease (CAD) is the most common and life threatening cardiovascular complication of type 2 diabetic (T2D) subjects. Risk for CAD is high among diabetic subjects by the factor of 2 to 4 as compared to non diabetic subjects. Peroxisome proliferator-activated receptor-gamma (PPAR.) promotes insulin stimulated tyrosine phosphorylation of insulin receptor substrate 1 (IRS1). PPAR. and IRS1 have been implicated to play a key role in insulin resistance. As T2D, obesity and cardiovascular diseases are characterized by insulin resistance, genes involved in the insulin resistance pathway are important candidate genes for CAD. Pro12ala (PPAR.) and G972R (IRS1) polymorphisms have been extensively studied in T2D and CAD patients in different populations with conflicting results. In the present study, association of Pro12Ala and G972R polymorphism in T2D and CAD patients of Punjabi population (North India) was investigated in 444 subjects divided into four groups 122 healthy controls,103 T2D+CAD, 117 T2D only and 102 patients only with CAD. Binary logistic regression was applied to test the association of risk factors with T2D, T2D+CAD and CAD only after adjusting for age, sex and BMI. The minor allele (G) frequency for Pro12ala polymorphism was observed to be 11.5%, 12%, 6.9% and 4.6% in controls; T2D+CAD, CAD only and T2D only group, respectively. In case of G972R polymorphism the minor allele (A) frequency in controls, T2D+CAD, CAD and T2D was observed to be 4.9%, 3.4%, 5.9 % and 4.3%, respectively. G972R polymorphism was found to be insignificant in all the groups ($p>0.05$) whereas in case of pro12ala statistically significant difference ($p<0.05$) was observed in the allele frequencies of T2D patients with controls, and T2D+CAD groups. CC homozygotes (Pro) at PPARG P12A appeared more likely to be susceptible to T2D than GG (Ala) carriers. Thus, C allele seems to increase susceptibility of CAD in T2D patients in Punjabi population.

337F

Pulmonary thromboembolism and the role of genetic thrombophilic gmutations in Iranian patients. *K. Ansarin[1], E. Sakhinia[2,4], A. Poursadegh Zonouzi[3], A. Firoozi[4], N. Karimi Ansari[4], Y. Heshmat[4], A. Asghari Haghie[4], M. Ghaffari[1].* 1) Tuberculosis and Lung diseases, Tabriz University of Medical Sciences, Tabriz, Iran; 2) Molecular Medicine Division, Department of Biochemistry, Faculty of Medicine, Tabriz, Islamic Republic of Iran,; 3) Department of Cellular and Molecular Biology, Faculty of science, Azarbaijan University of Tarbiat Moallem, Tabriz, I.R.Iran, Tabriz, Islamic Republic of Iran,; 4) Tabriz Genetic Analysis Centre (TGAC), Sheikh Alraeis Clinic, Specialized & Subspecialized Outpatient Clinic of Tabriz Medical Sciences University, Tabriz, I.R.Iran, Tabriz, Islamic Republic of Iran,.

Venous thromboembolism (VTE) is a common vascular disease that includes deep venous thrombosis and pulmonary thromboembolism. It is one of the leading causes of mortality across the world; with an annual incidence range from 1-5 cases per 1000 individuals. Various investigations examined the prevalence of thrombophilic gene mutations in patients who have suffered from a pulmonary thromboembolism. We aimed to determine the association of specific inherited thrombophilias with pulmonary thromboembolisms. A total of 85 subjects were included in this study: 53 patients who had been affected with a pulmonary thromboembolism and 32 healthy controls in the North West of Iran. Total genomic DNA was extracted from 2 ml EDTA anti-coagulated blood samples by a standard salting-out method and amplified by PCR using gene-specific primers. Amplification Refractory Mutation System (ARMS-PCR) method was used in the identification of PAI-1 (-675 I/D, 5G/4G), Factor XIII (Val34Leu), Betafibrinogen (-455G/A), Factor VII (Gln353Arg), Glycoprotein Ia (807C/T) and tPA (intron h D/I) gene mutations. Our data indicates that there were statistically significant differences in PTE patients compared with controls, when studying the frequency of these specific gene mutations. In conclusion, we propose that compound thromboplilic gene mutations rather than a specific gene mutation can be a risk factor for the occurrence of pulmonary thromboembolisms; however, further studies on a larger scale are required in order to gain a better understanding of the role of these gene mutations in pulmonary thromboembolism.

338F

Integration of Genomewide Linkage and Metabolomics Identifies Metabolic Quantitative Trait Loci (mQTL). *S. Shah[1, 2,3], X. Qin[2], C. Haynes[2], Z. Dowdy[1], J. Bain[3], J. Johnson[2], R. Stevens[3], C. Newgard[3], S. Gregory[2], V. Kraus[1], E. Hauser[2], W. Kraus[1].* 1) Department of Medicine, Duke Univ Med Ctr, Durham, NC; 2) Center for Human Genetics, Duke University Medical Center, Durham, NC; 3) Sarah W. Stedman Nutrition and Metabolism Center, Duke University, Durham, NC.

BACKGROUND. Many heritable common, complex diseases are metabolic in nature and the genetic architecture of disease risk is incompletely characterized. Metabolomic profiling of peripheral blood small molecular metabolites has been successfully used to identify disease biomarkers and novel mechanisms of disease, and have been shown to be heritable in plants, animals and humans. We therefore hypothesized that using metabolic profiles as quantitative traits for a genomewide linkage study would identify metabolic quantitative trait loci (mQTL), which could then serve as novel disease genetic markers. METHODS. Biological samples and clinical data were obtained on 365 family members from a large, multi-generational, multiethnic family (N=3000 total family members). Whole genome genotyping was done using the Illumina SNP linkage chip of ~5700 SNPs. Mass-spectroscopy based profiling of 45 acylcarnitines, 15 amino acids and traditional methods were used for measurement of three ketone-related metabolites was performed in frozen plasma. Variance components as implemented in the Sequential Oligogenic Linkage Analysis Routines (SOLAR) program was used to calculate heritabilities of each metabolite as well as for calculation of multipoint linkage odds (LOD) scores, using a polygenic model adjusted for sex. RESULTS. We found significant heritabilities for many metabolites, the most significant including the amino acids histidine (0.91), arginine (0.65), and the acylcarnitines C5-OH/C3-DC (0.54), C10:3 (0.72), and C20-OH/C18-DC (0.52). Genomewide linkage analysis of individual metabolite levels identified several candidates for metabolic quantitative trait loci (mQTL), including C14:1-OH/C12:1-DC acylcarnitine (chromosome 4, multipoint LOD [MLOD] 3.07) and C14 acylcarnitine (chromosome 12, MLOD 3.16); and the amino acids ornithine (chromosome 1, MLOD 2.79), citrulline (chromosome 4, MLOD 2.57), and glutamate/glutamine (chromosome 16, MLOD 2.64) CONCLUSIONS. We have identified metabolic QTL (mQTL) by integration of genomewide linkage and metabolomic profiling. Given previous findings of these metabolites serving as disease biomarkers, these mQTL may be harboring genes mediating disease risk through metabolic pathways. Investigations into this genetic architecture are ongoing.

339F

Smoking modifies the effect of lipoprotein lipase gene polymorphism on serum HDL-C concentration in Japanese general population. *H. Kitajima[1,4], K. Kurotani[2], K. Ohnaka[3], R. Takayanagi[4], K. Yamamoto[1].* 1) Division of Genome Analysis, Research Center for Genetic Information, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; 2) Department of Preventive Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; 3) Department of Geriatric Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; 4) Department of Medicine and Bio-Regulatory Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Genetic and lifestyle factors regulate serum high-density lipoprotein cholesterol (HDL-C) concentration. Recently, genome-wide association (GWA) studies have identified loci influencing HDL-C. Epidemiological studies have also reported lifestyle factors regulating HDL-C. The aims of this study are to verify the SNPs identified in the previous GWA studies and to identify the gene-lifestyle interactions that modify the level of HDL-C in the members of the Japanese general population, who have different genetic backgrounds and lifestyles from Caucasians. We selected 15 SNPs of which MAF were over 0.10 in the HapMap JPT among the SNPs shown to be significantly associated with HDL-C concentration in GWA studies. The selected SNPs were located on the *CETP*, *APOA5*, *LIPC*, *LPL*, *LIPG*, *ABCA1*, *LCAT*, *ANGPTL4*, *APOB*, *GALNT2*, *NR1H3*, *MADD-FOLH1*, *FADS*, *MVK-MMAB*, or chromosome 17p13.3 locus. We genotyped these SNPs for 9,281 subjects of the Fukuoka Cohort study in Japan with TaqMan assay. The seven SNPs, rs3764261 (*CETP*), rs662799 (*APOA5*), rs1800588 (*LIPC*), rs10503669 (*LPL*), rs2156552 (*LIPG*), rs4149268 (*ABCA1*), and rs2338104 (*MVK-MMAB*), were significantly associated with HDL-C adjusted for sex, age, BMI, current drinker, current smoker, physical activity, carbohydrate intake, protein intake, saturated fatty acid (SFA) intake, and n-3 highly unsaturated (long-chain) fatty acid (HUFA) intake. The effect size of additive model was 9.7, 7.7, 4.5, 5.7, 3.7, 2.7, and 1.5 mg/dl, and P-value was 2.4×10^{-80}, 1.9×10^{-58}, 5.5×10^{-27}, 1.9×10^{-18}, 2.6×10^{-9}, 5.3×10^{-9}, and 0.0016, respectively. A significant interaction (P = 0.00082) between rs10503669 (*LPL*) and current smoking was observed after adjustment for covariates. There was no significant SNP-SNP interaction among the seven SNPs. The adjusted means of HDL-C were 56.1 mg/dl, 56.7 mg/dl, and 56.6 mg/dl among current smokers and 60.4 mg/dl, 63.9 mg/dl, and 66.2 mg/dl among non-current smokers in risk allele homozygosity, heterozygosity, and non-risk allele homozygosity of rs10503669, respectively. The results indicate that the positive effect of the non-risk allele of rs10503669 on HDL-C level is negated by smoking. In summary, the seven loci were confirmed to be significantly associated with HDL-C in the Japanese general population. The effect of rs10503669 (*LPL*) on HDL-C might be modified by current smoking.

340F

Vitamin D dependent effects of APOA5 polymorphisms on HDL cholesterol and triglycerides. *B.H. Shirts[1], M.T. Howard[2], S.J. Hassedt[2], M.N. Nanjee[3], S. Knight[4], J.F. Carlquist[4], J.L. Anderson[4], P.N. Hopkins[3], S.C. Hunt[3].* 1) Dept of Pathology, Univ Utah, Salt Lake City, UT; 2) Dept of Human Genetics, Univ Utah, Salt Lake City, UT; 3) Cardiovascular Genetics Division, Dept of Internal Medicine, Univ Utah, Salt Lake City, UT; 4) Cardiovascular Dept. Intermountain Medical Center.

Background: Gene-environment interactions are thought to play a role in HDL levels. Here we identify a novel interaction between vitamin D (25OHD) and the APOA1-APOA5 locus that influences HDL and possibly triglyceride levels. Methods: We used maximum likelihood models to evaluate the interaction of dietary 25OHD and rs3135506 on HDL and triglyceride levels in 1060 individuals from Utah families and a subgroup of individuals with winter measures, when serum 25OHD is lowest and most correlated with dietary 25OHD. We replicated analysis using 2890 individuals from the Family Heart Study and used the combined sample to estimate heritability attributable to gene-environment interaction. Both seasonal enrichment for deficiency and higher seasonal correlation between the dietary and serum 25OHD could account for more significant results in winter subsamples, so we evaluated interactions in 1627 individuals from the Intermountain Healthcare Angiographic Registry with serum 25OHD measures. We hypothesized that if stronger winter interactions were due to higher correlation of dietary and serum 25OHD, linear interactions would be more apparent with serum 25OHD measures. We performed meta-analysis of all samples to synthesize results. We identified putative vitamin D receptor binding sites modified by linked SNPs and used luciferase expression assays to confirm allele-specific, 25OHD-dependent APOA5 promoter activity. Results: The minor rs3135506 allele was associated with lower HDL cholesterol in individuals with low winter dietary 25OHD (interaction p<0.001 Utah, p=0.002 FamHS). The proportion of HDL heritability attributable to rs3135506 with the interaction was 1.5% compared with 0.7% attributable to rs3135506 alone. Linear interaction of serum 25OHD and rs3135506 was not significant for HDL or triglyceride levels, and meta-analysis showed the interaction only at deficient 25OHD levels (interaction p=0.01 HDL, p=0.04 triglycerides). The rs10750097`G' allele near the APOA5 promoter was identified as a putative causative polymorphism, and was associated with 25OHD-dependent changes in APOA5 promoter activity in HEP3B and HEK293 cells (p<0.01). Conclusions: SNP rs3135506 interacts with dietary 25OHD influencing HDL cholesterol and is linked to vitamin D receptor binding site modifying rs10750097 in the APOA5 promoter. Targeted therapy may increase HDL by 7% and decrease triglycerides by 11% in 25OHD deficient carriers.

341F

Replication and Discovery of Lipid-Related Loci Exhibiting Heterogeneity by Sex in the Population Architecture Using Genomics and Epidemiology (PAGE) Study. *K. Taylor[1], K. North[1,2], C. Carty[3], L. Dumitrescu[4], L. Hindorff[5], F. Schumacher[6], M. Quibrera[1], S. Cole[7], S. Buyske[8], P. Buzkova[9], K. Brown-Gentry[4], N. Franceschini[1], L. Wilkens[10], D. Duggan[11], C. Eaton[12], B. Cochran[13], C. Haiman[6], L. Le Marchand[10], C. Kooperberg[3], D. Crawford[4,14], M. Fornage[15,16], The PAGE Study.* 1) Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Carolina Center for Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC; 3) Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 4) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 5) Office of Population Genomics, National Human Genome Research Institute, Bethesda, MD; 6) Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA; 7) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 8) Department of Statistics and Biostatistics, Rutgers University, Piscataway, NJ; 9) Department of Biostatistics, University of Washington, Seattle, WA; 10) Epidemiology Program, University of Hawaii Cancer Center, University of Hawaii, Honolulu, HI; 11) Integrated Cancer Genomics Division, The Translational Genomics Research Institute, Phoenix, AZ; 12) Department of Family Medicine and Community Health, Alpert Medical School of Brown University, Providence, RI; 13) Sponsored Programs, Baylor College of Medicine, Houston, TX; 14) Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN; 15) Division of Epidemiology, School of Public Health, University of Texas Health Sciences Center at Houston, TX; 16) Institute of Molecular Medicine, University of Texas Health Sciences Center at Houston, TX.

Background. The successes of genome-wide association studies (GWAS) in mapping over 100 loci associated with high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels have advanced our understanding of genomic influences on common diseases. However, the translation of such knowledge into clinical and public health applications requires exploration of the epidemiological architecture of these variants. For example, the environmental specificity of such effects has largely been unexplored. We investigated whether GWAS-identified SNPs previously associated with lipid traits exhibited heterogeneity by sex in the multi-ethnic Population Architecture Using Genomics and Epidemiology (PAGE) study. **Methods.** We genotyped 49 GWAS-identified SNPs associated with one or more lipid traits in at least two PAGE studies and across four population groups. We performed a meta-analysis testing for SNP associations with fasting HDL-C, LDL-C, and ln(TG) levels in self-identified European American (~20,000), African American (~9,000), American Indian (~6,000), and Mexican American/Hispanic (~2,500) adults, regardless of lipid-lowering medication use. A Chi-square heterogeneity test (1 df, resulting in P_{het}) was used to determine whether the effect of each SNP differed significantly by sex. **Results.** Examining 49 SNPs across three phenotypes, there was evidence for heterogeneity by sex (Bonferroni-corrected P_{het}<0.001) for six SNPs across five loci: *HNF4A, PLTP, HMGCR, LDLR*, and *APOA1*. Whereas heterogeneity by sex at *HMGCR* rs12654264 had been previously noted for total cholesterol, heterogeneity was novel for the other loci. Heterogeneity for the *APOA1* and *LDLR* SNPs was observed in multiple ancestrally diverse populations with consistent effect estimates. For *LDLR, PLTP*, and *HNF4A*, the sex-combined, meta-analyzed main effect P-value did not indicate an association, but a meta-analysis allowing for heterogeneity by sex was statistically significant (main effect P<0.001). **Conclusions.** We replicated and discovered several sex-specific effects of SNPs on fasting lipid concentrations in the ancestrally diverse PAGE study population. Our findings suggest a complex architecture for lipid-related traits and emphasize the need for considering context-specific population differences in interpreting the increasing body of genetic associations emerging from well-replicated GWAS.

342F

Investigation of Heterogeneity by Sex in Central Adiposity-Related Phenotypes in a Meta-Analysis of 42820 Individuals of European Descent. *S. Yoneyama[1], K.C. Taylor[1], C.S. Fox[2], B.J. Keating[3], L.A. Lange[1], K.L. Monda[1], Y. Gong[4], A.P. Reiner[5], H. Shen[6], E. Smith[7], M. Lanktree[8], S. Padmanabhan[9], S. Rafelt[10], T. Johnson[11], T. Gaunt[12], Y. Guo[3], S. Grant[3,13], L. Speilotes[14], S. Redline[15], G. Burke[16], H. Taylor[17], G. Papanicolaou[18], J. Liu[17], J. Ordovas[19], C. Jaquish[2], N. Heard-Costa[20], I. Heid[21], M. Gorski[21], K. North[1], E.W. Demerath[22], Candidate Gene Association Resource consortium.* 1) University of North Carolina, Chapel Hill, NC; 2) NHLBI (NIH/NHLBI), Bethesda, MD; 3) Children's Hospital of Philadelphia, Philadelphia, PA; 4) University of Florida, Gainesville, FL; 5) University of Washington, Seattle, WA; 6) University of Maryland, Baltimore, MD; 7) UC San Diego School of Medicine, La Jolla, CA; 8) The University of Western Ontario, London, Ontario, Canada; 9) University of Glasgow, Glasgow, Scotland; 10) University of Leicester, Leicester, UK; 11) Queen Mary, University of London, London, UK; 12) School of Social and Community Medicine, Oakfield Grove, Bristol, UK; 13) University of Pennsylvania, Philadelphia, PA; 14) Massachusetts General Hospital, Boston, MA; 15) Harvard Medical School, Boston, MA; 16) Wake Forest School of Medicine, Winston-Salem, NC; 17) University of Mississippi Medical Center, Jackson State University, Jackson, MS; 18) NIH, Bethesda, MD; 19) Tufts University, Boston, MA; 20) Boston University School of Medicine, Boston, MA; 21) Universität Regensburg, Fakultät Medizin, Regensburg, Germany; 22) University of Minnesota, Minneapolis, MN.

Background. Previous genome-wide association studies (GWAS) have identified loci associated with central adiposity. Gene-sex interactions are of particular interest for this phenotype because adiposity patterns differ markedly by sex. However, appropriate interaction identification methods in genetic epidemiology are still under development. In an effort to identify sex-specific genetic effects, we implemented two distinct analytical methods in a meta-analysis of 42820 European descent individuals. **Methods.** We conducted association analyses of waist circumference (WC), WC adjusted for BMI (WCadjBMI), and waist-to-hip ratio adjusted for BMI (WHRadjBMI) in 25776 females and 16975 males from the Candidate Gene Association Resource (CARe) consortium and ten other population-based studies. Individuals were genotyped using a cardiovascular disease-related gene-centric array, which includes ~50K densely mapped SNPs across ~2K genes. Each trait was modeled as a function of age, study site, and ancestry. We conducted a fixed-effects, inverse variance-weighted meta-analysis, stratified by sex. Heterogeneity by sex was assessed using a Wald Chi-square test (1df, yielding P_{het}). We ranked the results two ways: first by main effect association P-values (lowest P_{assoc}), and second by heterogeneity P-values (lowest P_{het}). We then compared the top 10 findings from these two approaches. **Results.** Using the first method (lowest P_{assoc}), we detected varying levels of heterogeneity across sex for all phenotypes, with P_{het} ranging from $1.2x10^{-4}$ to 0.97. Directions of effect were consistent across sex but with differing magnitudes. Using the second method, P_{het} values were lower, ranging from $1.3x10^{-5}$ to $3.7x10^{-4}$. The effect coefficients for males and females generally had opposite directions of association, but standard errors were larger than those reported using the first method. The two methods yielded different results with just one locus in common, rs2299656 in *CACNA1C*, for WCadjBMI. For this locus, there was a notable negative association among males (beta, SE = -0.12, 0.03; $P=5.5x10^{-6}$), but no effect was noted in females (0.02, 0.02; P=0.51) ($P_{het}=1.2x10^{-4}$). **Conclusions.** Two methods of evaluating heterogeneity across sex identified distinct loci. Replication studies are needed to distinguish real from spurious interactions. Moreover, it is clear that new methods to evaluate gene-environment interactions will be required.

343F

Autoimmune myocarditis induced by coxakie virus,suggesting novel loci on chromosome17. *R.M.T. Issa.* molecular diagnostics, institute of genetic engineering and biotechnology, cairo, Egypt.

Myocarditis is a major cause of heart failure.Disease is often initiated by pathogen insult in the heart.Enterovirus, coxsackie is the most prevelant.Following viral infection,disease progression to autoimmune myocarditis occurs only in genetically susceptible individuals. we use chromosome substitution strain(css)mice, to identify chromosome that contain autoimmune myocarditis.we used chromosome 17 to confer susceptibility to viral induced myocarditis.There are four susceptibility loci within the chromosome.The first locus in the distal portion of the chromosome,and moderately confer disease susceptibility.The second locus contain the MHC locus and TNFalpha.Two novel loci between 4 1cM and 16 cM,these loci contain no gene previously associated with disease development. However both contain a type 1 diabetes susceptibility locus suggesting the presence of global auto immune susceptibility genes.

344F

Association of Adiponectin Plasma Levels in African Americans with SNPs from Two Arrays. *S.G. Buxbaum[1], S. Musani[2], M. Allison[3], J. Kizer[4], S. Redline[5], J.G. Wilson[2], A. Bidulescu[6].* 1) Jackson Heart Study, Jackson State Univ, Jackson, MS; 2) Jackson Heart Study, University of Mississippi Medical Center, Jackson, MS; 3) Department of Family and Preventive Medicine, University of California, San Diego School of Medicine, La Jolla, CA; 4) Weill Cornell Medical College, Ithaca, NY; 5) Division of Sleep Medicine, Harvard Medical School, Boston, MA; 6) Morehouse School of Medicine, Cardiovascular Research Institute and Department of Preventive Medicine and Community Health, Atlanta, GA.

INTRODUCTION: Adiponectin is a moderately heritable biomarker of systemic inflammation and insulin resistance and is a putative predictor of subclinical and clinical cardiovascular disease. Adiponectin levels also have been associated with asthma control and may be associated with rheumatoid arthritis. Four cohorts with adiponectin measurements that included African Americans were analyzed to identify genetic variants associated with adiponectin levels. **METHODS**: Using both the Affymetrix 6.0 (Affy) and the ITMAT Broad-CARe (IBC) SNP arrays, we performed a meta-analysis that included up to 2487 African-Americans for association with log-Adiponectin blood plasma level adjusted for age, sex and body mass index. The IBC chip comprises densely typed SNPs in over 2000 candidate genes for CVD pathways. Samples were from the Candidate gene Association Resource (CARe) cohorts and included data from Jackson Heart Study, the Multi-Ethnic Study of Atherosclerosis (MESA), the Cleveland Family Study (CFS) and the Cardiovascular Health Study (CHS, IBC data only). The thresholds for genome-wide significance were 5×10^{-8} for the Affy data and 2.2×10^{-6} for the IBC data. **RESULTS**: No SNPs were observed to be genome-wide significant in either array. The strongest association for the Affy-based analysis (N=2375) adjusting for global ancestry was at rs4686807 at 3q27.3, $p = 6.01 \times 10^{-7}$. Further adjustment for local ancestry modestly increased the strength of association (1.69×10^{-7}). The most strongly associated SNP in the IBC array (N=2487) was at 6p21.1 with a p-value of 1.37×10^{-5}.

CONCLUSION: Although not genome-wide significant, these analyses confirmed a known locus for adiponectin and identified a potential novel locus. The association at 3q27.3 is of interest because this SNP is within the allergic/atopic asthma related QTL 45 and rheumatoid arthritis QTL 6. Further, the closest gene in RefSeq is *ADIPOQ*, 21.1 kbp away. The association at 6p21.1 is within a gene, *TREM2*, coding for a membrane protein that is a receptor for tyrosine kinase binding protein (TYROBP), a protein that may be involved in signal transduction, bone modeling, brain myelination, and inflammation. *TREM2* is included in the GO pathway 0004872 for receptor activity. Similar to the Affy SNP, this IBC SNP is within the allergic/atopic asthma related QTL 34 and osteoarthritis QTL 22. These findings require replication in other cohorts and a meta-analysis is ongoing.

345F

Family based association analysis of variants in activating FCgR with Kawasaki Disease (KD) susceptibility, IVIG refractoriness, and coronary artery disease. *S. Shrestha[1], H.W. Wiener[1], A. Shendre[1], J.C. Edberg[2], M.A. Portman[3].* 1) Department of Epidemiology, University of Alabama at Birmingham; 2) Division of Clinical Immunology and Rheumatology, Department of Medicine, University of Alabama at Birmingham; 3) Department of Pediatrics, University of Washington, Seattle Children's Hospital.

Kawasaki disease (KD) is the leading cause of acquired heart disease in children. FCg receptors (FCgR) modulate action of gamma-globulin, and the frequencies of single nucleotide polymorphisms (SNPs) in these FCgR genes also vary by race. Trios from three populations (156 Caucasians, 43 Asians, and 52 Hispanics) were genotyped for 11 single nucleotide polymorphisms (SNPs) in FCgRIIA, FCgRIIIA and FCgRIIIB, all located at chromosome position 1q23 within a span of about 200kb, by pyrosequencing using a nested polymerase chain reaction (PCR) approach to ensure gene-specific amplification. In this hypothesis-driven study, we performed the transmission disequilibrium test (TDT) using FBAT on individual SNPs to assess the associations for susceptibility to Kawasaki disease, IVIG refractoriness (IVIG-R) - defined by AHA guidelines, and coronary artery disease (CAD) - defined by persistence of coronary artery Z-score > 2.5 or aneurysm > 6 weeks after IVIG. Race was defined by the principle component analysis using 155 Ancestry Informative Markers (AIMs) and the analyses were limited to families where all members were from the same race. In the additive TDT model, the Fc. RIIA+559 variant showed excess A allele (Histidine) transmission from parents ($z = 3.12$, $p = 0.001$) to KD patients in 182 informative trios. This effect was consistent separately in 26 informative Asian ($z = 2.34$, $p=0.02$) and 105 informative Caucasian families ($z = 2.04$, $p = 0.04$). Likewise, excess transmission of the same A allele in Fc. RIIA+559 occurred among IVIG non-responders (156 informative trios; $z = 2.78$, $p= 0.005$), and mostly in Asians (25 informative trios, $z = 2.66$, $p=0.008$) and patients with CAD ($n = 43$, $z = 2.45$, $p = 0.01$), and mostly in Hispanics (12 informative trios, $z = 2.14$, $p = 0.03$). Other variants examined did not indicate any significant associations. Variants in Fc. RIIA+559 alter recognition of ligand where; the A variant binds human IgG2 with high affinity than the G variant. In our study, excess transmission of the more effective Fc. RIIA+559A variant is related to increased KD susceptibility, IVIG refractoriness, and CAD persistence in different races.

346F

Synergistic effect of ANRIL and BRAP polymorphisms on ankle-brachial index in a Taiwanese population. *P.C. Tsai[1], Y.C. Liao[2,3,4], T.H. Lin[5,6], E. Hsi[1,2], Y.H. Yang[1,7], S.H. Juo[1,8,9].* 1) Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 2) Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 3) Section of Neurology, Taichung Veterans General Hospital, Taichung, Taiwan; 4) Department of Neurology, National Yang-Ming University School of Medicine, Taipei, Taiwan; 5) Division of Cardiology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 6) Department of Internal Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 7) Department of Oral Hygiene, Kaohsiung Medical University, Kaohsiung, Taiwan; 8) Department of Neurology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 9) Department of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan.

Objectives: Single nucleotide polymorphisms (SNPs) on chromosome 9p21 are found to be associated with peripheral artery disease (PAD) in several studies, but have not been investigated in Asians. PAD was often diagnosed by ankle-brachial index (ABI) value. The aim of the present study is to evaluate the effects of SNPs on 9p21 region alone with the BRAP gene on ABI in a Chinese population. Methods: A total of 739 high-risk subjects were enrolled. SNP rs11066001 on BRAP and three SNPs (rs1333040, rs2383207 and rs1333049) on 9p21 were genotyped. The genotype-phenotype associations were tested by multivariate regression analysis. The combined effects of 9p21 and BRAP were evaluated by both multiplicative and additive models. The gene-environmental interaction between genes and smoking was examined. Results: SNP rs2383207 on 9p21 was significantly associated with lower ABI values (adjusted p=0.02). Similar to our previous findings, G allele of rs11066001 on BRAP was associated with lower ABI values (adjusted p=0.017). A dose-responsive relationship between ABI values and the number of risk alleles from both significant SNPs was observed (trend p =0.0003). The association between ABI and combined genetic effect was stronger in ever-smokers than in non-smokers. A significant interaction between genes and smoking were found (adjusted p=0.043). Conclusion: Chromosome 9p21 was associated with ABI in a Taiwanese population. An additive effect between variants on 9p21 and BRAP were shown. The combined genetic effect might be enhanced by smoking exposure. The findings of potential gene-gene interaction and gene-environment interaction are interesting, but need further investigation.

347F

Genetic and molecular characterization of variants associated with circulating interleukin-18 levels and cardiovascular disease. *T. Zeller[1,3], R.B. Schnabel[1,3], J. Dupuis[2], P.S. Wild[3], R. Castagne[4], J. Baumert[5], J. Kruppa[6], A. Schillert[6], B. Thorand[5], T. Munzel[3], K.J. Lackner[7], A. Ziegler[6], W. Koenig[8], E.J. Benjamin[2], L. Tiret[4], S. Blankenberg[1,3].* 1) Department of Cardiology, Medical University Hamburg-Eppendorf, Hamburg, Hamburg, Germany; 2) National Heart, Lung and Blood Institute's and Boston Univ's Framingham Heart Study, Boston, MA, USA; 3) Medical University Center Mainz, Department of Medicine 2, Mainz, Germany; 4) INSERM UMRS 937, Pierre and Marie Curie University and Medical School, Paris, France; 5) Helmholtz Zentrum Muenchen, Neuherberg, Germany; 6) Insitut für medizinische Biometrie und Statistik, IMBS Lübeck, Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Lübeck, Germany; 7) Medical University Center Mainz, Institute of Clinical Chemistry and Laboratory Medicine, Mainz, Germany; 8) Department of Internal Medicine 2, Cardiology, University of Ulm Medical Center, Ulm, Germany.

Interleukin-18 (IL-18) is a pleiotropic cytokine centrally involved in the immune response. A role of IL-18 in cardiovascular disease has been suggested. In a recent genome-wide association study we explored the genetic underpinnings of circulating IL-18 levels and identified a novel locus around in the NLRC4 (NLR family, CARD domain containing 4) gene. NLRC4, a member of the NOD-like receptor family, is a critical component of the inflammasome involved in caspase-1 and IL-18 activation. The aim of this study was to further explore the genetic and molecular basis of the relation of the NLRC4 locus to IL-18 levels and subsequently to cardiovascular diseases. As NLRC4 is involved in the activation of caspase-1, we investigated the functional consequences of genetic variations identified by GWAS analysis in relation to caspase-1 activity in PBMCs and IL-18 serum levels in healthy individuals (n=398). Carriers of the allele associated with lower IL-18 levels showed reduced capase-1 activity. Further, analyses of linkage disequilibrium (LD) structures identified two different LD blocks associated with IL-18 levels: one LD block encompassing single nucleotide polymorphisms (SNPs) spanning genes NLRC4, SLC30A6 and SPAST, and one LD-block encompassing SNPs spanning genes YIPF4 and BIRC6. Additional expression quantitative trait loci (eQTL) analysis showed association of SNPs located in the 5'and 3' UTR of NLRC4 (rs479333 p=2.41x10-5 and rs430759 p=1.08x10-5), as well as downstream of SPAST (rs7601267 p= 4.92x10-5) to expression of NLRC4 transcript levels, indicating different regulatory regions involved in the regulation of NLRC4 expression. Investigating the genetic and molecular basis of the NLRC4 locus revealed different regulatory regions involved in NLRC4 expression and function of the protein and provides further insights into the genetic basis of circulating IL-18 levels and its relation to cardiovascular disease.

348F

Mutations in *ACTA2* in a British cohort of TAAD patients. *G. Arno[1], J.A. Aragon-Martin[1], S. Harris[1], A. Saggar[2], M. Jahangiri[3], A.H. Child[1].* 1) Cardiac and Vascular Sciences, St George's University of London, London, United Kingdom; 2) Clinical Genetics Unit, St George's University of London, London, United Kingdom; 3) Department of Cardiothoracic Surgery, St George's Healthcare NHS Trust, London, United Kingdom.

Background: Thoracic aortic aneurysm and dissection (TAAD) is a feature of several genetic conditions, such as Marfan (MFS) and Loeys-Dietz (LDS) syndromes. However, there is a growing body of work suggesting that non-syndromic TAAD is also of genetic origin. Furthermore, up to 21 % of non-syndromic cases of TAAD have been found to be familial. Recently studies have implicated mutations in *ACTA2* (MIM#102620) as a cause of 14-21% of familial and 2.5-3.8% of sporadic TAAD. It was the aim of this study to determine the frequency of *ACTA2* mutation in a consecutive series of TAAD patients attending a British MFS cardiac genetics clinic. Diagnosis was based on pedigree, physical examination, echocardiogram, CT and MRI studies and operative reports. **Method:** A total of 78 UK patients (62M:16F, mean age 48.16, ±13.43) with known TAAD who did not fulfill the revised Ghent criteria for MFS, and with no demonstrable mutations in *FBN1* or *TGFBR2* were recruited to this study. These patients were screened for mutations in all exons of *ACTA2* including intron/exon boundaries. **Results:** Novel non-synonymous missense mutations were identified in 3/78 (3.85%) probands. These mutations comprised: in exon 3, p.Arg64Lys (c.191G>A); in exon 6 p.Arg179Cys (c.535C>T) affecting the same amino acid as a previously reported TAAD mutation (p.Arg179His); in exon 7 p.Lue244Phe (c.732G>T). None of the mutations were found in 100 control chromosomes or are reported in any SNP databases. **Conclusion:** This study supports data from previous studies that link mutations in *ACTA2* with TAAD. In our cohort of UK patients, the detection rate is similar to that found in previous studies of consecutive sporadic cases of TAAD and lower than that found for familial cases. This reflects the mixed familial and sporadic nature of the UK patient population studied.

349F

Identification of rare variants in familial hypoalphalipoprotenemia using exome sequencing. *N. Gonzaludo[1,2], P.L.F. Tang[2], C. Pullinger[2], M. Malloy[2], J. Kane[2], P.Y. Kwok[2].* 1) Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, CA; 2) Cardiovascular Research Institute, University of California, San Francisco, San Francisco, CA.

Hypoalphalipoproteinemia (HA) is characterized by decreased serum high-density lipoprotein cholesterol (HDL-C) levels (< 10th percentile for age and sex) and low apolipoprotein A-1 (APOA1). HDL demonstrates antiatherogenic properties and mediates removal of cholesterol from the artery wall through reverse cholesterol transport. Low HDL-C is a risk factor for coronary artery disease and is a key element in the metabolic syndrome. Serum HDL-C levels have heritability estimates of 40-60%. While common variants in lipid metabolism-related genes are associated with HA, family-based studies suggest the disorder is polygenic, and the primary genetic causes of familial HA are still largely unknown. Rare variants and private mutations, in addition to common variants in candidate genes, may account for variations seen within a family. In order to assess the genetic basis of familial HA, we analyzed a family of four with HA using whole exome sequencing. Levels of HDL-C varied among family members, with the son displaying severely low levels (as low as 4 mg/dL). While less severely affected, the parents and a daughter also displayed low levels of HDL-C. Exome sequencing was performed using paired-end sequencing on Illumina HiSeq 2000. We used Illumina TruSeq Exome Enrichment for capture, targeting 201,121 exons from 20,794 genes. For each individual, approximately 50 million sequence reads were generated, with a median base coverage of ~50X and 85% of reads having at least 10x coverage. Of these reads, ~80% aligned to hg19 version of the human genome. We identified ~35,000 SNPs per individual, with 5% not reported in dbSNP version 130. Preliminary analysis shows a common non-synonymous variant in ABCA1 (V825I) in the 3 most affected individuals. Deficiency in ABCA1 is known to result in HA or Tangier disease. Additionally, previously reported SNPs in approximately 20 genes related to lipid metabolism were found in all individuals. All family members had reference alleles for known SNPs in APOA1. While these common variants may explain some portion of the phenotype, we suspect that rare variants in the son may be responsible for his severely low HDL-C levels. Current analysis is underway to identify such variants, as well as interacting genes that may be causal for the severe phenotype in the male child. Findings from our analysis may help to elucidate the genetic basis of this disorder.

350F

Mutation spectrum of aortic hypoplasia-vascular calcification syndrome by whole exome sequencing. *I.J. Kullo, K. Ding, K. Shameer.* Division of Cardiovascular Diseases, Mayo Clinic, Roichester, MN.

We conducted whole exome sequencing (WES) in two affected siblings with premature vascular disease and their unaffected parents. The affected siblings had aortic hypoplasia and diffuse arterial calcification, calcific aortic valve stenosis, and premature cataracts. The male sibling presented with hypertension at age 17 and the female sibling presented with cataract at age 30. Both were subsequently noted to have a hypoplastic aorta with extensive calcification, features that were not present in the parents. Using WES, we investigated the mutation spectrum of the two siblings and their parents. Exome capture was performed using the SureSelect Human All Exon 50 MB System (Agilent Technologies). Sequencing was performed using Illumina Hiseq 2000™ platform and 100 base paired-end reads were generated. A bioinformatics pipeline was created for variant discovery, including quality control, sequence alignment, base quality score recalibration, and variant calling and filtering. The analysis-ready variants were annotated using SeattleSeq Annotation server, including both known (dbSNPs) and private variants. Analysis of depth of coverage indicated that at least 80% of the exonic regions were present >8 times in the four patients. We assumed a recessive pattern of inheritance and identified 10 missense mutations in 10 genes and one splice-site mutation in one gene, segregating in the affected siblings but not in the unaffected parents. Further studies are underway to confirm the causal mutation that led to aortic hypoplasia-vascular calcification in the two siblings.

351F

A Genome-Wide Association Scan of Lipids and Lipoprotein subclasses in African Americans Including the Sea Island Gullah. *W.M. Chen[1], Y. Huang[1], F. Chen[1], K.L. Keene[1], J. Divers[2], J.C. Mychaleckyj[1], U. Nayak[1], X. Hou[1], D.L. Kamen[3], K.J. Hunt[3], I.J. Spruill[3], J.K. Fernandes[3], M. Cushman[4], W.T. Garvey[5], M.M. Sale[1].* 1) Center for Public Health Genomics, University of Virginia, Charlottesville, VA; 2) Dept. Biostatistical Sciences, Wake Forest University, Winston-Salem, NC; 3) Medical University of South Carolina, Charleston, SC; 4) University of Vermont; 5) Dept. Nutrition Science, University of Alabama at Birmingham, AL.

To identify genetic basis of variations in lipids and lipoprotein subclasses in populations with African ancestry, we conducted a GWAS scan of lipoprotein subclasses, measured using Liposcience's NMR-based Lipoprofile assay, in 3,888 participants, including 1,492 Gullah-speaking African Americans from Project SuGAR in families living on the coastal islands of South Carolina (the Gullah) and 2,396 African Americans residing in the Southeast from REGARDS study. The lipoprotein subclasses include 24 heritable quantitative traits. The variance component model adjusting for sex, age, diabetes status, lipid medication, and ancestry principal components was used to take into account family structure in the association analysis at each of 851,705 autosome and X-chromosome SNPs. All traits were transformed to be normally distributed according to their normal quantiles prior to association analysis. There was no inflation in our GWAS scan results (GC lambda ≤ 1.01 in all scans). The strongest association was at the *CETP* gene on chromosome 16 for trait HDL ($P=1.1\times10^{-21}$) and the variant explained 2.7% of total phenotypic variance. This gene was also associated with large HDL particles (HLP, $P=2.6\times10^{-13}$), NMR calculated HDL cholesterol (NHC, $P=3.2\times10^{-12}$), LDL mean particle size (LZ, $P=2.5\times10^{-8}$) and HDL mean particle size (HZ, $P=7.2\times10^{-8}$). The second strongest association was at the *APOE* gene on chromosome 19 for trait triglyceride ($P=1.4\times10^{-13}$), and this gene was also associated with LDL particles (LDLP, $P=2.4\times10^{-10}$), medium HDL particles (HMP, $P=3.9\times10^{-10}$), LDL ($P=1.0\times10^{-9}$), and total cholesterol ($P=6.2\times10^{-8}$). We confirmed association at the *PLTP* gene on chromosome 20 for trait small HDL particles (HSP, $P=9.1\times10^{-8}$). In addition to the successful replication of *CETP, APOE* and *PLTP* genes for lipid traits in African Americans, we also identified a novel association at *SNTG1* gene (chromosome 8) for small VLDL particles (VSP, $P=3.9\times10^{-9}$) and marginally significant association at *ARNT2* gene (chromosome 15) for Lipoprotein insulin resistance (LPIR, $P=7.4\times10^{-8}$). Additional genetic variants with associations of lower statistical significance will be further investigated by meta-analysis with other GWAS studies. These data advance our understanding of the genetic influences on lipoprotein subclass phenotypes in people of African ancestry, and provide the basis for studies assessing the role of these genes in mediating cardiometabolic disease risk in African Americans.

352F

Genome-wide association analyses in up to 181,171 individuals reveal 14 new loci associated with heart rate. *M. den Hoed[1], T. Esko[2], M. Eijgelsheim[3], D.M. Evans[4], I.M. Nolte[5], A.V. Segre[6], Y.S. Cho[7], H.J. Westra[8], R.E. Handsaker[9], D.P. Strachan[10], T. Johnson[11], K. Stefansson[12], M.C. Cornelis[13], P.F. O'reilly[14], C. Ladenvall[15], N.R. Poulter[16], D. Cusi[17], P. van der Harst[18], W. Zhang[19], D. Albanes[20], Z. Kutalik[21], O.T. Njajou[22], T. Tanaka[23], A.U. Jackson[24], P. Salo[25], R.J.F. Loos[1], HRgen consortium, RRgen consortium, Global BPgen consortium and CARDIoGRAM consortium.* 1) Epidemiology Unit, Medical Research Council, Cambridge, Cambridgeshire, United Kingdom; 2) Estonian Genome Center and Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia and Estonian Biocenter, Tartu, Estonia; 3) Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands; 4) MRC CAiTE centre, School of Social and Community Medicine, University of Bristol, Bristol, UK; 5) Unit of Genetic Epidemiology & Bioinformatics, Department of Epidemiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; 6) Center for Human Genetic Research and Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts, USA and Program in Medical and Population Genetics, Broad Institute of Harvard and Massachusetts Institute of Technology, Cambrid; 7) Center for Genome Science, National Institute of Health, Seoul, Korea; 8) Department of Genetics, University Medical Center Groningen and University of Groningen, Groningen, The Netherlands; 9) Department of Genetics, Harvard Medical School, Boston, Massachusetts, USA and Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA; 10) Division of Population Health Sciences and Education, St George's, University of London, UK; 11) Clinical Pharmacology and the Genome Centre, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry and NIHR Cardiovascular Biomedical Research Unit, Queen Mary University of London, London, UK; 12) deCODE genetics and Faculty of Medicine, University of Iceland, Reykjavik, Iceland; 13) Nutrition, Harvard School of Public Health, Boston, MA, USA; 14) Department of Epidemiology and Biostatistics, Imperial College London, London, UK; 15) Department of Clinical Sciences, Diabetes and Endocrinology, Lund University and Lund University Diabetes Centre, Malmö, Sweden; 16) ICCH, Imperial College London, UK; 17) Department of Medicine, Surgery & Dentistry, University of Milano and Filarete Foundation, Milano, Italy; 18) Department of Cardiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; 19) Epidemiology and Biostatistics, Imperial College London, London, UK; 20) Division of Cancer Epidemiology and Genetics, US National Cancer Institute, Bethesda, Maryland, USA; 21) Department of Medical Genetics, University of Lausanne, Lausanne, Switzerland; 22) Department of Medicine, Institute for Human Genetics, University of California, San Francisco, California, USA; 23) Clinical Research Branch, National Institute on Aging, Baltimore, Maryland, USA; 24) Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Michigan, USA; 25) Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland and The Institute of Molecular Medicine, University of Helsinki, Helsinki, Finland.

A higher resting heart rate has been associated with an increased risk of cardiovascular diseases and (cardiovascular) mortality. To date, genome-wide association studies have identified seven loci that showed associations with heart rate. We aimed to identify novel genetic loci by examining associations between ~2.7 million SNPs and resting heart rate in up to 92,364 individuals of white European and Indian Asian descent. Variants from 42 loci ($p<1x10^{-5}$) were followed up in up to 88,823 additional individuals of white European descent. Combined analyses in up to 181,171 individuals confirmed associations with heart rate for variants in 21 loci ($p<5x10^{-8}$); we identified 14 new loci and confirmed the seven previously established loci. A genetic predisposition score, comprising the number of heart rate increasing alleles in the most significant signals for the 21 loci, was significantly associated with heart rate in 4,000 12-year old children (beta=0.35±0.06 beats/min/effect allele, $p=2.8x10^{-9}$) and in 5,053 adults (beta=0.47±0.07 beats/min/effect allele, $p=1.9x10^{-11}$). Some variants tagged copy number variants (in *C6orf170*, near *GNG11*/*GNGT1*), missense variants (in *MYH6*, *UFSP1*, *KIAA1755*, *CCDC141*, *B3GNT7*, *TFPI*) and variants in transcription factor binding sites (*C6orf204*, *GJA1*) ($r^2>0.8$). Five variants showed association with expression levels of 11 nearby transcripts in blood (*C11orf10*, *FADS1*, *BEST1*, *FTH1*, *FADS2*, *RAB3IL1*, *C6orf204*, *TRIP6*, *NCL*, *SNORD20*, *GNG11*, $p<1.6x10^{-3}$). Many of the 21 loci contain known regulators of angiogenesis (*EPHB4*, *TIMP1*, *PLXNA2*, *TFPI2*, *CALCRL*), calcium metabolism (*FADS1*/*FADS2*, *PLN*), cardiac contractility (*MYH6*/*MYH7*, *TTN*), pace making (*GJA1*, *HCN4*, *CHRM2*), and cardiac development (*FLRT2*, *NKX2-5*). Mutations in many of these positional candidate genes have previously been associated with cardiovascular diseases. Pathway analyses (MAGENTA) confirmed enrichment for genes involved in cardiomyopathies. None of the 21 most significant SNPs showed significant associations with risk of coronary artery disease and myocardial infarction, while four variants associated with increased heart rate tended to be associated with reduced systolic blood pressure (in/near *GJA1*, *PLXNA2*, *FLRT2*, *TFPI*, $p<0.05$). In conclusion, many of the confirmed loci harbour genes that point towards plausible and promising pathways that may provide new insights in the aetiology of heart rate regulation and potentially also cardiovascular risk factors and diseases.

353F

Functional Analysis of the TRIB1 Locus in Coronary Artery Disease (CAD). *A. Douvris, P. Lau, O. Jarinova, T. Naing, R. McPherson.* University of Ottawa Heart Institute, Ottawa, Ontario, Canada.

Genome-wide association studies (GWAS) have identified several common genetic variants associated with CAD and CAD risk factors. The TRIB1 locus (8q24.13) is a novel locus associated with plasma TGs, LDL-c, and CAD risk. As part of the Ottawa Heart Study, we have demonstrated that the relationship of this locus to CAD risk is entirely mediated by effects on plasma lipids. Trib1 is a regulator of MAPK activity, and recently, it has been has been shown that Trib1 also regulates hepatic lipogenesis and VLDL production in mouse models. However, the functional relationship between common single nucleotide polymorphisms (SNPs) at the TRIB1 locus and plasma lipid traits is unknown. Specifically, the TRIB1 locus as identified by GWAS comprises a cluster of SNPs significant for TGs, LDL-c, and CAD within an intergenic region 25kb to 50kb downstream of the TRIB1 coding region. By phylogenetic footprinting analysis, we identified an evolutionarily conserved region (CNS1) within the risk locus. DNA sequencing revealed that this region harbors two common SNPs in tight linkage dysequilibrium with GWAS risk SNPs and that also associate significantly with CAD. We investigated the regulatory potential of CNS1 using various luciferase reporter assays in HepG2 cells and demonstrate that this region has promoter activity and that the risk allele of one SNP in this region significantly reduces luciferase activity. Furthermore, a database search for ESTs within the risk locus revealed an EST directly downstream of CNS1, which we believe to be its promoter. We performed 3'/5' RACE using HepG2 RNA and identified multiple variants of this EST. We hypothesize that this EST is a long non-coding RNA since it lacks any significant ORF. Given that it is an intergenic RNA, and has proximity to TRIB1, we hypothesize that it may function to regulate the expression of the neighboring genes. Further, our promoter studies indicate that one of the risk alleles may contribute to decreased levels of this non-coding RNA, thereby constituting a link between the GWAS risk locus, plasma lipids, and CAD.

354F

A genome-wide association study of essential hypertension in an Australian population using a DNA pooling approach. *J.Y. Fowdar[1], Y. Lu[2], S. Macgregor[2], L.R. Griffiths[1].* 1) Genomics Research Centre, Griffith Health Institute, Griffith University, Gold Coast, Queensland, Australia; 2) Qld Statistical Genetics Laboratory, Queensland Institute of Medical Research, Brisbane, Queensland, Australia.

Essential hypertension (EH) is the leading cause of cardiovascular disease and is implicated in more than 7 million deaths each year from ischaemic heart disease and stroke. The prevalence of hypertension worldwide has reached epidemic proportions with more than 1 billion people suffering from the condition. Elucidating the underlying mechanisms of EH is therefore highly important for improved treatment and prevention strategies. We conducted a genome-wide assocation study (GWAS) using Illumina 1M-Duo SNP arrays on an Australian Caucasian population using DNA pools. DNA-pooling has been shown to significantly reduce the cost of array-based GWAS. 409 cases and 409 age-, sex-, and ethnicity-matched controls were combined into separate DNA pools consisting of equimolar amounts of each DNA sample. Four replicates of each pool were hybridized to the SNP arrays (a case and a control pool on each duo array) according to the manufacturer's protocol, and scanned using an Illumina Beadarray station. Raw fluorescence intensity data was processed for quality control and pooled allele frequencies were calculated. The following seven markers were found to have the highest p-values: rs34870220 (4.32E-07), rs4836667 (5.54E-07), rs1928277 (6.76E-07), rs1599961 (6.86E-07), rs11170043 (7.09E-07), rs12711538 (7.72E-07) and rs11177752 (8.08E-07), although none of them reached genome-wide statistical significance. These markers were located in or close to the following genes respectively: ASGR1, PRRX2, NHSL1, NFKB1, KRT7, GLI2 and LRRC10. The markers for NFKB1 and GLI2 were then genotyped on individual samples using Taqman assays (ABI 7900HT). Both markers were in Hardy-Weinberg equilibrium. Both allele and genotype frequencies for NFKB1 (p=0.01 and p=0.04 respectively) and GLI2 (p=0.001 and p=0.004 respectively) were found to be significantly different between the hypertensive and normotensive populations. The remaining five markers are in the process of being genotyped on individual samples. A gene-based association test, which is more appropriate for pathway analysis, is currently being conducted to investigate potential candidate gene pathways and the implicated SNPs for NFKB1 and GLI2 will be investigated in an independent hypertensive sib-pair population.

355F

Evidence for several genetic variants affecting lipoprotein (a) level on chromosome 6q25-26 in the Old Order Amish. *M. Fu, W. Lu, Q. Gibson, X. Shi, J. O'Connell, B. Mitchell, A. Shuldiner.* Dept Med, Div Endocrinology, Univ Maryland, Baltimore, MD.

Lipoprotein (a) [Lp(a)] is an independent risk factor for atherosclerosis-related events such as coronary artery disease, stroke, and restenosis after angioplasty. We performed a genome-wide association scan (GWAS) for genetic variants that associated with serum Lp(a) levels in the Old Order Amish, a relatively homogenous population in terms of both genetic ancestry and lifestyle characteristics. Using the Affymetrix 500K chip, we successfully genotyped 382,935 single-nucleotide polymorphisms (SNPs) in 861 subjects from the Heredity and Phenotype Intervention (HAPI) Heart Study, which were used to impute all autosomal SNPs in HapMap (~2.5 million SNPs) by MACH (version 1.0.15), using the publicly available phased haplotypes from HapMap (release 22, build 36, CEU population) as a reference panel. We identified 81 common variants showing significant association with age-, age2- and sex-adjusted Lp(a) level (P =5 x 10-8 to 2.83 x 10-25). The 81 common variants span ~3.3 Mb on chromosome 6q25-26 and were within or flanking 26 genes including LPA. Common variants in four genes (SNX9, FNDC1, IGF2R, and AGPAD4) were significantly associated with Lp(a) levels; however, no SNP in LPA itself was significantly associated with Lp(a) levels. Together, these variants explained 14.2% of the variation in Lp(a) levels. Linkage disequilibrium analysis revealed that these significantly associated SNPs distribute into different blocks suggesting that multiple genes on chromosome 6q25-26 may regulate Lp(a) levels. We further analyzed these variants with LDL, HDL, triglyceride, aortic calcification score, coronary calcification score, and diastolic carotid luminal diameter. After adjusting for multiple comparison, a number of the common variants associated with Lp(a) were also significantly associated with diastolic carotid luminal diameter (rs1982291; P < 5 x 10-5). Association of the SNPs with Lp(a) in this region have been reported in other populations. In conclusion, we have confirmed that common variants on chromosome 6q25-26 are significantly associated with Lp(a) levels and diastolic carotid luminal diameter. Studies characterizing further variation in the genes in this region and their functional consequences on Lp(a) levels are warranted.

356F

A GWAS from the Vitamin Intervention for Stroke Prevention (VISP) clinical trial identifies variants that influence measures of one carbon metabolism. *K. Keene[1], F. Chen[1], W.M. Chen[1], F.C. Hsu[2], J. Mychaleckyj[1], K. Doheny[3], E. Pugh[3], H. Ling[3], C. Laurie[4], S. Gogarten[4], E. Bookman[5], B. Worrall[1], M. Sale[1], GARNET.* 1) University of Virginia, Charlottesville, VA; 2) Wake Forest University School of Medicine, Winston Salem, NC; 3) Johns Hopkins University School of Medicine, Baltimore, MD; 4) University of Washington, Seattle, WA; 5) National Human Genome Research Institute, Bethesda, MD.

Every 40 seconds, someone in the United States will have a stroke. Unfortunately, nearly 40% of stroke survivors will suffer a recurrent stroke within 5 years of their first stroke. The concept that genetics influence stroke risk is well supported by epidemiological studies. We have performed a Genome-wide association study (GWAS), using the Illumina Omni 1M SNP platform, in 2,100 stroke patients from the Vitamin Intervention for Stroke Prevention (VISP) trial. VISP was a controlled, double blinded clinical trial, designed to determine whether the daily intake of high dose folic acid, vitamins B6 and B12 reduce recurrent cerebral infarction. Extensive quality control (QC) measures were performed, resulting in a total of 737,081 SNPs for analysis. We have performed genome wide association analyses for baseline quantitative measures of homocysteine (baseline and post methionine load measures), folate, Vitamins B12 and B6, using linear regression approaches implemented in PLINK. Multidimensional Scaling (MDS), implemented in KING, was utilized to address confounders due to population substructure. Inverse normal transformation was performed for each of the quantitative traits, prior to analysis. Genome wide association analyses were performed using age, sex and the top 10 principal components as covariates. There is no inflation in our GWAS scan results (GC lambda ≤ 1.01 in all scans). We have observed several associations that exceed genome wide significance (≤10 -8). The strongest association was observed with a non-synonymous SNP (nsSNP), located in the CUBN gene, for baseline Vitamin B12 (P=1.76×10-13; beta=-0.22). Two additional CUBN intronic SNPs were also strongly associated with B12 (P= 2.92×10-10; beta=-0.19 and 4.11×10-10; beta=-0.18). A second nsSNP, located in the TCN1 gene, was also associated with baseline measures of Vitamin B12 (P= 5.148×10-11; beta=-0.29). Additionally, we identified several hits on chromosome 6, in the CNPY3-GNMT-PEX6 gene region, that were associated with post methionine load homocysteine measures (P values ranging from 2.14×10-13 to 9.95×10-9). Our GWAS scans have successfully replicated associations for SNPs in the ALPL gene for measures of Vitamin B6. Our GWAS study has identified novel associations while also replicating previous GWAS hits for genes that contribute to measures of post methionine load homocysteine, Vitamins B12 and B6.

357F

Genome-wide Association Study, Meta-Analysis and Linkage Study of von Willebrand Factor and Gamma-Prime Fibrinogen Plasma Levels in a Healthy Young Cohort. *A. Ozel[1], K. Desch[2], D. Siemieniak[3], D. Ginsburg[1,3], J. Li[1].* 1) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 2) Department of Pediatrics, University of Michigan, Ann Arbor, MI; 3) HHMI, University of Michigan, Ann Arbor, MI.

von Willebrand factor (VWF) is an abundant plasma glycoprotein that regulates hemostasis. Gamma-prime fibrinogen (GPF) is an isoform of fibrinogen and a component of the blood-clotting complex. Its role in cardiovascular risk prediction has been recently proposed. Plasma levels of VWF and fibrinogen are highly variable among healthy people, with ~70% and ~50% of their variance, respectively, attributable to inherited factors. We performed genome-wide association studies on a healthy sibling cohort of 1152 subjects (ages 14-35) in 502 sibships, focusing on the European subset (940 subjects), using >800,000 genotyped SNPs (Illumina Omni1-Quad) and ~7.5 million imputed SNPs (based on the 1000 Genomes Project data). Plasma VWF and GPF levels were determined by Alphalisa (Perkin Elmer) assay; and heritability (h^2) was 68% and 54%, respectively. VWF plasma levels show significant association with variants at the ABO locus ($p\sim10^{-36}$), consistent with previous reports. Suggestive evidence of association and linkage is found near the VWF gene and a number of other loci. Preliminary linkage analysis identified the ABO locus on chromosome 9 and novel loci on chromosomes 2, 7 and 21. The latter 3 loci were not detected in the current or previously reported association studies. Similar analyses of GPF levels reveal significant association and linkage signals in the fibrinogen gene cluster on chromosome 4 ($p\sim10^{-126}$, LOD>9) and a novel locus on chromosome 16 ($p\sim10^{-5}$, LOD>4). A meta-analysis of VWF involving the Atherosclerosis Risk in Communities Study (ARIC) data confirms several previously identified loci, with strong agreement in the direction of the effects. Replication in another healthy young cohort of Irish origin is underway. These results provide new insight into the regulation of hemostasis, may identify novel genetic modifiers of bleeding and thrombosis risk, and suggest that linkage analysis of blood clotting traits may reveal novel loci undetectable by association studies. The study is supported by NIH grant HL039693 and is part of the GENEVA (Gene Environment Association Studies) Consortium.

358F

Genome-wide association analysis identifies TYW3/CRYZ and NDST4 loci associated with circulating resistin levels. *Q. Qi[1], C. Menzaghi[2], S. Smith[3], L. Liang[4], J. Ding[3], T. Harris[3], V. Trischitta[2, 5], F. Hu[1, 4, 6], Y. Liu[3], L. Qi[1, 6].* 1) Department of Nutrition, Harvard School of Public, Boston, MA; 2) Research Unit of Diabetes and Endocrine Diseases, IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo, Italy; 3) Division of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC; 4) Department of Epidemiology, Harvard School of Public Health, Boston, MA; 5) Department of Experimental Medicine, Sapienza University of Rome, Italy; 6) Channing Laboratory, Department of Medicine, Harvard Medical School, Boston, MA.

Resistin has been associated with insulin resistance, inflammation, and risk of type 2 diabetes and cardiovascular disease. However, little is known about the genetic determinants for circulating resistin levels. We conducted the first genome-wide association (GWA) study on the plasma resistin levels in individuals of European ancestry drawn from the two independent studies: the Nurses' Health Study (NHS, N=1587) and the Health, Aging and Body Composition Study (HABC, N=1661). The SNPs identified in the GWA analysis were replicated in an independent cohort of Europeans (N=626). Gene expression quantitative trait loci (eQTL) analyses were performed to examine the positional candidate genes of the identified loci. We also measured resistin mRNA levels in 47 type 2 diabetic subjects. We identified two novel loci near NDST4 gene (4q25) and TYW3/CRYZ gene (1p31), associated with resistin levels at a genome-wide significant level, best-represented by SNP rs13144478 (P=6.19×10-18) and SNP rs3931020 (P=6.37×10-12), respectively. In eQTL analyses, we observed a significant cis association between the SNP rs3931020 and CRYZ gene expression levels (P=3.68×10-7) (SNP rs13144478 or related SNPs near NDST4 gene was not available in the gene expression database). A SNP rs277369 in strong LD with rs3931020 (r2=0.711) showed the most significant association with CRYZ gene expression levels (P= 6.81×10-24). We also found another SNP rs1969111 (in strong LD with rs3931020, r2=0.783) significantly associated with TYW3 gene expression levels (P=1.86×10-8). Moreover, the SNPs rs3931020 was significantly associated with resistin mRNA levels (P=0.02). Our results suggest that genetic variants in TYW3/CRYZ and NDST4 loci may be involved in regulation of circulating resistin levels.

359F

Genome-wide association studies identify 15 loci with novel associations with plasma fibrinogen levels. *M. Sabater-Lleal[1], J. Huang[2], The FibrinoGEN Consortium.* 1) Atherosclerosis research Unit, Karolinska Institutet, 17176-Stockhom, Sweden; 2) National Heart, Lung and Blood Institute's Framingham Heart Study, Framingham, MA 01702.

Introduction: Fibrinogen is a major coagulation protein under strong genetic regulation with a central role in wound healing and thrombosis, and it also represents an important marker for acute inflammation. In addition, high fibrinogen concentration is an independent risk factor for cardiovascular disease, including coronary artery disease (CAD), stroke, and venous thromboembolism (VTE). Two recent meta-analyses of genome-wide association (GWA) studies confirmed associations with *FGB* and revealed new candidate loci harboring regulatory variants affecting plasma fibrinogen concentration. However, the known variants explain only a small part of the variability of this phenotype and more associated variants are likely to exist. **Methods and Results**: Here we present the largest meta-analysis for fibrinogen GWA studies, including a discovery analysis in 91,435 individuals of European ancestry from 28 cohorts plus further analysis in an additional 8,307 African Americans from 7 cohorts, in total analyzing around 2.7 milion SNPs. Overall, we have identified 23 genome-wide significant loci for fibrinogen levels, among which 8 represent replications of previously described associations and 15 are newly identified fibrinogen-associated loci. Secondary analysis conditional on the 23 most strongly associated SNPs revealed the presence of 2 independent signals on *IRF1* locus. Candidate genes at the newly identified fibrinogen-associated loci include inflammation genes (*LEPR*, *IL1R1*) and transcription activators (*ATF1, JMJD1C*). Validation of these findings in African American samples is ongoing. In addition, we are currently examining the newly identified loci in association with VTE and CAD. **Discussion**: The present study represents by far the largest effort aiming at the identification of novel loci that underlie variation in plasma fibrinogen concentration. Our results could help to understand the regulation of plasma fibrinogen levels as a quantitative trait conferring susceptibility to cardiovascular disease.

360F

Genome-wide Association Study of Vascular Inflammatory Markers Suggests Novel Candidate Genes Associated with Atherosclerosis and Coronary Artery Disease. *B. Suktitipat[1,3], D. Vaidya[2], L.R. Yanek[2], T.F. Moy[2], R.A. Mathias[2], J.H. Young[1,2], L.C. Becker[2], D.M. Becker[2], A.F. Wilson[3], M.D. Fallin[1].* 1) Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 2) Department of Medicine, the Johns Hopkins Medical Institutions, Baltimore, MD; 3) Genometric Section, Inherited Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Baltimore, MD.

Background: Inflammatory biomarkers in atherosclerosis, such as CRP, IL-6 and MCP-1, have been associated with the risk of coronary artery disease (CAD). In this study, we performed genome-wide association analyses to study all three inflammatory markers in both African Americans and European Americans with the goal to identify novel genetic susceptibility to inflammation and atherosclerosis. **Methods:** Healthy members of European-American and African-American families from the GeneSTAR study, which originally selected families of early-onset CAD patients, were included in this study (556 families, 2065 individuals, mean age 44 ± 12; 42% men). Genotypes from the Illumina human 1Mv1_c SNPchip were screened for association with serum inflammatory marker levels with generalized linear models adjusted for age and sex; Generalized Estimating Equations (GEE) were used to accommodate the family correlations. These statistical analyses were confirmed in family-based variance-components analysis. **Results:** The strongest associations were seen between MCP-1 levels and *FARS2* ($p = 1.6\times10^{-7}$) and *MACROD2* ($p = 1.6\times10^{-7}$). The strongest association for CRP was for a SNP near *BDH1* ($p = 8.3\times10^{-7}$); and the strongest associations with IL-6 levels were at SNPs near *ZNF385D* ($p = 1.5\times10^{-6}$). Additional genetic variants with highly suggestive results ($p \le 1\times10^{-5}$) were found in *BACH2*, *SAP130*, *DENND1B*, *KIF26B*, *SVOPL*, *CDH23*, *C14orf145* for CRP levels; *TAP2*, *CNTN4*, *KIAA0391* for IL-6 levels; and *EPHB2*, *RAD51L1*, *COTL1* for MCP-1 levels. No overlapping signals across the three inflammatory markers reached genome-wide statistical significance despite their biological relationship. However, highly suggestive genetic association signals implied more similar biological control between IL-6 and CRP, than for MCP-1. In addition, previously published GWAS signals were replicated between MCP-1 and *DARC*, and between CRP and variants in *CRP*, *HNF1A*, and *GCKR* ($p \le 0.05$). **Conclusion:** Several suggestive SNP associations implicated plausible novel candidate genes for inflammatory markers and replicated previously reported findings. Further investigation of these signals is warranted and may help identify novel pathways associated with atherosclerosis and CAD.

361F

Cytokine (IFN-. , IL-6, TNF-(, TGF-) 1 and IL-10) genotyping in Turkish Children with acute rheumatic fever. *N. Col Araz[1], S. Pehlivan[2], O. Baspinar[3], S. Oguzkan Balci[2], T. Sever[2], A. Balat[4].* 1) University of Gaziantep, Faculty of Medicine, Department of Pediatrics, Gaziantep, Turkey; 2) University of Gaziantep, Faculty of Medicine, Department of Medical Biology and Genetics, Gaziantep, Turkey; 3) University of Gaziantep, Faculty of Medicine, Department of Pediatric Cardiology Gaziantep, Turkey; 4) University of Gaziantep, Faculty of Medicine, Department of Nephrology, Gaziantep, Turkey.

Objectives of Study: Acute rheumatic fever (ARF) is a delayed immunologically mediated sequel of throat infection by group A,) -hemolytic streptococci. It is a multisystem inflammatory disease which can effect of different tissues including synovial joints and cardiac valves. Recently, it has been shown that inflammatory cytokines may play a pathogenic role in ARF. The objective of this study was to investigate the potential associations between IFN-. , IL-6, TNF-(, TGF-) 1 and IL-10 gene polymorphisms and childhood ARF. Method: Thirty-eight ARF patients and 40 age-and sex-matched healthy controls were analyzed for 8 polymorphisms in 5 different genes. DNA was extracted from whole blood by standard salting out method. Cytokine genotyping was performed by polymerase chain reaction sequence-specific primer methods. The polymorphisms analyzed in the present study were IFN-. (+874), IL-6 (-174), TNF-((-308), TGF-) 1 (+10, +25) and IL-10 (-1082 , -819 , -592). The results were statistically analyzed by calculating odds ratios (OR) and 95% confidence intervals (CI) using chi-square test. Results: The distribution of genotypes and allele frequencies were compared with the groups. ARF patients had significantly higher frequencies of IFN-. (+874) polymorphism in both TT genotype (p=0.0002, OR:8.10 [95 % CI:2.40-27.26]) and T allele (p=0.0004, OR:3.01, CI: [1.57-5.78]). No statistically significant differences were observed between groups in genotype and allele frequencies of IL-6, TNF-(, TGF-) 1 and IL-10 genes (P>0.05). There were also no statistically significant differences in the haplotypes of ARF patients and controls (P>0.05). Conclusion: We conclude that IL-6, TNF-(, TGF-) 1 and IL-10 polymorphisms are not associated with ARF. However, both TT genotype and high expression IFN-. gene (+874) may have a higher risk for ARF in Turkish children.

362F

Genetic risk scores related to leukocyte telomere length predict incident cardiovascular disease, the Framingham Heart Study. *SJ. Hwang[1,2], JM. Murabito[1,5], SL. Neuhausen[6], SC. Hunt[7], EN. Smith[9], M. Mangino[4], T. Spector[4], AL. Fitzpatrick[3], G. Berenson[9], NJ. Schock[10], JC. Bis[3], BM. Psaty[3], A. Aviv[8], D. Levy[1,2].* 1) Framingham Heart Study, Framingham, MA; 2) Center for Population Studies, National Heart, Lung, and Blood Institute, Bethesda, MD; 3) Department of Epidemiology, University of Washington, Seattle, WA; 4) Department of Twin Research and Genetic Epidemiology at King's College London. London UK; 5) Divisions of Cardiology and Epidemiology, Boston University School of Medicine, Boston, MA; 6) Department Population Sciences, The Beckman Research Institute of the City of Hope, Duarte, CA; 7) Cardiovascular Genetics Division, Department of Internal Medicine, University of Utah School of Medicine, Salt Lake City, UT; 8) Center of Human Development and Aging, New Jersey Medical School, Newark, NJ; 9) Center for Cardiovascular Health, Tulan University, New Orleans, LA; 10) Center for Biomedical Informatics, University of California, San Diego CA.

Shorter leukocyte telomere length (LTL) has been reported to be associated with higher risk of cardiovascular disease (CVD). Genome-wide association studies (GWAS) have identified common genetic variants associated with LTL. We used the results of GWAS meta-analysis to identify 30 independent SNPs associated with LTL at $p < 1 * 10^{-5}$ to create an LTL genetic risk score and tested the associated of the LTL risk score with new-onset of major CVD events (recognized myocardial infarction, coronary insufficiency, or coronary disease death) in participants from the Framingham Heart Study. The study sample included 4078 participants free of major CVD at time of DNA collection. Generalized estimation equation (GEE) was applied to test the significance of the association between risk score and new-onset CVD, adjusting for CVD risk factors and familial correlation. During 40,994 person-years of follow up, a total of 279 (7%) individuals developed a first major CVD event. Each 1 standard deviation increase in the risk score (mean 30, SD 3) carried a 1.14-fold higher risk of CVD (95% confidence interval, 1.008-1.289; p-value =0.036). These results highlight the potential causal relation of LTL dynamics in the pathogenesis of CVD. The prognostic utility of the LTL genetic risk score warrants confirmation.

363F

A deep-sequencing approach to identify new genes involved in Hereditary Hemorrhagic Telangiectasia (HHT). *M. EYRIES[1,2], J. ROUME[3], F. COULET[1,2], C. LONJOU[4], M. GERMAIN[5], P. LACOMBE[3], T. CHINET[3], J.H. BLONDEL[3], I. BOURGAULT[3], G. LESUR[3], A. OZANNE[3], B. RAFFESTIN[3], D.A. TREGOUET[5], F. SOUBRIER[1,2].* 1) Oncogenetics and Angiogenetics laboratory, Pitié-Salpétrière Hospital, AP-HP, Paris, France; 2) UMRS 956, UPMC-Paris 6, INSERM, Paris, France; 3) HHT pluridisciplinary clinics, Hôpital Ambroise par&, AP-HP, Boulogne France; 4) Bioinformatics and biostatistics platform, UPMC-Paris6, Paris, France; 5) UMRS 937, UPMC-Paris6, INSERM, Paris, France.

Mutations of the Endoglin (ENG) and Activin A receptor type II-like kinase-1 (ACVRL1) genes are known to be the major genetic factors of HHT. Mutations in SMAD4, causing HHT in association with juvenile polyposis, have also been described. However, mutations are not found in about 20% of HHT cases. We investigated a pedigree where the proband was reported to have epistaxis, telangiectasias, pulmonary arteriovenous malformation and a familial history of HHT with 8 affected or potentially affected relatives over 4 generations. Direct sequencing and large rearrangements analysis of ACVRL1, ENG and SMAD4 did not identify any mutation in these genes. A multipoint linkage analysis was performed in this pedigree and confirmed that these 3 gene regions are unlikely to explain the familial segregation of the disease. Following the proof-of-concept that new causal genes for mendelian disorders can be identified by exome sequencing of a small number of affected individuals, we designed a two-generational filtering strategy specially adapted to a genetic disease transmitted as an autosomal dominant trait with complete penetrance. This strategy includes an intra-familial filtering step comparing sequenced data from unaffected and affected parents to their affected offspring. We performed peripheral blood DNA sequencing of all protein-coding regions (exome) in 4 selected individuals of the family using Agilent SureSelect exome hybridization capture and the Illumina DNA sequencing platform. Around 35,000 variants (SNP and INDEL) were identified in each individual. 70 candidate variants were left after successive filtering steps including 2 nonsense variants and one missense variant predicted to be deleterious and located in a gene involved in angiogenesis. These variants are very good candidates to be the causal variant responsible for HHT disease in this family and co-segregation study is now in progress to confirm the implication of one of these candidates.

364F

Multigenic etiology of CHD with heterotaxy involving PCD and cilia genes. *Y. Li[1], H. Yagi[1], E. Onuoha[1], M. Barmada[2], M. Tsang[1], CW. Lo[1].* 1) Department of Developmental Biology, University of Pittsburgh, School of Medicine, Pittsburgh, PA; 2) Department of Human Genetics, University of Pittsburgh, Graduate School of Public Health Pittsburgh, PA.

Introduction: Patients with congenital heart disease (CHD) and heterotaxy show high postsurgical morbidity/mortality, with some developing respiratory complications. We recently showed CHD patients associated with heterotaxy can exhibit respiratory ciliary dysfunction (CD) similar to that of patients with primary ciliary dyskinesia (PCD) - a ciliopathy with airway mucociliary clearance defects. As both PCD and heterotaxy have a 1 in 10,000 incidence, the finding of CD in heterotaxy patients likely reflects the dual role of motile cilia in airway clearance and left-right patterning. Thus, we hypothesize heterotaxy may arise from mutations in PCD genes and other cilia related ciliome genes. **Methods and Results:** CHD patients with heterotaxy, 13 with CD and 13 without CD (no-CD), were sequence captured followed by SOLiD sequencing with average 40X coverage to identify sequence variants in all 13 known PCD causing genes and nearly 1000 other ciliome genes. Coding variants were filtered against dbSNP and the 1000 Genomes databases, and novel coding variants (NCV) were validated by Sanger sequencing. Analysis of the 13 PCD genes revealed 10 NCV in 7 CD patients, and 5 NCV in 3 no-CD patients. The 3 PCD patients had 5 NCV, with 2 siblings having homozygous CCDC39 mutation (1072delA) known to cause PCD. Interestingly, CD-heterotaxy patient 9002 was heterozygous for the most common pathogenic DNAI1 mutation, IVS1+2_3insT, a disease NCV not seen in the general population. As PCD is a recessive disorder, heterozygous mutation cannot alone cause disease. We evaluated NCVs found in DNAH6, a motor dynein gene not yet demonstrated to cause PCD. DNAH6 NCVs were found in 9002 as well as one other CD and one PCD patient, but not in any no-CD patient. Dnah6 morpholino (MO) knockdown in zebrafish embryos showed laterality defects and phenotypes consistent with heterotaxy, suggesting a role in CD and heterotaxy. Further experiments are underway to explore functional synergy between DNAI1 and DNAH6 by observing effects of co-administration of low doses of both MO in zebrafish. **Conclusion:** Our study showed heterotaxy patients with CD can have heterozygous NCVs in PCD genes, including known disease causing DNAI1 mutation. Functional analysis with zebrafish MO showed DNAH6 knockdown can cause heterotaxy. We propose DNAH6/DNAI1 double heterozygous mutation may contribute to CD and heterotaxy. Our results provided evidence for multigeneic etiology of CD and heterotaxy.

365F

Variants in the inflammatory *IL6* and *MPO* genes modulate stroke susceptibility through main effects and gene-gene interactions. *A.M. Vicente[1, 2, 3], H. Manso[1, 2, 3], T. Krug[2,4], J. Sobral[2,4], G. Gaspar[1], S. Oliveira[2,4], J.M. Ferro[4,5].* 1) Instituto Nacional Saude DRJ, Lisbon, Portugal; 2) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 3) Center for Biodiversity, Functional & Integrative Genomics BIOFIG, Lisbon, Portugal; 4) Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal; 5) Serviço de Neurologia, Hospital de Santa Maria, Lisbon, Portugal.

A complex interplay between genetic background, clinical and life-style factors and the environment is expected to ultimately regulate the onset, acute phase and outcome of stroke. There is substantial evidence that inflammation within the Central Nervous System contributes to stroke risk, and known clinical risk factors for stroke, like atherosclerosis, diabetes, obesity, hypertension, and peripheral infection, are associated with an elevated systemic inflammatory profile. The inflammatory response is equally of major importance in recovery and healing processes after stroke. In this study we tested the genetic association of major inflammatory players *IL1B* (2q14), *IL6* (7p21), *TNF* (6p21.3) and *MPO* (17q23.1) with stroke susceptibility and with stroke outcome at three months, in a population sample of 672 patients and 530 controls, adjusting for demographic, clinical and life-style risk factors and/or stroke severity parameters. The apparent complexity of the inflammatory mechanisms in stroke, and the multiplicity of players involved suggest a concerted process, in which implicated molecules interact to tightly regulate each other. We therefore examined both independent gene effects and the occurrence of gene-gene interactions among the tested inflammatory genes in stroke risk and stroke recovery. Two *IL6* and one *MPO* SNP were significantly associated with stroke risk after multiple testing correction ($0.022<\text{corrected}P<0.042$), highlighting gene variants of low to moderate effect in stroke risk. An epistatic interaction between the *IL6* and *MPO* genes was also identified in association with stroke susceptibility ($P=0.031$ after 1000 permutations). In the subset of 546 patients assessed for stroke outcome at three months using the modified Rankin Scale (mRS), we found one *IL6* haplotype associated with stroke outcome (corrected$P=0.024$). In the present study we present supporting evidence for a role of the *IL6* and *MPO* inflammatory genes in stroke susceptibility, and show that stroke risk is modulated by main gene effects together with clinical and life-style factors as well as by gene-gene interactions. Our findings are compatible and strengthen previous genetic and biological observations, highlighting the need of further functional studies, particularly in view of the possible utility of IL-6 as a diagnostic and/or prognostic biomarker for stroke.

366F

Genetic Analysis of Lipidomic Profiles in Mexican American Families. *C. Bellis[1], J.E. Curran[1], J.M. Weir[2], M.A. Carless[1], J.B. Jowett[2], M.C. Mahaney[1], T.D. Dyer[1], H.H.H. Göring[1], A.G. Comuzzie[1], L. Almasy[1], P.J. Meikle[2], J. Blangero[1].* 1) Department of Genetics, Texas Biomedical Research Institute, San Antonio, TX; 2) Baker IDI Heart and Diabetes Institute, Melbourne, VIC, Australia.

Lipids are a highly diverse class of molecules with essential roles in cellular structure, signaling, and energy storage. Deregulation of lipid metabolism can lead to the development of various diseases including Alzheimer's, diabetes, and atherosclerosis. These lipid molecules may represent endophenotypes that are closer to the gene action than classical lipid markers, and extremely valuable for genetic analysis. Traditional measures of plasma lipids such as HDL-C, LDL-C and total triglyceride represent relatively crude summary measures that are actually composed of multiple components. We hypothesize that by disentangling lipid complexity and focusing on more biologically simple canonical lipid species we may more rapidly identify causal disease genes. Using a unique existing resource, the San Antonio Family Heart Study, we have identified and quantified 356 different canonical lipid species in 1202 Mexican Americans from 40 large pedigrees. Quantitative genetic analysis and genome-wide association analyses using over one million SNPs were performed to examine potential genetic factors involved in lipidomic profile variation. Analyses have revealed statistically significant heritabilities for 349 of the 356 measured lipid species (when controlling for age and sex). Maximal heritability h2=0.61 (p<1x10-15) was calculated for DHC 24:1 (dihexosylceramide) while the average calculated heritability (h2= 0.346) across the spectrum of lipids assayed illustrates a strong genetic component. Genome-wide association analyses revealed many significant QTL localizations for these canonical lipid phenotypes. Most notably, we identified a region on chromosome 11 exhibiting a highly significant genome-wide association with PC 36:4b (phosphatidylcholine) levels (p=5.9×10-68). The peak SNP accounted for ~25% of the variance in PC 36:4b and also showed significant genome-wide association with an additional 18 quantitated lipid components, including 14 other PC species. Residing in this particular region is the human FADS1- FADS3 gene cluster harboring polymorphisms previously shown to influence circulating fatty acids. Our results suggest that these canonical lipid measures represent phenotypes closer to gene action than those of classical lipid markers.

367F

Genetic modifiers of the vasculopathy caused by elastin haploinsufficiency act by extrinsic noncomplementation. *B.A. Kozel[1], R.H. Knutsen[2], L. Ye[1], C.H. Ciliberto[2], T.J. Broekelmann[2], R.P. Mecham[2].* 1) Department of Pediatrics, Division of Genetics and Genomic Medicine, Washington Univ Sch Med, St. Louis, MO; 2) Department of Cell Biology and Physiology, Washington Univ Sch Med, St. Louis, MO.

Background. Elastin haploinsufficiency causes the cardiovascular complications associated with Williams syndrome and isolated SVAS. Alterations in expression of the non-mutated ELN allele and genetic modifiers have been proposed as explanations for the variable vascular phenotype associated with this disease. Using the Eln+/- mouse, we sought to identify the source of this variability. Methods and Results. Following outcrossing of C57Bl/6J Eln+/- mice to four inbred strains, two backgrounds were identified whose cardiovascular parameters deviated significantly from the parental strain. Progeny of the C57Bl/6J; Eln+/- x129X1/SvJ F1 were more hypertensive and their arteries less compliant. In contrast, Eln+/- animals crossed to DBA/2J were protected from the pathologic changes associated with elastin insufficiency. Among the Eln+/-s, aortic elastin and collagen content did not correlate with quantitative vasculopathy traits, suggesting the variability seen in these mice was elastin-independent. Quantitative trait analysis performed on F2 C57 Eln+/- x129 intercrosses identified highly significant peaks on chromosome 1 (LOD 9.7) for systolic blood pressure and on chromosome 9 (LOD 8.7) for aortic diameter. Additional peaks were identified that affect only Eln+/- animals, including a region upstream of Eln on chromosome 5 (LOD 4.5). Bioinformatic analysis of the QTL peaks revealed several interesting candidates, Ren1, Ncf1, and Nos1; genes whose functions are unrelated to elastic fiber assembly, but whose effects may synergize with that of elastin insufficiency to predispose to hypertension and stiffer blood vessels. The candidates identified here are known to affect cardiovascular health in humans and are pharmacologically modifiable. Conclusions. Genes that do not directly affect elastin expression strongly influence the presentation of vascular disease in Eln halpoinsufficient mice. To date, it has not been possible to increase in vivo elastin deposition. However, the cumulative risk of pathology in extrinsic noncomplementation is brought about by interplay among affected genes. Consequently, identification of modifiers may allow better risk modification for these patients through the use of pharmacotherapies aimed at pathways unrelated to elastin production.

368F

Investigation of genome-wide associated regions for triglycerides in the Mexican population. *D. Weissglas[1], JS. Sinsheimer[1], L. Riba[2], B. Bernal-Barroeta[3], D. Gomez[3], W. Zamudio[3], M. Ordoñez-Sánchez[2], ML. Rodríguez-Torres[2], I. Cruz-Bautista[3], RM. Cantor[1], CA. Aguilar-Salinas[3], T. Tusie-Luna[2], P. Pajukanta[1].* 1) Dept. of Human Genetics, UCLA, Los Angeles, CA; 2) Unit of Molecular Biology and Genomic Medicine, IIB-UNAM, INCMNSZ, Mexico; 3) Dept. of Endocrinology and Metabolism, IIB-UNAM, INCMNSZ, Mexico.

Background: Mexicans have a high predisposition to elevated triglyceride levels (TGs) and premature coronary artery disease (CAD). However, this population has been underinvestigated in genomic studies, and to date no genome-wide association (GWA) studies for lipids have been performed in Mexicans. **Methods and Results:** We evaluated whether 24 loci surpassing genome-wide significant threshold ($P < 5 \times 10^{-8}$) for TGs in a recent GWA meta-analysis of Europeans also confer a risk in Mexicans. The median size and number of SNPs per region was 171kb and 31 SNPs, respectively, and in total 3,409 SNPs from associated regions were genotyped in 2,245 Mexican hypertriglyceridemia cases and controls. We observed significant association with $P < 2.5 \times 10^{-3}$ at ten of the loci: ANGPTL3, APOA1, APOB, CETP, CILP2, GCKR, HLA, LPL, MLXIPL, and TIMD4. Our strongest signal was obtained with SNP rs964184 near APOA1 (OR=1.8; $P=1\times10^{-19}$). This SNP has also surpassed genome-wide significant threshold for TGs and CAD in Caucasian GWA studies. However, it is substantially more prevalent in Mexicans (28%) than in Caucasians (12%), and thus of greater attributable risk in Mexicans. Interestingly, except for rs964184 and the missense SNP rs1260326 in GCKR (OR=1.4; $P=2\times10^{-7}$), for all other loci the strongest signal was obtained with a different SNP than in the Europeans ($0<r^2<0.85$), suggesting that Mexicans may assist in localizing the actual susceptibility variants or possess population-specific risk variants in these loci. For instance, the intergenic SNP rs6873137 (OR=1.2; $P=5\times10^{-4}$) between TIMD4 and HAVCR1 is in stronger linkage-disequilibrium with a missense variant in HAVCR1 in Mexicans than in Europeans ($r^2=0.74$ versus 0.52), suggesting that HAVCR1 is more likely the functional gene accounting for this uncharacterized genome-wide significant signal. **Conclusions:** It is important to identify the common variants contributing to the increased susceptibility to hypertriglyceridemia in Mexicans. We show significant associations for ten GWA loci for TGs in Mexican hypertriglyceridemia case-control study samples. The strongest associated SNP, rs964184, is more prevalent in Mexicans than in Europeans and thus may contribute to the high predisposition to dyslipidemias in Mexicans.

369F

Genetic basis of familial hypercholesterolaemia. *A.C. Alves[1, 2], M. Bourbon[1,2].* 1) Cardiovascular Research Group, R&D Unit, Dept Health Promotion and Chronic Diseases, INSA, Portugal; 2) 2 Center for Biodiversity, Functional & Integrative Genomics (BioFIG).

Familial hypercholesterolemia (FH) is a genetic condition characterized by a high cholesterol concentration in the blood. The most frequent causes of FH are inherited defects in the Low Density Lipoprotein Receptor gene (LDLR) but, in a small percentage of patients, mutations in the apolipoprotein B gene (APOB) and in the propotein convertase subtilisin/kexin type 9 gene (PCSK9) are also responsible for FH. These 3 genes are currently studied in the "Portuguese FH study". From the 563 families with a clinical diagnosis of FH studied only 41% of these have a mutation in one of the 3 studied genes, so other gene defects must exist to explain the cause of hypercholesterolemia in the remaining families. The aim of this study was the exclusion of previously unidentified LDLR and APOB gene defects in 65 severely affected patients, as well as the exclusion of mutations in LDLRAP1 and CYP7A1 genes in patients with possible recessive hypercholesterolemia. The whole sequencing of LDLR and APOB genes of the 65 index patients, without mutations in LDLR or PCSK9 genes or in fragments of exon 26 and 29 of APOB gene was performed. A pool of the 65 DNAs was sequenced by pyrosequecing with custom design primers and a total of 227688 nucleotide reads were obtained, corresponding to a mean coverage of 35x/fragment/individual. CYP7A1 and LDLRAP1 genes were analysed by PCR and direct sequencing. A total of 87 alterations were detected by pyrosequencing. More than half were previously described SNPs and 32 were novel possible pathogenic variants. The majority of these variants (25) were in exons 26 and 29. From the 32 novel variations identified by pyrosequencing only 4 were found by Sanger sequencing. Three alterations (2 novel and 1 described) were found by Sanger sequencing and were not detected by pyrosequencing. Three SNP's were also studied do to their low alleles estimated. After family studies of these 10 variants, 3 alterations did not co-segregate in the family, 4 alterations were not possible to verify co-segregation and 3 of the alterations found are possibly mutations causing disease, but functional studies are required to prove pathogenicity. No mutations were found in LDLRAP1 and CYP7A1 genes in 10 patients with possible recessive hypercholesterolemia. Patients, in whom it was not possible to find the genetic cause of the hypercholesterolaemia, will require further studies, since all show a severe clinical phenotype of FH.

370F

Serum microRNA-21 and microRNA-221 as potential biomarkers for stroke. *M. Lu[1], P. Tsai[2], Y. Wang[2], Y. Liao[1], H. Lin[3], R. Lin[3], S. Juo[2].* 1) Grad Inst of Medicine, Kaohsiung Med Univ, Kaohsiung, Taiwan; 2) Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 3) Department of Neurology, Kaohsiung Medical University, Kaohsiung, Taiwan.

Background Stroke is one of the leading causes of death and disability worldwide. microRNAs are endogenous non-coding small RNAs that silence gene expression via post-transcriptional inhibition of their target mRNAs. Recent studies revealed that microRNAs can participate in the pathogenesis of stroke, such as endothelial dysfunction, neointimal hyperplasia, and inflammation. We aimed to find some microRNAs as potential biomarkers for stroke by exploring the association between the circulating microRNAs and stroke. Methods A total of 149 stroke patients and 163 non-stroke subjects were recruited in this study retrospectively. The diagnosis of stroke was diagnosed by both clinical neurological examinations and brain CT/MRI. Carotid doppler was performed to measure the extent of the carotid intima thickness (IMT) in the non-stroke subjects. Among non-stroke subjects, a carotid plaque score$\checkmark$ 3 was defined as atherosclerosis, and a plaque score=0 was served as healthy controls. The expression of serum microRNAs were detected by real-time PCR. Results The expression of serum miR-21 was highest in the stroke group, followed by atherosclerosis group, and lowest in the healthy controls (adjusted p=0.0021). On the contrary, miR-221 was significantly decreased in the stroke patients compared with either the atherosclerotic or healthy subjects (adjusted p=0.0005). The association between the severity of vascular disease and serum miRNAs only existed in men (p-trend =0.0011 for miR-21; p-trend =0.0259 for miR-221) but not in women. A predictive model including five risk components (miR-21, miR-221, age, diabetes, and hypertension) was built to assess the possibility of stroke in men. The best predictive model achieved 92.7% positive-predicted rate and 89.3% negative-predicted rate for stroke. Conclusion Circulating miR-21 and miR-221 were potential biomarkers for stroke in men.

371F

The up-regulation of mir-221 in adipose tissue is associated with the development of phenotype of obesity. *Y. Wang[1], W. Chou[1], X. Su[1], S. Juo[1,2].* 1) Medical Genetics, Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Department of Medical Research, Kaohsiung Medical University Hospital.

Background: Obesity and associated diseases are becoming a health-concerned issue as a result of imbalanced diet, especially given the current trend for increases in childhood obesity. Adipose tissue undergoes a dramatic expansion in obesity; however, recent reports indicate the number of adipocytes is established during childhood and stays constant during adulthood. Previous in vitro researches demonstrate specific intracellular microRNAs play a role in regulating adipogenesis. In our study, the aim is to investigate the expression profile of microRNAs in a dynamic obesity model. Methods: We utilized male mice (C57BL/6J) and induced obesity by feeding them with high-fat diet (HFD, 53.3% of total calories from fat) in comparison with chow diet in the control group. During the treatment course, intra-peritoneal glucose tolerance test were repeated to observe glucose and insulin responses at the ends of 4 week, 8 week and 12 week. Afterwards, both serum and epididymal fat tissue were collected to analyze both biochemical tests of lipid profile and the expression patterns of microRNAs reported in association with adipogenesis by real-time PCR. Results: During the dynamic growth of adipose tissue, only mir-221 expression was progressively up-regulated in mice fed a high-fat diet (total n=12) when comparing chow diet treated group (total n=8). The increases were 1.53-fold and 2.75-fold in 8-week and 12-week treatment, respectively (p=0.01). Furthermore, the level of mir-221 in adipose tissue correlated positively with body weight (BW) at sacrificed dates (r=0.580, p=0.003), BW change within treatment course (r=0.503, p=0.02), epididymal fat weight (r=0.553, p=0.005), and the ratios of epididymal fat weight to BW (r=0.535, p=0.007). Moreover, mir-221 expression in adipose tissue also showed positive correlations with blood glucose level at 60min after intra-peritoneal injection of 2mg/g glucose (r=0.506, p=0.03). However, there were no changes in expression patterns of tested adipogenic miRNAs in blood circulation between two groups in different time points. Conclusion: The up-regulation of mir-221 is associated with the dynamics of adipose tissue growth and possibly related to obesity induced insulin resistance. We are investigating the molecular mechanism of mir-221 in regulation of fat mass expansion.

372F

Genetic polymorphisms associated with hypertension in a sample population of Calabar and Uyo, Nigeria. *C.I. ANUMUDU[1], M.E. KOOFFREH[1], L. KUMAR[2].* 1) ZOOLOGY, UNIVERSITY OF IBADAN , IBADAN, Nigeria; 2) International Institute of Tropical Agriculture Ibadan.

Recent research into the molecular genetics of hypertension has the aim of identifying and associating the gene variants of different polymorphisms with hypertension as well as their interaction with environmental factors. This study was designed to determine the frequency and association of gene polymorphisms of the RAAS and ANP with hypertension in residents of Calabar and Uyo, Nigeria. A case-control study design was used, and 1308 participants were recruited into the study, 612 patients and 696 controls. The M235T allele, Insertion/Deletion allele, A1166C allele and C664G allele polymorphisms were investigated using polymerase chain reaction and restriction enzyme digestion to determine allele frequency. Hypertensinogenic factors such as dietary habits, physical activity, smoking and drinking habits were assessed using questionnaires. Descriptive statistics, chi-square, multiple regression analysis and odds ratio were used to analyze data obtained at a probability of 0.05. The frequency of the three genotypes of the M235T allele in both patient and control groups respectively were 88% and 92% (MM); 9% and 7.5% (MT); 0.3% and 0.7% (TT). The Insertion/Deletion allele had a frequency of 45% and 39% for the deletion genotype, the Insertion/Deletion genotype was 43% and 49% while the insertion genotype was 12% in both control and patient populations. For the A1166C allele, 99% of the study population had the wild type AA genotype and 1% was AC heterozygous carriers of the mutation. Only the CC genotype was observed for the C664G allele in the study population. There were no significant differences between the genotype frequencies in hypertensives and the control groups for all the polymorphisms under consideration. Age was a predictor for Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) in the hypertensive group r=0.604 SBP, r=0.594 DBP, p=0.005; and for DBP in the control group r=0.542. Gender, body mass index, AGT, ANP and ACE genotypes were not predictors for SBP and DBP in the hypertensive group. The Insertion/Deletion allele was a risk factor for hypertension odds ratio 1.15 at 95% CI, (0.92-1.46). Higher carriage of insertion/deletion allele was associated with an increased risk of developing hypertension. There was no association between the frequency of the M235T, A1166C and the C664G alleles and hypertension, suggesting other loci or environmental factors are involved in the disease outcome.

373F

Identification of polymorphisms in NPBWR2 gene associated with hypertension. *J. Klovins[1], R. Petrovska[1], G. Latkovskis[2], V. Pirags[3], H.B. Schioth[4], I. Kalnina[1].* 1) Latvian Genome Ctr, Latvian Biomedical Research and Study Ctr, Riga, Latvia; 2) 2Latvian Centre of Cardiology, Pauls Stradins Clinical University Hospital, Riga, Latvia; 3) University of Latvia and Department of Endocrinology, Pauls Stradins Clinical University Hospital; 4) Department of Neuroscience, Uppsala University, BMC, Box 593, SE751 24, Uppsala, Sweden.

NPBWR1 and NPBWR2 are receptors of neuropeptide B (NPB) and neuropeptide W (NPW). This neuropeptide system is thought to have a role in regulating feeding behaviour, energy homeostasis and neuroendocrine function, however its role in humans remains unknown. The aim of the study was to characterize the genetic variance of NPBWR1 and NPBWR2 and investigate the possible correlation of identified polymorphisms with number of available traits and phenotypes. In total 6 polymorphisms were indentified in both gene loci from direct sequencing of 100 individuals. Genotype based association was performed in randomly selected 1500 individuals from Genome Database of Latvian Population. Among 10 different traits included in this analysis the hypertension was significantly associated with one missense SNPs from NPBWR1 gene (rs33977775) and one missense SNP from NPBWR2 gene (rs4809401). These SNPs were genotyped in additional group of 600 hypertensives and 600 validated controls. rs4809401 were significantly associated with presence of hypertension (P=0.0016). We also present functional importance of three identified missense variants in NPBWR1 and NPBWR1 variants on cAMP inhibition in HEK-293 cells.

374F

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375F

Genome-wide Association Study of Cardiac Structure and Systolic Function in African Americans: the Candidate Gene Association Resource (CARe) Study. *E. Fox[1], S. Musani[1], M. Barbalic[2], H. Lin[3], K. Ogunyankin[4], N. Smith[5], A. Kutlar[6], W. Post[7], D. Dries[8], C. Duarte[9], S. Kardia[10], D. Arnett[9], D. Paltoo[11], D. Farlow[12], R. Vasan[3], Candidate Gene Association Resource (CARe) Study Working Group.* 1) Department of Medicine, University of Mississippi Medical Center, Jackson, MS; 2) Human Genetics Center, University of Texas Health Science Center, Houston , TX; 3) Department of Medicine, Boston University School of Medicine, Boston, Massachusetts; 4) Northwestern University School of Medicine; 5) University of Washington; 6) Medical College of Georgia; 7) John Hopkins University schoool of Medicine; 8) University of Pennsylvania School of Medicine; 9) University of Alabama School of Public Health; 10) University of Michigan School of Public Health; 11) Division of Cardiovascular Sciences, National Heart, Lung, and Blood Institute, NIH; 12) Broad Institute.

Background. Variation in cardiac structure and function is unexplained by established environmental factors and may be related to genetics. Using data from four community-based cohorts of African Americans (AA), we tested the association between genome-wide markers (SNPs) and cardiac phenotypes in the Candidate-gene Association REsource (CARe) study. **Methods**. Among 6,765 AA, we related age, sex, height and weight-adjusted residuals for nine cardiac phenotypes (assessed by echocardiogram or MRI) to 2.5 million SNPs (1.0 million genotyped using Affymetrix SNP array 6.0 and the remainder imputed). The primary analysis was conducted within each cohort and meta-analyzed across cohorts using inverse variance weights. 4.0E-7 was used as the prespecified threshold based on one expected false positive. We investigated replication in cohorts of African ancestry and one large consortium of European ancestry (EchoGEN). **Results**: Variants in 4 genetic loci reached genome-wide significance: one locus on chromosome 8 for left ventricular mass (LVM - p=1.43E-07); one locus on chromosome 17 for left ventricular internal diastolic diameter (LVIDD - p=1.68E-07); one locus on chromosome 10 for interventricular septal wall thickness (IVST- p= 2.57E-08); and one locus on chromosome 13 for ejection fraction (EF - p=4.02E-07). In addition, we found associated variants were enriched in three signaling pathways that were involved in cardiac remodeling. None of the 4 genome-wide loci replicated in a meta-analysis of cohorts composed of individuals of African ancestry. None of 3 available loci replicated in the EchoGEN consortium. **Conclusion**: In a genome wide association study of AA, we identified 4 genetic loci significantly related to LVM, IVST, LVIDD, and EF. Supplemental analysis found that associated variants were enriched with genes in three signaling pathways for cardiac remodeling. None of the top 4 loci replicated in cohorts of African and European ancestry suggesting that additional large-scale studies are warranted for these complex phenotypes.

376F

Enriched population from Lebanon improves the understanding of Genetic and Environmental effect on Cardiovascular Disease in the Levant and beyond. *J.-B. CAZIER[1], J.L. DAVIES[2], S. YOUHANNA[3], D.E. PLATT[4], C. HOLMES[1,2], D. GAUGUIER[5], P.A. ZALLOUA[3,6].* 1) Wellcome Trust Centre of Human Genetics, University of Oxford, Roosevelt Drive, Oxford, OX3 7BN, United Kingdom; 2) Department of Statistics, University of Oxford, 1 South Parks Road, Oxford, OX1 3TG, UK; 3) Lebanese American University, School of Medicine, Beirut 1102 2801, Lebanon; 4) Bioinformatics and Pattern Discovery, IBM T. J. Watson Research Centre, Yorktown Hgts, NY 10598, USA; 5) INSERM, UMRS872, Centre de Recherche des Cordeliers, 15 Rue de l'école de Médecine, 75006 Paris, France; 6) Harvard School of Public Health, Boston, MA 02215, USA.

We have collected a unique cohort of 6000 individuals from Lebanon with extensive and precise phenotypic and genotypic information. This cohort is of very special relevance for the improvement of the understanding of cardiovascular diseases and its interaction both in the Levant and how it relates to the rest of the world. The four main coronary arteries were visualized from different angles by angiography and the extent of stenosis in these vessels was assessed and recorded by percentage. Trained healthcare professionals collected further data on the socio-demographic background of the patients. With 2000 individuals genotyped on the Illumina 610-660 chips, this cohort is the largest genotyped patient population from the Levant. This enabled the first Genome-Wide-Association of the region which relative small cohort confirmed association loci found with far larger meta-analysis. Furthermore the extent and quality of the data collected at both genetic and phenotypic level allows us to put in perspective published findings made essentially in cohorts of European and Asian descents. Most importantly, this population from the Levant is ideally placed, geographically and historically, at the centre of the three most common HapMap groups of Europe, Asia and Africa. This unique position should make the Middle East the common denominator of Asian and European dispersion out of Africa, an ideal place to identify old, and common, variants. Furthermore the recent admixture in this population allows for a further refinement in the identification of variants between new and old contributions. We made use of this specificity to explore the stratification of the population by Principal Component Analysis and Admixture Mapping and applied a new robust statistical method to detect susceptibility gene-environment interactions in structured but closely related sub-populations. We looked for evidence of heterogeneous genetic associations across environmental background, either genetic or physiological (e.g. HDL, LDL), and demonstrate the importance of the region in a worldwide context. In conclusion, we have shown that our enriched cohort from Lebanon, not only can improve the understanding of cardiovascular diseases in the region, but also provide extremely valuable information for genomic studies beyond the classic framework of European, Asian and African populations.

377F

Whole-exome DNA re-sequencing in French Canadians with dilated cadiomyopathy using pre-exon capture barcoding. *M. Beaudoin[1], K.S. Lo[1], M. Schlogel[1,2], L. Robb[1], N. Laplante[1], J-C. Tardif[1,3], M. Talajic[1,3], G. Lettre[1,3].* 1) Montreal Heart Institute, Montreal, Quebec, Canada; 2) Laboratory of Human Molecular Genetics, de Duve Institute, Université catholique de Louvain, Belgium; 3) Universite de Montreal, Faculte de Medecine, Montreal, Quebec, Canada.

Dilated cardiomyopathy (DCM) is characterized by left ventricular dilatation, systolic dysfunction, myocyte death, and myocardial fibrosis. Environmental stimuli, such as viral infection, chronic alcohol consumption, or hypothyroidism can lead to DCM. Studies in families also indicate a strong genetic determinant for DCM, with mutations in more than 40 genes already linked to the development of DCM. Despite this knowledge, a large fraction of familial DCM cases remain unexplained after screening the known DCM genes. Next-generation DNA re-sequencing (NGS) offers new opportunities to find causal mutations in heritable pathologies. Already, NGS has been used successfully to find etiological mutations in several human Mendelian diseases and syndromes. In this project, we tested the use of whole-exome DNA re-sequencing to find novel DCM genes. We selected eight French-Canadian DCM patients from the Montreal Heart Institute Biobank using the following two criteria: (1) they had tested negative for mutations in LMNA, MH7, and TNNT2, and (2) they had to have at least one affected sib or child to enrich for DCM patients with segregating mutations. To minimize costs associated with exon capture, four genomic DNA were barcoded before being captured with a single Agilent's SureSelect Human All Exon 50Mb kit. Libraries were prepared according to standard protocols and sequenced on the Applied Biosystems SOLiD4 sequencer (4 exomes per slide). Sequence quality control and analysis was performed using a robust pipeline built around established software: BWA, SAMtools, Picard, GATK, and PLINKSEQ. Preliminary analysis of the first whole-exome sequence data suggests that our "pre-exon capture barcoding" protocol works relatively well: we obtained a mean coverage of 35X (54% of the targeted exonic sequences covered at ⁄ 20X) and identified 24,148 high-quality DNA sequence variants, including 2,837 variants not previously reported in databases (transition-to-transversion ratio=2.79). Analysis of the whole-exome sequence data for the eight DCM patients will be presented.

378F

Impact of *LIMK1, MMP2 and TNF-*(variations for intracranial aneurysm in Japanese population. *S.-K. Low[1,2], H. Zembutsu[1], A. Takahashi[3], N. Kamatani[3], P.-C. Cha[1], N. Hosono[4], M. Kubo[4], K. Matsuda[1], Y. Nakamura[1].* 1) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; 2) Genetics Division, National Cancer Center Research Institute, Tokyo, Japan; 3) Laboratory for Statistical Analysis, Center for Genomic Medicine, The Institute of Physical and Chemical Research (RIKEN), Kanagawa, Japan; 4) Laboratory for Genotyping Development, Center for Genomic Medicine, The institute of Physical and Chemical Research (RIKEN), Kanagawa, Japan.

Genetic factors are known to have an important role in intracranial aneurysm (IA) pathogenesis. The purpose of this study is to identify single-nucleotide polymorphisms (SNPs) that are associated with IA in Japanese population. A total of 2050 IA patients and 1835 controls recruited in Biobank Japan, The University of Tokyo were used in this study. In all, 45 SNPs in 24 genes encoding proteins, which have been considered to be possible risk factors to IA pathogenesis, were genotyped using multiplex PCR-invader assay. Association analysis was evaluated by logistic regression analysis before and after adjustment of age, smoking and hypertension status. This case-control association study revealed a SNP, rs6460071 located on *LIMK1* gene (*P*=0.00069) to be significantly associated with increased risk of IA. In addition, two SNPs, rs243847 (*P*=0.00086) and rs243865 (*P*=0.00090), on matrix metallopeptidase 2 (*MMP2*) gene and one SNP rs1799724 (*P*= 0.0026) on tumor necrosis factor-((*TNF*-( ) gene, are marginally associated with IA in male- and female-specific manner, respectively. In conclusion, a large-scale case-control association study was conducted to verify genetic variations associated with IA in Japanese population. This study gave insights on the importance of stratified analysis between genders, and suggested that the underlying mechanism of IA pathogenesis might differ between females and males.

379F

Genetic Susceptibility to Ischemic Stroke in Moldavians. *E. Mocan[1,2], O. Odainic[1], V. Nacu[1], S. Protopop[1], L. Lysiy[1], N. Barbacar[2].* 1) The State Medical and Pharmaceutical University , Chisinau, Chisinau, Moldova, Republic Of; 2) The Institute of Genetics and Plant Physiology, Chisinau, Moldova, Republic of.

Present research represents the first generalized study of genetic markers for Moldavian IS patients and for a control group of Moldavians similar gender distribution. The frequencies of allele and genotypes for 9 functionally significant SNP were determined in FII, FV, GP1BA, PAI-1, ALOX5AP, PDE4D and ACE genes. We examined samples of blood and genomic DNA from 159 Moldavians IS patients, age 53±12 yr. The control group consisted of 90 Moldavians without IS, age 37±9.7 yr. SNPs were detected with conventional allele-specific fluorescence PCR in real-time protocols (Taqman®) and insertion-deletion polymorphism of ACE gene by simple PCR. Statistically significant differences were found in biochemical parameters such as total cholesterol, triglycerides, HDL-cholesterol in group with ARE compared with the control group. All genotypes and alleles were in Hardy-Weinberg equilibrium. The groups showed no significant differences in the frequencies of individual alleles and genotypes for any studied polymorphism. When subjects were stratified by sex and age, the genetic effect was only evident in men of middle age but not in women of same age and men older 54 years attacked with IS. The statistically significant association with IS and D allele carrying of the ACE gene insertion-deletion polymorphism at men of middle age (till 54 years) were demonstrated with the odds ratio for men carriers of D allele was 3.53 (95%, CI: 1.05-10.86, p=0.026). Also significant correlation were observed between carrying of D allele and next clinical parameters such as hypertension (r=0.3, p=0.02), total cholesterol (r=0.24, p=0.03), LDL-cholesterol (r=0.25, p=0.02) and triglycerides (r=0.26, p=0.02). The prevalence of the studied polymorphisms of the fibrinolytic, coagulation and inflammation systems did not differ significantly between patients and control group that demonstrates an absence of genetic association of studied variants with IS in Moldavian population. But synergism was observed between ACE DD genotype, male sex and environmental risk factors. The genetic structure of the Moldavian population is poorly studied, and these data could be interesting in the historical-cultural context. Acknowledgments: This work was supported by grant ASM-RFBR 08-04-90121, MYSSP-1403 program MRDA-CRDF. We would like to thank the Institute of Molecular Genetics of RAS, Russia; especially we are deeply grateful to S.Limborska, P.Slominsky and E.Bondarenko for support.

380F

ATP2B1 gene polymorphisms are associated with susceptibility to hypertension. *Y. Tabara, K. Kohara, T. Miki.* Ehime University Graduate School of Medicine, Toon, Japan.

Hypertension is one of the most common complex genetic disorders. We have previously described 38 single nucleotide polymorphisms (SNPs) with suggestive association with hypertension in Japanese individuals. In this study we extend our previous findings by analyzing a large sample of Japanese individuals (n=14,105) for the most associated SNPs. We also conducted replication analyses in Japanese of susceptibility loci for hypertension recently identified from GWAS studies of European ancestries. Association analysis revealed significant association of the ATP2B1 rs2070759 polymorphism with hypertension ($p=5.3*10^{-5}$, allelic OR 1.17, 95% CI 1.09-1.26). Additional SNPs in ATP2B1 were subsequently genotyped, and the most significant association was with rs11105378 (OR 1.31 (1.21-1.42), $p=4.1*10^{-11}$). Association of rs11105378 with hypertension was cross-validated by replication analysis with the Global BPgen consortium data-set (OR 1.13 (1.05-1.21), $p=5.9*10^{-4}$). Mean adjusted systolic BP was highly significantly associated with the same SNP in a meta-analysis with individuals of European descent ($p=1.4 *10^{-18}$). ATP2B1 mRNA expression levels in umbilical artery smooth muscle cells were found to be significantly different among rs11105378 genotypes. Seven SNPs discovered in published GWAS were also genotyped in the Japanese population. In the combined analysis with replicated three genes, FGF5 rs1458038, CYP17A1, rs1004467 and CSK rs1378942, odds ratio of the highest risk group was 2.27 (95% CI 1.65 to 3.12, $p=4.6*10^{-7}$) compared with the lower risk group. In summary, this study confirmed common genetic variation in ATP2B1 as well as FGF5, CYP17A1 and CSK to be associated with BP levels and risk of hypertension.

381F

The use of alternative regulatory elements and the problem of missing heritability. *E.A. Ruiz-Narvaez[1,2].* 1) Slone Epidemiology Center, Boston University, Boston, MA; 2) Department of Epidemiology, Boston University, Boston, MA.

Genome-wide association studies (GWAS) have identified a multitude of single nucleotide polymorphisms (SNPs) associated with a wide spectrum of human phenotypic traits. However, the SNPs identified so far do not explain much of the expected phenotypic variation due to genetic factors and they are poor predictors of the occurrence of disease. Proposed explanations for the missing heritability include rare genetic variants, structural variants, gene-environment and gene-gene interactions. I recently advanced the hypothesis that there is person-to-person variation in the use of alternative regulatory elements (e.g. gene promoters) and this new source of variation may explain part of the missing heritability. In the present work I develop a simple mathematical model to explore the biologic consequences of the proposed hypothesis. Major implications of the hypothesis are: i. The total genetic variation due to observed genetic variants will be smaller than the heritable variance of the phenotypic trait. ii. For a particular causal SNP its observed genetic effect will be smaller than its actual genetic effect. iii. The presence of more than one causal SNP may be hidden due to linkage disequilibrium in conjunction with the use of alternative regulatory elements. iv. True genetic variation may completely be hidden if the use of alternative regulatory elements is not taken into account. A particular example of the proposed hypothesis would be the use of alternative gene promoters. It is known that more than 50% of human genes have more than one promoter, with an average of 3.1 promoters per gene. I discuss in the present work the biologic plausibility of the proposed hypothesis based on recent published evidence regarding the use of alternative gene promoters, as well as new paths of research to elucidate this mechanism for missing heritability.

382F

A variant in the promoter of MUC5B is a genetic predictor for survival in patients with idiopathic pulmonary fibrosis. *Y. Zhang[1,2], T. Richards[1,2], K. Gibson[1,2], K. Lindell[1,2], N. Kaminski[1,2].* 1) Dept Med, Div PACCM, Univ Pittsburgh Sch Med, Pittsburgh, PA; 2) Dorothy P. and Richard P. Simmons for Interstitial Lung Disease, Univ Pittsburgh Sch Med, Pittsburgh, PA.

Statement of Purpose: Idiopathic pulmonary fibrosis (IPF) is a complex genetic disease Recently, a SNP in the promoter of MUC5B, rs35705950, was reported to be associated with pulmonary fibrosis (Seibold et al. NEJM 21;364(16):1503-12. 6; Zhang et al. NEJM. 21;364(16):1576-7). In this study, we performed genotype-outcome analyses for rs35705950 to determine genotype correlations with survival in a large IPF cohort. **Methods:** Genetic and survival correlation analyses were performed using a IPF cohort from the University of Pittsburgh. The diagnosis of IPF was based on clinical, physiological, and high-resolution computed tomography (HRCT) findings in accordance with ATS/ERS criteria; surgical lung biopsy results were the basis of diagnosis in 53.7% of the patients. Only US non-Hispanic Whites were included. Genotype-outcome correlation analyses were performed using the R environment and graphing package. **Results:** The demographic and clinical characteristics as well as rs35705950 genotypes for the IPF cohort were reported previously (Zhang et al. NEJM. 21;364(16):1576-7). Survival analysis demonstrated that the T allele was protective with a median overall survival of 4.86 years for patients with GT and TT genotypes compared to 2.35 years for individuals with GG phenotype. The unadjusted hazard ratio for the GT+TT genotypes was 0.587 (p=0.0018) and the age, gender, and baseline forced vital capacity (FVC) adjusted hazard ratio was 0.664 (p=0.016). In addition, correlation of transplant-free survival with rs35705950 genotypes demonstrated a 2.85 years transplant-free survival for patients with GT and TT genotypes versus 1.61 years for patients with GG genotype. The hazard ratio for transplant-free survival of the GT+TT genotypes was 0.661 (p=0.0043, unadjusted) and 0.750 (p=0.047) after adjusted for age, gender, and baseline FVC. **Conclusion:** We identified significant correlations of the rs35705950 T allele (GT + TT genotypes) with better overall and transplant-free survivals in IPF. Interestingly, the rs35705950T allele was both associated with disease in case-control analysis and protective for survival in patients with IPF. Validations of our finding in additional IPF cohorts are warranted and underway. Molecular dissecting of the rs35705950 SNP in IPF will lead to better understanding the roles of MUC5B in the pathobiology and disease progressions of IPF. Prediction of clinical outcomes with genetic variants may result in better management of IPF.

383F

The Human Caveolin 1 Gene Upstream Purine Complex and Multiple Sclerosis - A Replicated Link. *A. HEIDARI, M. Ohadi, H. Darvish, R. Pazhoomand, M. Behmanesh, R. Meshkani.* Genetics Research Center, University of Social Welfare , Tehran, Iran.

The caveolin 1 gene (CAV1) is over-expressed in experimental animal models of multiple sclerosis (MS). Increased expression of this gene has also been reported in the Alzheimer's disease (AD) brain. Loss of this gene, on the other hand, has recently been reported to be associated with neruodegeneration. We have recently reported skew in the homozygote haplotypes of the human CAV1 gene -1.5kb upstream purnie complex in patients afflicted with MS and late-onset AD vs. controls. In order to examine reproducibility of those findings, we sequenced the region in independent groups of MS patients (n=120) and controls (n=150). We report two novel extreme homozygote haplotyes at 86-bp and 142-bp in the patients vs. controls ($p<0.00001$). The range of homozygote haplotypes in the controls was detected at between 106-bp to 122-bp. The above haplotypes were also detected in the previously reported cases of late-onset AD. Following pooling of the neurodegenerative and nonneurodegenerative subjects studied for the human CAV1 purine complex, nineteen haplotypes were found to be homozygous in the neurodegenerative, and not in the control pool ($p<0.000001$). Overlapping haplotypes between MS and AD patients strengthen the role of this region as a common etiological factor in the pathophysiology of neurodegenerative disorders, possibly through inflammatory mechanisms. The CAV1 purine complex GGAA and GAAA motifs contain binding sites for numerous inflammatory transcription factors including the Ets, STAT, and IRF family members. Further work on the functionality of this region will shed light on the downstream events to the linked haplotypes.

384F

Multipoint Linkage analysis using dense SNPs in large pedigrees. *C. Sun, G. Gao.* Biostatistics Dept, Virginia Commonwealth University, Richmond, VA.

Linkage analysis usually is based on sparse markers such as microsatellite markers. Although linkage analysis using dense single-nucleotide polymorphism (SNP) markers has been implemented in small pedigrees, but it is still challenging to be implemented in large pedigrees because existing methods (such as the well known software Loki) for identity-by descent (IBD) probability estimation can be computationally intensive. We have developed an identity-by descent (IBD) matrix estimation method using dense SNP markers in large pedigrees based on using a conditional enumeration haplotyping algorithm to identifying a subset of haplotype configurations with high likelihoods. The haplotyping algorithm eliminates haplotype configurations with relatively low conditional probabilities by use of a threshold for the conditional probabilities of ordered genotypes at every unordered marker and a threshold for the ratio of the conditional probability of a haplotype configuration to the largest conditional probability of all haplotype configurations. We incorporate the estimated IBD matrices into a two-stage variance component method for quantitative trait loci (QTL) mapping. We have done extensive simulation studies to compare our IBD matrix estimation method with Loki by their performance in QTL mapping. We simulated pedigree data with dense markers by using the structure of a Hutterite pedigree. Let d denote genetic distance between adjacent markers. Simulation results show that our methods and Loki generated very similar estimation of the pre-set QTL position. For a large number of dense markers with distance $d \leq 1$cM, Loki could be computationally intensive while our method took reasonable computing time. When $d \leq 0.25$cM, IBD matrices estimated by Loki (with 1,000,000 iterations) were singular at many putative QTL positions while the IBD matrices estimated by our methods were singular at only a few positions. From our simulation studies, we also found that for the same chromosome region, using more SNP markers in the region can generate more accurate estimate of the pre-set QTL position. We simulated a chromosome region of 50cM. We compared the results by using 50 SNPs (with d =1cM) in the region with those by using 100 SNPs (with d =0.5cM). Using 100 SNPs generated sharper and higher peaks (around the true QTL position 24.5cM) in the average log-likelihood test statistic curves.

385F

A holistic approach to studying complex diseases using *Gentrepid*. *S. Ballouz[1,2], J.Y. Liu[1], M. Oti[3], B. Gaeta[2], D. Fatkin[4,5], M. Bahlo[6], M.A. Wouters[7].* 1) Structural and Computational Biology, The Victor Chang Cardiac Research Institute, Sydney, NSW, Australia; 2) School of Computer Science and Engineering, Faculty of Engineering, University of New South Wales, Kensington, NSW, Australia; 3) Centre for Molecular and Biomolecular Informatics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 4) School of Medical Sciences, University of New South Wales, Kensington, NSW, Australia; 5) Molecular Cardiology and Biophysics Division, The Victor Chang Cardiac Research Institute, Sydney, NSW, Australia; 6) Bioinformatics Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia; 7) School of Life and Environmental Sciences, Deakin University, Geelong, VIC, Australia.

Disease manifestation and presentation is a highly varied and complex phenomenon. The level of complexity of a disease is determined by multiple factors that include the allelic spectrum, genetic architecture and gene epistasis. These are further compounded with a multitude of regulatory and environmental effects. Monogenic and oligogenic diseases are much simpler to understand mechanistically, whilst polygenic diseases stretch the limits of our current knowledge of disease aetiology and of molecular interplay and regulation. In order to dissect the possible molecular mechanisms of complex disease, we have analysed genetic data obtained from genome-wide association studies. We looked at predicted candidate genes from our *Gentrepid* tool. Using what we know of protein pathways, protein-protein interactions and protein domains of the known disease genes to base the predictions (*seeded* approach), we then also took a blind approach (*ab initio*) that searches for enrichment of these annotations from multiple implicated genetic loci. Our results show that both the *seeded* and *ab initio* methods are capable of extracting known disease gene mechanisms. We also believe that novel implications from the *ab initio* approach are highly significant and require further molecular validation. In particular, our case study on coronary artery disease was able to extract novel LDL-like receptor proteins and cell-ECM adhesion factors. With the advent of whole exome and genome sequencing of disease and cancer genomes, even more data will be present to analyse. Therefore, it still remains highly important to be able to dissect the aetiology of diseases at a mechanistic level using current knowledge and more importantly a less biased and more holistic approach. *Gentrepid* is freely available as a webserver at https://www.gentrepid.org.

386F

Genetic association studies with fetal hemoglobin levels and eight clinical complications in African-American sickle cell disease patients. *G. Galarneau[1], S. Coady[2], M. Pandey[2], B. Moore[2], N. Jeffries[2], D. Paltoo[2], A. Guasch[3], A. Kutlar[4], G. Lettre[1], G. Papanicolaou[2], The CARe Project.* 1) Montreal Heart Institute and Université de Montréal, Montréal, Québec, Canada; 2) National Heart, Lung and Blood Institute, Bethesda, MD, USA; 3) Emory University, Atlanta, GA, USA; 4) Medical College of Georgia, Augusta, GA, USA.

Sickle cell disease (SCD) is a monogenic disease caused by mutations in the) *-globin* gene, and results in many severe complications. Previous genetic studies carried out in SCD patients have so far focused on the regulation of fetal hemoglobin (HbF) because it is a strong modifier of morbidity and mortality in this population. However, few well-powered studies have attempted to identify genetic associations with the main clinical complications of SCD. As part of the NHLBI CARe Project, we genotyped 1,514 African Americans with SCD from the Cooperative Study of Sickle Cell Disease (CSSCD) on the IBC genotyping array, a gene-centric platform that contains 47,092 SNPs that cover genetic variation in ~2,100 genes relevant for heart, blood and lung diseases. Using imputation, we increased the total number of SNPs queried to 237,643. HbF levels were analyzed with linear regression. Pain crisis and acute chest syndrome were analyzed as quantitative traits using Poisson regression to account for the skewed distribution of the phenotypic data. Leg ulcer, priapism, osteonecrosis, renal failure, stroke and mortality were analyzed as dichotomous phenotypes using Cox proportional hazards regression models. Three loci had been previously identified as associated with HbF levels: *BCL11A* on chromosome 2, the *HBS1L-MYB* intergenic region on chromosome 6, and the) *-globin* locus on chromosome 11. As expected, we strongly validated the associations at *BCL11A* (rs1427407 ; P-value=2.1×10^{-41}) and the) *-globin* locus (rs3759074; P-value=6.2×10^{-9}). The *HBS1L-MYB* intergenic region was not covered by the IBC array. We did not identify SNPs that reach genome-wide significance level for any of the eight clinical complications tested, which is not surprising given our limited power. However, for these phenotypes, we observed a three- to eight- fold enrichment of SNPs with P-values less than 1×10^{-5} (2.4 expected by chance), suggesting an enrichment of true signals at the top of our results. We selected the most promising markers for replication in a panel of 315 independent African Americans with SCD. Replication results will be presented.

387F

GWAS follow-up mutation screen and expression analysis implicate *ARHGAP29* as a novel candidate gene for nonsyndromic cleft lip/palate. *E.J. Leslie[1], M.A. Mansilla[1], L.C. Biggs[1], K. Schuette[1], S. Bullard[2], T.X. Zhang[3], M. Cooper[4], M. Dunnwald[1], A.C. Lidral[2], D. FitzPatrick[5], M.L. Marazita[4], T.H. Beaty[3], J.C. Murray[1].* 1) Department of Pediatrics, University of Iowa, Iowa City, Iowa, USA; 2) Department of Orthodontics, University of Iowa, Iowa City, Iowa, USA; 3) Department of Epidemiology, School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA; 4) Center for Craniofacial and Dental Genetics, Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; 5) Medical Research Council Human Genetics Unit, Western General Hospital, Edinburgh, UK.

Nonsyndromic cleft lip and/or cleft palate (NSCL/P) is a common birth defect with complex etiology reflecting the action of multiple genetic and/or environmental factors. Genome wide association studies have successfully identified novel loci associated with NSCL/P including a locus on 1p22.1. The peak association signals at this locus are near or in the *ABCA4* gene, mutations in which cause a range of retinal disorders. Neither expression analysis nor mutation screening support a role for ABCA4 in the etiology of NSCL/P, so we investigated an adjacent gene, *ARHGAP29*, encoding Rho GTPase activating protein 29. ARHGAP29 has preferential activity toward RhoA, which is involved in many functions related to cellular shape, movement, and proliferation, all critical for craniofacial development. Sequencing of *ARHGAP29* in 1064 individuals with NSCL/P and 357 unrelated controls from the Philippines and the United States (US) revealed one nonsense and eleven missense variants. The cumulative frequency of rare coding variants (defined by a minor allele frequency less than 0.5%) from both populations was greater for cases than controls (0.38% vs. 0.19%), but this difference is not statistically significant (p=0.29). Expression analysis using a mouse model demonstrated that *Arhgap29* is present in the epithelium and mesenchyme of the medial and lateral nasal processes and the mandibular processes at E10.5 as well as the oral and medial edge epithelia and palatal mesenchyme at E14.5. We tested the most associated SNP (rs560426) near *ABCA4* and *ARHGAP29* for genetic interaction with other candidate genes including *IRF6*, 8q24, and *MAFB*, identifying a possible interaction with IRF6 (rs2235371) (p=0.04), which is supported by reduced expression of Arhgap29 in the oral epithelium of a mouse deficient for *Irf6*. These data suggest a novel pathway for clefting involving the transcription factor *IRF6* interacting with the Rho pathway via *ARHGAP29*. The combination of genome wide association, rare coding sequence variants, craniofacial specific expression, and interactions with a known clefting gene, *IRF6*, support a role for *ARHGAP29* in NSCL/P. Additional sequencing of *ARHGAP29* and putative regulatory elements will be needed to identify other rare and common variants contributing to NSCL/P. Further study of the genetic and molecular interactions between *ARHGAP29*, *IRF6* and other candidate genes in model systems will further elucidate the role of *ARHGAP29* in craniofacial development.

388F

Whole-genome sequencing and haplotype characterization of an extended multiplex kindred with inflammatory bowel disease. *A.P. Levine[1], L. Jostins[2], G.W. Sewell[1], P.J. Smith[1,3], L.B. Lovat[3], A.P. Walker[1], J.C. Barrett[2], A.W. Segal[1].* 1) Division of Medicine, University College London, London, WC1E 6JJ, United Kingdom; 2) Statistical and Computational Genetics, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, United Kingdom; 3) Department of Gastroenterology, University College London Hospitals NHS Foundation Trust, London, NW1 2BU, United Kingdom.

The study of large families with many affected individuals is a powerful method for identifying rare disease causing variants that might contribute to the “missing heritability”. To date, this approach has been of limited value in inflammatory bowel disease (IBD) owing to the moderate size of families described and the polygenic architecture of IBD. We have characterized an extended multiplex kindred of Ashkenazi Jewish descent comprising over 40 individuals with IBD spanning seven cities, from a total of over 800 individuals across four generations. This is far in excess of the 6 affected individuals expected given the prevalence of IBD of ~0.75% in this population. To assess the extent to which the increased prevalence is due to a high load of known IBD risk loci, we genotyped 160 family members for 38 previously associated variants. The family has a significant enrichment of known risk variants, but simulations show that this does not explain the large number of cases observed. We genotyped all affected and 60 unaffected family members for 300K SNPs. To cope with the size of the family, we implemented a novel parallel method that combines pairwise identity-by-descent calculation and haplotyping in selected overlapping subfamilies to reconstruct haplotype sharing over the entire pedigree. No genome-wide significant linkage was found, consistent with a complex disease. We therefore conducted a joint linkage and high-throughout sequencing experiment to identify rare moderate-to-high penetrance variants. We performed whole-exome sequencing in 6 affected individuals and used the haplotype sharing information within the family to identify genomic regions that could harbor causal variants. We filtered variants on microarray gene expression in monocyte-derived macrophages and rectal biopsies from patients, population frequency and functional prediction. We present a number of candidate variants and describe bioinformatic and experimental procedures for variant follow-up. As no candidate variant seemed to fully explain the heightened disease prevalence, whole-genome sequencing is being performed on an additional 8 selected individuals from a subfamily prioritized for the lowest *a priori* disease risk. This will allow full identification of coding and non-coding variants in all shared haplotypes. This family and its associated datasets provide an unprecedented resource for studying the profile and segregation of common and rare disease variants in IBD.

389F

Exome sequencing in two families of Dutch origin segregating for celiac disease. *A.M. Szperl[1], I. Ricaño-Ponce[1], J.K. Li[2], P. Deelen[1], A. Kanterakis[1], V. Plagnol[3], F. van Dijk[1], HJ. Westra[1], G. Trynka[1], C.J Mulder[4], M. Swertz[1], H.Ch. Zheng[2], C. Wijmenga[1].* 1) Genetics, UMCG, Groningen, Groningen, Netherlands; 2) Department of BioMedical Research, Research & Cooperation Division, BGI-Shenzhen, Shenzhen, China; 3) UCL Genetics Institute, University College London, London, UK; 4) Department of Gastroenterology, VU Medical Center, Amsterdam, the Netherlands.

Celiac disease (CeD) is a complex genetic disorder triggered by the environmental factor gluten, in genetically susceptible individuals. HLA and associations to 39 non-HLA loci explain around 40% of the heritability of CeD. We hypothesized that part of the hidden heritability could be due to low-frequency and rare variants that influence expression of protein and which map to exons and splice sites. Such causal variants could be more prominent in multi-generation families in which private mutations may co-segregate with the disease. We performed a combined linkage analysis and exome sequencing approach in two Dutch families, one family covered three generations and the other four, containing 23 patients in total that show an autosomal dominant segregation of CeD. The families showed linkage to five different regions of the genome (4q32.3-4q33, 6q25.3, 8q24.13-8q24.21, 9p13 and 10q23.1-10q23.32). In both families we then selected two affected individuals separated by 4 or 5 meiotic steps for sequencing. We investigated the linkage regions and the remaining exome for causal nonsense variants. In the first family we identified 12 nonsense mutations with a low frequency (MAF < 10%) present in both individuals, but none mapped to the linkage regions. Two nonsense variants in the CSAG1 and KRT37 genes were found to be present in all affected individuals. We are currently validating all the candidate nonsense variants from the second family by Sanger sequencing. The variants co-segregating with CeD in both families will also be examined in our cohort of >10,000 CeD cases and controls.

390F

Genetic association analysis of CIITA polymorphisms with nasal polyps pathogenesis in Korean asthma patients. *J.S. BAE[1], C.F.A Pasaje[1], C.-S. Park[3], H.D. Shin[1,2].* 1) Life Science, Sogang university, Seoul, Korea; 2) Department of Genetic Epidemiology, SNP Genetics, Inc., Seoul, Republic of Korea; 3) Division of Allergy and Respiratory Medicine, Soonchunhyang University Bucheon Hospital, Bucheon, Republic of Korea.

Nasal polyps are abnormal lesions arising mainly from the nasal mucosa and paranasal sinuses. By functioning as a positive regulator of MHC class II transcription, the human class II, major histocompatibility complex, transactivator (CIITA) gene is speculated to be involved in the presence of nasal polyps in asthma and aspirin-hypersensitive patients. To investigate the association between CIITA and nasal polyposis, 18 single nucleotide polymorphisms (SNPs) were genotyped in 467 asthmatics of Korean ancestry who were stratified further into 158 aspirin exacerbated respiratory disease (AERD) and 309 aspirin-tolerant asthma (ATA) subgroups. From pairwise comparison of the genotyped polymorphisms, 11 major haplotypes (frequency > 0.05) were inferred and selected for further association analysis. Differences in the frequency distribution of CIITA variations between polyp-positive cases and polyp-negative controls were determined using logistic analyses. Results reveal that five CIITA SNPs were significantly associated with the presence of nasal polyps in the overall asthma group (P = 0.006-0.05, OR = 0.53-2.35, using various modes of genetic inheritance). In addition, significant associations were also observed between the genetic variants tested for the presence of nasal polyps in AERD (P = 0.01-0.02, OR = 2.45-2.66) and ATA (P = 0.001-0.05, OR = 0.45-2.61) patients. The conclusions derived from the study are preliminary and may provide useful insights on the pathogenesis of nasal polyps. (This abstract will be submitted to a journal before the October meeting.).

391F

Familial Autoimmune Disease among Children with MS in the Canadian Pediatric Demyelinating Disease Study. *L. Zahavich[1], M. McGowan[1], C. Guimond[2], I. Yee[2], D.L. Arnold[3,4], A. Bar-Or[3,4], R.A. Marrie[6], B. Banwell[1,5], A.D. Sadovnick[2].* 1) The Hospital for Sick Children, Toronto, Canada; 2) The University of British Columbia, Vancouver BC; 3) The Montreal Neurological Institute, Montreal QC; 4) McGill University, Montreal QC; 5) The University of Toronto, Toronto ON; 6) University of Manitoba, Winnipeg, MB.

Background: The etiology of Multiple Sclerosis (MS) is thought to involve a complex interaction between genetic and environmental factors. Associations have been shown between susceptibility to MS and immune-response genes. Between 25-40% of children with acute acquired demyelination (ADS) of the CNS will ultimately be diagnosed with MS. Objective: To explore whether family history of MS or other autoimmune disorders helps distinguish children destined for MS from those with a monophasic CNS illness. Methods: As part of a 23-site prospective, observational Canadian Pediatric Demyelinating Disease Study, genetic-epidemiology data was acquired by a genetic counsellor through standardized interviews of biological parents, legal guardians, and/or grandparents of children presenting with ADS. Data collected included prenatal factors and demographic and health (particularly autoimmune diseases including rheumatoid arthritis, juvenile diabetes, lupus erythematosus, IBD and thyroiditis) information on the proband, proband's parents and grandparents. Results: Of 302 enrolled children with confirmed demyelination, 216 (71.5%) have completed the genetic interview to date and 47 of these (21.8%) have been diagnosed with MS (conferred by second clinical demyelinating attack or by MRI confirmation of dissemination in time). Among children with monophasic ADS, 107 (63.7%) reported a family history of autoimmune disease (AID). This is lower than for those with MS, where 36 (76.6%) had a family history of AID (p=0.089). A family history of IBD was most associated with a diagnosis of MS (p=0.045) followed by rheumatoid arthritis (p=0.069). Of children with MS, 8 (17.0%) reported a family history of MS. This is similar to children with monophasic ADS where 26 (18.8%, p=0.79) described a family history of MS. Conclusions: Our previous work has described familial autoimmune disease in children with ADS, however this new data provides insight into the difference in familial AID between children with monphasic ADS and those with MS. Preliminary results suggest that children with MS have a higher familial rate of AID. Of note, the strongest association of AID for patients with MS was IBD. The increased frequency of IBD in families of children with MS suggests that despite the involvement of different organ systems, there is a shared genetic factor increasing one's risk for AID. Future studies are ongoing exploring possible susceptibility loci in children with IBD and MS.

392F

The AcB61 and AcB60 recombinant congenic strains of inbred mice: susceptibility and resistance to a murine model of human Typhoid fever. *S.C. Beatty[1,2], M. Roy[3], J.C. Loredo-Osti[4], D. Malo[5].* 1) Complex Traits Group, McGill University, Montreal, QC; 2) Human Genetics, McGill University, Montreal, QC; 3) Department of Equine Internal Medicine, University of Calgary, Calgary, AB; 4) Department of Mathematics and Statistics, Memorial University, St. John's, NFLD; 5) Department of Medicine, McGill University, Montréal, QC.

Survival following acute infection with *Salmonella* Typhimurium, a systemic infection modelling human typhoid fever, is dependent upon a coordinated and genetically complex immune response. Previously our laboratory identified a locus, *Ity5* (chr2) in (AcB61x129S6)F2 mice, linked to a susceptibility to *Salmonella* infection of unknown aetiology.Using a fully informative F2 intercross we have validated the presence of the *Ity5* locus in (A/Jx129S6)F2 mice. In addition to *Ity5* we have a identified a second locus linked to resistance to Salmonella infection, *Ity8* (chr15) in (AcB60xDBA/2J)F2 mice. Using genome-wide transcription profiling and fine mapping, informed through the exploitation of bioinformatic resources, we have identified candidate genes underlying the *Ity5* and *Ity8* loci. Using both DNA and RNA sequencing, as well as analysis of gene expression (Q-PCR) we have characterized the impact of positional candidate genes for both *Ity5* and *Ity8* on the host-response to *Salmonella* infection. Through the characterization of the *Ity5* and *Ity8* susceptibility and resistance loci these results provide new insight into the pathogenesis of Salmonella infection and further illuminate the genetic elements of the host immune response to the murine model of Typhoid fever.

393F

Deep sequencing and pooled association testing of 259 candidate genes reveals a role for rare variants associated with durable control of HIV-1 replication. *P.J. McLaren[1, 2], E.A. Stahl[1, 2], M. Rivas[2,3], F. Pereyra[4], N. Gupta[2], B.D. Walker[4], P.I.W. de Bakker[1,2,5], The International HIV Controllers Study.* 1) Department of Medicine, Harvard Medical School, Boston, MA; 2) Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA; 3) Oxford Centre for Diabetes, Endocrinology & Metabolism, Oxford, UK; 4) Ragon Institute of MGH, MIT and Harvard, Charlestown, MA; 5) Department of Medical Genetics, Division of Biomedical Genetics, University Medical Center, Utrecht, Netherlands.

Background: Low-frequency variants are poorly interrogated by GWAS, though likely influence complex phenotypes. A recent GWAS of HIV control demonstrated a major role for common variants within the MHC and no significant associations elsewhere. Here we used pooled next-generation sequencing of select genes to test rare sequence variants for association to HIV control.

Methods: We sequenced the coding exons of 295 candidate genes known to impact the HIV life cycle, host response to HIV or with suggestive GWAS P-values in 499 HIV controllers (virus load <2,000 copies per mL) and 500 progressors (median virus load >50,000 copies per mL) of European ancestry. We used principal components from GWAS data to match cases to controls. We constructed 40 case pools and 40 control pools of 12 or 13 individuals each, captured their exons by in-solution hybridization and sequenced them on the Illumina HiSeq. Variants were called using the Syzygy algorithm and filtered based on several quality control heuristics. We used likelihood ratio tests to assess single variants, and permutation-based burden tests per-gene and across all genes to detect differences in variant frequencies between controllers and progressors.

Results: Sequence data was obtained for ~450 Kb of exon target with a median coverage of >250x. After variant calling and quality control, 2,005 SNPs were analyzed. No single variant reached study-wide significance using likelihood ratio tests ($P>1x10^{-4}$). Burden tests (including C-alpha and variable threshold tests) of variants across the entire gene set showed significant association (study-wide permutation P-values < 0.01) and an enrichment of variants occurring exclusively in HIV controllers ($P<0.05$). Three genes, *SOCS1*, *ACCN1* and *WWOX* (implicated in HIV replication by genome-wide transcriptional profiling, or with suggestive GWAS P-values) achieved significant burden test results after correcting for multiple comparisons ($P<3x10^{-4}$ for all).

Conclusions: Next-generation sequencing of candidate genes shows differential proportions of rare variants in HIV controllers compared to progressors. This analysis implicates novel non-MHC genetic factors in HIV control, highlighting a role for low-frequency variants not detected by GWAS. These encouraging preliminary findings are now being replicated.

394F

Polymorphisms in *MC3R* promoter and *CTSZ* 3`UTR are associated with tuberculosis susceptibility. *M. Möller[1], L.A. Adams[1], A. Nebel[2], S. Schreiber[2], L. van der Merwe[3,4], P.D. van Helden[1], E.G. Hoal[1].* 1) DST/NRF Centre of Excellence for Biomedical Tuberculosis Research / MRC Centre of Molecular and Cellular Biology, Faculty of Health Sciences, Stellenbosch University, Western Cape, South Africa; 2) Institute of Clinical Molecular Biology, Christian-Albrechts-University, Kiel, Germany; 3) Biostatistics Unit, Medical Research Council, Tygerberg, South Africa; 4) Department of Statistics, University of Western Cape, South Africa.

We have validated the association of two genes on chromosome 20q13.31-33 with tuberculosis susceptibility. A previous genome-wide linkage study performed by Cooke *et al* identified the genes melanocortin-3-receptor (*MC3R*) and cathepsin Z (*CTSZ*) as possible candidates in tuberculosis susceptibility. MC3R has been implicated in obesity studies and is known to play a role in many biological systems including the regulation of energy homeostasis and fat metabolism. CTSZ has been detected in immune cells, such as macrophages and monocytes and it is hypothesized that the protein may play a role in the immune response. In our South African population, a case-control study confirmed the previously reported association with a SNP in *CTSZ* and found an association in *MC3R* with a SNP not previously implicated in tuberculosis susceptibility. Six single nucleotide polymorphisms (SNPs) in *MC3R* and eight in *CTSZ* were genotyped and haplotypes were inferred. SNP rs6127698 in the promoter region of *MC3R* (cases = 498, controls = 506) and rs34069356 in the 3`UTR of *CTSZ* (cases = 396, controls = 298) both showed significant association with tuberculosis susceptibility ($p = 0.0004$ and <0.0001, respectively) indicating that pathways involving these proteins, not previously researched in this disease, could yield novel therapies for tuberculosis.

395F

Investigating single nucleotide polymorphisms and interactions of candidate genes involved in susceptibility to tuberculosis in a South African population. *C. Wagman, M. Möller, E.G. Hoal.* Division of Molecular Biology and Human Genetics, MRC Centre for Molecular and Cellular Biology and the DST/NRF Centre of Excellence for Biomedical TB Research, Faculty of Health Sciences, Stellenbosch University, Tygerberg 7505, South Africa.

Tuberculosis (TB) is an infectious disease, primarily caused by the bacterium *Mycobacterium tuberculosis*, which is estimated to have infected a third of the world's population and has resulted in a worldwide incidence of more than 8 million cases per year and a mortality rate of 2 million individuals each year. In developing countries, such as South Africa, TB has been a public health problem for a long time. According to the latest World Health Organisation report, South Africa is ranked 5th in the world with regards to TB incidence. Although a large portion of the population is infected with *M. tuberculosis*, only 10% of those infected will develop active disease while the majority of the population effectively control the bacterium. Many studies have shown that genetic factors are involved in the disease and numerous studies have identified host susceptibility genes in TB. The Macrophage Scavenger Receptor with Collagenous Structure (*MARCO*); Surfactant Protein D (*SP-D*); Toll-like Receptor 2 (*TLR2*) and *CD14* genes have been shown to play crucial roles in TB. We investigated the role of ten single nucleotide polymorphisms (SNPs) in the *MARCO* (rs6761637; rs1318645; rs3943679 and rs2119112) and *SP-D* (rs1923537; rs1923538; rs1923539; rs2243639; rs721917 and rs2255326) genes in susceptibility to TB in the South African Coloured population. This population is genetically diverse, as there is genetic input from several populations, and this makes it possible to evaluate the association of unique markers with TB in a single population. A case-control association study was done which included approximately 400 pulmonary TB patients and 400 healthy controls. Samples were genotyped with ARMS-PCR and TaqMan™ SNP genotyping assays and genotypes were confirmed by sequencing. Gene interaction analysis was performed with SNPs from *MARCO*, *SP-D*, *CD14* and *TLR2*. The R statistical program was used to analyse results, which were all adjusted for age and sex. We found that five SNPs (rs1923537; rs2255326; rs1318645; rs3943679; rs2119112) were associated with TB susceptibility in the Coloured population. A number of haplotypes occurred more frequently in cases than controls and there was an indication that SNPs from genes interacted with SNPs from the same gene as well as the other genes in question. These results suggest that variants in *MARCO* and *SP-D* and interactions between these genes and *CD14* and *TLR2* may affect susceptibility to TB in the South African population.

396F

QTL analysis, pathway analysis and consomic mapping show genetic variants of Tnni3k, Fpgt or H28 control susceptibility to viral myocarditis. *S. Wiltshire, G.A. Leiva-Torres, S.M. Vidal.* Human genetics, Mcgill University, Montreal, QC, Canada.

Coxsackievirus B3 (CVB3) infection is the most common cause of viral myocarditis. The pathogenesis of viral myocarditis is strongly controlled by host genetic factors. While certain indispensible components of immunity have been identified, the genes and pathways underlying natural variation between individuals remain unclear. Previously, we isolated the Viral Myocarditis Susceptibility 1 (Vms1) locus on chromosome 3, which influences pathogenesis. We hypothesized that confirmation and further study of Vms1 controlling CVB3-mediated pathology, combined with pathway analysis and consomic mapping approaches, would elucidate both pathological and protective mechanisms accounting for natural variation in response to CVB3 infection. Vms1 was originally mapped to chromosome 3 using a segregating cross between susceptible A/J and resistant B10.A mice. To validate Vms1, C57BL/6J-Chr 3A/NaJ (CSS3) were used to replicate susceptibility compared to resistant C57BL/6J (B6). A second segregating F2 cross was generated between these confirming both the localization and effects of Vms1. Microarray analysis of the four strains (A/J, B10.A, B6 and CSS3) illuminated a core program of response to CVB3 in all strains that is comprised mainly of interferon stimulated genes. Microarray analysis also revealed strain specific differential expression programs and genes that may be prognostic or diagnostic of susceptibility to CVB3 infection. A combination of analyses revealed very strong evidence for the existence and location of Vms1. Differentially expressed pathways were identified by microarray and candidate gene analysis revealed Fpgt, H28 and Tnni3k as likely candidates for Vms1. .

397F

Genetic Analysis of a Rat Model of Aerobic Capacity and Aging. *Y. Ren[1], L.G. Koch[2], S.L. Britton[2], N. Qi[3], C.F. Burant[3], M.K. Treutelaar[3], J. Li[1].* 1) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 2) Department of Anesthesiology, University of Michigan, Ann Arbor, MI; 3) Department of Internal Medicine, University of Michigan, Ann Arbor, MI.

We have established two outbred lines of rat for studying the biological basis of running capacity and metabolic traits. The two lines, high capacity runners (HCR) and low capacity runners (LCR), were developed by selectively breeding for high and low inborn endurance running capacity. HCRs and LCRs are maintained as heterogeneous cohorts using a rotational mating scheme to minimize inbreeding. After 27 generations, the two lines differed by 7-fold in running capacity, and diverged in many other physiological measures and health indicators, including metabolic syndrome, body weight, oxygen consumption, and life span. Our goal is to use HCR/LCR as a model system for dissecting the genetic and functional mechanisms of endurance, metabolism, and accelerated aging. We genotyped genomic DNA for 142 rats representing the breeding members of both lines at the 5th, 14th, and 26th generations by using the Affymetrix Rat Mapping 10K GeneChips that cover >10K SNPs. Principal Component Analysis (PCA) reveals an increase in genetic divergence between the two lines over the three generations analyzed. The clusters also became tighter over time, reflecting increasing levels of fixation. As expected, genomewide heterozygosity decreased over time in both lines, ranging from 0.37 to 0.34 in HCRs, and 0.37 to 0.36 in LCRs. The coefficient of inbreeding showed an increasing trend across generations. The heritability of running capacity was much higher in the HCR line for two possible reasons: (1) the low runner trait approaches its lower limit in early generations, reducing the effectiveness of selection; and (2) more genetic loci exist for high aerobic capacity and were progressively exposed to selection as “buffering” loci came to fixation. Haplotype structures showed significant differences between the HCR and LCR lines, supporting our hypothesis that different alleles are differentially enriched among the two divergent lines. Ongoing analysis will compare the patterns of genetic drift and selective sweep in both lines, and determine the heritability of related traits such as body weight and aging phenotypes, across 28 generations. An animal model such as HCR/LCR has the inherent advantage of a known genealogy, controlled environment, accessibility of multiple tissues for functional analyses, and has the potential to accelerate the understanding of polygenic human traits, including aging and late-life diseases.

398F

Conditioning on associated alleles increases power to detect linkage and gene-gene interaction. *B. Corso[1,2], R.L. Subaran[2,3], D.A. Greenberg[2,3].* 1) Applied Health Science, University of Pavia, Pavia, Italy; 2) Division of Statistical Genetics - Biostatistics, Columbia University, Mailman School of Public Health; 3) Department of Psychiatric, New York State Psychiatric Institute Columbia - Presbyterian Medical Center.

Background Complex diseases are likely influenced by multiple interacting genes. However, proving that gene-gene interaction exists between loci is difficult. Many existing methods can be computationally intensive and are often underpowered. We tested whether interaction can be reliably established between two epistatically interacting loci (A & B) compared to two loci that independently produce the trait (heterogeneity). We hypothesized that observing only offspring that carry an allele strongly associated with the trait at locus A significantly increases power to detect linkage at locus B compared to analysis where all offspring are observed. Furthermore we predict that such a comparison can yield evidence of an interaction between the two loci. **Methods** We generated linkage data for a disease caused by two separate, epistatically-interacting loci and tested evidence for linkage. A marker allele near disease locus A was in strong linkage disequilibrium (LD) with the disease allele at A. There was no disease-marker association for locus B. We then removed (pruned) all offspring without the locus A-associated marker allele and reanalyzed the dataset for linkage, thus stratifying the data on the presence of the locus A disease allele. For comparison, we did the same two analyses on data simulated under a two locus heterogeneity model where each locus caused disease independently (i.e., no inter-locus interaction). **Results** Under epistasis, when the associated marker is in strong LD (D' > 0.8) with the disease allele at locus A, removal of all offspring not carrying the associated marker *increased* the Hlod score at locus B. Under heterogeneity, this stratification strategy *lowered* the Hlod score at locus B compared to analysis of the unpruned data. We show that if the Hlod score increases by 0.5 or more after pruning, this indicates epistasis with 93% sensitivity over multiple epistatic disease models (DD, DR, RD, RR). When heterogeneity was simulated, the Hlod for the pruned families is significantly lower than for the unpruned data and the average specificity across all disease models is 98%. Sensitivities: RR=100%; DD=82%; DR=91%; RD=96%. Specificities: RR=DD=RD=100%; DR=92%. **Conclusion** We propose that the stratification scheme we tested (1) can increases the power to detect epistatically interacting loci; (2) can, with good specificity and sensitivity, distinguish whether linkage signals are the result of gene-gene interaction or heterogeneity.

399F

Genome-wide meta-analysis of autosomal SNP differences between men and women. *V. Boraska[1,2], Autosomal sex differences meta-analysis group.* 1) Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom; 2) University of Split School of Medicine, Soltanska 2, 21000 Split, Croatia.

Introduction: The male-to-female sex ratio at birth is constant across world populations, ranging between 1.02-1.08 (102-108 male to 100 female live births), with an average of 1.06 for populations of European descent. Sex ratio is considered to be affected by numerous biological and environmental factors, and has been suggested to have a heritable component. The aim of the present study is to investigate the presence of common-allele modest effects at autosomal variants that could explain the observed sex ratio at birth. We also conducted a simulation study to assess the probability of observing significant allele frequency differences at autosomal markers between men and women. Methods: We conducted fixed and random-effects large-scale genome-wide scan (GWAS) meta-analysis across 51 studies, comprising overall 114,863 individuals (61,094 women and 53,769 men) of European ancestry. Allele frequencies were compared between men and women for directly-typed and imputed common (minor allele frequency >0.05) variants within each study. Forward-time simulations for unlinked neutral biallelic loci were performed under the demographic model for European populations. Results and Discussion: We did not detect any genome-wide significant ($p<5x10^{-8}$) autosomal common SNP differences between men and women in the meta-analysis. We also observed no frequency differences between men and women in our simulation of 1.3 million common loci in a cohort matching the study sample. Conclusion: This large-scale investigation across ~115,000 individuals shows no detectable contribution from common genetic variants to the observed skew in sex ratio. The absence of sex-specific differences is useful in guiding genetic association study design, for example when using mixed controls for sex-biased traits.

400F

Additive genetic variation in risk to schizophrenia tagged by common SNPs in an admixed African American population. *T. de Candia[1, 2], S.H. Lee[3], J. Yang[3], P.M. Visscher[3], N.R. Wray[3], M.C. Keller[1, 2], P.V. Gejman[4], D.F. Levinson[5].* 1) Department of Psychology & Neuroscience, University of Colorado at Boulder, CO, USA; 2) Institute for Behavioral Genetics, University of Colorado at Boulder, CO, USA; 3) Queensland Institute of Medical Research, 300 Herston Road, Brisbane, Australia 4006; 4) Department of Psychiatry and Behavioural Sciences, Evanston Northwestern Healthcare Research Institute, Evanston, IL, USA; 5) Department of Psychiatry and Behavioral Sciences, Stanford University, Palo Alto, CA 94304-5797, USA.

Schizophrenia is a highly heritable disorder (~.7 to .8) with a prevalence rate of ~1%, but with prominent variation between populations (McGrath, 2006) . However, to date, few studies have specifically examined heritability in African American (AA) populations. We used a recently developed method to determine the proportion of additive genetic variance in liability to schizophrenia tagged by common SNPs in the AA (n=1286 cases and 973 controls) Molecular Genetics of Schizophrenia sample. We also undertook a number of additional analyses, including the exclusion of SNPs that predict African ancestry, to explore the impact, if any, of population stratification on these results. Using 760803 genome-wide SNPs that passed rigorous quality control procedures, we estimate the variation in liability to schizophrenia associated with these SNPs after controlling for potential confounds to be at least .25 (SE .13). These results are in line with previous studies using samples of European Ancestry (EA). Given that common SNPs capture a considerable proportion of heritability, this indicates that common causal variants are likely important factors in the etiology of schizophrenia. Reference: McGrath JJ. Variations in the incidence of schizophrenia: data versus dogma. Schizophr Bull 2006;32:195-7.

401F

Multi-ethnic studies in complex disease: towards better insight in disease mechanisms. *E. Festen[1,2], J.Y. Fu[2], C. Wijmenga[2].* 1) Dept of Gastroenterology, UMCG, Groningen, Groningen, Netherlands; 2) Dept of Genetics, UMCG, Groningen, Groningen, Netherlands.

Genome-wide association studies (GWAS) have become a powerful tool to identify genetic variants underlying complex human traits and have greatly improved our understanding of complex disease. Although the history of GWAS is still short, the course of the development of GWAS seems to consist of four consecutive stages: 1) single GWAS performed per research institute with samples taken exclusively from Caucasian populations; 2) merging of multiple GWAS in meta-analyses by research institutes joining forces in international consortia; samples are still taken from Caucasian populations; 3) GWAS on non-Caucasian populations; 4) establishment of global consortia for GWAS on multi-ethnic groups simultaneously. Currently GWAS are heading from stage 1 and 2, to stage 3 and 4. Complex traits are characterized by marked genetic heterogeneity. This heterogeneity poses a major challenge in complex disease research, but also yields an opportunity for multi-ethnic mapping. The recent advance in GWAS on East Asian populations allows us to compare the genetic architecture of complex traits between Caucasians and East Asians. Based on this comparison we construct a proposal on analysis strategies for multi-ethnic GWAS. For this study we selected four traits for which we extracted the risk loci reported from Caucasian and East Asian GWAS from the GWAS database (www.genome.gov/gwastudies). We analyzed 34 susceptibility loci for type 2 diabetes, 21 loci for systemic lupus erythematosus, 47 loci for ulcerative colitis and 157 loci for height. For each trait we observed that 50%-100% of the susceptibility loci identified in East Asians were shared with Caucasians. However, we also observed that 25%-55.6% of the association signals at the shared loci were independent between populations (R2< 0.2 in each population). This suggests that diseases etiology is common between populations but risk variants can be population-specific. The population-specific variants could result from natural selection, genetic drift and from recent mutation after population immigration. When analyzing multi-ethnic GWAS population-specific variants will significantly reduce the power of the study. Therefore we propose gene-based meta-analysis to pool population-specific signals at the shared loci. Gene-based meta-analysis is a complementary approach to signal variant-based meta-analysis that is also targeted at shared association signals.

402F

Common variants show predicted polygenic effects on height in the 1% tails of the distribution, except in extremely short individuals. *OL. Holmen[1], Y. Chan[2,3,4], A. Dauber[3,4], L. Vatten[5], AS. Havulinna[6], F. Skorpen[7], K. Kvaløy[1], K. Silander[6,8], T. Nguyen[4], C. Willer[9], M. Boehnke[9], M. Perola[6,8,10], A. Palotie[2,8,11,12], V. Salomaa[6], K. Hveem[1], TM. Frayling[13], JN. Hirschhorn[2,3,4], MN. Weedon[13].* 1) HUNT Research Centre, Department of Public Health and General Practice, Norwegian University of Science and Technology, Levanger, Norway; 2) Harvard Medical School, Department of Genetics, Boston, Massachusetts, United States of America; 3) Broad Institute, Cambridge, Massachusetts, United States of America; 4) Children's Hospital, Boston, Massachusetts, United States of America; 5) Department of Public Health and General Practice, Norwegian University of Science and Technology, Trondheim, Norway; 6) National Institute for Health and Welfare, Helsinki, Finland; 7) Department of Laboratory Medicine, Norwegian University of Science and Technology, Trondheim, Norway; 8) Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland; 9) Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan, United States of America; 10) Estonian Genome Project, University of Tartu, Tartu, Estonia; 11) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom; 12) Department of Medical Genetics, University of Helsinki and University Central Hospital, Helsinki, Finland; 13) Genetics of Complex Traits, Peninsula Medical School, University of Exeter, Exeter, United Kingdom.

Common genetic variants have been shown to explain a fraction of the inherited variation for many common diseases and quantitative traits, including height, a classic polygenic trait. The extent to which common variation determines the phenotype of even highly heritable traits such as height is uncertain, as is the extent to which common variation is relevant to individuals with more extreme phenotypes. To address these questions, we genotyped SNPs at ~160 loci previously associated with height in 1,214 individuals from the top and bottom extremes of the height distribution (tallest and shortest ~1.5%), drawn from ~78,000 individuals from the HUNT and FINRISK cohorts. We found that common variants still influence height at the extremes of the distribution: more common variants were nominally associated with height in the expected direction than is consistent with chance ($p < 5 \times 10^{-28}$) and the odds ratios in the extreme samples were broadly consistent with the effects estimated previously in population-based data. To examine more closely whether the common variants have the expected effects, we calculated a weighted allele score (WAS), which is a weighted prediction of height for each individual based on the previously estimated effect sizes of the common variants in the overall population. The average WAS was consistent with expectations in the tall individuals, but was not as extreme as expected in the shortest individuals ($p<0.006$), indicating that some of the short stature is explained by factors other than common genetic variation. The discrepancy was more pronounced ($p<10^{-6}$) in the most extreme individuals (height <0.25 percentile). In additional simulations, we showed that the observed WAS was consistent with a number of different models incorporating rare genetic variants (or rare non-genetic factors) that decrease height. In conclusion, our data suggest that common genetic variants are associated with height at the extremes as well as across the population, but that additional factors start to predominate around the shorter 0.25% extreme.

403F

A comprehensive assessment of the power to detect low-frequency risk alleles through imputation in genome-wide association studies of Northern European populations. *B. Howie[1], C. Fuchsberger[2], H.M. Kang[2], A. Mahajan[3], A. Morris[3], M. Stephens[1] on behalf of the 1,000 Genomes Project and the Genetics of Type 2 Diabetes (GoT2D) Consortium.* 1) Department of Human Genetics, University of Chicago, Chicago, IL, USA; 2) Department of Biostatistics, University of Michigan, Ann Arbor, MI, USA; 3) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK.

Through a combination of large disease cohorts and improvements in DNA sequencing and genotyping technologies, it is now possible to extend genome-wide association studies (GWAS) to variants that exist at low frequency (0.5-5%) in a population. One affordable way to test such variants is to sequence them in hundreds of samples, then use statistical imputation methods to extrapolate them to thousands of GWAS samples. A major challenge in designing and interpreting this kind of study is to know how much power is lost through imperfect imputation. Researchers have tried to address this question through cross-validation in existing GWAS datasets, but such comparisons are hard to generalize because of SNP ascertainment bias and limited sampling of the genome. Here, we use data from the GoT2D Consortium to overcome these limitations and provide the first comprehensive assessment of imputation power for low-frequency variants throughout the genome. GoT2D is investigating the genetic architecture of type 2 diabetes (T2D) through a combination of low-coverage shotgun sequencing, deep exome sequencing, and 2.5M SNP array genotyping of 1,325 cases and 1,325 controls of Northern European ancestry. We use this dataset to evaluate imputation accuracy by masking GoT2D genotypes and imputing them from a reference panel of 762 European haplotypes from the 1,000 Genomes Project (1kGP). We find that the 1kGP haplotypes, which were estimated statistically from low-coverage sequence data, are nearly as effective for imputation as high-quality haplotypes obtained from array genotypes and family transmission data for the same samples. We observe high imputation coverage among common SNPs and a nearly linear relationship between coverage and MAF at low-frequency SNPs, where imputation from a 2.5M array captures (at R^2>0.8 between true and imputed genotypes) 76% of SNPs with MAF=5% and 41% of SNPs with MAF=1%. For SNPs with MAF<1%, coverage drops sharply to 17%, which actually overestimates genome-wide coverage because SNP discovery is incomplete at low frequencies; we are actively working to quantify this effect. We also quantify the ascertainment bias of using GWAS SNP chips in cross-validations: relative to SNPs on a 2.5M array, the mean R^2 between true and imputed genotypes at non-chip SNPs drops by 0.03 for MAF>5%, 0.08 for MAF= 3%, and 0.25 for MAF<1%. These results provide valuable benchmarks that we will update as larger reference panels are generated by 1kGP and GoT2D.

404F

Dissecting the genetic and disease heterogeneity in complex traits: Crohn's Disease as an example. *N. Maniatis[1], H. Elding[1], W. Lau[1], T. Andrew[2], D. Swallow[1].* 1) RESEARCH DEPARTMENT OF GENETICS, EVOLUTION AND ENVIRONMENT, UNIVERSITY COLLEGE LONDON, LONDON, United Kingdom; 2) Department of Twin Research & Genetic Epidemiology, Kings College London, London, United Kingdom.

The very small contribution of many genes that has been reported so far in GWA studies for complex diseases has led some to believe that GWA studies have been an expensive waste of time. However for many complex traits, it is very likely that different risk genes are involved in different patients. Dissecting this genetic heterogeneity is thus of utmost importance for the identification of all the genes involved. Here we report an approach to this problem and also demonstrate methodological progress and the importance of using high-resolution genetic maps in identifying disease loci. We employ a multimarker approach to analyse many SNPs simultaneously within a genomic window. This takes directly into account the underlying structure of Linkage Disequilibrium (LD) by using genetic distances from LD maps rather than physical locations, and provides an estimated location of the causal variant. In addition, we genetically and phenotypically stratify the data to demonstrate independent effects. Here we give as an example our work on Crohn's Disease (CD) and on chromosome 16q that harbours *NOD2* (the first gene that was identified for CD using family studies). Several GWA studies had only detected *NOD2* despite the fact that linkage is still observed on 16q for families who do not have the common *NOD2* mutations. Using the WTCCC1 data on CD, we show genetic heterogeneity within the *NOD2* region itself and demonstrate the independent involvement of the neighbouring gene *CYLD* which is replicated with striking precision using an independent GWA data. *CYLD* encodes a deubiquitinating enzyme known to be down-regulated in CD. We also report for the first time significant association with the *CDH1/CDH3* genes encoding cadherins, and the *IRF8* gene-region, genes also known to be involved in inflammation/immune dysregulation. These two signals are also powerfully replicated only when we sub-classify the disease phenotype and/or ancestry/ethnicity. We thus show that disease phenotype and ancestry sub-classification can help to dissect the genetics of complex inheritance, and we demonstrate its impact on association mapping. This demonstrates that addition of phenotypic and other data to DNA banks is much more important than increasing their sample size. These results and the striking replication not only bring to light new genes involved in CD but also show how the complexity of complex inheritance can be understood, showing a promising way forward in disease-gene mapping.

405F

Genome-wide methylation profiling of Chronic Rhinosinusitis. *L. Mfuna Endam[1], A. Filali-Mouhim[1], C. Divoy[1], V. Tardif[1], M. Desrosiers[1,2].* 1) Department of Otolaryngology, Hôtel-Dieu Hospital, Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Montreal, PQ, Canada; 2) Department of Otolaryngology-Head and Neck Surgery, Montreal General Hospital, McGill University, Montreal, PQ, Canada.

Background: Chronic Rhinosinusitis (CRS) is a common inflammatory disorder of the upper airway affecting 10% of Americans. The etiology of CRS remains unknown but is believed to result for deregulated responses to bacterial pathogens on the sinus mucosa. In support of this, our group has identified a marked hyporesponsiveness to bacterial pathogens in cultured primary epithelial cells isolated from CRS patients. (Divoy, 2011) This downregualtion is not present on PBMCS isolated from the same patients, suggesting that a localised effect, possibly from a bacterial toxin. That this effect remains present in cultured cells suggest a potential epigenetic modification in genes of the immune response. Objective: To wished to verify if epigenetic changes are present in cultured primary epithelial cells obtained from CRS subjects by evaluating the methylation status of whole genome in primary nasal epithelial cells isolated from patients with CRS. Methods: Primary nasal epithelial cells were isolated from 11 patients with CRS and 8 controls (CTL) and raised until differentiated in an air-liquid interface. DNA was extracted using QIAmp DNA Mini kit and genome-wide DNA methylation was performed using Illumina Infinium HumanMethylation450 BeadChip. The level of methylation for each CpG site was measured by a quantity (Beta) ranging from 0 (no cytosines methylated) to 1 (all cytosines methylated). To identify differentially methylated CpG sites between the CRS and CTL individuals we used a t test. DeltaBeta was calculated as the average of beta values for CRS group minus the average of beta values for CTL group. We selected loci with |DeltaBeta|>0.2 and t test p value <0.05. Results: In CRS, hypermethylation was detected at 344 loci, while in CTL, hypermethylation was obtained in 50 loci. Pathways analyses using Ingenuity software implicated networks in cell signalling and cell death. Implicated functions were immunological disease, endocrine disorders, cell morphology, cell development, and tissue development and nervous system development and function. Conclusions: Methylation of genes of immune function is present in epithelial cells isolated from CRS subjects and may be responsible for the hyporesponsiveness to bacteria identified in these subjects. Correlation with functional data from expression studies will help assess functional impact of these changes.

406F

Finding all statistically significant genetic interactions in case-control datasets without using a supercomputer. *S. Prabhu, I. Pe'er.* Computer Sci, Columbia Univ, New York, NY.

The hundreds of genetic variants found by GWAS so far have told us very little about the genetic architecture of common diseases. These diseases are likely to possess a complex genetic etiology - harboring gene-gene interactions, among other possibilities. Yet, statistical and computational hurdles have limited the systematic characterization of interactions in genome-scale datasets. Existing approaches fall into 2 categories: either extremely time consuming (exhaustive tests) or incomplete (sub-genome scale). We present a powerful statistical technique - Probably complete and Approximately Correct (PAC) sampling - which overcomes the computational burden that has plagued interaction methodology so far. PAC sampling can exhaustively scan for interactions between millions of SNPs, without relying on incomplete information (like marginal association) to mitigate the search problem. Instead, we reduce our computational footprint by focusing only on those interactions which will pass the multiple-testing burden. In fact, our technique is several orders of magnitude faster than its contemporaries. For datasets of a million SNPs it is ~20X faster for all pairwise tests and ~200X faster for all 3-way tests, when compared to exhaustive search methods that are currently in use. To the best of our knowledge, ours is the only method that has the potential to comprehensively scan for 3-way interactions between millions of SNPs in feasible amounts of time. Most importantly, PAC sampling can provide guarantees of arbitrarily high power (e.g. 99% certain) that all statistically significant interactions in the dataset will be found. We have implemented our algorithm in java, and the software - SIXPAC - is available on our webpage (www.cs.columbia.edu/~snehitp). We ran SIXPAC on 7 common diseases (WTCCC data), scanning for interactions that cleared the Bonferroni significance threshold (1e-11). Our test statistics do not suffer from undue overinflation ($\lambda \sim 1.01$ to 1.06) on the 7 datasets. We observed a distinct landscape of interaction for each disease, and report finding (at least) 1 interaction in CAD, 1 in T1D, 2 in BD, 4 in T2D, 8 in RA, 4 in CD and 3 in HT. Crohns interactions have been validated, while others are as yet untested on other datasets. All interactions have been further confirmed using a regression based ANOVA test (interaction term pval <1e-9), verifying that the interaction effect cannot be attributed to marginal SNP associations.

407F

Screening for pathogenic sequence variants in early-onset and familial Crohn's disease. *N.J. Prescott[1], B. Lehne[1], K. Harrison[1], J. Knight[1], M.A. Simpson[1], P. Green[1], J. Sanderson[2], J.C. Mansfield[3], C.M. Lewis[1], M.E. Weale[1], T. Schlitt[1], C.G. Mathew[1].* 1) Department of Medical and Molecular Genetics, King's College London School of Medicine, London, UK; 2) Department of Gastroenterology, Guy's & St Thomas' NHS Foundation Trust, St Thomas' Hospital, London, UK; 3) Department of Gastroenterology and Hepatology, University of Newcastle upon Tyne, Newcastle Upon Tyne, UK.

Genome-wide association studies (GWAS) have delivered significant discoveries of common genetic variants that increase susceptibility to Inflammatory Bowel Disease. However, much of the genetic contribution to Crohn's disease (CD) remains unaccounted for. A possible source for this is rare disease-causing variants undetectable by GWAS, but with stronger genetic effects. Here, we have used massively parallel sequencing to attempt to elucidate such rare causal variation. We identified 531 candidate genes either located in GWAS hit regions or identified by gene ontology and network-based analyses as interacting with these genes. CD patients were selected for early-onset disease and/or a family history to enrich for causal mutations of higher penetrance. DNA samples from 475 CD cases and 481 controls were pooled and pool size was optimised to 24 using known individual genotypes at 150 SNPs. Coding exons and proximal promoters (1.2Mb total) were enriched from genomic DNA using a custom liquid capture array and then sequenced on an Illumina GAIIx. Variants were called from sequence reads using stringent criteria: base quality >20 per pool, variant seen in 3 or more pools at 1/n of reads per locus per pool (n = pooled chromosome number). We identified 2561 variants, 316 of which were novel and included 118 missense changes and 4 nonsense mutations. We performed variant-level and gene-level analyses, in which allele counts for individual variants, or combined allele counts for all rare variants (control MAF<5%) in a gene, were compared in cases and controls. We replicated associations with known CD risk variants NOD2 p.R702W, IRGM p.L105L, and ATG16L1 pT300A, and observed 2 rare protective variants in IL23R, p.G149R and p.V362I recently reported in a Belgian cohort (Momozawa et al 2011). In the top 20 ranked hits, the ratio of risk:protective effects was high (20:0 in rare variants, 14:6 in genes), despite ratios overall being closer to 1:1 (691:536 in all rare variants, 222:200 in all genes). 6 rare variants, 5 common variants and 5 genes survived Bonferroni correction for multiple tests. In 4 genes the variants conferred increased risk and in one they were protective. Our analyses are consistent with a contribution of rare variants to influencing risk in Crohn's disease. Follow-up studies of candidate genes and variants in large case control samples will be required to further validate their role in pathogenesis.

408F

Joint Ancestry and Association Testing in Admixed Individuals. *D. Shriner, A. Adeyemo, C. Rotimi.* National Human Genome Research Institute, Bethesda, MD.

Population structure is detectable in essentially all human populations given sufficiently large samples and dense panels of genomic markers. In genome-wide association studies, many techniques exist to control for population stratification resulting from analysis of multiple, ancestrally homogenous samples. For admixed samples, it is possible to test for both ancestry effects via admixture mapping and genotypic effects via association mapping. Here, we describe a joint test of ancestry and association for a sample of unrelated, admixed individuals. We first perform high-density admixture mapping using local ancestry. We then perform association mapping using stratified regression, wherein for each marker genotypes are stratified by local ancestry. In both stages, we use generalized linear models providing the advantage that this test can be used with any phenotype distribution (*e.g.*, binary or quantitative traits) with an appropriate link function. To define the alternative densities for admixture mapping and association mapping, we describe a method based on autocorrelation to empirically estimate the testing burdens of admixture mapping and association mapping. We then describe a joint test that uses the posterior probabilities from admixture mapping as prior probabilities for association mapping, capitalizing on the reduced testing burden of admixture mapping relative to association mapping. The sample size required to reach genome-wide significance via association mapping is reduced by a minimum of 63.7% given genome-wide significance via admixture mapping. We illustrate the gain in power through analysis of fasting plasma glucose among 922 unrelated, non-diabetic, admixed African Americans from the Howard University Family Study. We detected loci at 1q24 and 6q26 as genome-wide significant via admixture mapping. Using the joint test, we resolved the 1q24 signal into two intergenic regions. One region, upstream of the gene *FAM78B*, contains three binding sites for the transcription factor PPARG and one binding site for HNF1A, both previously implicated in the pathology of type 2 diabetes. The fact that both loci were discovered via admixture mapping may provide novel insight into the genetic architecture of fasting plasma glucose in persons of African ancestry.

409F

Unveiling and detecting the hidden heritability of complex disease genes. *G. Thomson.* Dept Integrative Biol, Univ California, Berkeley, CA.

Confirmed GWAS SNP associations with complex diseases show weak effects and explain little of the disease heritability even when many disease predisposing genes have been identified. Explanations for this encompass: heritability is overestimated; overly stringent statistical requirements; unidentified structural and rare variants; and predisposing variants are not in sufficient linkage disequilibrium (LD) with the SNPs studied. While all are potentially contributing factors, the aim of this study was to investigate a simpler overriding solution: Does the multi-locus nature of complex diseases itself contribute to this phenomenon, and if so by how much, and how to take account of this? A spectrum of multi-locus models of disease susceptibility, specifying *within* and *between* locus effects, were analyzed. *Except for the case of multiplicative penetrance values between loci, all models show decreasing marginal single locus odds ratios (ORs) and heritability with increasing numbers of disease loci.* This follows since individuals *not* carrying the risk variant at a single locus under study have increased risk when they carry disease predisposing genotypes at other loci. In many cases the resulting marginal effects correspond to those in the range of validated, albeit weak, single SNP associations. For example, if one gene predisposes to disease (recessive with sporadics, allele frequency 0.5) and has an OR of 4; this exact same disease model will result in marginal ORs of 2.2, 1.4, and 1.2, for corresponding symmetric 3, 10, and 20 gene models (with a between gene additive penetrance model). Thus, measuring only single locus marginal effects, the true heritability may be well hidden. Unmasking of this effect is achieved by considering pairwise, and then progressively increasing, numbers of SNP genotypes simultaneously based on the strongest effects at each step: the ORs, chi-squares, and overall heritability progressively increase beyond the combined marginal effects, and significant heterogeneity is seen in marginal ORs based on the risk level averaged over the other disease predisposing loci. Concomitant study of significant effects of markers in LD with each other reduces the number of SNPs studied at each step. Thus, with appropriate increase in the numbers of SNPs analyzed simultaneously, including effects that do not reach marginal single locus genome wide significance, the heritability and penetrance values are much higher and closer to true values.

410F

An Assessment of the Individual and Collective Effects of Variants on Height Using Twins and a Developmentally-Informative Study Design. *S.I. Vrieze[1], M. McGue[1], M.B. Miller[1], L.N. Legrand[1], N.J. Schork[2], W.G. Iacono[1].* 1) University of Minnesota, Minneapolis, MN; 2) The Scripps Translational Science Institute The Scripps Research Institute 3344 North Torrey Pines Court, Room 306 La Jolla, CA 92037.

In a sample of 3187 twins and 3294 of their parents we sought to replicate association of both individual variants and a genotype-based height score involving 180 common genetic variants with adult height identified recently by the GIANT consortium. We also sought to extend this association in two important ways. First, longitudinal observations on height spanning pre-adolescence through adulthood in the twin sample allowed us to investigate the separate effects of the previously identified SNPs on pre-pubertal height and pubertal growth spurt. We show that the effect of the SNPs identified by the GIANT consortium is primarily on prepubertal height. Only one SNP, rs7759938 in LIN28B, was found to be significantly associated with pubertal growth. Second, we show how using the twin data to control statistically for environmental variance can provide insight into the ultimate magnitude of SNP effects and consequently the genetic architecture of a phenotype. Specifically, we computed a genetic score by weighting SNPs according to their effects as assessed via meta-analysis. This weighted score accounted for 9% of the phenotypic variance in prepubertal height, but 14% of the corresponding genetic variance. Longitudinal samples will be needed to understand the developmental context of common genetic variants identified through GWAS, while genetically informative designs will be helpful in accurately characterizing the extent to which these variants account for genetic, and not just phenotypic, variance.

411F

Whole Exome Sequencing of 42 Crohn's Disease Patients and Identification of Rare Variants associated with Crohn's Disease. *D. Ellinghaus[1], T. Jiang[2,3], B. Stade[1], A. Keller[4], E. Ellinghaus[1], C. Sina[6], Y. Bromberg[5], X. Liu[2], Q. Liu[2], F. Jiang[2,3], P. Rosenstiel[1], S. Schreiber[1,6], A. Franke[1].* 1) Institute of Clinical Molecular Biology, Christian-Albrechts-University, Kiel, Germany; 2) BGI-Shenzhen, Shenzhen, China; 3) Genome Research Institute, Shenzhen University School of Medicine, Shenzhen, China; 4) Department of Human Genetics, Saarland University, Saarbrücken, Germany; 5) Department of Microbiology, School of Environmental and Biological Sciences, Rutgers University, New Brunswick, New York, USA; 6) First Medical Department, University Clinic S.-H., Campus Kiel, Germany.

Crohn's disease (CD) is one of the most important subphenotypes of common idiopathic inflammatory bowel disease (IBD) with high relative risk of siblings to affected individuals (3s ~ 25-42). A recent meta-analysis of six distinct genome-wide association studies (GWAS) established 71 CD susceptibility loci as genome-wide significant. However, the 71 loci described so far account for only a limited amount (~ 23.2%) of CD heritability. This suggests that the more common (>5% allele frequency) GWAS tag SNPs are accompanied by many large effect low-frequency variants. High-throughput sequence capture methods coupled with next generation sequencing (NGS) technologies offer the opportunity to extract almost the complete variation in regions of interest which may help to elucidate the missing genetic contribution to disease susceptibility, in our case CD. We captured the exomes of 42 german CD patients, one HapMap trio and 3 german unrelated healthy control individuals by means of the NimbleGen 2.1M Human Exome Array, and subsequently sequenced on the Illumina Genome Analyzer (GA) platform. In total, 39,370 megabases (Mb) of mappable sequence was generated and was mapped to target region for our 48 individuals, with an average read depth on target of 24.77 per individual exome. On average, approximately 96.92% of targeted bases were successfully covered. We identified 117,957 SNPs in our 48 individuals, including 59,076 coding and splice site SNPs (cSNPs + ssSNPs). On the basis of existing filtering strategies that had been demonstrated to work well for monogenic disorders, we developed a strategy approach that incorporates association results from the recent CD meta-analysis in combination with previously developed in silico mutation effect prediction algorithms. Selected rare variants were then subjected to a replication phase using large and independent case-control collections. The replication results will be presented at the conference.

412F

Is CADPS2 involved in autism and intellectual disability? *G. Tortora[1], P. Magini[1], S. Miccoli[1], E. Bacchelli[2], F. Minopoli[1], A. Battaglia[3], L. Mazzone[4], G. Romeo[1], E. Maestrini[2], E. Bonora[1], C. Graziano[1].* 1) U.O. Genetica Medica, Policlinico S Orsola-Malpighi, Università di Bologna, Bologna, Italy; 2) Dipartimento di Biologia Evoluzionistica Sperimentale, Università di Bologna, Bologna, Italy; 3) Stella Maris Istituto Scientifico per la Neuropsichiatria dell'Infanzia e dell'Adolescenza, Calambrone (Pisa), Italy; 4) U.O. Neuropsichiatria Infantile, Dipartimento di Pediatria, Università di Catania, Catania, Italy.

We describe a family where brother and sister show mild intellectual disability, behavior abnormalities and epilepsy, with normal brain imaging and no major dysmorphic features. Karyotype is normal, as well FMR1 analysis in the male patient. Their mother, who died of cancer at 48 years of age, and a maternal aunt are both reported to have behavioral abnormalities, mild intellectual disability, but no seizures. Two maternal uncles are healthy. In both patients, array-CGH analysis revealed a 250 kb deletion of chromosome 7q31.32 encompassing one single gene, CADPS2. The father does not carry the deletion, indicating likely maternal inheritance. The deletion was excluded in one of the maternal uncles. CADPS2 is a calcium binding protein that regulates the exocytosis of synaptic and dense-core vesicles in neurons and neuroendocrine cells. It is a candidate gene for autistic disorders, since it maps in the autism susceptibility region AUTS1 on chromosome 7q31.32, which also corresponds to a candidate region for mood disorders. Knockout mice show an autistic-like phenotype and some ASD (Autism Spectrum Disorders) patients show a possible defect in CADPS2 splicing. The identification of a cryptic deletion encompassing one single gene in 2 siblings affected by intellectual disability gives further evidence of CADPS2 involvement in brain development and function. We performed, therefore, a mutation screening in 2 cohorts of patients with ASD and with intellectual disability, who did not carry any genomic rearrangement as far as array-CGH analysis could detect. Detailed results will be presented concerning the identified single nucleotide variants.

413F

Meta-analysis of Genome-Wide Association Studies Yields New Loci and Insight into the Genetic Architecture of the Extremes of Anthropometric Traits. *S.I. Berndt[1], S. Gustafsson[2], R. Mägi[3], E. Wheeler[4], A. Ganna[2], A. Scherag[5], E.K. Speliotes[6,7], M.F. Feitosa[8], D. Meyre[9,10], K. Monda[11], C.M. Lindgren[3], K.E. North[11,14], M.I. McCarthy[3,12], R.J.F. Loos[13], E. Ingelsson[2] for the GIANT Consortium.* 1) Division of Cancer Epidemiology & Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA; 2) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; 3) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 4) Welcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom; 5) Institute for Medical Informatics, Biometry and Epidemiology, University Duisburg-Essen, Essen, Germany; 6) Department of Internal Medicine, Division of Gastroenterology, and Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, Michigan, USA; 7) Broad Institute, Cambridge, Massachusetts, USA; 8) Division of Statistical Genomics in the Center for Genome Sciences Washington University, School of Medicine St. Louis, Missouri ,USA; 9) CNRS-8199-Institute of Biology, Pasteur Institute, Lille, France; 10) McMaster University, Hamilton, Canada; 11) Department of Epidemiology, University of North Carolina, Chapel Hill, North Carolina, USA; 12) Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Churchill Hospital, Oxford, United Kingdom; 13) MRC Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, United Kingdom; 14) Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, North Carolina, USA.

Anthropometric traits have a strong genetic component with heritability estimates between 40-70% for body mass index (BMI) and 80-90% for height; however, established loci only account for a fraction (1-10%) of the phenotypic variance of these complex traits. It has been hypothesized that the extremes of the distributions for these traits are enriched for genetic loci and may have a distinct genetic architecture compared to the general population. To explore the genetic contribution of the extremes (defined as the upper and lower 5th percentile) of BMI, height, and waist-hip ratio and clinical classes of obesity (including overweight and obesity classes I, II, and III), we conducted meta-analyses of ~2.8 million SNPs from 49 genome-wide association studies of European ancestry totaling from 7,962 cases and 8,106 controls (extreme BMI) to 93,015 cases and 65,840 controls (overweight) for these traits. The most promising loci from each meta-analysis ($P < 5 \times 10^{-6}$) were taken forward for replication. Preliminary analyses of the combined stages identified 10 novel loci ($P < 5 \times 10^{-8}$), including two loci for extreme height (*H6PD, RSRC1*), three for obesity class II (*HS6ST3, AK5/ZZZ3, PCDH8P1*), three for obesity class I (*GNAT2, CACNA1D, MRPS33P4*), and two for overweight (*RPTOR, LOC644456/EIF3FP3*). Several loci for obesity were located near genes expressed primarily in the brain (e.g., *CACNA1D, AK5*), suggesting a neuronal influence, whereas the loci for overweight were near genes involved in other processes, such as mTOR signaling (e.g., *RPTOR*). All of the novel loci discovered for the extremes and obesity classes were nominally associated with the trait as a continuous measure in the general population (N = 123,865) but at a lesser significance level (P range: 0.003 - 1.4×10^{-5}). A polygenetic risk score including all independent SNPs associated with BMI (at different P-value thresholds) revealed that significantly more of the variance was explained for the extremes of BMI and obesity class II than for BMI as a continuous measure in the population (variance explained, 20%, 10% and 5%, respectively), suggesting a greater genetic influence on the extremes. Investigations are underway to evaluate haplotypes and additional signals at known BMI and height loci in the extreme samples to explore allelic heterogeneity. In conclusion, this study identifies additional loci and provides novel insights into the genetic architecture of the extremes of anthropometric traits.

414F

Integrating linkage and association data through a weighted test statistic increases power to detect association. *A.L. Peljto, T.E. Fingerlin.* Epidemiology, University of Colorado Denver, Aurora, CO.

Genome-wide association studies often combine familial cases of disease from linkage studies with sporadic cases to achieve the large samples necessary for detecting low-penetrance alleles. Most such studies include only a single case from each family. Family-based association tests cannot be applied in this case and case-control association tests ignore family-specific allele-sharing information that may be available, which results in an inefficient use of resources and loss of power for this common study design. We propose a weighted test of association that increases power by integrating family-specific linkage information with association data across the genome. We have previously shown that including familial cases based on family-specific allele sharing can increase the disease allele frequency in the cases, even when there is no evidence for linkage. This indicates that there is valuable information in allele sharing scores genome-wide. Common strategies for combining linkage and association information either require significant evidence for linkage, or combine results in an ad-hoc manner by identifying regions with overlapping evidence. By contrast, our method directly combines family-specific linkage and association data by giving more weight to familial cases with evidence for excess allele sharing at the position of the variant being tested. To assess the performance of our method, we simulated genetic data for familial cases, singleton cases, and controls under various genetic models. We then compared our weighted test of association to the standard case-control test using case samples that included varying proportions of familial cases.

RESULTS: We show that our method has increased power compared to the standard method, and that power increases were greater when the proportion of familial cases was higher. Power increases were greatest for models with modest genetic effects where there was no evidence for linkage, as is the case for many complex traits. For example, in a dominant model with a genotype relative risk of 1.25, power increased by 1.6% - 6.2% in samples with 10% - 50% familial cases. Our method provides a framework for tests of association applicable to GWAS and resequencing studies that are more statistically efficient, while leveraging the tremendous resources that have been devoted to recruiting, describing, and genotyping familial linkage cohorts.

415F

A new whole-genome genotyping array of almost 4.5 million SNPs based on data from the 1000 Genomes Project. *M.A. Eberle, J. Stone, L. Galver, M. Hansen, C. Tsan, D. Segale.* Dept Bioinformatics, Illumina, Inc, San Diego, CA.

The 1000 Genomes Project is creating a comprehensive catalogue of human variation down to ~1% minor allele frequency. We now have progressed from the HapMap database which contained genotypes for ~3.5 million SNPs genotyped across 270 samples of European, African and Asian descent to the 1000 Genomes database which contains ~40 million SNPs and short indels categorized across almost 1200 samples. Data from the 1000 Genomes Project is being used to develop the next generation of arrays that will be used to search for disease associations in whole genome association studies. Compared to whole genome sequencing these arrays are limited to a significantly smaller portion of the total genome - e.g. the latest Illumina array (the Omni5) can simultaneously assay ~4.5 million markers per sample versus sequencing which assays almost 3 billion bases. Through careful design and because the majority of the genome is not polymorphic in a reasonable number of samples much of the total genomic information can still be included in a relatively small number of markers. The measure commonly used to quantify the amount of the total genomic SNP information contained by a particular array is commonly referred to as the genomic coverage and is the fraction of SNPs highly correlated ($r^2>0.8$) to the SNPs on the array. Genotypes from the 1000 Genomes Project can be used to assess the genome-wide coverage of these new arrays, but because the quality of the genotypes from low-depth sequencing is error prone coverage is systematically underestimated when calculated this way. For example, the OmniExpress genotyping chip which contains ~732k markers covers ~66% of the common variants in the YRI samples when LD is calculated using the HapMap genotypes. An identical calculation (e.g. coverage of the SNPs common in the HapMap samples) performed using the 1000 Genomes genotypes estimates the coverage at 58% - a reduction in the estimated coverage of ~13%. The drop in coverage for the JPT+CHB and CEU and samples is less significant - 2.5% and 5% respectively. We will calculate the coverage of the Omni5 based on the most recent data from the 1000 Genomes. Also, because this calculation will be an underestimate we will use the array genotypes to assess the LD biases overall and also as a function of both population and minor allele frequency.

416F

The first genetic animal model for idiopathic scoliosis. *K. Gorman[1], J. Parent[2], R. Amahadi[2], K. Pohl[2], F. Ali[2], K. Bandwait[2], R. Wallis[2], E. Jin[2], G. Handrigan[2], J. Christians[2], F. Breden[2], A. Moreau[1].* 1) Biochemistry, Université de Montréal;Viscogliosi Laboratory in Molecular Genetics of Musculoskeletal Dis, Montreal, Quebec, Canada; 2) Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada.

Idiopathic-type scoliosis (IS) accounts for 80% of all human spinal curvatures and affects on average 3%-4% of the pediatric population. The deformity imposes a substantial cost on the healthcare system through physician care, bracing treatments, and spinal fusion surgeries. The etiology remains unknown because of phenotypic variability, a curve pathogenesis that coincides with variable growth dynamics, and the lack of an animal model with a similar phenotype. Although there have been numerous genetic studies for IS, causative genes have not been confirmed. The proposed research uses model teleosts to study the basic biology of heritable idiopathic-type spinal curvature. Considering that humans and teleosts share many genes with similar tissue and temporal expression characteristics, we hypothesize that the basic biology is conserved between human and teleost heritable idiopathic-type spinal curvature syndromes. In order to test the feasibility of teleosts as models for human idiopathic-type spinal curvature, we characterized the non-induced guppy curveback phenotype using the same parameters that are used for IS. We demonstrated that as with the human IS syndrome, the curveback spine has no congenital abnormalities that explain curvature; has similar concave/convex biases such as vertebral thickening at the curve apex; has a female bias for severe curves; is associated with disproportionate allometric growth; and has a similar scope of phenotypic variability. Based on these and other phenotypic parallels, we propose curveback as the first genetic/developmental animal model for IS. To identify genes that are associated with curvature in curveback, and candidates for IS, we mapped a single major effect QTL explains 100% of the susceptibility for curvature and approximately 80% of the variation for curve magnitude. Identification of a major QTL for curvature is a first step in understanding the genetics of this type of deformity and will lead to the identification of important pathways associated with spinal integrity. We describe how the curveback model is being applied to help understand the genetic basis of IS as well as phenotypic complexity.

417F

Disruption of imprinted regions in a mouse model of fetal alcohol spectrum disorders (FASD). *B. Laufer, E. Diehl, M. Kleiber, K. Janus, E. Wright, S. Singh.* Department of Biology, University of Western Ontario, London, ON.

Fetal Alcohol Spectrum Disorders (FASD) remain the leading cause of preventable birth defects and cognitive deficits in North America. We have developed a C57BL/6J mouse model of voluntary ethanol consumption in which pregnant mice show a 70% preference for 10% ethanol over water. The offspring of these mothers show developmental and behavioral abnormalities, including learning deficits as young adults (60 day old). Using this model, RNA and DNA from the whole brains of ethanol-exposed offspring (and matched controls) were used on mouse-specific arrays (gene expression, microRNA and DNA methylation) to assess alterations in genome-wide gene expression (including noncoding RNAs) and DNA methylation at early adulthood (postnatal day 70). Results show that exposure to alcohol during neurodevelopment has a long-lasting effect on neurometabolomics. There is a significant ($p<0.05$) up-regulation of noncoding RNA from both known mammalian clusters of small nucleolar RNA (snoRNA) encoding genes, *Snrpn* through *Ube3a* (Human 15q11-q13/Murine 7qC) and *Dlk1* through *Dio3* (Human 14q32.2/Murine 12qF1). These clusters are neuronally expressed, with the *Dlk1-Dio3* cluster containing an abundance of brain-specific microRNAs. The clusters are localized to imprinted loci and are associated with several neurodevelopmental disorders that exhibit developmental, behavioral and physical deficits similar to FASD. Furthermore, integral parts of the spliceosome (U6 and U2 splicesomal RNAs as well as *Prp2*) were significantly ($p<0.05$) up-regulated. Following confirmation by qPCR of select coding and non-coding RNAs and bisulfite sequencing of methylated regulatory regions, further research will evaluate the role of *Snrpn-Ube3a* cluster, which is known to play a unique role in the alternative splicing of the serotonin receptor *5htr2c*. These results suggest a novel relationship between imprinting, non-coding RNAs and alternative splicing in the etiology of FASD. Potentially, an epigenetic feedback loop may maintain these alterations in gene expression and splicing caused by prenatal alcohol exposure, providing a mechanism for long-term neural dysfunction leading to distinct and persistent FASD phenotypes.

418F

Substance abuse and addiction measures in the PhenX Toolkit for use in genomic and epidemiologic studies. *K. Conway[1], D.B. Hancock[2], M. Scott[3], A. Agrawal[4], A. Hussong[5], W. Bickel[6], J. Bjork[1], P. Wakim[1], E. Augustson[7], T. Hendershot[2], D. Nettles[2], M. Phillips[2], J. Pratt[2], W. Huggins[2], T. Grant[2], D. Maiese[2], H.A. Junkins[8], L. Farrer[9], E.M. Ramos[8], L.C. Strader[2], C.M. Hamilton[2], K.J. Sher[9].* 1) National Institute on Drug Abuse, Bethesda, Maryland, MD; 2) RTI International, Research Triangle Park, NC; 3) National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland, MD; 4) Washington University in St. Louis, St. Louis, MO; 5) University of North Carolina at Chapel Hill, Chapel Hill, NC; 6) Virginia Tech Carilion Research Institute, Roanoke, VA; 7) National Cancer Institute, Bethesda, MD; 8) National Human Genome Research Institute, Bethesda, MD; 9) University of Missouri, Columbia, MO.

The PhenX (consensus measures for **Phen**otypes and e**X**posures) Toolkit (https://www.phenxtoolkit.org/) offers 295 high-quality, well-established, standard measures of phenotypes and exposures for use in genome-wide association studies and other large-scale genomic and epidemiologic studies. Currently, the Toolkit addresses 21 research domains (fields of research), including an Alcohol, Tobacco and Other Substances domain with 14 measures related to substance abuse and addiction (SAA) (e.g., cigarettes per day). There was a recognized need for additional SAA measures in the Toolkit, so a project was launched in early 2011 to create six "Specialty" Collections and one "Core" Collection of SAA-related measures. These new Collections will provide SAA and other investigators with common measures, and thus make it easier to compare or combine studies. It is desirable to replicate association findings by comparing results from different studies or to combine studies to create larger sample sizes, increasing statistical power and the ability to detect more subtle and complex associations. To provide guidance, a SAA Scientific Panel (SSP) of 10 academic and federal governmental scientists was assembled to select and define the initial scope of six specialty areas using a consensus process. The six specialty areas are: (1) Assessment of substance use and substance use disorders, (2) Substance-specific intermediate phenotypes, (3) Substance use-related neurobehavioral and cognitive risk factors, (4) Substance use-related psychosocial risk factors, (5) Substance use- related community factors, and (6) Substance use-related co-morbidities and health-related outcomes. Each of three Working Groups (WGs) composed of academic and government experts will address two Specialty Areas. The WGs will recommend up to eight high-priority measures for each Specialty Area. The WGs will use a consensus-based process that includes input from the scientific community as they select the measures for inclusion in the PhenX Toolkit. Outreach results will be reviewed by the WGs and considered in their final deliberations. The WGs present their selections for review and approval by the SSP and the PhenX Steering Committee. We present the rationale, criteria, scope, and preliminary results from the SSP and the SAA Working Groups. It is expected that some 48 SAA measures will be added to the Toolkit by spring 2012. *Supported by NHGRI and NIDA grants 5U01HG004597 and 3U01HG004597-03S3.*.

419F

Mapping Phenotype Variables to Support Cross-study Data Integration Among Genomic Studies. *C.-N. Hsu[1,2], C.-J. Kuo[2], C. Cai[1], S.A. Pendergrass[3,4], T.C. Matise[5], M.D. Ritchie[3,4], J.L. Ambite[1].* 1) USC/Information Sciences Institute, Marina del Rey, CA; 2) Institute of Information Science, Academia Sinica, Taipei, Taiwan; 3) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 4) Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN; 5) Department of Genetics, Rutgers University, Piscataway, NJ.

A wealth of biomedical data is available in public and private repositories. These data from multiple sources can be integrated to improve statistical power for a variety of genetic analyses. Consider the National Center for Biotechnology Information (NCBI) genome-wide association studies (GWAS) database of Genotypes and Phenotypes (dbGaP). Due to the use of non-standardized phenotype labels by different studies, it can be difficult to find studies that have comparable phenotypic measures. Similarly, consider Phenome-Wide Association Studies (PheWAS), a novel study approach used by the Population Architecture Using Genomics and Epidemiology (PAGE) network that investigates the association between genetic variation and an extensive range of phenotypes. In order to compare PheWAS results across studies to seek replication for significant genotype/phenotype associations, an important step is matching and mapping phenotypes across studies. Herein we present a machine learning solution to solve this challenging phenotype mapping problem. We have developed a computational approach which determines whether two phenotypes are in the same class from a training set of the results of manual phenotype matching. The key innovations include the use of weighted Jaccard features and term augmentation by dictionary lookup. We started with a set of 774 phenotype variables from five participating studies in our PAGE PheWAS study to train and evaluate different possible approaches. These phenotypes were manually mapped together into 106 classes. When compared to string similarity metric-based features, our solution improves the F-score from 0.59 to 0.73. With augmentation, we had further improvement in F-score to 0.89. For terms not covered by the dictionary, we used transitive closure inference and reached an F-score of 0.91, close to a level sufficient for practical use. We have found that our approach generalizes well to phenotypes not used in our training dataset. When used to map an independent set of 275 phenotypes from four studies, our program initially obtained an F-score of 0.78, low because 161 phenotypes could not be classified into the 106 known classes. After counting those mapped to semantically related existing classes as true positives, the F-score became 0.92. The results show that our solution is promising to significantly reduce the phenotype-matching burden on researchers when integrating data from multiple studies.

420F

Tackling the Challenges of Phenotyping Behavior for Genetic Epidemiological Studies. *R. Plaetke, V. Stegall.* Anthropology, University of Alaska, Fairbanks, AK.

A bottleneck in producing high-quality genetic epidemiological research is phenotyping. This aspect of genetic epidemiological research is re-visited for behavior genetics studies by discussing relevant literature & our study of positive affect in Alaskan Huskies as an example. To obtain measures with reduced bias, we apply the following guidelines: (1) Focus on phenotyping components of multidimensional (latent) behavioral traits. (2) Reduce subjectivity in data collection. (3) Aim for good phenotypic resolution. (4) Investigate reliability & validity of data. Ad (1): Based on the Big-Five Model, temperament is a multidimensional, latent trait. To investigate the evolutionary aspect of one dimension, extraversion, we first study positive affect, one of its components. We use an ethological approach & expose dogs to two tests measuring the effect of positive stimuli. To date, we have collected 3 continuous phenotypes & over 30 (qualitative) characteristics of dogs' behaviors from a total of 73 dogs (2 kennels). Ad (2): Data characterize dogs' body language & vocal expressions & are extracted from videos, allowing quality control & evaluation of subjectivity of observers. A Clever Hans Effect in one of the tests in which a tester interacts with the dogs could not be observed: Correlations between sum-of scores characterizing behaviors when the tester arrived in a kennel and scores derived from the test were high (r=0.91). Ad (3): Two continuous variables (dogs' intensity & speed: contacting the tester) continue to show variation & reconfirm an already observed heterogeneity in 2 kennels. Ad (4): We are currently revisiting one kennel of 17 dogs for two more times to test reliability of the tests. We will provide an overview about the concordance of these repeated measures. To test validity of both tests, we are in the process of interviewing 10 people, who will be asked to evaluate study-dogs' behaviors & describe their perception of positive affect. Often genetic studies of behavioral traits have been performed by interviewing owners, since these traits need repeated observations. To avoid owners' subjectivity in phenotyping, we consider an ethological approach as optimal. The joint evaluation of multiple behavioral tests & additional observations, as proposed in our study, may provide a solution for generating consistent, unbiased behavioral phenotypes & the need of a genetic epidemiological study for large sample sizes.

421F

Genome wide association studies in allergic disease - re-definition of endotypes helps identify true genetic associations. *A. Simpson[1], J.A. Curtin[1], J. Winn[2], C.M. Bishop[2], A. Custovic[1].* 1) The University of Manchester, Manchester Academic Health Science Centre, NIHR Translational Research Facility in Respiratory Medicine, University Hospital of South Manchester NHS Foundation Trust, Manchester, UK; 2) Microsoft Research Cambridge, Cambridge, United Kingdom.

Background: Much of the heritability of complex traits (e.g. atopy, asthma) remains unexplained by genome wide association studies (GWAS). One reason for this may be poor phenotype definition. Conventionally, atopy is defined as the presence of a positive skin test or specific IgE to any allergen. We propose that this diagnostic label encompasses a number of different endotypes. To test this, we applied machine learning approach to all available skin test and sIgE data collected from birth to age 8 years in an unselected birth cohort to cluster children into different atopy classes (Simpson et al. AJRCCM 2010;181:1200-6). Most atopic children clustered into 4 distinct classes, only one of which was associated with asthma (Multiple early sensitization class, comprising ~25% of children assigned as atopic using conventional criteria). We hypothesized that genetic associates of different endotypes will be characteristic; to test this, we compared the results of GWAS using “Conventional atopy” and “Multiple early sensitization class” as outcomes. **Methods:** DNA was genotyped using Illumina 610 quad chips. Following standard QC we imputed additional genotypes (IMPUTEv2.1.2) with 1000 genomes and Hapmap phase 3 as reference; we excluded SNPs with INFO <0.4 and MAF <0.05. GWAS was performed with probabilistic imputed genotypes (SNPTEST) with two different definitions of outcome: “Conventional atopy” (250 cases; 519 controls) and “Multiple early sensitization class” (105 cases; 513 controls). We defined genome wide significance as p<5X10-8. **Results:** Using the additive model, markedly different patterns of association were observed for the two outcomes. For “Conventional atopy” no SNP reached genome wide significance. In contrast, despite a lower number of cases, we observed significant association of 7 SNPs (5 regions) with “Multiple early sensitization class”: 2 SNPs on 3q25.2 (p<=1.84E-09) and 20p11.1-20q11.1 (p<=2.24E-09) and single SNPs on 6p12.1 (p=2.56E-08), 13q3 (p=4.70E-09) and 21q22.3 (p=2.24E-09). **Conclusions:** Atopy comprises multiple latent atopic vulnerabilities with different genetic risk factors. We identified 5 regions associated with precisely defined endotype of “Multiple early sensitization”, whereas no loci were associated with “conventional atopy”. Increasing the size of studied populations will not overcome the problem of phenotypic heterogeneity; better endotyping may help explain part of the “missing heritability” of complex diseases.

422F

Association of Polymorphisms in the ITPKC gene with Susceptibility to Kawasaki disease in Taiwanese children. *H. Chi[1,5,6], LY. Chang[2,5], FY. Huang[1,4], MR. Chen[1,6], NC. Chiu[1,6], HC. Lee[1,4], SP. Lin[1,3,6,7], WF. Chen[3], CL. Lin[3], HW. Chan[3], HF. Liu[3], LM. Huang[2,5], YJ. Lee[1,3,4].* 1) Pediatrics, Mackay Memorial Hosp, Taipei, Taiwan; 2) Pediatrics, National Taiwan University Hospital, Taipei, Taiwan; 3) Medical Research, Mackay Memorial Hospital, Tamshui, Taipei, Taiwan; 4) Pediatrics, Taipei Medical University, Taipei, Taiwan; 5) Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei, Taiwan; 6) Mackay Medicine, Nursing and Management College, Taipei, Taiwan; 7) Infant and Child Care, National Taipei College of Nursing, Taipei, Taiwan.

Kawasaki disease (KD) is a systemic vasculitis caused by unknown infectious agents, host immune dysregulation, and genetic susceptibility in children. Coronary artery lesions (CALs) complicate 15%-25% of cases of untreated KD. Inositol 1,4,5-trisphosphate 3-kinase C (ITPKC) is a kinase of inositol 1,4,5-trisphosphate (IP3), which negatively regulates T-cell receptor signal transduction. The aim is to investigate the association between ITPKC gene polymorphisms and the susceptibility to KD or with CALs in Taiwanese children. All patients with KD were from the Department of Pediatrics at Mackay Memorial Hospital and National Taiwan University Hospital, Taipei, Taiwan. A total of 494 unrelated Taiwanese children (282 boys and 212 girls) with KD were included, 181 of whom had CALs. Mean age at diagnosis was 1.9 ± 1.7 (0.1 - 10.2) years. A group of 614 (339 boys and 275 girls) unrelated, healthy, Taiwanese subjects with age at 9.1 ± 4.3 (0.1 - 20.0) years served as a control group. We selected 7 tag SNPs (rs2561531, rs2561528, rs890934, rs2303723, rs10420685, rs2561536, and rs2290693) and 1 putative functional SNP (rs28493229) to be genotyped by using the TaqMan Allelic Discrimination Assay. The Hardy-Weinberg equilibrium was assessed for each SNP in both the control and study groups by chi square analysis. The frequencies of ITPKC haplotypes and linkage disequilibrium (LD) between paired SNPs in controls and children with KD were estimated using Haploview 4.1. Corrected P (Pc) values < 0.05 were considered statistically significant. The results showed a significant association between genotype GG+AG of SNP rs2290693 and the susceptibility of KD (OR = 1.46, 95%CI 1.13-1.90, P=0.0042, Pc=0.0327) and CALs (OR = 1.69, 95% CI 1.16-2.47, P=0.0064, Pc=0.0498). Besides, significant associations were obtained between genotype CC+CG of SNP rs2561536 and the susceptibility of KD (OR = 1.42, 95%CI 1.12-1.81, P=0.0039, Pc=0.0311) as well as between genotype GG+GT of SNP rs890934 and the susceptibility of CALs (OR = 2.00, 95% CI 1.23-3.25, P=0.0046, Pc=0.0359). No statistically significant differences were found in the distribution of haplotypes between the controls and children with in KD patients with and without CALs and controls. This extensive tag SNP of ITPKC gene study showed that SNP rs2561536 and rs2290693 polymorphisms are associated with KD susceptibility and SNP rs890934 and rs2290693 polymorphisms are associated with CALs in the Taiwanese population.

423F

Common variant of the *TRIB2* influences central obesity. *K. Nakayama[1], A. Ogawa[1], Y. Ishizuka[1], Y. Yanagisawa[1], H. Miyashita[2], Y. Kagawa[3], S. Iwamoto[1].* 1) Jichi Medical University, Shimotsuke, Japan; 2) Jichi Medical University Hospital, Shimotsuke, Japan; 3) Kagawa Nutrition University, Sakado, Japan.

Human *TRIB1-3* encode the pseudokinase protein family that influence various biological processes including energy metabolism. Common variants of the *TRIB1* and the *TRIB3* were known to be associated with blood lipid profiles and insulin resistance, respectively. The *TRIB2* was reported to be involved in adipocyte differentiation and inflammatory responses in monocytes. However, for the *TRIB2*, there has been no report of variants that affect energy metabolism. We recruited 2,755 Japanese individuals from attendees of general health-checkups. Five tag SNPs in the *TRIB2* were tested for associations with BMI, waist circumstance (WC), BMI-adjusted WC, plasma triglycerides, LDL- and HDL-cholesterol, fasting blood glucose, and blood pressures. Another cohort consisted of 1,560 East Asians was used for replication analyses. Visceral and subcutaneous adipose tissues were obtained from 45 Japanese individuals and measured *TRIB2* expression level. 95 Japanese individuals were screened for sequence variants of *TRIB2* exons. Among tests of 5 SNPs for 8 traits, association of rs1057001, A to T transversion in the 3' UTR, and BMI-adjusted WC survived multiple testing corrections ($P < 0.0007$). Copy number of the minor T allele (allele frequency = 0.22) was associated with increased BMI-adjusted WC. The association of rs1057001 and BMI-adjusted WC was successfully replicated in the East Asian cohort ($P < 0.0019$). Moreover, rs1057001 was positively associated with BMI-adjusted waist-hip ratio ($P < 0.0082$) but not with hip circumstance ($P > 0.44$). rs1057001 showed genotypic differences of the *TRIB2* expression level in the adipose tissues, in which T/T homozygous individuals had higher expression levels ($P < 0.05$). We performed allele specific quantification of the *TRIB2* transcripts in 20 heterozygous individuals and confirmed the significant enrichment of the T allele in *TRIB2* transcripts. Moreover, the variant screening identified a proxy polymorphism ($r^2 = 1.0$) that was a TC indel near a poly adenylation signal of the *TRIB2*. The high expresser T allele was linked with TC insertion allele and this insertion type sequence was highly conserved among other mammalian homologues. The association between functional variant of the *TRIB2* and BMI-adjusted WC unveiled emerging roles of the *TRIB2* in abdominal specific adiposity. The *TRIB2* may confer risks of central obesity and metabolic syndrome in East Asians.

424F

CYP1A1(Ile462Val) genetic polymorphisms and uterine leiomyoma risk ina cohort of iranian women, a case- control study. *f. taghizade mortazaee, S. Miraj, E. farrokhi, N. shahinfard, M. Hashemzadeh, S. Ashori, K. Ghatre Samani.* , Cellular and Molecular Research Center, shahrekord, Select a Country.

Background: Uterine leiomyoma is benign solid tumors of smooth muscle and the most common type of gynecological tumor. it occurs in approximately 25%-30% of women over 30 years of age and the most common disorder leading to hysterectomy, therefore, has a significant effect upon health of women. Purpose: The aim of the study was to evaluate the association of CYP1A1(Ile462Val) polymorphisms with uterine leiomyoma in a cohort of women Charmahal va Bakhtiari province in southwest of iran . Methods: In this case - control study 156 women with clinically diagnosed uterine leiomyoma and 151 healthy normal subjects were investigated. The genetic distribution of CYP1A1 polymorphism at Ile462Val were analyzed by polymerase chain reaction - restriction fragment length polymorphism method. All the data were analyzed by SPSS.17 software, using chi squre test. Results: no significant differences was found in the frequency of Ile462Val (A>G) polymorphism between uterine leiomyoma patients and controls in Charmahal va Bakhtiari women (p>0.05). Conclusion: our findings suggest that the genotype of CYP1A1 Ile462Val was not associated with the increased risk of uterine leiomyomas in studied cases. Keywords: CYP1A1 . Uterine leiomyoma .polymorphism.

425F

Allele-specific transcriptional activity at type 2 diabetes-associated SNPs in regions of pancreatic islet open chromatin. *M.P. Fogarty[1], T.M. Panhuis[1,2], S. Vadlamudi[1], M.L. Buchkovich[1], K.J. Gaulton[1,3], K.L. Mohlke[1].* 1) Department of Genetics, University of North Carolina, Chapel Hill, NC; 2) Department of Zoology, Ohio Wesleyan University, Delaware, OH; 3) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK.

For many type 2 diabetes (T2D) susceptibility loci, the underlying functional variants and genes are unknown. As pancreatic islets are a critical tissue in T2D pathogenesis, we hypothesized that altered transcriptional regulation in islets could contribute to trait susceptibility at some loci. We used maps of predicted regulatory regions, including islet open chromatin, histone modifications and transcription factor ChIP-seq data, to distinguish potentially functional regulatory SNPs from marker SNPs in high linkage disequilibrium (LD). We selected variants in strong LD with GWA index SNPs (r^2>.8, CEU) at three T2D-associated loci (*JAZF1*, *TCF7L2*, *CDC123/CAMK1D*) for which an islet regulatory effect is plausible. Selected SNPs meeting these criteria and also present in an islet predicted regulatory region were tested for transcriptional activity using luciferase reporter assays in 832/13 insulinoma cells.

We successfully identified SNPs with allele-specific transcriptional enhancer activity for rs1635852 in intron 1 of *JAZF1* (*P*=7.8x10^{-5}), rs7903146 in intron 1 of *TCF7L2* (*P*=.005) and rs11257566 located between the *CDC123* and *CAMK1D* genes (*P*=.03). For rs1635852 and rs7903146, we confirmed differential luciferase activity in MIN6 insulinoma cells and identified allele-specific protein binding using gel shift assays. The specific DNA-binding proteins for both of these SNPs remain to be identified. At the *JAZF1* locus, the rs1635852 T2D protective allele (C) is associated with increased *JAZF1* transcript levels in eQTL analysis of adipose tissue (Voight et al, Nat Genet 42; 579), consistent with the direction of allele-specific enhancer activity. At the *CDC123/CAMK1D* locus, decreased luciferase activity with the rs11257655 T2D risk allele (C) is not consistent with the direction of eQTL's for both *CAMK1D* and *CDC123* in blood. Opposing directions may reflect cell-type specific regulatory mechanisms or indicate that other regulatory variants remain to be identified.

While further experiments are necessary to validate this initial evidence of functional variants associated with T2D, these results support epigenomic maps as a valuable resource to guide identification of variants with allelic differences in regulatory activity at complex trait loci.

426F

Exome Sequencing in Severe COPD Cases and Resistant Smoking Controls from COPDGene. *M.H. Cho[1], M. Emond[2], R.A. Mathias[3], J. Hokanson[4], T.H. Beaty[6], N. Laird[5], C. Lange[5], K.C. Barnes[3], M. Bamshad[2], J.D. Crapo[7], E.K. Silverman[1] on behalf of the NHLBI Exome Sequencing Project.* 1) Channing Lab, Brigham & Women's Hosp, Boston, MA; 2) University of Washington, Seattle, WA; 3) Johns Hopkins School of Medicine, Baltimore, MD; 4) Colorado School of Public Health, University of Colorado Denver, Aurora, Colorado; 5) Harvard School of Public Health, Boston, MA; 6) Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 7) National Jewish Medical and Research Center, Denver, Colorado.

Background: Chronic obstructive pulmonary disease (COPD), defined as airflow obstruction that is not fully reversible, is the third leading cause of mortality in the United States. In addition to common variants, rare protein-coding variants are also known to have a role in COPD susceptibility: alpha-1 antitrypsin deficiency, typically caused by a rare nonsynonymous SNP in SERPINA1, is a well-known Mendelian disorder characterized by severe COPD. We hypothesized that additional rare coding variants contribute to COPD susceptibility. Methods: We selected extremes of COPD susceptibility from among Non-Hispanic Whites from the multicenter COPDGene study: 132 cases of severe, early-onset COPD (age < 63.0, GOLD Stage 3-4 COPD) with emphysema on chest CT scan (>15% at -950 HU) and 127 resistant controls (age > 65.0, normal lung function) without emphysema (<5% at -950 HU) with similar cigarette smoking histories. Exome sequencing was performed using the Nimblegen SeqCap EZ V2.0 on the Illumina platform as part of the NHLBI Exome Sequencing Project LungGO. Sequences were aligned using BWA, and variants were called using the Genome Analysis Toolkit. Variant positions were annotated using Seattle-Seq and Polyphen2. Quality control checks included gender, missingness, and transition / transversion ratios. Case-control analysis was performed collapsing nonsynonymous and nonsense variants by gene using a fixed allele frequency threshold in cases and controls in a logistic regression framework, with secondary analyses using the variable threshold test and Polyphen2 weights. Results: After quality control, we identified a total of 162,757 SNPs (81,675 nonsynonymous or stop) with a TsTv of 3.11. Analysis of the most significant genes using a fixed cutoff included SUN2, PCDHA6, and RECQL; and using a variable threshold MGLL, C6ORF97, and RSPH6A. None of the genes were significantly associated after correction for multiple testing, and overlap of the most significant results between the analytical methods was modest. Conclusions: Analysis of exome sequencing in severe COPD and resistant smoking controls can identify novel candidate genes that may contain rare variants influencing COPD susceptibility. Sequencing of additional cases and controls and exploration of other rare variant analytical methods are underway.

427F

Admixture mapping in Puerto Rican and Mexican Latinos: Dense SNP genotype data identifies novel genetic risk factors for asthma. *L.A. Roth[1], D.G. Torgerson[1], C.R. Gignoux[1], J.M. Galanter[1], K. Drake[1], C. Eng[1], S. Huntsman[1], P. Avila[2], R. Chapela[3], J.G. Ford[4], J. Rodriguez-Santana[5], W. Rodriguez-Cintron[6], S. Sen[1], E.G. Burchard[1].* 1) University of California, San Francisco - San Francisco, CA; 2) Northwestern University - Chicago, IL; 3) INER - Mexico City, Mexico; 4) Johns Hopkins University - Baltimore, MD; 5) Centro de Neumologia Pediatrica - San Juan, Puerto Rico; 6) VA Medical Center - San Juan, Puerto Rico.

Asthma is a chronic respiratory disease that disproportionately affects certain racial and ethnic groups. In the United States the highest asthma incidence, morbidity, and mortality is in Puerto Ricans, and the lowest in Mexicans, highlighting heterogeneity within Latinos. The Genetics of Asthma in Latino Americans (GALA) study genotyped ~900,000 SNPs in 529 asthmatic children and 343 controls. All four subject grandparents had self-declared Mexican or Puerto Rican ancestry. Genomic ancestry estimated using clustering and PCA confirms that Puerto Ricans have a larger African ancestry proportions, whereas Mexicans have Native American ancestry proportions. Local genetic ancestry of European, African, and Native American origin estimated using LAMP exhibited considerable variation across loci and between individuals. We leveraged the genetic diversity between Puerto Rican and Mexican Latinos in GALA by performing genome-wide admixture mapping to identify novel asthma associated loci. We tested for differences in local ancestry between cases/controls rather than individual genotype, which presumably has more power over genotypic associations due to increased coverage from ancestry-LD, and may also identify regions more likely to contain population-specific rare variants. Our admixture mapping scan identified 62 candidate regions where differences in local ancestry were associated with asthma susceptibility at $p<10^{-3}$. Notably, we find that Native American ancestry is risk factor for asthma in Puerto Ricans at 1p13.2 ($p=5.1x10^{-4}$, OR=2.3), whereas African ancestry is a protective for asthma in Mexicans at 6q15 ($p=4.6x10^{-6}$, OR=0.56). To take advantage of the genetic variation produced by local ancestry and genotype, we performed a likelihood ratio test that examined their joint effect across the genome. We identified a locus associated with asthma (likelihood ratio test $p=4x10^{-7}$) at TCRA, the alpha chain of the T cell receptor, a commonly studied gene in asthma. Overall, our results suggest that the genetic architecture of asthma susceptibility may differ across genetically diverse Latino populations, and that incorporating estimates of local genetic ancestry enhances genetic association tests in admixed populations.

428F

Risk prediction for hypertension using gene-environment risk scores and a decision tree in a Korean genome-wide association study. *J.W. Park[1], I. Kim[1], S. Uhmn[2], J. Hwang[3], C. Shin[4], N.H. Cho[5], J.Y. Lee[3].* 1) Department of Medical Genetics, College of Medicine; 2) Department of Computer Engineering, College of Information and Electronic Engineering, Hallym University, Chuncheon; 3) Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Korea University Ansan Hospital, Ansan; 4) Department of Preventive Medicine, Ajou University School of Medicine, Suwon; 5) Center for Genome Science, National Institute for Health, Seoul, Korea.

Hypertension is one of the most common diseases influenced by complex interplay of multiple genetic and environmental factors. Conventional methods of multiple regression, genotype scoring, and reclassification, may be of limited use in risk prediction for complex disease due to difficulties of estimating multilayer interactions of gene-gene and gene-environmental factors. We performed a genome-wide association study using 352,228 SNPs for hypertension by comparison of 1,878 cases and 3,811 controls that were obtained from two population-based cohorts developed in South Korea. The SNPs showing significant association in the initial study were reanalyzed in an independent study composed of 215 cases and 737 controls. We compared the prediction abilities of five risk scores combined genetic and/or non-genetic risk factors and a machine-learning approach (i.e. decision tree, DT). We found the evidence for association with the SNPs located in seven genes including TSNARE1 (rs6583607, 8q24.3, $p=1.04 \times 10^{-7}$ under a dominant model). These 7 SNPs (i.e. rs16844620, rs7142394, rs9861122, rs6026886, rs7125196, rs6583607, and rs2074356) were evaluated for their genotype risk score (GRS) and weighted GRS (WGRS). Additionally, non-GRS (NGRS), weighted non-GRS (WNGRS), and weighted genetic-nongenetic risk score (WGNRS) were calculated. Adding the effect of non-genetic risk factors to the GRS increased the area under the curve (AUC) slightly (from 0.57 to 0.63). In the DT analysis, a prediction model composed of 5 SNPs and 3 non-genetic factors (i.e. age, BMI, family history) increased the overall accuracy to 67.4. In conclusion, the machine learning approach improves the ability to predict risk of hypertension. To develop more accurate risk prediction model for a complex disease, not only genetic factors but also non-genetic factors must be evaluated under a goodness-of fit genetic model.

429F

Variants of C-C motif chemokine 22 (*CCL22*) are associated with susceptibility to atopic dermatitis. *T. Hirota[1], H. Saeki[2], K. Tomita[1], K. Ebe[3], S. Doi[4], T. Enomoto[5], N. Hizawa[6], S. Takashi[7], T. Ebihara[7], M. Amagai[7], S. Takeuchi[8], M. Furue[8], N. Kamatani[9], M. Kubo[10], M. Tamari[1].* 1) Laboratory for Respiratory Diseases, CGM, RIKEN, Yokohama, Kanagawa, Japan; 2) Department of Dermatology, The Jikei University School of Medicine, Tokyo, Japan; 3) Takao Hospital, Kyoto, Japan; 4) Department of Pediatric Allergy, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Osaka, Japan; 5) Wakayama, Japan NPO Japan Health Promotion Supporting Network, Wakayama, Japan; 6) Division of Respiratory Medicine, Institute of Clinical Medicine, University of Tsukuba, Ibaraki, Japan; 7) Department of Dermatology, Keio University School of Medicine, Tokyo, Japan; 8) Department of Dermatology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; 9) CGM, RIKEN, Yokohama, Kanagawa, Japan; 10) Laboratory for Genotyping Development, CGM, RIKEN, Yokohama, Kanagawa, Japan.

Background: Atopic dermatitis (AD) is a pruritic and chronically relapsing inflammatory skin disease involving disturbed skin barrier functions, cutaneous inflammatory hypersensitivity and defects in the antimicrobial immune defense with a strong genetic background. CCL22 binds to CC-chemokine receptor 4 (CCR4), which is expressed by Th2 cells. CCL22 plays a crucial role in controlling the trafficking of Th2 cells into sites of allergic inflammation. But genetic influences of CCL22 on pathophysiology of AD are unclear. **Methods**: We searched for genetic variations in CCL22 region by PCR-directed sequencing. Seven tag SNPs were selected using the Tagger algorithm and genotyped by Taqman and invader methods. We performed association studies of CCL22 with two independent Japanese populations (919 AD patients and 1032 controls, and 1034 AD patients and 1004 controls, respectively) and combined the results using Mantel-Haenszel meta-analysis. We further conducted allele-specific transcript quantification (ASTQ) and electrophoretic mobility shift assay (EMSA) analyses of the susceptible SNPs. **Results**: We found that rs4359426 (Ala2Asp) SNP was associated with AD under an allelic model in the first population (P = .0070; OR, 1.29; 95% CI, 1.07-1.56) and the second population (P = .00034; OR, 1.40; 95% CI, 1.17-1.69). The direction of association of the SNP was similar in both of the populations. We combined the results using Mantel-Haenszel meta-analysis, and observed the association at rs4359426 (meta-analysis, P = .0000089; OR, 1.35; 95% CI, 1.18-1.54). The susceptible allele showed higher mRNA expression in ASTQ analysis. We also observed that the susceptible allele of rs223821 (in absolute LD with rs4359426) showed stronger signal intensity of the DNA-protein complex than non-susceptible allele in EMSA analysis. **Conclusion**: Polymorphisms of the CCL22 gene might be involved in AD in a gain of function manner.

430F

Exome Data Analysis for a Complex Disease Using a Novel Filtering Tool Reveals a Candidate Mutation. *B.-S. Petersen[1], B. Stade[1], B.D. Juran[2], J.K. Laerdahl[3], J.R. Hov[4], A. ElSharawy[1], K.N. Lazaridis[2], T.H. Karlsen[4], A. Franke[1].* 1) Institute of Clinical Molecular Biology, Christian-Albrechts-University, Kiel, Germany; 2) Division of Gastroenterology and Hepatology, Center for Basic Research in Digestive Diseases, Mayo Clinic College of Medicine, Rochester, Minnesota, USA; 3) Centre for Molecular Biology and Neuroscience (CMBN), Department of Microbiology, Oslo University Hospital Rikshospitalet, Oslo, Norway; 4) Norwegian PSC Research Center, Clinic for Specialized Surgery and Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway.

Exome sequencing is currently one of the most promising applications for next-generation sequencing in medical research. We have developed a pipeline for the filtering of variants from exome sequencing data for finding disease-relevant ones and here demonstrate its functionality using the example of a family with multiple individuals affected by a generally assumed polygenic disease. The familial clustering represents an opportunity to identify rare, high-penetrance polymorphisms that could shed light on the underlying pathogenic mechanisms. The family consisted of two affected sisters with an affected mother and unaffected father. Enrichment was performed using Agilent SureSelect v1 and sequencing was carried out on a SOLiD v4 sequencer. BioScope was used for alignment to the reference and SNPs were identified using two distinct algorithms. Further analyses were performed using our own tool snpActs which annotates the SNPs and displays predictions of various programs for the impact on protein structure and function as well as e.g. collecting information from dbSNP, the 1000 Genomes data, HGMD and calculating Grantham scores for a thorough overall picture. The average on-target coverage was 24.5x with ~19,000 genetic polymorphisms identified per exome. Using snpActs, it was possible to find a likely mutation segregating with the phenotype in the family. We filtered the data keeping non-synonymous/nonsense and splice-site SNPs present in all three affected individuals but not in the unaffected father, in dbSNP or any of the 200 available exomes of healthy controls (Ng et al. Nature 2009, Li et al. Nat. Genet. 2010) and a maximum frequency of 1% in the 1000 Genomes data. Comparing the remaining 84 SNPs with 10 predefined candidate genes revealed a nonsense SNP resulting in truncation of the protein involved in the same pathway as those of previously identified susceptibility genes. Functional and genetic follow-up studies are under way. This example shows that exome sequencing is not only applicable for monogenic diseases but also for cases of multiplex families of complex diseases. The main challenge is the filtering for finding those variants involved in disease manifestation. Our tool provides an ideal environment by bundling various sources of information in a single step and allowing their collective analysis. A user-friendly web-interface has been set up. At the conference, we will publicly release the tool and describe the pilot study in detail.

431F

Syntropy and Personalized Medicine. *V.P. Puzyrev.* Research Institute for Medical Genetics, Tomsk, Russian Federation.

In the “unbounded” phenome separate diseases and their clinical courses are usually analyzed in respect to association with genetic variants (GAS or GWAS), although the phenomenon of co-morbidity is a very specific feature of human morbidity in modern society. Co-morbidity, or syntropy (the term introduced by German pathologists M. Pfaundler and L. von Seht, 1921) which is a natural generic phenomenon itself, is defined as non-random combination of two or more pathological conditions (nosologies or syndromes) in an individual and his/her closest relatives, which has an evolutionary and genetic bases. A set of functionally interacting genes spread throughout the entire space of human genome, co-regulated and involved in common metabolic pathways for a given syntropy, are the syntropic genes (Personalized Medicine. 2010, 7:359-370). In fact, the concept of syntropy, suggested 90 years ago, is the source of the modern “diseasome” conception. Personalized medicine should take into account that genetic testing is necessary not only for single diseases, but for a disease complexes (syntropies). Both science and medical practice are in the very beginning of this route. On the current stage of genetic testing for common diseases development we proposed the guidelines/paradigms which can be useful to promote successes of personalized medicine: while moral and “absolute” knowledge represent an ideal world, clinical practice is a real world; genetic testing is a way toward something which will never be identified perfectly and never be a simple area of application and a simple subject of study; a reconstruction of mutual expectations of doctors, researchers, and patients is a prerequisite of successful advancement of genetic testing; clinical practice has to lean on evidence-based medicine, but the latter is a process of everlasting improvement for the guarantee of high-quality healthcare; genetic testing, not instead, but together with phenotypic markers can be accounted for in personalized prognosis, always probable. In traditional medicine “nosology” concept is basic. However, shared susceptibility (resistance) genes for different diseases (nosologies) can be a proof of other “pathological panorama” of diseases in contemporary human populations. The genetic approach to human diseases systematics will lead to reassessment of their classification which makes a direction towards personalized medicine practice more precise.

432F

Genome-wide analysis of imputed genotypes identifies chemokine receptor-1 (*CCR1*) as a novel susceptibility locus in Behçet's disease. *E.F. Remmers[1], G. Bertsias[1, 2], Y. Kirino[1], M.J. Ombrello[1], D. Ustek[3], C. Satorius[1], J. Le[1], A. Gül[3], D.L. Kastner[1].* 1) Inflammatory Disease Section, Medical Genetics Branch/NHGRI, Bethesda, MD; 2) Rheumatology, Clinical Immunology, and Allergy, Department of Internal Medicine, Faculty of Medicine, University of Crete, 2208, Heraklion, Greece; 3) Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Rheumatology, Istanbul University, Istanbul, Turkey.

Behçet's disease (BD) is a genetically complex disease characterized by recurrent inflammatory attacks affecting the orogenital mucosa, eyes, and skin. We have previously performed a genome-wide association study with 311,459 SNPs in 1,215 BD cases and 1,278 healthy controls from Turkey, and have identified independent associations with HLA-B*51, an additional MHC class I locus, *IL10*, and the *IL23R-IL12RB2* locus. In this study, we carried out whole-genome imputation, using as a reference, the genotypes of 96 of the Turkish healthy controls determined on Illumina HumanOmni1M-Quad SNP chips. SNPs were excluded for deviation from HWE ($p<5\times10^{-4}$), low call rate (<95%), and low MAF (<5%). Imputation was conducted using MACH v1.0.15 providing 814,474 SNPs for analysis in the 1,215 BD cases and 1,278 controls. Using a p-value cut-off of 1×10^{-5}, we identified 114 non-HLA gene SNPs suggestive of association with BD. One imputed SNP rs7616215 on chromosome 3, located ~38 kb from the 3' UTR of the chemokine receptor-1 gene (*CCR1*), (odds ratio [OR] = 0.71, $p=1.9\times10^{-8}$) exceeded genome-wide significance ($p<5\times10^{-8}$). Fine mapping of the *CCR1* region using Sequenom iPLEX assays confirmed the imputation results for rs7616215 and identified 2 additional SNPs in strong LD with rs7616215 that also exceeded genome-wide significance. The association of rs7616215 replicated in additional Turkish and Japanese BD cases and controls (in a meta-analysis of 2,007 cases and 2,187 controls, OR = 0.73, 95% CI 0.66-0.81, $p=3.1\times10^{-10}$). CCR1 belongs to the family of CC-motif chemokine receptors, is expressed on neutrophils, monocytes, and T lymphocytes, and binds several chemokine ligands, including CCL5/RANTES, CCL3/MIP-1(, and CCL4/MIP-1) . A role for CCR1 has been identified in several inflammatory conditions, such as rheumatoid arthritis, multiple sclerosis, and transplant rejection. ENCODE data indicate that rs7616215 resides in a putative regulatory genomic domain. Analysis of *CCR1* transcripts from the HapMap European (CEU), Chinese (CHB), and Japanese (JPT) subjects showed the disease-associated (T) allele correlated with significantly reduced *CCR1* expression ($p<0.03$). Similarly, we found reduced *CCR1* mRNA as a function of the number of rs7616215 risk alleles in purified peripheral blood CD14+ monocytes from healthy controls. We have identified *CCR1* as a novel susceptibility locus in BD, with potential implications for regulation of inflammatory responses in the context of disease.

433F

Experimental validation and search for polymorphism of insertions and deletions from comparisons of primates genomes. *D. Ferrer[1], A. Mazo-Vargas[1], C.T. Ruiz-Rodriguez[1], J.Cr Martínez-Cruzado[1], R.M. Stephens[2], C.A. Winkler[3], N. Volfovsky[2], T.K. Oleksyk[1], Ferrer.* 1) Biology Department, University of Puerto Rico-Mayaguez; 2) ABCC, SAIC-Frederick, NCI-Frederick, Frederick, MD; 3) Basic Science Program, SAIC-Frederick, NCI-Frederick, Frederick, MD 21702.

Comparisons between human and primate genomes are important to further understand the evolution of our own species. Indels, insertion or deletion fragments discovered by sequence alignments among closely related species or individuals are common in these comparisons. Preliminary pairwise comparison revealed 33,479 indels >10bp in reference genomes of human (Homo sapiens), and four primate species: chimpanzee (Pan troglodytes), gorilla (Gorilla gorilla), rhesus macaque (Macaca mulatta), and orangutan (Pongo abelii). The objective of this study was to validate the existence of indels found computationally either in the coding sequence or in the splice sites in vitro by PCR amplification and electrophoresis across all five species, as these have the largest potential impact on protein sequence. From 153 genes containing candidate indels, all have been tested in the laboratory. Of these, 30% have not been validated from human to chimpanzee and 15% from human to rhesus comparisons. Validation results suggest that computational analysis alone is not sufficient for the reliable discovery of insertions and deletions, and additional laboratory testing is required. We further tested HapMap populations for presence of ancestral/derived polymorphisms among the interrogated indels; polymorphic indels were further tested in the HGDP and maps were created of their worldwide distributions. Gene ontology analysis indicated that indels discovered in our study are overrepresented in genes involved in the nervous system, followed by the reproductive system. This is consistent with the current view of human evolution, in which selection operates more strongly on cognitive skills. Further analysis is required to understand the precise impact and differentiation of these genes. The discovered polymorphisms, due to their high impact, will serve as good candidates for the search for human disease associations.

434F

Recent accumulation of deleterious mutations in human populations. *E. Gazave[1], A.G. Clark[2], A. Keinan[1].* 1) Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY, USA; 2) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA.

Since the emergence Out-of-Africa, the human population has grown exponentially at a moderate rate (0.4% per generation). In the last 100 generations, this rate has progressively increased: from around 10% per generation 2000 years ago, it has reached the dramatic figure of 37% in the last 100 years. One genetic consequence of the rapid increase of the human population is the accumulation of rare variants arising from very recent mutations, that are observed in large sequencing datasets, such as in Coventry et al. (2010). The present work is motivated by the need to understand the nature of the very low-frequency polymorphisms that are present in our current population, as it may help to answer the fundamental question of how recent demography affects the architecture of genetic risk of chronic disease. Based on simulated data, we studied the interplay of demography and natural selection comparing two model populations, one following a classical model of European demography (with two population bottlenecks), and the other also experiencing a strong recent exponential growth. Our results show a clear difference between the distribution of the number and fitness effect of mutations present in the two populations: with explosive growth, the number of mutations per individual is increased. Although natural selection is expected to be more efficient in a larger population, our simulations predict that the rapid and recent growth has led to an accumulation of deleterious mutations, which are retained longer in the population. Contrary to what is seen in the baseline model with no growth, we observed no significant increase in the proportion of alleles with positive fitness effect among the very few mutations that go to fixation. The proportion of SNPs lost at each generation increases logarithmically over time, reflecting a complex relationship between the diversity present in the population before the expansion, the number of new mutations introduced at each generation, and the extremely low frequency of these new variants that are likely to be lost before they can increase in frequency. Altogether, our results suggest that the recent rapid population growth has produced an increase of the mutation load in human populations today. We highlight the challenge of differentiating between the genetic signature of recent neutral variants from those of rare deleterious mutations in the face of explosive growth of the magnitude experienced in recent human evolution.

435F

Natural selection and the allele frequency spectrum for the APOL1 gene. *G. Genovese, D.J. Friedman, M.R. Pollak.* Nephrology, Beth Israel Deaconess Medical Center, Boston, MA.

Two distinct protein coding changes in the APOL1 gene have been associated with kidney disease in people of African descent. It is likely that these changes arose to high frequency within the last 10,000 years due to conferring resistance to Trypanosoma Brucei Rhodesiense, an African parasite causing sleeping sickness and transmitted by the tsetse fly, similar to transmission of malaria. While the exact infectious agent responsible for the selective pressure has not been yet identified, analysis of the allele frequency spectrum for APOL1 in different African populations shows patterns indicating that natural selective pressures are geographically localized. Data from the 1,000 Genomes project show the existence of haplotypes at high frequency with diversity falling well outside the haplotypic variation of African haplotypes, consistent with introgression of a divergent APOL1 allele in the ancestral non-African population. While it is now known that APOL1 plays a role in the innate immune system, the specific roles played by this gene remain yet to be determined.

436F

Allele-level HLA A~B~DRB1 global haplotype frequency maps. *L. Gragert, E. Williams, M. Maiers.* Bioinformatics, National Marrow Donor Program, Minneapolis, MN.

Integrating analysis of human leukocyte antigen (HLA) genes in genetic studies has many challenges due to extreme polymorphism, high population privacy of alleles and haplotypes, and the unique DNA typing methods required. This study provides population specific allele-level reference HLA frequencies useful in uncovering associations of specific HLA variants with disease and/or drug interactions, and in revealing the origins and movement of global human populations. We analyzed HLA genotypes provided by 78 international registries representing 41 countries, members of Bone Marrow Donors Worldwide (BMDW), which list nearly 18 million volunteer donors and cord blood units available for unrelated stem cell transplantation. Allele-level HLA A~B~DRB1 haplotype frequencies were estimated by country using the expectation-maximization (EM) algorithm to resolve allelic and phase ambiguity from HLA genotype data. Haplotype frequencies were plotted by country using ESRI ArcGIS 9.3 software to create global maps. Global maps of HLA haplotype frequency are available through the National Marrow Donor Program (NMDP) HaploStats web application (http://www.haplostats.org). When HLA A-B-DRB1 genotype information from individual subjects is input at any level of typing resolution, the application displays the most likely allele-level haplotype pairs in different population categories, with each haplotype linked to a global map. The global maps offer a visual depiction of the worldwide geographic variation of allele-level HLA haplotypes, available for the first time. We observed that the majority of HLA haplotypes have a wide range of variation in frequency in the geospatial dimension, including a number of haplotypes that were private to small regions of the world. The availability of global HLA haplotype maps in HaploStats has direct application in unrelated donor selection for stem cell transplantation therapies and can also be useful in other genetic association or anthropological studies. While Europe has especially good coverage, many regions of the world are unrepresented in these maps due to a lack of stem cell registries in associated countries. Integration of published results from HLA population studies could expand the global coverage. Collection of more detailed population and regional information from BMDW registries could provide refinements to existing maps.

437F

Admixed human genomes reveal ancient and recent migration patterns. *S. Gravel, J.M. Kidd, J.K. Byrnes, A. Moreno-Estrada, S. Musharoff, F.M. De La Vega, C.D. Bustamante.* Genetics, Stanford University, Stanford, CA.

A substantial fraction of humans are admixed, having recent ancestry from distinct continental groups. We conduct a population genomic analysis of admixed individuals that combines local ancestry assignment with whole-genome sequencing data. We focus analysis on 50 individuals sequenced by Complete Genomics, including 4 Mexican-Americans, 4 African-Americans and 2 individuals from Puerto Rico. Combining these data with SNP genotype data from 130 additional samples, we employ a PCA-based method to infer the ancestral population of origin for each genomic location.

To study local ancestry patterns resulting from admixture, we developed a continuous Markov model approach where states include timing information of past migration events. This model remains tractable for a variety of time-dependent migratory models, and we develop an inference framework for parameter estimation and hypothesis testing within this model. We use this framework to infer the timing of admixture and to differentiate between punctual and continuous models of migration: using demographic models that are consistent with both historical records and genetic data, we find evidence for continuous migration patterns in both Mexican and African-American populations.

Whole genome sequence data further reveals that admixed populations contain an excess of low frequency variants compared to their less-admixed counterparts. This excess can be, for the most part, traced back to a simple, neutral sampling process, but affects the frequency distribution of functional variants. We further use our local ancestry assignments to evaluate times to the most recent common ancestors for different population pairs, and find that the results recapitulate the expansion of modern humans out of Africa. Taken together, whole genome sequencing and local ancestry assignment therefore permit inferences about long-term histories of unsampled ancestral populations and highlights recent historical demographic processes that altered patterns of variation observed in admixed populations.

438F

Divergences among 21 Native American populations suggesting a rapid prehistorical dispersion in Central and South Americas. *Y. He[1], R.W. Wang[1], R. Li[1], S. Wang[2], L. Jin[1,3].* 1) Chinese Academy of Sciences and Max Planck Society (CAS-MPG) Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China; 2) FAS Center for Systems Biology, Harvard University, 52 Oxford Street, Cambridge, MA 02138; 3) Ministry of Education Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institute of Biomedical Sciences, Fudan University, Shanghai 200433, China.

Genetic divergence of Native American populations plays a critical role in understanding the initial entry and early dispersion of human being in New World. Although investigations for classic genetic coefficient have provided valuable clues for divergence of Native American populations, a linear correlation between the coefficient and divergence time is susceptible in scenarios involving multiple populations in significant size expansion. Based on a four-population model, a measure for genetic divergence of populations, i.e. `time unit' was proposed in this report. Computer simulation showed the measure of serial divergence events kept a good linear correlation with time of the serial divergence events in complicate multi-population scenarios. Utilizing the novel method and microsatellite data of 21 Native American populations, we investigated genetic divergences of the Native American populations. Results showed genetic divergences between North American populations are greater than that between Central or South American populations. None of the divergences, however, is great enough to be a concrete evidence to support the two-wave or multi-wave migration model for the initial entry of human being. Genetic affinity of the Native American populations suggested that the populations could be divided into 4 groups without damaging their geographic relationship. Divergence of the population groups implicated that early dispersion of human being in America was more likely to be a multi-step procedure. And the divergences supported that there was a rapid dispersion of Native Americans in Central and South Americas right after a long-time standstill in North America.

439F

A coalescent model for genotype imputation. *L. Huang[1], E.O. Buzbas[2], N.A. Rosenberg[1,2].* 1) Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI; 2) Department of Human Genetics, University of Michigan, Ann Arbor MI.

Empirical studies have identified population-genetic factors as important determinants of the properties of genotype-imputation accuracy in imputation-based disease association studies. Here, we develop a coalescent model of imputation that we use to explore the theoretical basis for the ways in which these factors influence genotype-imputation accuracy. Under a two-population demographic model in which two populations diverged at some time in the past, we derive the expectation and variance of imputation accuracy in a study sequence sampled from one of the two populations, using reference sequences sampled either from the same population as the study sequence or from the other population. We analytically show that under this model, imputation accuracy-as measured by the proportion of polymorphic sites that are imputed correctly in the study sequence-increases in expectation with the mutation rate, the proportion of the markers in a chromosomal region that are genotyped, and the time to divergence between the study and reference populations. Each of these effects derives largely from an increase in information available for determining the reference sequence that is genetically most similar to the sequence targeted for imputation. We analyze as a function of the divergence time the expected gain in imputation accuracy in the target using a reference sequence from the same population as the target rather than from the other population. Together with a growing body of empirical investigations of genotype imputation in diverse human populations, our population-genetic modeling framework lays a foundation for extending imputation-based association techniques to novel populations that have not yet been extensively examined.

440F

Out of Africa migrations determine the distribution of deleterious alleles in diverse human genomes. *J. Kidd[1], B. Henn[1], S. Musharoff[1], M.C. Yee[1], G. Euskirchen[1], H.M. Cann[2], M. Snyder[1], C.D. Bustamante[1].* 1) Department of Genetics, Stanford University, Stanford, CA; 2) Fondation Jean Dausset - Centre d'Étude du Polymorphisme Humain Paris, France.

The primary demographic signature in many human populations is an ancient bottleneck associated with the Out-of-Africa expansion 50,000 years ago. We expect this event to have a lasting effect on existing patterns of genetic variation, including altered proportions of deleterious alleles in populations that have undergone severe bottlenecks. To test this hypothesis, we have sequenced full genomes to 6x-15x coverage from over 50 individuals from 7 divergent human populations included in the HGDP-CEPH Diversity Panel. These populations complement the 1000 Genomes Project by establishing a picture of genomic diversity in geographically and ethnically distinct indigenous groups from Namibia, Congo, Algeria, Pakistan, Cambodia, Siberia and Mexico. We identify alleles inferred to be deleterious using the predicted impact for protein-coding changes as defined by the PolyPhen-2 algorithm. Using the analysis framework implemented in the program dadi we fit demographic models based on a numerical solution of the diffusion equation representation of the allele frequency spectrum. Using these data, we test the effect of the age and strength of bottlenecks on the distribution of deleterious alleles and quantify the distribution of selective effects for variants observed in each population. We conclude that ancient demographic history, in particular severe bottlenecks, contribute to significant differences between human populations in their mutational load.

441F

Validating the authenticity of the pedigrees of Chinese Emperor CAO Cao of 1,800 years ago. *H. Li.* MOE Key Laboratory of Contemporary Anthropology, Fudan University School of Life Sciences, Shanghai, China.

Deep pedigrees are of great value for studying the Y chromosome evolution. However, the authenticity of the pedigree information requires careful validation. Here, we validated some deep pedigrees in China with full records of 70-100 generations spanning over 1,800 years by comparing their Y chromosomes. The present clans of these pedigrees claim to be descendants of Emperor CAO Cao (155AD-220AD). Haplogroup O2-M268 is the only one that is enriched significantly in the claimed clans ($P=9.323\times10^{-5}$, OR=12.72), and therefore, is most likely to be that of the Emperor. Moreover, our analysis showed that the Y chromosome haplogroup of the Emperor is different from that of his claimed ancestry of the earlier CAO aristocrats (Haplogroup O3-002611). This study offers a successful showcase of the utility of genetics in studying the ancient history.

442F

Using ancestry information and tests of natural selection to prioritize craniofacial candidate genes for admixture mapping. *D.K. Liberton[1], A.W. Bigham[2], X. Mao[3], B.P. McEvoy[4], M.D. Shriver[1].* 1) Anthropology, Pennsylvania State University, University Park, PA; 2) Department of Anthropology, University of Michigan, Ann Arbor, MI; 3) Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, PA; 4) Queensland Institute of Medical Research, Brisbane, Australia.

When performing candidate gene studies, prioritizing the genes to assay leads to more efficient use of resources. A set of candidate genes known to be involved in Mendelian craniofacial dysmorphologies will be tested against quantitative measures of normal human facial variation in an admixture mapping study. To determine the list of candidate genes to test, ancestry information as well as tests of natural selection were used to prioritize the genes. First, a list of 199 potential candidate genes associated with craniofacial variation was determined by searching OMIM for the terms “facial” and “craniofacial.” However, only SNPs that exhibit large frequency differences (high deltas) between particular parental populations are informative for admixture mapping studies. Therefore, all SNPs in these 199 genes were pulled from HapMap and then tested for the presence of ancestry informative markers (AIMs) with a frequency difference of greater than 50% between West Africans and Europeans as the high-delta threshold. Of the 199 OMIM candidate genes, only 58 genes had AIMs with high deltas. To further refine the candidate genes, a 500kb window surrounding each of the 58 candidate genes was tested for signatures of natural selection in the HapMap populations using three standard statistics: Tajima's D, the log of the ratio of the heterozygosities (lnRH), and Locus Specific Branch Length (LSBL). A gene was considered to show evidence of natural selection if all three statistics had significant values or if Tajima's D and either lnRH or LSBL had significant values. Because these genes will be tested for phenotypic effects in an admixed population of West African and European descent, a significant value in either Europeans or West Africans was required for inclusion of the gene in the candidate gene list. After testing, 30 out of 58 genes containing AIMs were found to exhibit signatures of natural selection. Nine out of 30 genes demonstrated significant evidence of natural selection in West Africans and 17 in Europeans. Additionally, four genes yielded significant results in both populations, although the proportion of significant SNPs in each window varied between the populations. These methods provide a means of prioritizing candidate genes for tests for phenotypic association via admixture linkage. Ancestry informative SNPs in these 30 selection-nominated candidate genes will be then tested for an association with normal human facial variation.

443F

Signature of negative natural selection in the human genome on sites that are not conserved across species. *K. Lohmueller, R. Nielsen.* Integrative Biology, University of California, Berkeley, CA.

Comparative genomic studies indicate that approximately 5% of the human genome is conserved across distantly related species. Based on these analyses, it has been suggested that about 5% of the genome is directly affected by negative natural selection. The common assumption is that the remaining 95% of the genome is neutrally evolving, though, to date, this assumption has not been explicitly tested. Here we test this assumption. To do this, we developed a novel approach to detect negative natural selection at sites of the genome that are not conserved across species. We applied this test to six European genomes sequenced by Complete Genomics. First, we removed sites that are conserved across distantly related species. Under the null hypothesis, the remaining sites should be neutrally evolving. However, we found that in genic regions of the genome, minor allele frequency was positively correlated with human-chimp divergence. Such a correlation is unexpected under models including only neutrally evolving sites. Instead, this correlation can be explained by varying amounts of negative selection across the genome. Regions with more sites under negative selection will have reduced human-chimp divergence and reduced minor allele frequency. Regions with fewer sites under negative selection have higher levels of divergence and higher allele frequencies. Thus, this result suggests that there are non-conserved sites that are directly affected by negative natural selection and that these sites are located near genes. Conversely, in regions of the genome that do not overlap with genes, there is no correlation between human-chimp divergence and minor allele frequency, suggesting that non-conserved sites in these regions are neutrally evolving. Finally, we examined the site frequency spectrum of single nucleotide polymorphisms (SNPs) at sites of the genome that are not conserved across distantly related species. Nonsynonymous, 5' UTR, and 3' UTR SNPs showed an excess of low-frequency alleles, as compared to intergenic SNPs, suggesting that the former have been affected by negative selection. We will present estimates of the fraction of the genome that is negatively selected within human populations. In conclusion, we have shown that sites of the human genome that are not conserved across species are not all neutrally evolving.

444F

A SNP array for human population genetics studies. *Y. Lu[1], T. Genschoreck[1], S. Mallick[2,3], A. Ollmann[1], N. Patterson[3], Y. Zhan[1], T. Webster[1], D. Reich[2,3].* 1) Affymetrix, Inc. Santa Clara, CA 95051; 2) Harvard Medical School Department of Genetics, Boston, MA 02115; 3) Broad Institute of Harvard and MIT, Cambridge, MA 02142.

High-density arrays that simultaneously genotype hundreds of thousands of single nucleotide polymorphisms (SNPs) can be a powerful tool in theory for population genetics studies, whether for learning about human history or natural selection. However, SNP arrays designed for medical genetics have been limited in their utility for population genetics because the polymorphisms on the arrays were discovered and selected for inclusion in a complicated manner that is difficult to model. This “ascertainment bias” has severely limited the types of population genetic analyses that can be carried out with SNP arrays. Here we report the first SNP array developed specifically for human population genetics studies. A total of 1.81 million candidate SNPs, all from genome locations covered by sequencing reads from Neandertals, Denisovans, and chimpanzees, were ascertained using a simple SNP discovery procedure first described by Keinan et al., 2007. The most important ascertainment involved using whole-genome shotgun sequencing data to discover differences between the two chromosomes carried by individuals from 11 populations (San, Yoruba, Mbuti, French, Sardinian, Han, Cambodian, Mongolian, Karitiana, Papuan, and Bougainville). This resulted in 13 panels of SNPs ascertained in simple ways that are appropriate for population genetic analysis. An Axiom® genotyping screen was carried out for 1.35 million of the SNPs and about 600,000 were validated on the Axiom platform, producing genotypes that passed rigorous quality thresholds appropriate for inclusion in a commercial array design. To facilitate joint analysis with other data sets that have been collected on diverse populations, the final array contains an additional approximately 80,000 SNPs that overlap between the Affymetrix SNP Array 6.0 and Illumina 650Y array. We genotyped 952 unrelated samples from 53 populations in the CEPH-Human Genome Diversity Panel (Cann et al., 2002), and have made the data freely available in the CEPH-HGDP database. In addition, we report on the genotyping of approximately 500 samples from 70 other diverse worldwide populations. Preliminary analyses demonstrate the promise of this SNP array for population genetics. As examples, we use the data to provide a new line of evidence for gene flow from Neandertals into modern humans, and to learn about the history of ancient African population that was ancestral to Yoruba and San Bushmen.

445F

Assigning Intra-European Ancestry to Identical-by-Descent Segments using a Large Database of Self-Reported Ancestry. *J.M. Macpherson, B.T. Naughton, C.B. Do, J.Y. Tung, J.L. Mountain.* 23andMe.com, Mountain View, CA.

For assignment of ancestry to a given genomic region, traditional autosomal ancestry analyses rely on probabilistic models of haplotype frequencies. This approach has been successful in assigning ancestry in individuals with ancestry from widely-separated geographic regions, for example in admixture mapping studies of African-American and Latino populations. However, this approach has difficulty in discriminating between haplotypes from more closely-related populations. Here we introduce a method for autosomal ancestry assignment using identical-by-descent (IBD) segments from a large database of individuals of European ancestry who have themselves provided information about their, their parents', and their grandparents' ancestry. The method is frequently able to identify the European countries of origin of segments in individuals of known ancestry correctly, which suggests its use in identifying the origin of segments in individuals of unknown ancestry. The method is based on the idea that, if an individual shares an IBD segment with an individual of uniform ancestry from a given country, the segment likely derives from that country. To guard against the possibility of erroneous or misleading ancestry information, we use a procedure based on principal components analysis to filter the dataset. We examined the concordance of the method's results with the individuals' own self-reported ancestry information; depending on the country of origin, the method correctly identifies European country of origin from 55% to 85% of the time, and correctly identifies European region of origin 65 to 100% of the time. We also explore the accuracy of the method in Ashkenazi Jewish individuals, finding 85% concordance in individuals with self-reported Ashkenazi Jewish ancestry. We conclude by analyzing how this method's coverage and accuracy depend on database size and mean population IBD sharing.

446F

Comparative population structure among three ethnic groups (Indian, Chinese, and Malay) from Malaysia and Singapore. *J.E. Molineros[1], K.H. Chua[2], A.J. Adler[1], K.M. Kaufman[1], S.K. Nath[1].* 1) Program of Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation, 825 NE 13th Oklahoma City, OK 73104; 2) Dept of Molecular Medicine,Faculty of Medicine,University of Malaya,50605 Kuala Lumpur, Malaysia.

Malaysia is a multi-ethnic country with three main ethnic groups (Malay (54%),Chinese (25%) and Indians (9%)). Our objectives were to elucidate population structure of 3 ethnic groups in Malaysia, and compare admixture proportions between Malayans and Singapore natives. We genotyped 575 individuals from Malaysia from 3 different ethnic origins (Chinese N=363; Malay N=173; and Indian N=39). We selected 1265 ancestral informative markers. We used 268 samples from the Singapore Variation Project ($N_{Chinese}$=96; N_{Indian}=83; N_{Malay}=89), 944 HGDP samples and 993 HAPMAP samples as reference. We estimated 10 principal components and used them to determine the optimal model for clustering between populations. We estimated admixture proportions and compared them between countries of origin. We attributed origin to each cluster by admixture proportion of the reference population in each cluster. Differences between ethnic groups were tested by t-test. Using all population data, the best admixture model was identified as the 6 ancestral population model; optimal classification of principal components had 6 clusters. Among the 6 admixture populations we identified primarily Chinese-Japanese, African, Polynesian, Caucasian, Indian, and Native American ancestries. Malaysian and Singapore Chinese were only significantly different on their African ancestry ($P=4.2x10^{-3}$). Indians were significantly different in the Indian, Caucasian and Chinese-Japanese ancestries ($P_{Indian}=3.1x10^{-2}$, $P_{Caucasian}=1.1x10^{-5}$, $P_{China}=3.3x10^{-4}$). Malays were significantly different in the Indian, Chinese-Japanese, African and Polynesian ancestries ($P_{Indian}=5.7x10^{-10}$, $P_{China}=2.3x10^{-8}$, $P_{Africa}=1.6x10^{-4}$, $P_{Polynesia}=2.8x10^{-2}$). This was confirmed by clustering, where samples from Singapore remained within the same cluster, and Malaysian samples clustered in separate groups. Malaysian-Chinese were split into the Han-Chinese cluster and the cluster including Malays, Dai Chinese and Cambodians. Indians clustered together with Gujarati and Pakistani. Indians and Malay were significantly differently admixed with the major difference coming from the Chinese-Japanese cluster and the Caucasian cluster. Difference in Malay admixture was primarily due to differences in Indian ancestry. These results suggest a difference in the history of admixture between individuals from Malaysia and Singapore.

447F

Comparison of the X Chromosome to Autosomes of Diverse Human Genomes and the Analysis of Sex-Specific Processes. *S. Musharoff[1], S. Ramachandran[2], J.M. Kidd[1], B.M. Henn[1], M.C. Yee[1], H.M. Cann[3], G. Euskirchen[1], M. Snyder[1], C.D. Bustamante[1].* 1) Genetics, Stanford University School of Medicine, Stanford, CA; 2) Department of Ecology and Evolutionary Biology, Brown University, Providence RI; 3) Fondation Jean Dausset - Centre d'Étude du Polymorphisme Humain Paris, France.

Analysis of the human X chromosome presents a unique opportunity to look at the effect of population genetic forces. Because the X chromosome is carried in two copies in females and in one copy in males, it has a smaller effective population size relative to that of the autosomes and is more affected by drift. Since males are effectively haploid on the X chromosome, there may be increased selection relative to the autosomes as has been previously shown in other organisms. The X chromosome is sensitive to sex-baised processes such as male-based migration or increased female reproductive success and comparison of the X chromosome to autosomes can reveal the information about demographic events that looking at either class of chromosomes alone cannot. To quantify the effect of sex-biased forces on patterns of genomic variation, we have sequenced full genomes to 6x-15x coverage from over 50 individuals from 7 divergent human populations included in the HGDP-CEPH Diversity Panel. Estimates of diversity, levels of heterozygosity, and TMRCA (time to most recent common ancestor) as well as the distribution of deleterious alleles compared between the X chromosome and autosomes reveal the effect of sex-biased forces. By inferring the demographic history of these populations, some of which have been isolated or have experienced a bottleneck, we seek to reconcile conflicting estimates of key population genetic parameters relating to the X chromosome including the effective population size of males and females. This is of fundamental importance to the reconstruction of human history and to analysis that depends on the estimate of human demographic parameters.

448F

PRDM9 binding targets occur within most human recombination hotspots. *N. Noor[1], J. Knight[1], A. Aricescu[3], G. McVean[1,2], S. Myers[1,2].* 1) Wellcome Trust Centre of Human Genetics, Oxford University, Oxford, United Kingdom; 2) Department of Statistics, University of Oxford; 3) Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford.

In humans, meiotic recombination and crossing over is an essential process required for correct segregation of chromosomes. Recombination clusters within 1 to 2kb regions of the genome called “hotspots”, enriched for the presence of a 13-bp sequence motif, CCNCCNTNNCCNC. Recent work by several groups has shown PRDM9 binds to this motif, and so controls human recombination hotspot positioning. However, a close motif match occurs in only 40% of hotspots, while recent work shows that PRDM9 somehow controls hotspot activity even for hotspots lacking a close motif match. To address how this occurs, we used EMSAs to explore binding characteristics of human PRDM9. In each of five previously identified hotspots without a close motif match, we observe strong in vitro PRDM9 binding to more degenerate, but recognisably 13-bp motif like sequences near the hotspot centre, offering an explanation for hotspot activity in these cases. Although we also show PRDM9 binds DNA in a highly selective manner, with certain single base pair changes essentially abolishing binding, it is capable of binding considerably degenerate sequences, raising the question of why only a subset of in vitro binding targets correspond to recombination hotspots. A likely explanation is a role for nucleosome positioning and nucleosome modifications. We report the results of the first genome-wide survey revealing the role of these features in interacting with PRDM9 binding to shape the human recombination landscape.

449F

Length distributions of identity by descent reveal fine-scale demographic history. *P. Palamara, I. Pe'er.* Dept. of Computer Science, Columbia University, New York City, NY.

Data-driven studies of identity-by-descent (IBD) were recently enabled by high resolution genomic data and IBD detection algorithms that scale well for large, densely typed cohorts. However, haplotype sharing currently represents an underutilized source of information for population genetics research. We present a closed form relationship between a population's demographic history and IBD sharing. Based on the distributions for coalescent events and the length of co-inherited haplotypes, we express the expected fraction of genome spanned by IBD segments in specific length intervals as a function of a population's demographic history. We derive a least squares, closed form solution for population size inference in a Wright-Fisher model, and an efficient hill climbing approach for complex demographies. The reconstructed population size is within 3% of the true size in 95% of our simulations for Wright-Fisher populations. For exponentially expanding or contracting populations, the reconstructed size is within 10% of the true size for the past 100 generations. We applied our inference procedure to two densely typed datasets: 500 Ashkenazi Jewish (AJ) individuals and 56 Kenyan Masai individuals (HapMap 3 dataset). Analyzing the AJ cohort we reconstruct a mild population expansion (r _ .02) during the past two millennia, leading to an ancestral population of ~60,000 individuals in the 12th century A.D. Following a severe bottleneck, we reconstruct ~300 individuals rapidly (r _ .28) expanding to ~4,000,000 in the past 34 generations. This demographic profile is consistent with previous genetic analysis and historical accounts of AJ demographic history. In the Masai cohort high levels of cryptic relatedness are detected, in agreement with recent reports. The spectrum of IBD sharing favors a demographic explanation of this phenomenon over the effects of sampling procedures. When a single population model is used, our methodology suggests a population bottleneck (r _ -.14) during the past 30 generations. We show how this effect can be explained by an alternative demographic model, where several small-sized demes (“villages”) interact through high migration rates, resulting in a pattern of shared long-range haplotypes that is compatible with that observed in the Masai cohort. This mismatch between single-population models and historically structured demographies may explain the unexpected abundance of runs of homozygosity within several populations.

450F

A combined approach to identifying preterm birth risk genes: admixture mapping and signatures of selection. *L.N. Pearson[1], A.W. Bigham[2], X. Mao[3], J.P. Kusanovic[4], R. Romero[4], J.F. Strauss 3rd[5], M.D. Shriver[1].* 1) Department of Anthropology, The Pennsylvania State University, University Park, PA; 2) Department of Anthropology, University of Michigan, Ann Arbor, MI; 3) Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, PA; 4) Perinatology Research Branch of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health in Bethesda, Maryland and Wayne State University, Detroit, MI; 5) School of Medicine, Virginia Commonwealth University, Richmond, VA.

Preterm birth is a complex phenotype that is a leading cause of infant mortality in the United States. Although there is a role for social and environmental factors in prematurity, it is increasingly understood that genes also contribute to risk. African-American women experience the greatest risk of preterm birth among U.S. populations, nearly twice that of European-American women. To prioritize a genotyping panel to test for association with preterm birth among African-American women, a combination of admixture mapping and selection screening, on both regions identified by admixture mapping and of candidate genes, was used. Admixture mapping analysis was conducted using a sample of 632 women of self-reported African-American ancestry recruited from Hutzel Hospital (Detroit), the Hospital of the University of Pennsylvania (Philadelphia) and Virginia Commonwealth University Medical Center (Richmond). Genotyping of cord blood or umbilical cords from the neonates of 371 women with confirmed preterm premature rupture of membranes (PPROM) and neonates from 261 women with normal term pregnancy outcomes was completed using a genome-wide panel of 1,509 ancestry informative markers (AIMs) designed for admixture mapping analysis in African-American populations. Using prior allele frequencies from three parental populations (West African, European and East Asian), Bayesian admixture mapping was conducted using the computer program ADMIXMAP. Regions on five chromosomes (2, 8, 11, 19 and 21) were identified as significantly contributing to risk of PPROM. Single nucleotide polymorphisms (SNPs) with significant allelic association within the chromosomal regions identified in the admixture mapping analysis were tested for signatures of selection using normalized Tajima's D, log of the ratio of heterozygosities (lnRH), and locus specific branch length (LSBL). Of the 26 SNPs analyzed, 6 show evidence for selection in West Africans. Additionally, preterm birth candidate genes identified from previous reports were assessed using the same tests of selection. Fourteen of the 85 candidate genes screened were found to show signatures of selection using all three tests. Combining admixture mapping with tests for signatures of selection is a useful method for identifying genes associated with phenotypes that vary among populations, like preterm birth.

451F

Increased Genetic Diversity observed among Inupiat Populations from the North Alaskan Slope. *J.A. Raff[1, 2], M. Rzhetskaya[1], L. Armstrong[1], M.G. Hayes[1, 3, 4].* 1) Division of Endocrinology, Metabolism, and Molecular Medicine, Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL; 2) Department of Anthropology, University of Utah, Salt Lake City, UT; 3) Center for Genetic Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL; 4) Department of Anthropology, Northwestern University, Evanston, IL.

Inupiaq speaking populations of the North American Arctic are characterized mitochondrially as possessing very little genetic diversity, often postulated to be due to a very recent, rapid migration of small, isolated groups, taking perhaps only 100-200 years to reach Greenland from the North Slope of Alaska. The Inupiaq speaking populations of Canada and Greenland investigated to date are virtually monomorphic for mitochondrial haplogroup A (the remainder are D [<3%]). However, very little is known genetically of the North Alaskan Inupiat populations, currently inhabiting the hypothesized source region for the migration eastward into Canada and Greenland. We investigated the mtDNA haplotype frequencies of Inupiat populations from the Alaskan North Slope, and discuss the placement of this population in the geographical and temporal context of North American Arctic Prehistory. Using standard methods, HVS1 of the mtDNA genome was sequenced using DNA extracted from saliva samples of 178 consenting adults residing in all eight communities that span the Alaskan North Slope. There is considerable variation in the pattern and frequencies of mtDNA haplogroups among the eight communities investigated although they hold the general North American Arctic pattern of haplogroup A being most common followed by haplogroup D. Most interestingly, the frequency of haplogroup D (10.7%) among the aggregate North Slope population is the highest observed among Inupiaq speakers in the North American Arctic. We also observed two instances (1.1%) of participants belonging to haplogroup C, which has never been reported for this region of the world. When compared to the haplotypes of their neighbors, the frequencies of A2a (52.8%) and A2b (32.4%) are most similar to the Siberian Eskimos. Haplogroup D3 (7.3%), which is found in among Greenland and Canada inhabitants, is also found in North Slope populations, as is D2 (3.4%), which is found among Aleut and Siberian Eskimos and ancient (Paleo-)Eskimos from the eastern Arctic. All modern and ancient Eastern Arctic haplotypes are present among modern North Slope populations, suggesting that the North Slope is a good source for Inuit populations further east. This study was funded by the National Science Foundation, grant number OPP- 0732857.

452F

Population genetics of Finland revisited - looking Eastwards. *K. Rehnström[1,2], K. Palin[1], T. Esko[3,4,5], E. Salmela[2,6], O. Kallioniemi[2], J. Kere[6,7], S. Limborska[8], B. Melegh[9], J. Klovins[10], A. Metspalu[3,4,5], V. Salomaa[11], A. Palotie[1,2,12], P. Lahermo[2], R. Durbin[1], S. Ripatti[2].* 1) Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom; 2) Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland; 3) Department of Biotechnology, Institute of Molecular and Cell Biology, Faculty of Science and Technology, University of Tartu, Tartu, Estonia; 4) Estonian Genome Centre of University of Tartu, Tartu, Estonia; 5) Estonian Biocentre, Tartu, Estonia; 6) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 7) Department of Biosciences and Nutrition, and Science for Life Laboratory, Karolinska Institutet, Stockholm, Sweden; 8) Institute of Molecular Genetics, Russian Academy of Science, Moscow, Russia; 9) Department of Medical Genetics and Child Development, University of Pécs, Pécs, Hungary; 10) Latvian Biomedical Research and Study Center, Riga, Latvia; 11) National Institute for Health and Welfare, Helsinki, Finland; 12) The Broad Institute, Cambridge, Massachusetts, United States of America.

We have previously reported that the genetic structure within Finland correlates well both with geography and known population history. While these studies have quantified the genetic distances between Finland and European neighbours to the south and the west, the influence of the Eastern and the Northern populations have not been described using genome-wide tools. Here we investigated the degree of Asian ancestry in Northern Europe. We also studied the genetic ancestry of geographic and linguistic neighbours of Finns, using genome-wide SNP data in a dataset comprising over 2200 individuals. First we quantied the proportions of European (represented by HapMap CEU) and Asian (HapMap CHB/JPT) genetic ancestry. Within Finland, the average Asian ancestry proportion varied from 2.5% in the Swedish speaking Finns to 5.1% in Northern Finland. The Saami population, being the indigenous inhabitants of Northern Finland, showed a surprisingly high proportion of Asian genetic ancestry (17.5%). We therefore hypothesize that, as genetic sharing between individuals in Northern Finland and Saami are higher than in other parts of the country, the Asian genetic ancestry in Finland could partly be through admixture with the Saami. Using a model-based estimation of individual ancestry, three ancestral populations provided a best fit for the combined Finnish and Saami dataset. Particularly, one of these ancestral populations was predominant in the Saami (average 78%), and higher in Northern Finland (average 14%) compared to the rest of the country (average 4%). Despite the fact that Finns are the closest relatives of the Saami of all populations included in this study, in general, our results show that language and genetics are only weakly related. The Finns are more closely related to most Indo-European speaking populations than to linguistically related populations such as the Saami. These analyses are currently being extended to sequence level variation using genome-wide sequence data for 100 Finns as part of the 1000 Genomes project, and 200 further individuals from the North-Eastern Finnish subisolate of Kuusamo. These 200 individuals provide good power to identify founder haplotypes within this isolate. Next, we aim to investigate the power to extend the imputation of haplotypes to the rest of Northern Finland as well as to the rest of the country.

453F

Coal: A Coalescent Simulator Capable of Modeling Faster than Exponential Population Growth. *M. Reppell, S. Zoellner.* Department of Biostatistics, University of Michigan, Ann Arbor, MI.

Recent human sequencing projects with greater than 10,000 samples have discovered an extensive amount of very rare genetic variation with frequencies $<10^{-4}$. The estimated number of singleton and doubleton variants in these studies significantly exceed the predictions of a both a neutral Wright-Fisher and a model of exponential population growth. After sequencing 2 genes in 13,715 individuals, Coventry et al. estimated an expected number of singleton sites ~5 times greater and doubleton sites ~4 times greater than predicted under a neutral Wright-Fisher model at sites free from selection. Theory suggests a sample from a population growing at an explosive, faster than exponential rate, may display the quantity of rare variation seen, because the majority of mutation events in a sample from such a lineage would occur after the sample's pairwise MRCAs. Very rare variants are likely candidates for functional alleles as they are least affected by purifying selection. Development and refinement of new statistical tools aimed at uncovering the role of very rare genetic variants in trait and disease etiology depends on the ability to simulate data with an accurate distribution of rare variants. Current programs used to simulate human genetic data perform well when simulating common variation in humans. However these programs cannot accurately simulate the excess of rare variation present in human sequence data. Here we present a coalescent model of explosive, faster than exponential growth which generates the number of rare variants presently seen in human samples. By expressing the rate of change in a population's size as a function of its current size we are able to model samples from a population growing at a faster than exponential rate. We have implemented this model in a coalescent simulation program which generates haplotypes sampled from a population which has undergone such explosive growth. By comparing the site frequency spectrum and linkage disequilibrium patterns in simulated haplotypes to patterns in real sequence data we evaluate how well our program generates realistic data. Additionally, the model parameters which allow us to simulate human sequence data provide estimates of the growth rates responsible for the quantity of rare variation seen. In summary, we provide an easy to use tool which can rapidly produce realistic simulated human sequence data and answer questions about the impact of faster than exponential growth on a population.

454F

Exome Sequencing in a Diverse Set of Individuals Identifies Potentially Deleterious Alleles at Unexpected Frequency in Qatar, the Migration Crossroads of Arabia. *J.L. Rodriguez-Flores[1], J. Fuller[1], N. Hackett[1], J. Salit[1], J.A. Malek[2], M.A. Ziki[2], G. Hoffman[3], L. Omberg[3], A. Jayoussi[4], M. Zirie[4], R.G. Crystal[1], J.G. Mezey[1,3].* 1) Department of Genetic Medicine, Weill Cornell Medical College, New York, NY; 2) Department of Genetic Medicine, Weill Cornell Medical College in Qatar, Doha, Qatar; 3) Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY; 4) Hamad Medical Corporation, Doha, Qatar.

The allelic variation that distinguishes populations in the Arabian peninsula has not been studied in any depth. Qatar is a nation situated at the crossroads of Africa and Eurasia with a diverse population representative of ancient and recent migration in the region, hence it is ideal for sampling the spectrum of allelic variation in Arabia. Here, we used deep exome sequencing of Qatari individuals to identify potentially deleterious alleles at unexpected frequency when compared to European, Asian, and African populations sampled by the 1000 Genomes Project (1000G). Our strategy was to select Qatari individuals of primarily Arabian, Persian, or African ancestry, a diverse sample representing three major migrations in the region. Our analysis identified SNPs and other allelic variants that are either novel or at unexpected frequency in Qatar when compared to one, two or three neighboring continents (Europe, Asia, Africa). Where possible, we imputed local haplotypes around the exons using 1000G individuals as a reference panel, and determined the most likely continental origin of haplotypes flanking the enriched SNPs. Using a combination of disease and drug metabolism databases, including HuGE, OMIM, HGMD, PharmGKB and prediction tools, including SIFT and PolyPhen-2, we identified the subset of these alleles that show evidence for being potentially deleterious or that have been previously associated with disease. A number of these cases were consistent with the epidemiological profile of Qatar and reflect the rich migration history of Arabia. Among others, this included a SNP previously associated with type 2 diabetes in Asian populations that has an extreme frequency in Qatar with a 1.4x odds ratio for the risk allele in the Qatari individuals of Arabian and Persian ancestry, a result consistent with the high incidence of diabetes among the Qatari people. Our study demonstrates that next-generation exome data collected for a small sample of individuals can be used to reveal haplotypes of possible medical importance in populations understudied by genome sequencing efforts, providing an empowerment mechanism for building internal genomic health initiatives based on samples of diverse populations.

455F

Survey of 20,000 human Y chromosomes shows that the population genetic characteristics of deletions involving AZFc and the rates at which they arise by new mutations are surprisingly diverse. *S. Rozen[1,2], J.D. Marszalek[1,3,4], K. Irenze[5], L. Brown[1,3,4], H. Skaletsky[1,3,4], K. Ardlie[5,6], D.C. Page[1,3,4].* 1) Whitehead Institute, Cambridge, MA; 2) Duke-NUS Graduate Medical School, Singapore; 3) Howard Hughes Medical Institute; 4) Department of Biology; Massachusetts Institute of Technology, Cambridge, MA; 5) Genomics Collaborative, SeraCare Life Sciences, Cambridge, MA; 6) Broad Institute, Cambridge, MA.

The Y-chromosome's AZFc region is subject to four types of recurrent deletion. One of these, the b2/b4 deletion, removes most of the region and always severely impairs spermatogenesis. Three others, the “partial AZFc deletions" affect less of the Y chromosome, and many studies have focused on their influence on spermatogenesis. To better understand the population genetics of deletions involving AZFc, we assessed their prevalence in five populations and a total of 20,884 men unselected for sperm count or fertility. These results provide new insights into the relationships between the prevalence of the deletions, the rates at which they arise by new mutations, and natural selection. The prevalence of b2/b4 deletions across the five populations, 0.04%, is the lowest of four types of deletions. As b2/b4 deletions are almost never transmitted without assisted reproduction, their prevalence approximates the rate at which they arise by new mutation. The gr/gr deletions are the most common in our sample, with a prevalence of 2.4%. Based on this prevalence and the risk of severely impaired spermatogenesis in men with gr/gr deletions, we infer that they arise at a rate similar to new b2/b4 deletions. Deletions in a third group, the b1/b3 deletions, are almost are rare as b2/b4 deletions, 0.1%. Our analysis suggests that these deletions arise by new mutation < 1/10th as often as b2/b4 deletions. Further analysis suggests that deletions in a fourth group, the b2/b3 deletions, also arise very infrequently by new mutation. The population genetic characteristics of deletions involving AZFc and the rates at which they arise by new mutations are surprisingly diverse.

456F

Ancient exome sequencing of human remains from Teopancazco, a neighborhood of the Teotihuacan Mesoamerican city. *K. Sandoval[1], B.A. Áglvarez-Sandoval[2], A. Moreno-Estrada[1], F.M. De La Vega[1], L.R. Manzanilla[3], T. Harkings[4], C.D. Bustamante[1], R. Montiel[2].* 1) Genetics, Stanford, Stanford, CA; 2) Laboratorio Nacional de Genómica para la Biodiversidad, CINVESTAV-IPN. Km. 9.6 Libramiento Norte Carretera Irapuato - León. Irapuato, Guanajuato, Mexico; 3) Instituto de Investigaciones Antropológicas, Universidad Nacional Autónoma de México, Mexico City, Mexico 04510; 4) Genetic Systems, Life Technologies, Beverly, MA 01915.

Mesoamerica is the cradle of some of the most complex and advanced civilizations of the New World. Its geographic location has been a key factor for acting as a natural corridor between North and Central-South America, thus becoming home not only of the initial and subsequent human migration waves into the continent but also of the many cultures that flourished later on. Today, this large geographical area continues to exhibit one of the highest cultural, linguistic, genetic and archeological diversity in the Americas. Of particular interest is the archeological site of Teotihuacan as it was the largest city in the pre-Columbian Americas. Around 500 CE, it may have had more than 125,000 inhabitants, placing it among the largest cities of the world in this period. The ethnicity of the inhabitants of Teotihuacan is a subject of debate. Possible candidates are the Nahua, Otomi or Totonac ethnic groups, but it has also been suggested that Teotihuacan was a multiethnic city. In order to shed light on their population history reconstruction, and as part of the multidisciplinary project Teotihuacan: elite and government, directed by Linda R. Manzanilla, we have collected 100 samples from human remains from the Teotihuacan neighborhood of Teopancazco and succesfully exctracted genomic DNA for 20 samples derived from bone and tooth. These samples are used to prepare whole-genome sequencing libraries to obtain mitochondrial variants and exon capture libraries for high-coverage human-specific sequence. The objective is to reconstruct the genetic variability of an ancient Native American population. By collecting genome-wide sequencing data of a pre-Columbian population we will be able to provide a non-biased reference panel of the Native American gene pool present in the Americas prior to the European contact. Comparison of these variants with contemporary genomic data from putatively related populations will be valuable to reveal important historic and genomic processes shaping the genome of extant Native Americans and to evaluate the proportional contribution of pre-Columbian diversity into present day ethnic groups and admixed Latino populations; as well as to discover functionally relevant and disease-related variants that were specific to Native Americans prior to the European contact. Given the scale of this effort, our project represents unparalleled challenges in the field but is paving the path on genome-wide ancient DNA studies in the Americas.

457F

A simple test for historical gene flow between populations based on shared genotype data for two individuals. *A. Scally, B. Yngvadottir, Y. Xue, Q. Ayub, C. Tyler-Smith, R.M. Durbin.* Wellcome Trust Sanger Institute, Hinxton, CB10 1SA, United Kingdom.

The increasing accessibility of genome sequence data means we are now able to sample genome-wide sequence diversity in many human and non-human primate populations throughout the world. Genetic analyses have shown that while divergence and separation are common features in the evolutionary history of these populations, so too are subsequent migration and gene flow between them. However, the inference of gene flow is often complicated by the use of a numerical demographic model in which the null hypothesis is overspecified. Here we derive a simple test for historical gene flow based on the sharing of heterozygous sites between two individuals from different populations, making a minimal set of assumptions about the demographic history of the two populations. In particular we make no assumptions about the time of separation or the size of either population since separation. The test uses genotypes called in each individual relative to an outgroup (e.g. chimpanzee for human data), and does not require phase information. We demonstrate this test and assess its robustness using coalescent simulations, and we apply it to publicly available data collected from individuals from modern human populations in Europe, Africa and Asia. We also apply it to data from two species of Gorilla, showing evidence for gene flow between the subspecies since separation, even though they are now morphologically distinct and separated by 1000 km in central Africa.

458F

Variants in SNAP25 are targets of natural selection and influence verbal performances in women. *M. Sironi[1], R. Cagliani[1], S. Riva[1], C. Marino[1], M. Fumagalli[1], M.G. D'Angelo[1], V. Riva[2], G.P. Comi[3], U. Pozzoli[1], D. Forni[1], M. Caceres[4,5], N. Bresolin[1,3], M. Clerici[6,7].* 1) Bioinformatics, IRCCS Eugenio Medea - Associaz, Bosisio Parini, Italy; 2) The Academic Centre for the study of Behavioural Plasticity, Vita-Salute San Raffaele University, Milan, Italy; 3) Dino Ferrari Centre, Department of Neurological Sciences, University of Milan, Fondazione Ca' Granda IRCCS Ospedale Maggiore Policlinico, 20122 Milan, Italy; 4) Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain; 5) Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain; 6) Chair of Immunology, Department of Biomedical Sciences and Technologies LITA Segrate, University of Milan, 20090 Milano, Italy; 7) Fondazione Don C. Gnocchi, IRCCS, 20148 Milano, Italy.

Descriptions of genes that are adaptively evolving in humans and that carry polymorphisms with an effect on cognitive performances have been virtually absent. SNAP25 encodes a presynaptic protein with a role in regulation of neurotransmitter release. We analysed the intra-specific diversity along SNAP25 and identified a region in intron 1 that shows signatures of balancing selection in humans. The estimated TMRCA (time to the most recent common ancestor) of the SNAP25 haplotype phylogeny amounted to 2.08 million years. The balancing selection signature is not secondary to demographic events or to biased gene conversion, and encompasses rs363039. This SNP has previously been associated to cognitive performances with contrasting results in different populations. We analysed this variant in two Italian cohorts in different age ranges and observed a significant genotype effect for rs363039 on verbal performances in females alone. Post hoc analysis revealed that the effect is driven by differences between heterozygotes and both homozygous genotypes. Thus, heterozygote females for rs363039 display higher verbal performances compared to both homozygotes. This finding was replicated in a cohort of Italian subjects suffering from neuromuscular diseases that do not affect cognition. Heterozygote advantage is one of the possible reasons underlying the maintenance of genetic diversity in natural populations. The observation that heterozygotes for rs363039 display higher verbal abilities compared to homozygotes perfectly fits the underlying balancing selection model. Although caution should be used in inferring selective pressures from observed signatures, SNAP25 might represent the first description of an adaptively evolving gene with a role in cognition.

459F

Phylogeography of R1a1 Y-chromosomal haplogroup and genetic history of Indo-Europeans. *V.A. Stepanov, V.N. Kharkov.* Institute for Medical Genetics, Tomsk, Russian Federation.

Recent discussion of the prehistoric spreading of the Indo-European language group has generally concentrated on two alternative hypotheses: so-called “Kurgan Culture” hypothesis, which places the homeland of proto-Indo-Europeans to the Steppe of Eastern Europe, and alternative hypothesis of the spread of farmers from the Near East (Anatolia) to Europe in the Neolithic times. Y-chromosomal haplogroup R1a1, lineage is thought to have originated in the Eurasian Steppes north of the Black and Caspian Seas, seems to be associated with the Kurgan culture. Three geographic areas with the highest frequency of R1a1 haplogroup were revealed: Eastern Europe; Southern Siberia and Hindustan where the highest diversity of microsatellite haplotypes was observed. Phylogenetic analysis of microsatellite haplotypes demonstrates the presence of three corresponding major clusters with the age of the generation of haplotytic diversity of 7.2-12.5 ky. The highest diversity in Hindustani is related to the presence of haplotypes of Indo-Pakistani and Southern Siberian clusters in the population from India and Pakistan, probably due to relatively recent migrations from Central Asia. The age of the cluster admittedly brought to Hindustan from Central Asia / Southern Siberia is 3,9 +/- 1,3 ky. Probably, the primary center of the generation of diversity and expansion of R1a1a was the territory of the Eastern European Steppe. With the spread of of R1a1 carriers, secondary centers of genetic diversity and population expansions were formed in the Southern Siberia and Hindustan.

460F

A Genetic Simulation Approach to Prehistoric Population Events in Finland. *T. Sundell[1,2], J. Kammonen[1], M. Heger[1], J. Palo[3], P. Onkamo[1].* 1) Department of Biosciences, University of Helsinki, Finland; 2) Department of Philosophy, History, Culture and Art Studies, Archaeology, University of Helsinki, Finland; 3) Hjelt Institute, Department of Forensic Medicine, University of Helsinki, Finland.

Due to Finland's geographical location and settlement history, the country has been a genetic isolate. Contemporary studies show that there is still a slightly reduced genetic diversity among the Finnish population. This diversity reduction and the specific `Finnish Disease Heritage' could be explained by founder effects and bottlenecks. Archaeologically, there is evidence for fluctuation of population size, including bottlenecks at 4100-3800 BP and 1500-1300 BP.

Together with population size estimates based on archaeological data, we use methods and ideas from genetics in order to assess the existence and size of possible prehistoric population bottlenecks. Well-preserved ancient organic remains entailing aDNA are practically non-existent in Finland due to the naturally acidic soil. Thus, we employ population simulations to follow genetic changes over hundreds of generations and evaluate the effects of Neolithic population bottleneck on the assumed Finnish gene pool. Here, in continuation of our previous research, we apply both forward and coalescent simulations with mtDNA and Y chromosomal haplotypes to trace back the possible population history behind the present day genetic diversity in Finland.

Our new model simulation begins at 11,000 BP and the prehistoric Finnish population is simulated with two archaeologically justified bottlenecks. We split the population into geographic sub-populations, added gender-specific migration as well as migration waves from neighbouring populations, compatible with archaeological phenomena. To follow the assumed demographic events as realistically as possible, we added minor constant gene flow from three background populations: Archaic European, Archaic Scandinavian and Saami. We have obtained actual ancient mtDNA haplotype frequencies from published aDNA studies of prehistoric European populations and used them as a proxy. According to recent studies, there are considerable differences in haplotype distributions of hunter-gatherers and early farmers.

The preliminary results indicate that bottlenecks substantially reduce genetic diversity, at least in the narrowest models and a surprisingly small constant gene flow from background populations clearly outweigh the effects of larger, short-term migration waves.

461F

Molecular analysis of an ancient Thule population at Point Barrow, Alaska. *J.C. Tackney[1], J.A. Raff[1,2], J.B. Coltrain[1], A.M. Jensen[3], D.H. O'Rourke[1].* 1) Department of Anthropology, University of Utah, Salt Lake City, UT; 2) Division of Endocrinology, Metabolism, and Molecular Medicine, Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL; 3) UIC Science LLC, Barrow, AK.

Nuvuk was an Iñupiat village at Point Barrow, Alaska. It was continuously inhabited for at least 1300 years until it was lost to coastal erosion in the last century. Archaeologically recovered burials from Nuvuk have calibrated radiocarbon ages between 1187 and 1579AD. Molecular samples were collected in situ by genetic analysts prior to full burial exposure by archaeologists. mtDNA hypervariable segment I sequences indicate that the ancient Nuvuk individuals belong predominantly, but not exclusively, to mtDNA haplogroup A2. All ancient Nuvuk sequences reported here have been replicated from independent extracts in our laboratory.

Contemporary Iñupiat/Inuit populations and ancient Thule studied to date are nearly monomorphic for mtDNA haplogroup A2, although lineage heterogeneity exists. Individuals from Nuvuk can be confidently assigned to multiple sublineages of haplotypes A2b and A2a, as well as to haplotypes A2-root and D3. This is consistent with the standard view of the ancestral/descendant relationship between the prehistoric Thule and modern Iñupiat/Inuit populations. However, the mitochondrial sequence diversity at Nuvuk is noticeably lower than that observed in comparably aged ancient samples we have also sequenced from the Alaska Peninsula and Eastern Aleutians.

These results confirm that a subset of Beringian-specific mtDNA haplotypes were carried by the early Thule prior to their dispersal across the North American arctic, in what was likely the final major event in an extended period of later Holocene circumarctic migrations/gene flow between Asia and North America. The uniquely late expansion and limited dispersal of these mtDNA haplotypes in the Americas emphasizes the need to assay the full mtDNA genome, the Y chromosome, and various autosomal markers in these ancient samples. Next generation technologies will permit access to a richer genetic record of prehistoric arctic populations.

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462F

Synthesis of autosomal and gender-specific genetic structures of the Uralic-speaking populations. *K. Tambets[1], S. Rootsi[1], M. Metspalu[1], B. Yunusbayev[1,2], E. Metspalu[1], A.M. Ilumäe[1], M. Reidla[1], K. Dibirova[3], O. Balanovsky[3], I. Evseeva[3], L. Osipova[4], E. Khusnutdinova[2], R. Villems[1].* 1) Evolutionary Gen, Estonian Biocentre, Tartu, Estonia; 2) Institute of Biochemistry and Genetics, Ufa Research Centre, Russian Academy of Sciences, Ufa, Russia; 3) Research Centre of Medical Genetics of Moscow, Laboratory of Human Population Genetics, Moscow, Russia; 4) Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia.

The variation of uniparentally inherited genetic markers - mitochondrial DNA (mtDNA) and non-recombining part of Y chromosome (NRY) - has suggested somewhat different demographic scenarios for the spread of maternal and paternal lineages of North Eurasians, in particular those speaking Uralic languages. The west-east-directed geographical component has evidently been the most important factor that has influenced the proportion of western and eastern Eurasian mtDNA types among Uralic-speakers. The palette of maternal lineages of Uralic-speakers resemble that of geographically close to them European or Western Siberian Indo-European and Altaic-speaking neighbours. However, the most frequent in North Eurasia NRY type N1c, that is a common patrilineal link between almost all Uralic-speakers of eastern and western side of the Ural Mountains, is rare among Indo-European-speakers, with a notable exception of Latvians, Lithuanians and North Russians. In this study the information of genetic variation of uniparentally inherited markers in Uralic-speaking populations from 13 Finno-Ugric and 3 Samoyedic speakers is combined with the results of their genome-wide analysis of 650 000 SNPs (Illumina Inc.) to assign their place in a landscape of autosomal variation of North Eurasian populations and globally. The genome-wide analysis of the genetic profiles of studied populations showed that the proportion between western and eastern ancestry components of Uralic-speakers is concordant with their mtDNA data and is determined mostly by geographical factors. Interestingly, among the Saami - the population which is often considered as a genetic outlier in Europe - the dominant western component is accompanied by about one third of the eastern component, making the Saami genetically more similar to Volga-Finnic populations than to their closest Fennoscandian-East Baltic neighbors. The high frequency of pan-northern-Eurasian paternal lineage N1c among Saami cannot explain this phenomenon alone - genetic ancestry profiles of autosomes of other Finnic- and Baltic-speaking populations, who share the high N1c with the Saami, do not show a considerable eastern Asian contribution to their genetic makeup.

463F

Novel coding variation at TYRP1 explains a large proportion of variance in the hair colour of Solomon Islanders. *N.J. Timpson[1], E. Kenny[2], M. Sikora[2], A.M. Estrada[2], C. Eng[3], S. Huntsman[3], E.G. Burchard[3], M. Stoneking[4], C.D. Bustamante[2], S. Myles[2,5], The Solomon Islands Pigmentation Collection.* 1) MRC CAiTE Ctr/SSCM, Bristol Univ, Bristol, United Kingdom; 2) Department of Genetics, School of Medicine, Stanford University, Stanford, USA; 3) Department of Medicine, University of California, San Francisco, USA; 4) Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany; 5) Nova Scotia Agricultural College, Truro, Nova Scotia, Canada.

The Solomon archipelago comprises over 1,000 islands located east of Papua New Guinea and has a population noted for wide variation in hair pigmentation. 1200 samples were collected from 16 centres and hair colour measured in donors by spectrophotometer. Analysis of 589,241 single nucleotide polymorphisms across a subset of 42 dark haired and 43 blond haired individuals revealed a signal for pigmentation driven by 27 markers on 9p23 at the TYRP1 gene (rs13289810; OR=29.5, p=1.11e-19, lambda=1.03). There were no systematic differences in ancestry between dark and blond haired participants indicating that this variation is unlikely to be due to recent introgression from other populations. Sequencing of TRYP1 showed complete conservation of this locus bar nucleotide 5,888(NG_011750), which was homozygous C in dark haired individuals and T in blonds. The resulting CGC->TGC missense mutation changes the 93 amino acid in exon 2 from an Arginine to a Cystine. Genotyping of TYRP1(93C/T) in all samples and analysis showed that in a recessive model including sex, age and local geography, there was a -1.67(-1.76, -1.50) standard deviation difference in hair colour by genotype groups (p=3.5e-106) equating to ~40% variance in this trait. Genotyping in the Human Gene Diversity Panel showed TYRP1(93C/T) to be essentially private to the Solomon Islanders. TYRP1(93C/T) shows similarity to a Tyrp1 allele in the brown^light laboratory mouse where progressively lightened hair with age is due to premature melanocyte death. It is coincidental to observe another Arg to Cys substitution in humans and whilst in a different location, one would predict that a missense change creating a new Cys residue in the amino-terminal region of the protein would yield a similar phenotype. In humans, complete loss of function for Tyrp1 is known to cause rufous albinism. This is one of the only examples of a genomewide association study implicating causal variation directly, of a common local variant of functional effect being absent in other human populations and is one of the largest phenotypic effects attributable to a common polymorphism. Reasons for the maintenance of this variation are unclear, however this finding prompts the notion that we may find other large (disease causing) effect variants that are population specific and that our results are a call to arms to expand medical genomics to underrepresented populations.

464F

Estimation of Population Histories Incorporating Admixture using Genome-Wide Variation Data. *M. Tsai[1], G. Belloch[2], R. Ravi[3], R. Schwartz[4].* 1) CMU-Pitt Ph.D. Program in Computational Biology, Carnegie Mellon University, Pittsburgh, PA; 2) Department of Computer Science, Carnegie Mellon University, Pittsburgh, PA; 3) Tepper School of Business, Carnegie Mellon University, Pittsburgh, PA; 4) Department of Biological Science, Carnegie Mellon University, Pittsburgh, PA.

Learning how modern human populations have arisen, dispersed, and intermixed since we emerged as a species is one of the central questions in anthropology and human genetics. Past models for learning divergence times and ancient admixture proportions have often been limited to a few loci and perform poorly when applied to genome-wide genetic variation data. With the advent of large-scale genome variation studies, methods able to take advantage of large numbers of variations are needed. We previously developed a method to infer an overall tree of population history that has maximum consensus with a large number of genomic variants, using information theoretic techniques to automatically adjust the complexity of the tree based on the amount and quality of data available. Here, we describe a novel two-step inference model designed to extend that prior work to incorporate admixture and other sources of reticulate evolution. Rather than inferring the parameters directly from the molecular data, we first learn a model of population divergence using our prior consensus tree algorithm and use that model to categorize the timing of variations with respect to potential admixture events. We then apply a coalescence-based Metropolis-Hastings sampler to learn divergence times and admixture proportions around the putative association events. To evaluate the algorithm, we generated simulated data consisting of a two parental and one admixed population model where individuals from two parental populations P_1 and P_2 migrated to form admixed population at time t(generations with (admixture fraction and the two parental populations diverged from an ancestral population t_d generations ago. Empirical evaluation of our method using simulated data with different t(, t_d, and (parameters shows good performance of the method at inferring the correct parameters from realistic data set sizes. Comparison of our method with alternatives in the literature shows improved accuracy in admixture estimation. Results from the analysis of the HapMap Phase II dataset are shown consistency with prior literature on human population dynamics. While these are relatively simple scenarios, they nonetheless provide promising evidence that our method will provide a viable strategy for learning more detailed and accurate histories of admixed population groups from large genetic variation data sets.

465F

An automated method for deriving mitochondrial DNA (mtDNA) haplogroups based on changes within the Hypervariable Regions. *V.L. Vance, J.J. Bryan, M.R. Szczepanski, A.B. Carter, C.L. Mouritsen.* Research and Development, Sorenson Genomics, Salt Lake City, UT.

In 2009, van Oven and Kayser described a comprehensive phylogenetic tree of human mtDNA variation which has been made accessible at www.Phylotree.org. This phylogenetic tree is based on both coding and control (hypervariable) regions. Van Oven and Kayser identified the variances from the revised Cambridge Reference Sequence (rCRS) which define an individuals' haplotype and corresponding mtDNA haplogroup. A new computer-based method has been developed for assigning mtDNA haplogroups using the variances and haplogroup nomenclature described by van Oven and Kayser. This new method makes use of Structured Query Language (SQL) and a mathematical algorithm that allows for the reliable determination of one's haplogroup based solely on mtDNA sequence from the Hypervariable Regions (HVR). The SQL-based algorithm combines a database search process with a method that walks stepwise through the phylogenetic tree, which is rooted with rCRS at the first position. Using a novel scoring method to account for the number and stability of the markers that define each haplogroup, an individual's HVR differences from rCRS are compared with the haplogroup designations defined in the mtDNA Phylotree. The algorithm has a high degree of reliability even when potential “back-mutations” and/or recent mutations are observed at key haplogroup defining positions, in which case a haplogroup is assigned based on likelihood and match criteria thresholds defined within the algorithm. In instances of ambiguous calls, the algorithm has the ability to select the nearest parental haplogroup in the tree. This new method was validated by comparing the haplogroups assigned by our method to the haplogroups assigned by van Oven and Kayser for samples with mtDNA haplotypes published in Phylotree. This comparison showed concordance of our method to be greater than 95%. Use of our system can accurately and quickly estimate over 800 different mtDNA haplogroups across the mtDNA tree using only rCRS differences within Hypervariable Region 1, over 1000 haplogroups using Hypervariable Regions 1 and 2, and nearly 1100 haplogroups using Hypervariable regions 1, 2 and 3. Since Phylotree is continually being updated as new data are published, we have incorporated a parsing tool that allows the program to be updated as the science progresses.

466F

Height-associated loci contribute to genetic differences between African Pygmies and their non-Pygmy neighbors. *P. Verdu[1], T.J. Pemberton[1], N.S.A. Becker[2], A. Froment[3], B.S. Hewlett[4], S. LeBomin[2], C. Willer[5], N.A. Rosenberg[1,6,7], E. Heyer[2].* 1) Department of Human Genetics, University of Michigan, Ann Arbor, Michigan, USA; 2) CNRS-MNHN-Université Paris7, UMR 7206 Ecoanthropology and Ethnobiology, Paris, France; 3) IRD-MNHN, UMR 208 Patrimoines locaux, Paris, France; 4) Department of Anthropology, Washington State University, Vancouver, Washington, USA; 5) Department of Biostatistics, Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan, USA; 6) Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, Michigan, USA; 7) The Life Sciences Institute, University of Michigan, Ann Arbor, Michigan, USA.

African Pygmy populations have the shortest average adult height among worldwide human populations. These hunter-gatherer populations live in close socioeconomic association with nearby non-Pygmy agricultural populations that have noticeably greater average adult height. In search of genetic loci associated with height differences between Pygmies and non-Pygmies, we genotyped 358 individuals from 13 Central African Pygmy populations and 169 individuals from 7 neighboring non-Pygmy populations for ~200,000 SNP markers on the Illumina Cardio-Metabo BeadChip, which incorporates a large number of height-associated SNPs originally identified in populations of European descent. In an admixture analysis, we find that estimated non-Pygmy admixture in Pygmy individuals is positively correlated with individual height in males (r=0.350, P=0.002) and in females (r=0.451, P=0.003). A combined test of SNP-by-SNP P-values for allele frequency difference between Pygmies and non-Pygmies finds that the collection of 949 known height-associated SNPs on the chip has a greater difference between Pygmies and non-Pygmies (P=8.94×10^{-13}). This set of height-associated SNPs also has a greater allele frequency difference between Pygmies and non-Pygmies than do any of 10,000 frequency-matched SNP sets from the full SNP dataset (P<10^{-4}). These results provide evidence that some of the loci contributing to the difference between African Pygmies and non-Pygmies are among those loci previously identified as height-associated in Europeans.

467F

Methods for demographic inference using the transition matrix of allelic types from diploid genomic sequences. *y. wang, k. lohmueller, r. nielsen.* University of California at Berkeley, Berkeley, CA.

We developed a novel population genetic method for inferring demographic parameters from pairs of diploid genomes. Our method uses information from both linkage disequilibrium (LD) patterns and differences in allele frequency between populations. We summarized the genomic information by numbers of different transitions between allelic types. Starting from the fact that there are three possible genotypes (homozygous ancestral, heterozygous and homozygous derived) at each site for one genome, we defined seven pair-wise allelic types at polymorphic sites, for a pair of diploid genomes. These allelic types contain information about the extent of population differentiation between the two genomes. For example, using genomes from highly differentiated populations, there would be many sites where the two individual genomes were homozygous for different alleles. The transition pattern from one allelic type to another allelic type moving along a chromosome is a measure of LD across the genome. Transitions between very different allelic types indicate that there is little LD in the genomic region. We tabulated the number of the 49 different possible transitions from a pair of genomes and applied standard coalescent simulation to estimate demographic parameters. Our method explicitly models variation in recombination rates including recombination hotspots, and further takes sequencing errors into account. We evaluated the method by simulations and by applying it to whole-genome sequences of seven Asian, European and African individuals.

468F

Validation of ancestry informative markers in Brazilian Amerindians and ancestry estimates in quilombo remnant communities. *C.E.V. Wiezel[1], M.R. Luizon[2], S.M.B. Sousa[3], K. Abe-Sandes[4], I.R. Souza[5], Y.C.N. Muniz[5], C.T. Mendes-Junior[6], A.L. Simões[1].* 1) Department of Genetics, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazi.l; 2) Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil; 3) State University of Santa Cruz, Ilhéus, Bahia, Brazil; 4) Departament of Genetic, State University of Bahia, Salvador, Brazil; 5) Departament of Cell Biology, Embryology and Genetic, Federal University of Santa Catarina, Florianópolis, Brazil; 6) Departamento de Química, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brasil.

Brazilian quilombo remants are Afro-derived communities founded mainly by fugitive slaves between the 16th and 19th centuries. Each community has its own cultural and ancestral characteristics, including heterogeneous contribution of non-African people. Ancestry Informative Markers (AIMs) exhibit high allele frequency differences between ancestral populations, and can be useful to characterize populations that vary in degree of admixture and to estimate individual and population ancestry estimates. However, it is important to consider the most suitable ancestral population frequencies when generating admixture estimates. Then our goal was to characterize the frequencies for 12 AIMs (FY, RB, LPL, AT3, Sb19.3, APO, PV92, CKMM, DRD2, MID93, MID52 e MID575) in Amerindians from Brazilian Amazon in order to generate proper population and individual ancestry estimates in six quilombo remnants characterized by distinct demographic histories of formation: Mimbó, Sítio Velho, Gaucinha, Barra, São Gonçalo and Valongo; and five urban population samples: Teresina, Jequié, Pernambuco, São Paulo and Florianópolis. African and European genotypes were kindly provided by Dr. Mark Shriver from PSU. Genotyping was performed by PCR and PCR-RFLP and fragments analyzed by PAGE and silver staining. African Ancestry Indexes (AAI) was obtained by dividing the African component by the sum of the European and Amerindian components, obtained from STRUCTURE software. The comparison of AAI medians were performed by non-parametric tests using GraphPad. Ancestry estimates were performed using ADMIX software and Principal Component Analysis (PCAs) using MVSP software. The 12 AIMs were sufficient for an adequate discrimination among the considered ancestral populations. Three totally divergent clusters that correspond to the European, African and Amerindian populations were obtained without any overlap, each group clustered in one of the vertices of the STRUCTURE triangular plot. PCAs indicated that the first component groups the African population and the quilombo remnants. Although there is a clear AAI heterogeneity within and between groups, the AAI medians were not different among quilombo remnants. All ancestry estimates were trihybrid except for São Gonçalo. African estimates ranged from 51% in Valongo to 92% in Barra, and from 0.9% in Florianópolis to 46% in Jequié for urban populations. These estimates were more accurate than those obtained by autosomal STRs. Support: FAEPA, CNPq.

469F

Remarkably little homozygosity in first generation mixed race individuals. *J. Wilson[1,2], C. O'Dushlaine[3,4], R. Fraser[1], R. McQuillan[1].* 1) Centre for Population Health Sciences, Univ Edinburgh, Edinburgh, United Kingdom; 2) EthnoAncestry Ltd, Howden St, Edinburgh, United Kingdom; 3) Psychiatric and Neurodevelopmental Genetics Unit, Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts, USA; 4) Stanley Center for Psychiatric Research, The Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA.

Long runs of homozygosity (ROH) are ubiquitous features of human genomes. There is, however, wide variation in the number and length of ROH across individuals and populations due to their diverse demographic histories: sub-Saharan African populations are the least homozygous and Native South Americans the most. Using genomic analysis we here demonstrate the remarkable lack of homozygosity in mixed race individuals: first generation (F1) West African x European individuals are the least homozygous of all, with an order of magnitude fewer ROH than commonly observed in other populations. Their parents may not share ancestors after the Out of Africa movement, ~70,000 years ago. Populations may be ranked by increasing mean number of ROH: (1) F1 West African x European; (2) F1 Afro-Caribbean x European; (3) Afro-Caribbean; (4) sub-Saharan African (excluding hunter-gatherers), F1 European x East African, F1 European x East Asian, Mauritian and Seychellois; (5) F1 European x South Asian; (6) “Backcross” Eurasian mixed race individuals; (7) other Eurasians; and (8) Native North Americans. Afro-Caribbeans have on average ~20% European ancestry, and these haplotypes generate ROH in offspring with Europeans. F1 European x East Asian individuals carry a similar complement of ROH to sub-Saharan Africans, emphasising the great genetic diversity in Africa. The variance in the number of ROH was highest for Native North Americans: NROH was strongly inversely correlated with the large and variable genomic estimates of European admixture. The reduction in genome-wide homozygosity in mixed race individuals will reduce their risk for diseases with a recessive genetic component.

470F

Genetic relationship among ethnic minority groups in South America from the viewpoint of autosomal and Y chromosomal STRs. *T. Yamamoto[1], T. Ito[1], T. Gomyoda[1], K. Saijo[2], I. Danjoh[2], Y. Nakamura[2].* 1) Dept. of Legal Med. & Bioethics , Nagoya University, Nagoya, Aichi, Japan; 2) Cell Engineering Div., RIKEN BRC, Tsukuba, Ibaraki, Japan.

RIKEN RBC, a public cell bank in Japan, preserves a valuable cell collection called “the Sonoda-Tajima Cell Collection” where cell samples collected from a variety of ethnic minority groups across the world especially from South America. A part of the samples were immortalized with Epstein-Barr virus, and the B-lymphoblastoid cell lines (B-LCLs) were established and provided to researchers. Alternatively, it is important to investigate the genetic relationship and structure among the present ethnic groups in South America to obtain the information about the peopling of the Americas. It is also valuable to investigate the origin of and the degree of admixture of male-linage to analyze the markers on Y chromosome (Y-chr). Here, DNA extracted from the 431 B-LCLs originated from the 32 ethnic groups were genotyped for 21 autosomal short tandem repeat (STR) loci using 2 multiplex typing systems. Based on the Fst genetic dictance, we constructed a Neighbor-Joining tree, and the topology was concordant with the geographical distribution, and in particular, Sanuma and Ye'kuana tribes in Venezela, Ticuna, Chipaya and Quechua tribes, Lengua and Nivacle tribes in Chaco Province in Paraguay formed each cluster, respectively. Genetic structural analysis revealed very isolated ethnic groups such as Sanuma, Ye'kuana, Ticuna, Chipaya tribes, and Chaco-Lengua and Nivacle tribes. Alternatively, 184 male DNA out of 431 were haplotyped for 17 Y-chr STRs (Y-STRs) using a commercially released kit. 160 haplotype(HT)s were observed, and all of them were searched through a web worldwide database (db) (YHRD; Y-STR haplotype reference database) including about 32000 HTs for 17 Y-STRs. As a result, totally 12 HTs matched and about 50 neighbor HTs were observed in the db, and one HT originated from Canar tribe in Ecuador and another HT from an unknown tribe in Ecuador were matched to one from Germany and Korea respectively, which suggests different population histories through the Beringia from East Asia and through the northeast America from the European Continent. The other HTs, however, were observed in African, Hispanic, native American and admixed populations in South America. Further examination would be necessary.

471F

Evaluation of *ATOH7* and *RFTN1* in Primary Open Angle Glaucoma. *M. Zhang[1], J.H. Chen[1,2], D. Wang[1], Y. Zheng[1], H. Chen[1,2], L. Chen[2], C.P. Pang[2].* 1) Joint Shantou International Eye Center, Shantou, Guangdong, China; 2) Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong, China.

Purpose: The atonal homolog 7 (*ATOH7*) and raftlin, lipid raft linker 1 (*RFTN1*) genes have recently been identified by genome-wide association studies to be associated with glaucoma-related parameters of optic nerve area and vertical cup-to-disc ratio (VCDR). Their link with the occurrence has not been reported. We investigated *ATOH7* and *RFTN1* sequence variations in primary open angle glaucoma (POAG). **Methods:** The study cohort contained 171 POAG patients and 200 controls recruited in Shantou, a city in southeast China. *ATOH7* sequence variations were screened by direct DNA sequencing of polymerase chain reaction-amplified UTRs, exons and splice regions. Logistic regression implemented by the R statistical language was performed for detected SNP. In silico tools were used for predicting the impact of any missense variant. **Results:** No coding mutations were detected in both patients and controls in *ATOH7* although 5 SNPs were detected, including 4 SNPs in *ATOH7* and 1 SNP in *RFTN1*. None of them deviated from Hardy Weinberg Equilibrium ($P > 0.05$). They did not show association with POAG statistical significance ($P > 0.05$). However, the *ATOH7* SNP rs7916697 showed strong interaction with the *RFTN1* SNP ($P = 0.007$, sex-adjusted $P = 0.020$). The combination of rs7916697 CC genotype and rs690037 AA genotype exhibited a strong protective effects (OR = 0.28, 95%CI: 0.11-0.71; sex-adjusted OR = 0.31, 95%CI: 0.12-0.83). **Conclusions:** No coding variants have been detected in *ATOH7*. However, the strong protective effect exerted by the interaction between the two VCDR-related genes but not by single SNP indicated multi-factorial effect in the complex genetics of glaucoma.

472F

A Computationally Fast Bayesian Semi-parametric Algorithm For Inferring Population Structure And Adjusting For Case-control Association Tests. *S. Ghosh[1], A. Majumdar[1], S. Bhattacharya[2], A. Basu[3].* 1) Human Genetics Unit, Indian Statistical Institute, Kolkata, India; 2) Bayesian and Interdisciplinary Research Unit, Indian Statistical Institute, Kolkata, India; 3) National Institute of Biomedical Genomics, Kalyani, India.

Genome-wide case-control association studies have been successful in identifying novel variants involved in the pathogenesis of complex disorders. However, the problem of population stratification remains a major limitation of such studies. While methods have been developed (e.g., Genomic Controls, STRUCTURE along with STRAT, EIGENSTRAAT) to infer on population structure and correct for stratification in the tests for association, the estimation of the number of underlying subpopulations (K), which is of additional interest from an evolutionary perspective, has not been adequately addressed, except in STRUCTURE. In order to circumvent the problem of estimation of parameters in high dimensional spaces, STRUCTURE adopts an ad hoc approach of Bayesian deviance that tends to overestimate K and may lead to reduced power in detecting association. We have developed a Bayesian semi-parametric approach in the lines of Bhattacharya (2008) to estimate population structure under the assumption that K is random. The model is complemented by a summarization of the clustering data generated by the MCMC based on an elegant “Central Clustering” approach developed by Mukhopadhyay et al. (2011). Our approach has several advantages over STRUCTURE, the most prominent being a substantial reduction in computational time. Based on extensive simulations under a set-up of no admixture and an unlinked set of markers, we find that our method not only provides more accurate estimates of K compared to STRUCTURE, but also never overestimates the parameter. The test for association using STRAT is marginally more powerful for our clustering method compared to STRUCTURE after controlling for the overall false positive rate. We analyzed the Human Genome Diversity Panel data using our model and obtained very good clustering of the individuals in the panel. In the presence of admixture, the method can be modified by efficient designing of the Hierarchical Dirichlet Process.

473F

Population Structure and Genetic Diversity Revealed by DNA Microsatellites of Han Population Through a Comparison with Populations Worldwide. *F. Guan[1,2,3], S. Li[1,2,3], S. Wei[1,3], H. Zhang[1,2], J. Feng[1,2], X. Gong[1,2], R. Su[1,2].* 1) Key Laboratory of National Ministry of Health for Forensic Sciences, College of Medicine, Xi'an Jiaotong University, Xi'an, China, 710061; 2) Key Laboratory of Environment and Genes Related to Diseases, Ministry of Education, Xi'an, China, 710061; 3) Institute of Human Genomics and Forensic Sciences, Xi'an, China, 710061.

This study examined genetic diversity and population structure of Han population including 33997 individuals from 31 Han subpopulations. Each of sampled individual was genotyped for 13 microsatellites spread across most autosomes, which are core loci for the Combined DNA Index System (CODIS). To detect the relationship between Han population and world's populations, we analyzed these date jointly with similar data of 12 other populations worldwide, available from Allele Frequency Database (ALFRED), including Japanese, Pakistani, Turks, Colombian, Brazilian, Russians, Australian Aborigines, European Americans, Tunisian, Navajo, African Americans and Equatorial Guinean. Through a series of analyses, including principal components analysis, structure analysis and analysis of molecular variance, our results clearly shown that there is a continuous gradient feature in the structure of two-dimensional West and East groups, which is different from the previous studies, and based on the percentage (9.251%) of genetic diversity among different subpopulations and the percentage (19.135%) of genetic variation between West and East groups, it is believed that there is a certain degree of genetic similarities among subpopulations in the Han gene pool, and there is obvious difference between West and East groups, which means that it's well supported by DNA molecular genetics to divide Han population into West and East groups. In addition, through comparing the autosomal gene bank of Han population with that of different reference populations worldwide, the results of neighbour-joining tree show that there is the grouping of Han population in one cluster, while such a cluster in the genetic variation of other populations worldwide is independent of and different from the Han population. The reason might be that these Han subpopulations have experienced the gene flow of certain extent in the history, which coincides with the facts that there are several large-scale migrations in history. Actually, it should be more cautious to perform population deduction based on the data of several loci which don't cover the whole genome, because too few loci may result in a statistically significant, but incorrect clustering. Anyway, we provided a large amount of forensic genetic data and it's very useful in relevant population genetics and forensic studies.

474F

Population Substructure in the Sea Island Gullah African Americans. *Y. Huang[1], WM. Chen[1], X. Hou[1], U. Nayak[1], J.C. Mychaleckyj[1], F. Chen[1], K.L. Keene[1], K.H. Lok[3], D.L. Kamen[2], K.J. Hunt[2], I.J. Spruill[2], J.K. Fernandes[2], W.T. Garvey[3], M.M. Sale[1].* 1) University of Virginia, Charlottesville, VA; 2) Medical University of South Carolina,Charleston,SC; 3) University of Alabama at Birmingham,Birmingham,AL.

African populations are the most ancient and diverse on Earth, with a complex genetic substructure due to a long history of migration and admixture. African Americans (AA) are the admixed descents of African and European ancestors and are more genetically susceptible than European Americans to certain prevalent complex diseases. This renders an understanding of African ancestry critical not only in terms of evolution but also for disease based analyses. We used Multidimensional Scaling (MDS) and Principal Component Analysis (PCA) to analyze the population substructure in 384 unrelated Sierra Leone Africans and 877 unrelated Gullah AAs from Sea Island Genetic African American Family Registry (SuGAR), the Center of Biomedical Research Excellence (COBRE) for Oral Health at MUSC and the Systemic Lupus Erythematosus in Gullah Health (SLEIGH) Study. In total, 103731 LD-pruned GWAS SNPs that are also present in 130 Stanford-HGDP and 592 Hapmap III founders were included in the analysis. Four distinct linguistic and lifestyle groups were found. They include West Africans (Sierra Leone populations, Yoruba (YRI) and Mendenka), 3 distinct populations in the South and Southwest (Biaka Pygmies, Mbuti Pygmies and San), Khoisans in the East (Luhya in Webuye(LWK), Maasai in Kinyawa(MKK) and North East Bantu in Kenya) and North Africans in the Northwest (Mozabite). Substructure was also observed within Sierra Leone samples with the three largest subgroups being Mende, Temne and Creole. In addition, the Mendenka samples from Senegal were found to distinct from all other African populations in MDS analysis. Consistent with migratory and residential history, the Gullah AA samples from SuGAR, COBRE and SLEIGH exhibit similar ancestry, and all carry higher (~89%) global African ancestry than what is typically reported for AA (~80%). Compared to YRI and Sierra Leone African samples, the African ancestry of Gullah AAs is closest to Creole in Sierra Leone Africans, while genetically in-between all other Sierra Leone Africans and YRI. These findings may contribute to further understanding of the Sea Islands Gullah population's ancestral origins. Similar finding were also obtained using model-based ancestry estimation software ADMIXTURE.

475F

People of the British Isles: An analysis of fine-scale population structure in a UK control population. *S. Leslie[1], B. Winney[1], G. Hellenthal[2], S. Myers[2], A. Boumertit[1], T. Day[1], K. Hutnik[1], E. Royrvik[1], D. Lawson[3], D. Falush[4], P. Donnelly[2], W. Bodmer[1].* 1) Department of Oncology, University of Oxford, Oxford, United Kingdom; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 3) Department of Mathematics, University of Bristol, Bristol, United Kingdom; 4) Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany.

There is a great deal of interest in fine scale population structure in the UK, both as a signature of historical immigration events and because of the effect population structure may have on disease association studies. Although population structure appears to have a minor impact on the current generation of genome-wide association studies, it is likely to play a significant part in the next generation of studies designed to search for rare variants. A powerful means of detecting such structure is to control and document carefully the provenance of the samples involved. Here we describe the collection of a cohort of rural UK samples (The People of the British Isles), aimed at providing a well-characterised UK control population that can be used as a resource by the research community as well as providing fine scale genetic information on the British population. So far, some 4000 samples have been collected, the majority of which fit the criteria of coming from a rural area and having all four grandparents from approximately the same area. Three thousand samples were genotyped on the Illumina 1.2M and Affymetrix v6.0 platforms as part of WTCCC2. Using a novel clustering algorithm that takes into account linkage disequilibrium structure, approximately 3000 of the samples were clustered, using these comprehensive genotyping data, into more than 50 groups purely as a function of their genetic similarities without any reference to their know locations. When the appropriate geographical position of each individual within a cluster is plotted on a map of the UK, there is a striking association between clusters and geography, which reflects to a major extent the known history of the British peoples. Thus, for example, even individuals from Cornwall and Devon, the two adjacent counties in the southwestern tip of Britain, fall into different, but coherent clusters. Further details of this comprehensive analysis of the genetic structure of the People of the British Isles, together with a description of the provenance of the samples, will be give in the presentation. We believe that this is the first time that such a detailed fine scale genetic structure of a population of generally very similar individuals has been possible. This has been achieved through, on the one hand, a careful geographically structured collection of samples and, on the other hand, an approach to analysis that takes into account fully the linkage disequilibrium structure of the population.

476F

Nine forensic STRs are sufficient to determine genetic ancestries of Chinese populations. *S. Li[1,2], F. Guan[1,2], F. Gao[1,4], H. Gui[1,2], J. Wang[3], T. Doetschman[4], P. Stambrook[5].* 1) The Key Laboratory of National Ministry of Health for Forensic Sciences, College of Medicine, Xi'an Jiaotong University, Shaaxi 710061, China; 2) The Key Laboratory of Environment and Gene Related to Disease of Ministry of Education, College of Medicine, Xi'an Jiaotong University, Shaaxi 710061, China; 3) Department of immunobiology, University of Arizona, AZ 85724,USA; 4) BIO5 Institute and Dept of Cell Biology & Anatomy, University of Arizona, Tucson, AZ 85724,USA; 5) Department of Molecular Genetics, College of Medicine, University of Cincinnati, Cincinnati, Ohio 45267, USA.

Several indigenous ethnic populations in the Northwest region of China display clear differences in their cultures and social customs, perhaps as a result of geographic isolation and different linguistic traditions. Extensive trade and other interactions between these populations over the last two millennia probably also facilitated the admixture of different gene pools. Forensic identification of these populations often involves the genotyping of 16 STR loci (D3S1358, TH01, D21S11, D18S51, PentaE, D5S818, D13S317, D7S820, D16S539, CSF1PO, PentaD, vWA, D8S1179, TPOX, FGA, and AMEL). Since allele frequency distributions differ between these populations, however, it would be desirable to optimize the most effective set of genetic markers for genotyping. To further explore the evolutionary relationship between 13 ethnic populations residing in northwest China, and to reveal the features of population admixture, 9 most-commonly employed CODIS loci (D3S1358, TH01, D5S818, D13S317, D7S820, CSF1PO, vWA, TPOX, FGA) were selected for genotyping and further analysis. Multiple statistic methods were employed to explain the differentiation among populations and to explore the potential gene flow. The results suggest that populations living in Xinjiang Province typically show significant difference ($p<0.05$) from those residing in other provinces, while another five ethnic populations residing in Gansu and Qinghai Provinces show no significant difference from major populations such as Han, Hui and Mongolian. These data support the existence of high-level gene flows and population admixture in Gansu and Qinghai Provinces but not in Xingjiang Province . The Mantel test suggested that geographic factors contributed more significantly than linguistic differences ($p<0.05$), which explains the total variance of 21.58%.

477F

Genome-wide patterns of admixture among US Hispanic/Latino populations of Caribbean-descent. *A. Moreno Estrada[1], P. Ortiz Tello[1], M.L. Cuccaro[2], S. Gravel[1], R.J. Martinez[2], J.L. McCauley[2], D.J. Hedges[2], R.W. Morris[2], J.K. Byrnes[1], F. Zakharia[1], S. Acevedo[3], P.J. Norman[4], Z. Layrisse[5], P. Parham[4], C.D. Bustamante[1], E.R. Martin[2].* 1) Department of Genetics, Stanford University, Stanford, CA; 2) Center for Genetic Epidemiology and Statistical Genetics, University of Miami Miller School of Medicine, Miami, FL; 3) Department of Biology, University of Puerto Rico, Mayaguez, PR; 4) Department of Structural Biology, Stanford University, Stanford, CA; 5) Center of Experimental Medicine “Miguel Layrisse”, Venezuelan Research Institute (IVIC), Caracas, Venezuela.

Cryptic population structure and admixture are common confounders in genome-wide association and medical resequencing studies. Accounting for differences in ancestry, especially in case/control designs, is critical for proper analysis and interpretation of studies with multi- and trans-ethnic samples. Genomic studies of Hispanics/Latinos reveal that they are a highly genetically heterogeneous admixed group with strong variation in the proportions of African, European, and Native American ancestry. While Mexican populations have been characterized genomically to some extent, genetic studies of populations from the Caribbean and South America have been largely underrepresented. To that end, we have densely genotyped 252 samples from parent-offspring triads of Caribbean-descent sampled in South Florida, US, including Puerto Ricans, Cubans, Dominicans, Haitians, Hondurans and Colombians. We combined these SNP data with other publically available genomic resources, including HapMap and 1000 Genomes data, as well as Native American SNP data from putatively ancestral populations. Global ancestry proportions, estimated using the ADMIXTURE clustering algorithm, revealed substantial variation in admixture proportions among and within Caribbean populations. To assign local, ancestry-specific haplotypes across the genome, we implemented a novel PCA-based admixture deconvolution approach (PCADMIX) for three ancestral populations. Given the optimal accuracy obtained from trio phasing, we were able to determine exact posterior probabilities along each chromosome and measure the length of tracts attributable to distinct ancestries. We observed patterns consistent with continuous influx of African ancestry across the islands and a pulse migration across populations in contact with the continental landmass. By contrast, we find less evidence for continuous flow of Native American ancestry (characterized by shorter ancestry tracts), compatible with a single migration event between 15-20 generations ago and no further contribution of Native Americans into the Caribbean. We have also used local ancestry estimates to scan the genome for ancestry-specific enriched regions potentially indicative of natural selection. Insights gained from the application of new statistical methods for admixture deconvolution will improve the design and genetic analysis of medical genomic studies in Hispanics/Latinos and other complex admixed groups.

478F

Population Structure of Native Hawaiians in the Multiethnic Cohort Study. *R. Saxena[1], SK. Kim[2], CR. Gignoux[2], A. Lum-Jones[3], H. Wang[3], CA. Haiman[4], BE. Henderson[4], LN. Kolonel[3], L. Le Marchand[3], DO. Stram[4], I. Cheng[3].* 1) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 2) Institute for Human Genetics, University of California, San Francisco, San Francisco, CA; 3) Epidemiology Program, University of Hawai`i Cancer Center, Honolulu, HI; 4) Department of Preventive Medicine, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA.

The patterns of population structure and admixture in Native Hawaiians are understudied and quantification of their underlying population structure is imperative for providing a proper framework when conducting genome-wide association studies in this population. Unlike previous studies which have focused on ancestry informative markers (AIMs), we utilized high resolution genome-wide SNP array data and/or mitochondrial genomes to complement AIMs data when characterizing 403 self-reported Native Hawaiians with varying degrees of Native Hawaiian heritage. To calculate each individual's proportion of genetic ancestry, we conducted an unsupervised analysis of population structure by implementing ADMIXTURE. For genome-wide SNP array data, we detected 78% Native Hawaiian, 11.5% Caucasian, and 7.8% Asian origins for individuals who reported full Native Hawaiian heritage. For individuals who reported 75% and 50% Native Hawaiian heritage, we estimated a mean Caucasian ancestral proportion of 20.3% and 50.1% and Asian ancestral proportion of 22.3% and 43.6%, respectively. For whole mitochondrial genome data, we characterized each individual's haplogroup and found 99% of Native Hawaiians belonging to the B4 lineage and one Native Hawaiian of the Q1 haplogroup among those who reported full Native Hawaiian heritage. Our findings here represent the most comprehensive study of the genetic ancestry of Native Hawaiians to date.

479F

A Major Semitic Genetic Contribution to the Brazilian Population. *D. Schlesinger[1,2], L. Kimura[2], M. Naslavsky[2], L. Grinberg[3,4], R. Mingroni-Netto[2], M. Zatz[2], Brazilian Aging Brain Study Group.* 1) Brain Institute, Albert Einstein Jewish Hospital, Sao Paulo, Brazil; 2) Human Genome Research Center, University of São Paulo, Brazil; 3) Brazilian Aging Brain Study Group (LIM22), University of São Paulo Medical School; 4) Memory and Aging Center, Department of Neurology, UCSF.

We have genotyped 547 inhabitants of São Paulo, Brazil, for 90 ancestry-informative markers (analyzed using Structure 2.3.3) that have previously been shown to distinguish individuals with Semitic and European ancestry. Estimated Central-South Asian (CSA) ancestry corresponded to 29%, larger than the African cluster (19%). The percentage of CSA ancestry within the Caucasian cluster (44%) was comparable to Semitic groups (Israelis, Druze, Palestinians) of the Human Genome Diversity Project. CSA ancestry was also more frequent in Brazilian subjects with higher African and Amerindian ancestry (colonial/pre-colonial populations) ($p<0.001$ for each). We then genotyped 35 relatively isolated descendants of African slaves, the Quilombolas. The quilombo population had higher CSA/Caucasian ratios compared to the São Paulo urban population (66% vs. 42%, $p<0.001$). These results suggest, in agreeance with historical evidence, that the likely Semitic ancestry arrived in Brazil during the colonial (1500-1822) period rather than the recent European immigration wave (1822-today). Further studies should elucidate whether this ancestry in São Paulo is derived from Portuguese “New Christians” fleeing persecution by the Inquisition or a Moor genetic component of the Portuguese population.

480F

Improved inference of relationships and consanguinity in CEPH pedigrees using identity-by-state combined with identity-by-descent. *E. Stevens[1], G. Heckenberg[2], J. Baugher[3], T. Downey[2], J. Pevsner[4,5].* 1) Program in Human Genetics, Johns Hopkins School of Medicine, Baltimore, Maryland, 21205, USA; 2) Partek Inc., St. Louis, Missouri, 63141, USA; 3) Program in Biochemistry, Cellular, and Molecular Biology, Johns Hopkins School of Medicine, Baltimore, Maryland, 21205, USA; 4) Department of Neurology, Hugo Moser Institute at the Kennedy Krieger Institute, Baltimore, Maryland, 21205, USA; 5) Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, Maryland, 21205, USA.

It is essential that specified relationships in large, population-based datasets are correctly annotated as related or unrelated. While many methods exist to assess genetic relatedness between two individuals, we developed a novel approach to infer identity-by-descent (IBD) and combined it with an existing identity-by-state (IBS) approach. This combined method also elicits information about the population structure. We estimated IBD based on the observed IBS values across all chromosomes using a windowed approach. We validated our approach against the methods of PLINK, RELPAIR, and PREST using a set of 186 Centre d'Étude du Polymorphisme Humain (CEPH) individuals in 14 families. An explicit feature of the CEPH collection is that these multigenerational families represent reference panels of known relatedness, consisting mostly of three-generation pedigrees. We identified unexpected relatedness between nominally unrelated grandparents both within and between pedigrees. In some cases, the estimated Cotterman's coefficient of relatedness K1 (IBD1) exceeded 0.1 indicating recent ancestry (consistent with one-sixteenth sharing) between either grandparents or parents. In several cases, significant K2 (IBD2) values in parent-child and second-degree relationships also indicated potential consanguinity within the pedigrees. Consistent with an interpretation of inbreeding, we identified corresponding regions of homozygosity in the offspring of related or consanguineous parents. Our findings support a 1999 report, by Broman et al., that several CEPH families had regions of homozygosity consistent with autozygosity. We found additional intrapedigree sharing among four CEPH/Utah families and interpedigree sharing within two other CEPH/Utah families. Our IBD method has the capacity to analyze datasets with thousands of samples, and improves IBD estimation compared to similar programs without the need for a priori information about haplotypes or relatedness between individuals. When combined with IBS we provide an intuitive and practical graphical approach. The application of this approach could improve genome-wide association, linkage, heterozygosity, and other population genomics studies that rely on SNP genotype data.

481F

Barriers to Neutral Introgression Generated by Local Adaptation and Sex-Specific Incompatibility. *M.K. Uyenoyama[1], D. Fusco[2].* 1) Dept Biol, Duke Univ, Durham, NC; 2) Computational Biology and Bioinformatics, Duke Univ, Durham, NC.

Adaptation to local conditions within subpopulations balanced by migration can maintain polymorphisms for variants that affect fitness in certain ecological contexts. We addressed the effects of such polymorphisms on the rate of introgression of neutral marker genes, possibly genetically linked to targets of selection. This analysis serves as the basis for the development of a method designed to use the pattern of neutral variation throughout the genome to infer the location and characteristics of targets of local selection. We proposed a scaling of backward migration rates that explicitly incorporates linkage and selection for local adaptation and show that it accurately determines the waiting time between migration events at neutral sites traced back in time along randomly sampled lineages. We studied two major types of selection regimes: those that promote local monomorphism (purifying or disruptive selection) and those that promote local polymorphism (overdominance or purifying selection balanced by meiotic drive). In agreement with previous work by Barton and Bengtsson and others, we found that the former type generates barriers to neutral introgression that increase with linkage to targets of selection. However, the latter type generates only weak barriers, and in fact meiotic drive can enhance transmission of genes borne by migrants beyond residents. Viewing interspecific hybrid incompatibility as an extreme form of local selection, we explored the effects on neutral introgression of sex-specificity in crossover rates, expression of incompatibility, or transmission. The magnitude of the reproductive barrier differs among genomic regions, even in the absence of physical linkage of neutral markers to targets of selection or of functional epistasis among incompatibility factors. Our partitioning of variation in relative reproductive rate showed associations between sex and incompatibility and between sex and neutral markers. Concordant sex-specific incompatibility (e.g., greater impairment of male hybrids or longer map lengths in females) permits higher rates of introgression and discordant incompatibility lower barriers.

482F

A quantitative comparison of the similarity between genes and geography in worldwide human populations. *C. Wang[1], N.A. Rosenberg[1,2,3].* 1) Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI; 2) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 3) Life Science Institute, University of Michigan, Ann Arbor, MI.

Multivariate statistical techniques such as principal components analysis (PCA) and multidimensional scaling (MDS) have been widely used to summarize the structure of human genetic variation, often in easily visualized two-dimensional maps. Many recent studies have reported a visual similarity between geographic maps of individual or population locations and MDS or PCA maps of genetic variation inferred from single-nucleotide polymorphisms (SNPs). However, this similarity has primarily been evident only in a qualitative sense, and because different multivariate techniques and marker sets have been used in different studies, it has not been possible to formally compare datasets in terms of the levels of similarity with geography of MDS or PCA plots of genetic variation. In this study, we perform a systematic analysis to quantitatively evaluate the similarity of genes and geography in different geographic regions. We have therefore integrated genome-wide SNP data from over 100 populations worldwide, identifying a shared set of markers and employing the same multivariate analysis techniques to analyze datasets from different geographic regions. For each of a series of geographic regions, a Procrustes analysis approach is used to find an optimal transformation that maximizes the similarity between PCA maps of genetic variation and geographic maps of sampling locations. We consider examples consisting of populations from Africa, Asia, China, Europe, and a worldwide sample. We find that significant similarity between genes and geography exists in general at different geographic levels. Further, we find that the similarity is highest in our examples from Asia, and once isolated populations have been removed, Africa. Our results provide a broad picture of the geographic structure of human genetic variation across the world, supporting the view that geography plays a strong role in giving rise to human population structure.

483F

Epistatic selection between coding and regulatory variation in human evolution and disease. *T. Lappalainen, S.B. Montgomery, A.C. Nica, E.T. Dermitzakis.* Department of Genetic Medicine and Development, University of Geneva Medical School, Geneve, Geneve, Switzerland.

Interaction (non-additive effects) between genetic variants has been highlighted as an important mechanism underlying phenotypic variation, but the discovery of genetic interactions in humans has proved difficult. In this study, we show that the spectrum of variation in the human genome has been shaped by modifier effects that cis regulatory variation has on the functional impact of putatively deleterious protein-coding variants. We analyzed 1000 Genomes population-scale resequencing data from Europe (CEU) and Africa (YRI) together with gene expression data from arrays and RNA-sequencing for the same samples, as well as eQTL data based on RNA sequencing of B-cells, T-cells and fibroblasts of 55 individuals. We observed an underrepresentation of derived putatively functional coding variation on the higher expressed regulatory haplotype, which suggests stronger purifying selection against deleterious coding variants that have increased penetrance due to their regulatory background. Furthermore, the frequency spectrum and impact size distribution of common regulatory polymorphisms (eQTLs) appear to be shaped in order to minimize the selective disadvantage of accumulation of deleterious coding mutations on the higher expressed haplotype. We also describe how tissue-specific eQTLs leading to strong allelic imbalance of coding variants lead to differences in protein structure between the tissues of an individual. Interestingly, eQTLs explaining common disease GWAS signals showed an enrichment of putative epistatic effects, suggesting that some disease associations may arise from interactions increasing the penetrance of rare coding variants. Altogether, our results indicate that regulatory variation not only changes gene expression levels but also affects the penetrance of coding variants, which also implies that knowing the regulatory context is important for predicting the functional impact of coding variants. The specific type of genetic interaction described in this study is detectable from sequencing data in a genome-wide manner, and characterizing these joint effects may help to understand functional mechanisms behind genetic associations to human phenotypes - including both Mendelian and common disease.

484F

Genotype-based test in mapping cis-regulatory variants from allele-specific expression data. *J.-F. Lefebvre[1], E. Vello[1], B. Ge[2], S.B. Montgomery[3,4], E.T Dermitzakis[3,4], T. Pastinen[2,5,6], D. Labuda[1,7].* 1) Centre de Recherche du CHU Sainte-Justine, Université de Montréal, Montréal, Québec, Canada; 2) McGill University and Genome Québec Innovation Centre, Montréal, Québec, Canada; 3) Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, 1211 Switzerland; 4) Wellcome Trust Sanger Institute, Cambridge CB10 1HH, UK; 5) Department of Human Genetics, McGill University Health Centre, McGill University, Montréal, Québec, Canada; 6) Department of Medical Genetics, McGill University Health Centre, McGill University, Montréal, Québec, Canada; 7) Département de Pédiatrie, Université de Montréal, Montréal, Québec, Canada.

Understanding the impact of regulatory variation in gene expression is of particular interest in evolutionary genetics and genetic epidemiology. Regulatory variation in cis is readily detected in individuals showing uneven expression of a transcript from its two allelic copies, usually referred to as allelic imbalance (AI). Identifying individuals exhibiting AI permits mapping regulatory DNA regions and the underlying genetic variants. However, the existing mapping protocols require knowledge of the haplotypes and are therefore sensitive to phasing errors, which may hamper identifying cis-regulatory variants located far away from the regulated transcript. We introduce genotype-based mapping test that does not require haplotype-phase inference. It relies on partitioning individuals exhibiting AI phenotype and non-AI regulatory homozygotes in a 2x3 contingency table comparing three possible bi-allelic genotypes of all SNPs along the chromosome that expresses the transcript of interest. Its performance to detect linkage disequilibrium (LD) between a purported regulatory site and the genotyped SNPs locating this region was examined by analyzing both simulated and empirical AI data. In simulation experiments the genotype-based test outperforms the haplotype-based tests with increasing genetic distance separating the regulatory region from its regulated transcript. The haplotype based tests compared include the linear regression and the binomial test partitioning SNPs between the down and up regulated chromosomes in AI individuals. The genotype-based test performed equally well with the experimental AI data, from genome-wide cDNA hybridization arrays and from RNA sequencing, pinpointing candidate regulatory regions that are either close or away from the examined transcript. Circumventing the need of haplotype inference, the genotype-based test will suit AI analyses in population samples of unknown haplotype structure and will facilitate tracing cis-regulatory variants that are located far away from the regulated transcript.

485F

Genome-wide identification of neuronal targets for PTBP2 using RIP-seq. *P. Cormican, E.M Quinn, E.M Kenny, A.P Corvin, M. Gill, D.W Morris.* Neuropsychiatric Genetics Research Group, Dept. of Psychiatry and Institute of Molecular Medicine, Trinity College Dublin, Ireland.

RNA-binding proteins play a significant role in the regulation of mRNAs transcripts and facilitate differential gene expression patterns in a post-transcriptional context. One such RNA-binding protein, Polypyrimidine Tract Binding Protein 2 (PTBP2) is located in a strongly associatied region identified by the 2009 International Schizophrenia Consortium case-control study. PTBP2 is primarily a neuronal expressed gene and is involved in a range of cellular functions, including splicing, translational regulation, mRNA stability and transcription but as yet a comprehensive list of associated RNAs is not available. In this study we identify these associated mRNAs that co-precipitate with PTBP2 using RNA-immunoprecipitation and subsequent sequencing on an Illumina GAII. We employ two complementary tagging methods, His-Tag and anti-PTBP2 antibody to pull-down the protein-RNA interactors from a neuronal cell-line preparation. Examination of the overlap of enriched trancripts between these methods identifies ~1300 genes that comprise the PTBP2 associated transcriptome. Identification and classification of these associated mRNAs represents the next step in understanding the functional role of PTBP2 in neuronal cells and may potentially provide an insight into PTBP2s contribution to the pathogenesis of schizophrenia.

486F

Comparative study of the Y chromosome diversity in some ethnic groups living in Iran and populations of the Middle East. *L. Andonian[1], S. Rezaie[2], A. Margaryan[3], D.D. Farhud[4], K. Mohammad[1], K. Holakouie Naieni[1,5], M.R. Khorramizadeh[6], K. Nourijelyani[1], M.H. Sanati[7], M. Jamali[8], A. Berahmeh[6], N. Aalizadeh[9], L. Yepiskoposyan[3].* 1) Dept. of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran Iran; 2) Division of Molecular Biology, Dept. of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran Iran; 3) Human Genetics Group, Institute of Molecular Biology, National Academy of Sciences, Yerevan Armenia; 4) School of Public Health, Tehran University of Medical Sciences, Tehran Iran; 5) Iranian Epidemiological Association; 6) Dept. of Medical Biotechnology, School of Advanced Medical Technologies, Tehran University of Medical Sciences, Tehran Iran; 7) National Institute for Genetic Engineering and Biotechnology, Pajoohesh Blvd., 17 Km Karaj HWY, Tehran Iran; 8) Academic Member of Ministry of Health and Education, Tehran Iran; 9) Hormozgan University of Medical Sciences, Bandar Abbas Iran.

Background: The main goal of this study is to conduct a population genetic study of: a) Armenians living in Iran, in the context of general Armenian population; and b) Iranian Azeris, one of the biggest ethno-linguistic communities, in comparison with other Turkic-speaking populations of the Middle East (from eastern Turkey, Azerbaijan Republic and Turkmenistan). Methods: Buccal cells of 89 Armenian males from central Iran, the descendants of Armenians forcibly moved to Iran in the beginning of 17th century CE, and 105 Turkic-speaking Azeri males from north-west Iran (Tabriz) were collected by mouth swabs. The samples were screened for 12 Single Nucleotide (SNP) and 6 microsatellite markers on the non-recombining portion of the Y chromosome. The results of genetic typing were statistically analyzed using Arlequin software. Results: Iranian Armenians display a moderate level of genetic variation and are genetically closer to Western Armenians which is in agreement with historical records. Iranian Azeris demonstrate much weaker genetic resemblance with Turkmens (as putative source population) than with their geographic neighbors. Conclusion: Political, religious and geographic isolation had moderate influence on the genetic structure of modern Iranian Armenians during the last four centuries, which is expressed in lower diversity of their patrilineal genetic legacy. The imposition of Turkic language to the populations of north-west Iran was realized predominantly by the process of elite dominance,i.e. by the limited number of invaders who left weak traces in the patrilineal genetic history of Iranian Azeris.

487F

Refining the genetic effects of obesity-, dyslipidemia-, blood pressure-, hematology-associated variants on abdominal anthropometric measurements by constitution types. *S. Cha, H. Yu, S. Yu, J.Y. Kim.* Constitutional Med Res, Korea Institute of Oriental Medicine, Daejeon, Korea.

A Korea traditional tailored medicine, Sasang constitutional medicine, categorizes human beings into four types depending on states of physiological imbalances and responsiveness to herbal medicine; it has been also considered that each type may have different susceptibilities to metabolic disorders. Tae-eumin type, for instance, seems to be more susceptible to metabolic disorder like cardiovascular disease than other types, as it has been known for being sensitive to energy intake due to an imbalance toward preserving energy. One of main features for physiological imbalances developing metabolic disorder is the fat accumulation in abdomen. Therefore, the genetic variants associated with metabolic disorder could manifest different effects on the abdominal measurements according to the constitution types. Here, we examined the relationships between obesity-, dyslipidemia-, blood pressure, or hematology-associated polymorphisms and abdominal body mass in each Sasang constitution type. After the variants which had been reported in previous genome-wide association studies with the above disorders were genotyped with 2,328 Koreans, we estimated the effects of the variants on abdominal anthropometric measurements such as circumference and width as well as the respective ratio via multiple linear regression analyses in each consititution subgroup. The sets of polymorphisms affecting abdominal body mass were different between constitution types so that the variants significantly associated with abdominal increase in one type almost had no effects on the abdominal increase in the other types. The constitutional discrepancy for the association of genetic variants with accumulation in abdominal mass appeared to reflect the physique differences between constitution types, for example big Tae-eumin type and thin Soeumin type, as the major alleles of Tae-eumin type-associated variants showed the effects largely on the increases of abdominal phenotypes, whereas the variants associated in Soeumin type manifested the decreased effects on abdominal phenotypes. Our findings demonstrate that the genetic effects of certain polymorphisms involved in obesity, dyslipidemia, hypertension, or hematological disorder could be exerted in different manners for abdominal accumulation depending on constitution type. Therefore, classification of individuals based on Sasang constitution types would offer an effective assessing system for the risk of metabolic disorders.

488F

Searching for adaptive introgression of favorable alleles in Yemeni populations. *J.A. Hodgson[1,2], R.L. Raaum[2,3], A.M. Al-Meeri[4], C.J. Mulligan[5].* 1) Center for the Study of Human Origins, Department of Anthropology, New York University, New York, NY; 2) New York Consortium in Evolutionary Primatology, New York, NY; 3) Department of Anthropology, Lehman College and The Graduate Center, City University of New York, New York, NY; 4) Department of Biochemistry, Faculty of Medicine, Sana'a University, Sana'a, Yemen; 5) Department of Anthropology, University of Florida, Gainesville, FL.

Adaptive introgression may be an important source of evolutionary novelty in geographically dispersed taxa such as humans, though the extent of its role in recent human evolution remains poorly understood. Adaptive introgression occurs when beneficial alleles previously unique to one population enter into a new population and are selected to a frequency above the background admixture rate. In order to better understand the possible extent of adaptive introgression in recent human evolution, we performed a genome-wide study of admixture between two divergent modern human populations. Populations on the southern Arabian Peninsula are located at the nexus of trade between East Africa and Eurasia and have a long history of known admixture. For example, in Yemeni populations approximately 30% of mitochondrial lineages and 15% of nuclear lineages are of African origin. We collected high-resolution SNP genotype data from 62 unrelated Yemeni sampled across the country. The ancestry of chromosomal segments in this sample was estimated using a haplotype-based method (HAPMIX) with the HapMap Masaai (MKK) and Northern and Western Europeans (CEU) as source populations. We have previously shown the HapMap Masaai (MKK) to be the best available proxy for the source population of African ancestry in Yemen. We applied a hierarchical Bayesian model to evaluate the extent of adaptive introgression from African populations into the Yemeni sample. The pattern of introgression varied across the genome, with regions showing significantly higher and lower than expected introgression relative to genome-wide admixture. The possible functional complexes associated with these outlier regions of introgression may provide insight into expectations for earlier episodes of introgression in human evolution. For example, it has been suggested that admixture with Archaic human groups such as Neanderthals has played an important role in human evolution. However, no clear examples of adaptive introgression from archaic populations have yet been found in living humans. Our study of the genetic consequences of historical admixture on the Arabian Peninsula provides a modern analogue for earlier admixture events in human evolution.

489F

Association of PCSK1 rs6235 with Cardiovascular Disease Risks in Relation to Neck Circumference in Koreans. *H. Yu, J.Y. Kim, S. Yu, S. Cha.* Korea Institute of Oriental Medicine, Daejeon, Korea.

Common nonsynonymous variants in proprotein convertase subtilisin/kexin type 1 (PCSK1) converting prohormones into active-form hormones have been associated with obesity in European ancestry. In subsequent studies with Chinese populations, the gene variants have been associated with obesity as well as cardiovascular disease risk factors, which are known to be correlated with neck circumference. Here, we studied the association between a nonsynonymous variant and the cardiovascular disease risk in relation to neck circumference in Koreans. The genotype of rs6235 polymorphism was determined with 2,328 Koreans, and the genetic effects on phenotypes and diseases related with cardiovascular disease risk were statistically analyzed via regression analyses in two subgroups divided by the neck circumference cut-off points (39 cm for men; 34 cm for women) for overweight status (body mass index／ 25 kg/m2) using a receiver operating characteristics curve analysis. While we failed to replicate an association with obesity and related traits, we found the association of a minor allele of rs6235 with decreases in the prevalence of stroke (OR = 0.42, P = 0.038), ischemic heart disease (OR = 0.63, P = 0.015), and diabetes (OR = 0.57, P = 0.025) especially in the subjects with neck circumference values under the cut-off points. We newly researched the genetic effects of PCSK1 in relation with neck circumference. The results suggested that rs6235 polymorphism in PCSK1 may affect cardiovascular disease risk through the mediation of neck circumference, although this remains to be confirmed with other large populations.

490F

Association of TNF-(gene with Neurological manifestations of dengue in the Rondonia Population. *D. Delani[1], A. Andrade-Casseb[1], L. Cantanhede[1], A. Krauze[1], A. Simões[2], M. Guimarães[1], J. Farias[1].* 1) Universidade Federal de Rondônia, Porto Velho, Brazil; 2) Faculty of Medicine of Ribeirao Preto, University of São Paulo, Brazil.

TNF gene showed a significant association with severe forms of infection caused by Dengue virus (Sierra 2010). By an extensive variability of SNPs, it has become essential tool for genetic linkage studies or association in composition and frequency within and between different ethnic groups (Vejbaesya, 2009). Aiming to genotype the SNPs -238 and -308 located in the promoter region of this gene and the genetic association observed between individuals infected with dengue, we collected blood samples from 108 individuals living in Cacoal and Jaru, Rondonia's State. DNA extraction was performed using the protocol described by Higuchi and analysis of samples was by PCR and RFLP, followed by electrophoresis on PAGE 6%. Statistical data were obtained using the program GENEPOP (Version 4.0). The allele frequency was 0.936 for -238G, and 0.0640 for -238A, corresponding to that observed in European populations (0.068 and 0.932 respectively, NCBI, 2010). In dengue patients the presence of this mutant allele-238A is associated with protection (Oliveira 2004). In this study, no association was found (p> 0.05). -308G allele had a frequency of 0.878 and -308A of 0.122, similar to described for African-American (0.123 and 0.877 respectively, NCBI, 2010), confirming the ethnic heterogeneity of the study population. Unable to establish a biological basis of susceptibility and resistance in relation to TNF, even though there is data in the literature (Fernández, 2004). New molecular approaches and statistics will be performed for the association dengue / TNF can be analyzed in order to confirm whether or not the literature.

491F

The paternal and grand-paternal age effects on blood telomere length in Cebu, Philippines. *D.T.A. Eisenberg[1,2], J.B. Borja[3], C.W. Kuzawa[1,2], M.G. Hayes[1,4,5].* 1) Department of Anthropology, Northwestern University, Evanston, IL; 2) Cells 2 Society: the Center for Social Disparities and Health, Institute for Policy Research, Northwestern University, Evanston, IL; 3) Office of Population Studies Foundation, University of San Carlos, Talamban, Cebu City 6000, Philippines; 4) Division of Endocrinology, Metabolism and Molecular Medicine, Department of Medicine, Northwestern University Medical School, Chicago, Illinois; 5) Center for Genetic Medicine, Northwestern University, Chicago, Illinois.

Telomeres are repetitive DNA sequences (5'-TTAGGG in vertebrates) found at the ends of chromosomes. Telomere lengths (TL) shorten with age in proliferating human tissues due to the `end-replication problem' and oxidative stress. This shortening is implicated in senescence, with previous work suggesting that shorter TL impairs immune and cardiovascular function and results in increased mortality. Contrary to the TL shortening which occurs with age in human blood cells, several studies report that the TL in sperm of older men tend to be longer than that in younger men and, correspondingly, that offspring of older men inherit longer TL. We recently hypothesized that this paternal age effect on offspring TL is a mechanism for transmitting information about environmental experiences (age at reproduction) in recent generations to adaptively adjust offspring physiology. For the paternal age effect on TL to convey reliable information about past environments, it was predicted that the paternal age effect would exhibit a multi-generational character, providing integrated and thus more reliable information. This model leads to the prediction that grandfather age at the birth of the parent will be associated with longer TL in the grandchild (independent of any effects of paternal age). To test this hypothesis, TL from young men and women from Cebu, Philippines were measured from DNA extracts of blood using a qPCR method. Consistent with findings in European populations, having an older father was associated with longer TL in this population (n=799, p=0.02). Only a non-significant trend towards paternal grandfathers having grandchildren with longer TL was observed (n=122, p= 0.12). In analyses stratified by gender of the grandchild, older paternal grandfathers had grandsons with longer TL (n=69, p=0.03). No effect of maternal grandfather's age on grandchildren's TL was observed, which might be explained by past findings of reduced heritability of TL from mother to offspring when compared to paternal-offspring heritability. The effect sizes of the associations with paternal and grand-paternal ages suggest potentially biologically important magnitudes of effect. These findings provide the first evidence that grand-paternal age at conception of father might influence grandson's TL. This work was supported by NSF and Wenner Gren Foundation grants. DTAE is supported by a NSF GRF. DNA extracts generously provided by Karen Mohlke.

492F

The mobilome of *Callithrix jacchus* (the common marmoset). *M.K. Konkel[1], J.A. Walker[1], B. Ullmer[2], Q. Wang[1], R. Hubley[3], A.F.A. Smit[3], M.A. Batzer[1] for the Marmoset Genome Sequencing and Analysis Consortium.* 1) Louisiana State University, Department of Biological Sciences, 202 Life Sciences Building, Baton Rouge, LA 70803; 2) Louisiana State University, Department of Computer Science, Center for Computation and Technology (CCT), 216 Johnston Hall, Baton Rouge, LA 70803; 3) Institute for Systems Biology, Computational Biology, 1441 North 34th Street, Seattle, WA 98103.

The availability of the common marmoset (*Callithrix jacchus*) genome provides the first sequenced New World monkey (NWM) genome. The purpose of this study was to investigate the propagation and evolution of mobile elements in the NWM lineage through the lens of the common marmoset. In addition, our objective was to investigate the population genetic composition of common marmosets using polymorphic retrotransposon markers to determine the diversity of common marmoset colonies of three U.S. Primate Centers (New England, Southwest, Wisconsin). Our analysis of the *C. jacchus* draft genome assembly [CalJac 3.2] revealed that the common marmoset has an overall repeat composition similar to other sequenced and analyzed primate genomes (e.g. human, chimpanzee, orangutan, and rhesus macaque). In the lineage leading to the common marmoset, two primary non-LTR (long terminal repeat) retrotransposon families, long interspersed elements (LINE1, L1) and short interspersed elements (SINEs) contribute the bulk of lineage-specific insertions. The NWM-specific L1 lineage intersects with the human-derived L1 tree between L1PA6f and L1PA7, and evolved in a mostly linear manner. Using the CalJac 3.2 assembly, we reconstructed the *Alu* subfamily evolution in the NWM lineage leading to the common marmoset. The youngest *Alu* subfamilies are derivatives of *Alu*Ta15, a previously identified NWM-specific subfamily. Moreover, about half of the NWM-specific *Alu* elements appear to be derived from *Alu*Ta15 or its derivatives, indicating that a small burst of *Alu* retrotransposition coincided with the rise of *Alu*Ta15 and its derived subfamilies. *Alu* activity seems to have declined somewhat thereafter, causing the most recent *Alu* retrotransposition rate in common marmosets to appear slower than in humans and rhesus macaques. Our population genetic analysis with polymorphic *Alu* markers using the Structure software indicates the presence of population structure with at least two different clusters, indicating diversity among common marmosets. In addition, we investigated the evolution of *Alu* subfamilies with respect to the radiation of NWMs through the lens of the common marmoset. This study provides insights into the evolution of mobile elements in the lineage leading to the common marmoset and represents the first comprehensive analysis of the mobilome of a NWM species.

493F

***PDE4B* is a high myopia susceptibility gene likely by down regulating collagen synthesis in sclera.** *W. Chen[1], X. Zhou[2], C. Li[1], J. Qu[2], C. Zeng[1].* 1) The Laboratory of Disease Genomics and Individualized Medicine, Beijing Institute of Genomics, Chinese Academy of Sciences; 2) Wenzhou Medical College.

High myopia (HM) or `pathologic' myopia refers to short vision with refraction error less than -6.00 diopters or axial length higher than 26 mm. Previous pedigree and association analysis had identified several susceptible loci for HM. However, with its high heterogeneity and lacking of functional assays, the genetic mechanism underlying HM still remains uncertain.

In order to identify loci contributing to HM susceptibility in Han population, we conducted a two-stage study including whole genome association (GWA) analysis and functional assays. In attempt to have both large samples size and high SNP density in GWA analysis, we took a strategy of using pooled samples. Briefly, two batches of case pools (331 and 176 subjects) and control pools (144 and 150 subjects) were constructed by mixing equal amount of DNA in each group. Genotyping was then performed using Illumina 1M duo chip with 3 replicate arrays for each pool to reduce experimental bias. Candidate SNPs obtained from association analysis were then verified by individual genotyping with Sequenom MASSARRAY. In total, 9 loci associated with HM were obtained. We chose rs10889602 (pooling $p=1.38\times10^{-6}$; individual verification p=0.0198), which locates in the third intron of cAMP-specific phosphodiesterase 4B (*PDE4B*), for further replication study in consideration of the possible role of PDE4B in collagen synthesis. rs10889602 appeared to be truly correlate to HM in Han Chinese by association analysis using 1,606 cases and 1,509 controls in total from two Han cohorts ($p_{meta}=3.72\times10^{-3}$).

Next we explored if PDE4B is functionally involved in myopia formation. *PDE4B* expression in form-deprived myopic sclera of guinea pig was decreased compared to those in fellow eyes, whereas there was no reduced expression for other *PDE4* family members. The inhibition of PDE4B by subconjunctival injection of rolipram, a PDE4 inhibitor, induced myopia in normal eyes and exacerbated myopia in form-deprived eyes. Moreover, thinner scleral collagen fibrils were observed in form-deprived eyes treated with rolipram. PDE4B inhibition by rolipram down-regulated transcription of collagen I, III, and V as well as decreased collagen secretion in cultured human scleral fibroblasts. Our findings suggest that *PDE4B* is a susceptibility gene for high myopia and the inhibition of PDE4B contributes to myopia development by down-regulating scleral collagen synthesis.

494F

Mutation analysis of Parkin, PINK1 and DJ-1 and LRRK2 G2019S in European and North African families with parkinsonism. *S. Lesage[1,2,3], A. Honoré[1,2,3], C. Condroyer[1,2,3], M. Anheim[1,2,3], S. Klebe[1,2,3,4], E. Lohmann[1,2,3], C. Cazeneuve[3], A. Dürr[1,2,3], A. Brice[1,2,3], The French Parkinson's Disease Genetics Study Group.* 1) INSERM, UMR_S975 (Formerly UMR_S679), and Université Pierre et Marie Curie-Paris, Centre de Recherche de l'Institut du Cerveau et de la Moelle épinière, Paris, France; 2) CNRS, UMR 7225, Paris, France; 3) AP-HP, Pitié-Salpêtrière Hospital, Department of Genetics and Cytogenetics, Paris, France; 4) INSERM CIC-9503, Hôpital Pitié-Salpêtrière, Paris, France.

BACKGROUND: Parkinson disease (PD) is a frequent neurodegenerative disorder caused by loss of dopaminergic neurons in the substantia nigra. Three genes are responsible for autosomal recessive early-onset parkinsonism: Parkin, PINK1 and DJ-1. Although the common LRRK2 G2019S mutation was initially associated with dominant forms of PD, it is also found in a significant proportion of apparently autosomal recessive and sporadic patients. **AIM**: To assess the frequency of Parkin, PINK1, DJ-1 and the LRRK2 G2019S mutations in a large series of 880 families with parkinsonism. **PATIENTS AND METHODS**: We studied a series of 380 probands with familial and 500 with isolated PD. The families were of European descent (n=733), North African (n=117) and of diverse or unknown origins (n=30). All probands were screened for mutations in Parkin, PINK1, DJ-1 and the LRRK2 exon 41 using direct sequencing and semi-quantitative multiplex PCR or Multiplex Ligation-dependent Probe Amplification (MLPA) kits. In this study, we focused on probands with two mutations in recessive genes, except for the LRRK2 G2019S mutation. Segregation of the identified mutations was determined by analyzing all available DNA from affected and non-affected family members. The proportions of patients with Parkin mutations in function of the age at onset were examined in a total of 960 PD patients. **RESULTS**: We found 94 PD families with two Parkin mutations, representing a total of 11% (17% in familial forms and 6% in isolated cases). The proportion of patients with two Parkin mutations from PD families and from isolated cases decreased significantly with increasing age at onset, ranging from 53% and 28% before age 20 to 8% and 2% between 46 and 55 years, respectively. PINK1 mutations were identified in 9 families (1%) whereas no mutation in DJ-1 was found. In contrast, the LRRK2 G2019S mutation accounts for 5% of familial and 3% of isolated forms of PD. **CONCLUSION**: Parkin mutations are the most common genetic cause of parkinsonism in Europe and North Africa, particularly in early-onset familial PD. Although LRRK2 is known as a dominant gene, it plays a significant role in autosomal recessive forms of PD. In contrast, PINK1 and DJ-1 are more rarely implicated. This comprehensive mutation analysis of recessive genes and the LRRK2 G2019S in a large series of familial and isolated cases will be of major importance for molecular diagnosis to prioritize testing of the most common disease-causing genes.

495F

Prioritizing novel variants through whole-exome sequencing and pathway discovery in Alzheimer's Disease. *M.W. Logue[1,6], M. Schu[1], B.N. Vardarajan[1], J. Farrell[1], C.T. Baldwin[1,7], L.A. Farrer[1,2,3,4,5,6].* 1) Biomedical Genetics, Boston Univ Sch Med, Boston, MA; 2) Neurology, Boston Univ Sch Med, Boston, MA; 3) Ophthalmology, Boston Univ Sch Med, Boston, MA; 4) Genetics & Genomics, Boston Univ Sch Med, Boston, MA; 5) Epidemiology, Boston Univ Sch Public Health, Boston, MA; 6) Biostatistics, Boston Univ Sch Public Health, Boston, MA; 7) Center for Human Genetics, Boston Univ Sch Med, Boston, MA.

There is growing consensus that, apart from *APOE* isoforms, late-onset Alzheimer (AD) risk conferred by common mutations is likely to be small. However, next-generation sequencing methodologies may enable the identification of rare or private risk variants of moderate to high effect. In this study we sequenced whole-exomes of 7 African American AD patients from the MIRAGE study including two affected sib-pairs with additional family history of AD and three singleton AD patients. Whole exome capture was performed using the Agilent SureSelect Human All Exon kit. Sequencing was performed using the Illumina GA IIx platform. The 80 bp single end reads were aligned with the Burrows-Wheeler Aligner (BWA) program. Sequencing was completed with a 96% read alignment and 17.5x coverage within target regions. The variant calling of the aligned sequencing data was completed using a pipeline based on the Genome Analysis Toolkit. The variant calls were further filtered for mapping quality, sequence depth, and extent of homozygosity in the region. To ensure that called variants were real and to prioritize those more likely to be pathogenic, we focused on the novel 1,746 variants in 1,364 genes found in 2 or more subjects. Two of these genes, *ABCA7* and *CD33*, were previously implicated in GWAS studies of AD (the reported variants were 4 and 15 kb away from the sequenced novel SNPs respectively). Ingenuity pathway analysis (IPA) was able to create several densely connected gene pathways based on our list of 1,364 genes. One of the most interesting and heavily weighted pathways (28 of 35 pathway genes from the target list) links *CD33* and genes involved in Lipid Metabolism, Molecular Transport, and Small Molecule Biochemistry. As a next step to determine whether or not these new variants are involved in AD risk, we are currently genotyping and testing association with SNPs from these pathways in a large group of African American and Caucasian AD cases and controls.

496F

Lipid droplets and the pathogenesis of axonopathies caused by dominant mutations in *BSCL2*. *G. Mancuso[1], T. Rizo[2], E.I. Rugarli[1,2,3].* 1) Institute of Zoology, University of Cologne, Cologne, Germany; 2) Center for Molecular Medicine (CMMC), University of Cologne, Cologne, Germany; 3) Cologne Excellence Cluster on Cellular Stress Responses in Aging Associated Diseases (CECAD), University of Cologne, Cologne, Germany.

Long axons of central and/or peripheral motor neurons degenerate in several inherited neurological conditions, such as hereditary spastic paraplegia and Charcot Marie Tooth disease. With the identification of many causative genes for these conditions, we now appreciate that a considerable overlap can exist in term of genetic causes and involved cell pathways. Dominant mutations in *BSCL2* are responsible for a variety of axonopathies, which clinically manifest as Silver syndrome/spastic paraplegia 17, distal hereditary motor neuropathy type V, and Charcot-Marie-Tooth disease type 2 with predominant hand involvement. *BSCL2* encodes for seipin, an integral membrane protein of the endoplasmic reticulum involved in lipid droplet biogenesis. Loss-of-function recessive mutations of *BSCL2* cause Berardinelli-Seip syndrome, a severe form of lipodystrophy, and deletion of the orthologous gene in yeast causes a defect in lipid droplet formation. The neurological diseases associated with *BSCL2* are dominantly inherited and due to missense mutations (N88S and S90L) affecting two residues within a glycosylation motif. The current hypothesis is that glycosylation defect of seipin leads to protein misfolding, endoplasmic reticulum (ER) stress, and subsequent cell death by apoptosis. A gain-of-function pathogenic mechanism is also supported by the fact that seipin-N88S and seipin-S90L can complement a deleted yeast strain to the same extent as the wild-type human protein. We now provide evidence that seipin-N88S and seipin-S90L mutants decorate cup-shaped ER subcompartments in close contacts with lipid droplets. Live-imaging experiments suggest that these ER structures closely follow lipid droplet movements. Experiments are in progress to understand the functional significance of these cup-like structures and the effect of mutant seipin on cellular lipid metabolism. We propose that seipin-N88S and seipin-S90L mutations display a gain-of-function effect on some aspect of lipid droplet biogenesis, thus linking *BSCL2* axonopathies to dysfunction in lipid metabolism.

497F

Genetic interactions in diversified biological pathways predict hippocampal and entorhinal atrophy in Alzheimer Disease. *S.A. Meda[1,2], M.E. Koran[2,3,4], N.A. Restrepo[2,4], T.A. Thornton-Wells[1,2].* 1) Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN; 2) Center for Human Genetics & Research, Vanderbilt University, Nashville, TN; 3) Vanderbilt Medical Scientist Training Program, Vanderbilt University, Nashville, TN; 4) Human Genetics Ph.D. Program, Vanderbilt University, Nashville, TN.

Background: The complex genetic etiology of late onset Alzheimer disease (LOAD) is still largely unknown. A large portion of the missing heritability can be attributed to heterogeneity in clinical disease status and to the lack of statistical analyses exploring genetic interactions. We attempt to address the above issues by using quantitative intermediate phenotypes derived from MRI data, which capture a larger portion of the underlying trait variation, and by testing for gene-gene interactions (GxG) within known biological KEGG pathways. **Materials and Methods:** This study included 156 control, 281 MCI and 140 LOAD Caucasian subjects derived from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort. Brain volumes for hippocampus (HIP) and entorhinal cortex (ER) were derived using Freesurfer for their baseline and 12 month annual visits. Annual percent change for each region was used as a quantitative trait (QT), and 556,045 SNPs passed QC for further analysis. We tested all two-locus GxG within 212 KEGG pathways for association with both QTs using linear regression implemented in INTERSNP. This resulted in 151 million pairwise GxG models for each QT. Gender, APOE E4 and clinical status were included as covariates. **Results:** Atrophy rates for HIP ($p<1e-4$) and ER ($p<0.003$) were significantly different across groups and followed the expected direction (LOAD>MCI>controls). Results identified several significant (Bonf-corr (=0.05) two-locus associations with R HIP and R ER atrophy. A total of 143 GxG interactions in 34 KEGG pathways were associated with R HIP atrophy. Top pathways (with >=10 significant GxG) included calcium signaling (16), neuroactive ligand-receptor (12), long-term hippocampal potentiation (10), regulation of actin cytoskeleton (10) and gap junction (10). Similarly, 326 GxG in 41 KEGG pathways were significantly associated with R ER atrophy. Top associated pathways were calcium signaling (47), neuroactive ligand-receptor (41), axon guidance (20), glycan structure biosynthesis (23), purine metabolism (19), cell adhesion biosynthesis (15), prostrate-cancer (14) and focal adhesion (10). **Conclusion:** To our knowledge, this is the first study to explore association of pathway-based GxG interactions with cortical atrophy rates in LOAD. Findings support the interplay of specific biological networks (both known and novel in the context of LOAD) that contribute to atrophy rates in brain regions affected earliest in the disease process.

498F

The genetic contribution of dementia and Parkinson genes to Dementia with Lewy Bodies and Parkinson Disease with Dementia. *B. Meeus[1,2], A. Verstraeten[1,2], D. Crosiers[1,2,3], S. Engelborghs[2,4], M. Van den Broeck[1,2], M. Mattheijssens[1,2], K. Peeters[1,2], E. Corsmit[1,2], E. Elinck[1,2], B. Pickut[3], R. Vandenberghe[5], P. Cras[2,3], P.P. De Deyn[2,4], C. Van Broeckhoven[1,2], J. Theuns[1,2].* 1) Neurodegenerative Brain Diseases Group, Department of Molecular Genetics, VIB, Antwerpen, Belgium; 2) Institute Born-Bunge, University of Antwerp, Antwerpen, Belgium; 3) Department of Neurology, University Hospital Antwerp, Antwerpen, Belgium; 4) Department of Neurology and Memory Clinic, Hospital Network Antwerp Middelheim and Hoge Beuken, Antwerpen, Belgium; 5) Department of Neurology, University Hospitals Leuven and University of Leuven, Leuven, Belgium.

Dementia with Lewy bodies (DLB) and Parkinson Disease with Dementia (PDD) represent neurodegenerative dementias characterized by clinicopathological features that overlap with those of Alzheimer dementia (AD) and Parkinson disease (PD). Therefore, genetic commonalities between these disorders can be anticipated. Genetic research in a limited number of mostly isolated patients already supported a potential role for genes that are known to be implicated in classic forms of either AD or PD in the development of DLB and PDD. Despite strong hints for a genetic component in the etiology of both diseases a comprehensive mutation analysis of all major dementia and PD related genes in larger DLB and PDD cohorts is still missing. The prior aim of this study was to determine whether mutations in dementia and PD genes play a role in DLB and PDD.

Therefore, we systematically screened all major dementia and Parkinson disease genes in 99 DLB and 75 PDD patients for simple mutations in coding exons and regulatory regions previously reported to contain pathogenic mutations in AD, frontotemporal lobar degeneration (FTLD) or PD patients. In addition, copy number variations in *APP*, *SNCA* and *PARK2*, important dosage sensitive genes in AD and PD, were determined.

In the AD genes, we detected proven pathogenic missense mutations in *PSEN1* (A79V) and *PSEN2* (R71W) and two novel missense variants in *PSEN2* (V191E) and *MAPT* (R221Q). We identified one duplication mutation in *SNCA*, the *LRRK2* R1441C founder mutation and four novel heterozygous missense variants of which two in *PARK2* (A46S and R275W) and two in *PINK1* (P138L and S499C), with unknown pathogenicity.

It is not yet fully clear to which extent these genetic variants contribute to the pathogenesis of both disorders, but our data indicate a potential contribution to the genetic etiology of DLB and PDD though limited. Out results, however do support the hypothesis of a potential genetic overlap between diseases belonging to the Lewy body disease spectrum.

499F

Identification of the causative gene for SPG27 by Exome capture. *A. Noreau[1], S.L. Girard[1], A. Szuto[1], I.A. Meijer[1], P. Cossette[1], P.A. Dion[1,2], G.A. Rouleau[1,3,4].* 1) The Centre of Excellence in Neuroscience, CRCHUM, Montreal, Quebec, Canada; 2) Université de Montréal, Department of pathology and cellular biology, Montreal, Quebec, Canada, H3T 1J4; 3) University of Montreal, Faculty of Medicine, Department of Medicine, Montreal, Quebec, Canada, H3C 3J7; 4) Research Center, CHU Sainte-Justine, Montreal, Quebec, Canada, H3T 1C5.

Hereditary spastic paraplegia (HSP) is a motor neuron disease for which the key symptoms are lower limb spasticity and weakness because of progressive neurodegeneration events. The group of HSP is particularly heterogeneous because different modes of inheritance were observed (dominant, recessive and X-linked) as well as various levels of symptoms complexity; pure and complicated forms were described. To date, over 45 spastic paraplegia locus were identified and 21 genes identified. A previous linkage analysis on an autosomal recessive pure HSP family identified a new locus (SPG27) on the 10q22.1-10q24.1 region; a locus partially overlapping the SPG9 locus. We proposed to perform use an exome capture approach with the Agilent Technology SureSelect Human All Exon Kit (50MB) and proceed to resequencing with ABI SOLID4 DNA sequencer. Four affected individuals, the unaffected-carrier father and another unaffected sibling carrying the same disease-allele as the father will be used. As we may be looking for compound-heterozygote mutations, we will develop an algorithm to determine the parental origin of variants and the more promising mutations will be validated through Sanger sequencing. By this method, it will be possible to cover the 40 genes, find the causative mutation and maybe solve the SPG9 clue.

500F

Identification and Confirmation of an Exonic Splicing Enhancer Variation in Exon 5 of the Alzheimer Disease Associated PICALM Gene. *N.C. Schnetz-Boutaud[1], J. Hoffman[1], D.G. Murdock[1], M.A. Pericak-Vance[2], J.L. Haines[1].* 1) Center for Human Genetics Research and Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN; 2) The Hussman Institute for Human Genomics at the Miller School of Medicine, University of Miami, Miami, FL.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory and cognitive impairment and is the leading cause of dementia in the elderly. A growing number of genome wide association studies and subsequent replication studies have been recently published on late onset AD (LOAD), these studies identified several new susceptibility genes including phosphatidylinositol-binding clathrin assembly protein (PICALM) on chromosome 11. The aim of this study was to examine the entire coding sequence of PICALM to determine if the association could be explained by any previously undetected sequence variation. Such variation might explain, in whole or in part, the observed association. Therefore, we sequenced 48 cases and 48 controls homozygote for the risk allele for rs3851179 and of mixed APOE status. In this dataset, we did not find any new polymorphisms. However, rs592297 a known coding SNP that is part of an exonic splice enhancer region in exon 5 is in strong linkage disequilibrium with rs3851179. This SNP has altered heterozygosity and likely requires further study.

501F

Whole-exome sequencing confirms locus heterogeneity underlying Parkinson disease in an extended Amish family. *W.K. Scott[1,2], K. Nuytemans[1,2], G. Bademci[1,2], Y.J.K. Edwards[1,2], G.W. Beecham[1,2], M.F. Davis[3], A.C. Cummings[3], C.A. Jauregui[1,2], S.L. Lee[4], M.A. Pericak-Vance[1,2], J.L. Haines[3], J.M. Vance[1,2].* 1) Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL; 2) John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 3) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN; 4) Department of Neurology, Dartmouth Medical School, Lebanon, NH.

We studied 19 individuals with Parkinson disease (PD) and 2 with progressive supranuclear palsy (PSP) in a large Amish kindred from Indiana and Ohio. Two-point linkage analysis for PD across 13 subpedigrees generated LOD scores > 3 for 7 regions on 6 chromosomes. We next performed whole-exome sequencing (WES) to identify candidate genes in these regions of interest. WES is an economical strategy that addresses the possibility of locus heterogeneity suggested by the multiple significant linkage regions. Relationships among individuals with PD vary from siblings to more distant than 2nd cousins. We captured exomes using the Agilent SureSelect Human All Exon 50Mb Kit. Paired-end sequencing on the Illumina HiSeq2000 platform was performed on the captured DNA. Illumina analysis pipeline v1.7 and MAQ software v0.7.1 were used for analyzing the sequencing data. No single rare variant (RV; allele frequency < 5%) was shared by all individuals (19 PD & 2 PSP) or by all 19 PD patients, affirming the apparent locus heterogeneity suggested by linkage results. Further, no single functional variant was shared by all PD patients in the 1-LOD-unit down support interval surrounding the most significant multipoint LOD score peak for PD (a 12 Mb region of chromosome 9). Fourteen of the 17 genes in the region contained RVs in at least one individual. Taken together, these results indicate that finding the PD-associated RVs will require detailed examination of segregation in subpedigrees linked to each locus. Next-generation sequencing technologies provide new opportunities for rapid interrogation of large numbers of candidate genes in multiple linkage regions, such as those detected in this Amish kindred. The initial results here suggest that the familial aggregation of PD observed in this family is not due to the sharing of a single causal variant, but is due to multiple loci segregating in subsets of the pedigree. While the isolation of the Amish population (estimated inbreeding coefficient 0.015) likely reduces the number of PD-associated genetic variants, enough locus heterogeneity persists to render this pedigree a good model for PD in the general population.

502F

Duchenne/Becher like phenotype and L276I mutation in FKRP gene. *P. Spadafora, V. Andreoli, R. Cittadella, F. Trecroci, L. Maria.* Inst Neurological Sci, National Research Council, Mangone (CS), Italy.

Background and Objective. Mutations in the FKRP gene encoding for the fukutin-related protein are responsible for either a severe form of congenital muscular dystrophy (MDCIC) and the Limb girdle Muscular Dystrophy type 2I (LGMD2I). IperCKmia, cardiomyopathy, proximal muscles weakness and atrophy, calf hypertrophy, respiratory failure are common clinical features in the LGMD2I as well in the dystrophinopathies. The majority of LGMD2I patients follow a mild Becher-like course without showing severe gait impairment until late in their adulthood. Especially in western and northern Europe, the most frequent mutation identified in LGMD2I patients is 826C>A (L276I) located in FKRP gene. Homozygosity for L276I variant seems to confer a mild clinical phenotype, while compound heterozygosity for this mutation has been reported associated with a more severe clinical phenotype. On these grounds, we decided to perform a L276I mutation screening in patients from southern Italy who originally referred to our diagnostic laboratory with a suspect of Duchenne (DMD)/Becher (BMD) syndromes. Materials and methods. The study was performed on 106 sporadic cases resulted negative for exonic deletion/duplication of the DMD gene. The molecular analysis consisted of PCR amplification of the FKRP gene coding region containing the L276I variant followed by Bfa1 restriction enzyme digestion. The L276I mutation was confirmed by direct sequencing. Results. The selected FKRP mutation screening revealed one patient homozygous for L276I and two patients heterozygous for the same mutation. A full sequencing of exon 4 did not reveal further genetic mutations. Discussion and conclusions. The spectrum of clinical variability reported associated with LGMD2I ranges from severe early onset Duchenne-like muscular dystrophy to mild late onset Becher-like muscular Muscolar distrophy and existence of a frequent mutation (L276I) in Caucasian patients with LGMD2I, led us to analyze this mutation in 106 DMD/BMD sporadic patients with no rearrangement of the dystrophin gene. We identified the L276I mutation in three subjects (2,8%). This finding indicates the importance of screening for the L276I mutation also patients with clinical presentation of DMD/BMD muscular dystrophy.

503F

Alzheimer's disease: Novel Mutations in Genes Comprising the Gamma Secretase Complex. *P. St Jean[1], L. Li[1], J. Shen[1], M. Nelson[1], M. Ehm[1], J. Whittaker[2], A. Yeo[2], V. Mooser[3], J. Rubio[2].* 1) Genetics, GlaxoSmithKline, Inc, Res Triangle Park, NC; 2) Genetics, GlaxoSmithKline Inc, Stevenage, UK; 3) Genetics, GlaxoSmithKline Inc, Upper Merion, PA.

We sequenced the exons of 202 genes encoding known or potential drug targets in over 14,000 individuals identifying over 38,000 variants. Subjects were consented for the study of common diseases and medically-relevant traits including 679 Alzheimer's disease (AD) cases and 673 AD controls. AD cases had a mean age at onset of 72.9 years. To increase power, 6424 additional non-AD reference controls were selected from among the 14,000+ individuals using principal components to minimize the genetic distance between controls and cases. The focus of this abstract is the identification of rare functional variants in four genes (PSEN1, PSEN2, PSENEN and APP) that are involved in the gamma secretase complex taking advantage of the largest data set sequenced to date for these genes. Several analytical methods were used to investigate the effect of rare variants on disease endpoints including a binary collapsing method, SKAT and a variable allele frequency threshold method. Our primary endpoint was AD status. Within AD cases other endpoints were assessed including age at onset, Parkinsonism, psychosis and depression. Our most significant association with AD risk was PSENEN (p=2.66E-06). Of the 3 variants, R82Q, was present in 2 of 679 AD cases and 0 of 7097 controls. Both cases had onset at 65 years. This variant is predicted to be functionally damaging by both SIFT and Polyphen and has not been previously identified as an AD mutation. The other 2 variants, I80T and L29I, were each present in a single control. PSEN1 was our strongest association with age at onset (p=4.54E-10), underpinned by 4 variants (A79V, H163R, I213L, S365A) previously linked to Mendelian forms of AD. PSEN1 163R had been reported in early onset AD in French Canadian families, and in this study all 3 AD carriers were French Canadian with onset 40-44 years of age. PSEN1 213L was discovered in an early onset Polish AD family and our sole AD subject with this mutation was also of Polish/German ancestry with an onset of 40 years. A novel APP mutation predicted to be benign, V431L, and a novel PSEN2 mutation predicted to be damaging, S30F, were each present in a single AD case but no controls. Neither mutation was associated with any endpoints tested. The age at onset was 70 and 67 for subjects harboring APP 431L and PSEN2 30F, respectively. Our work adds to the body of knowledge of AD mutations present in genes in the gamma secretase complex, a target for the development of novel AD drugs.

504F

ROLE OF THE PPCS, PPCDC, PPAT, AND DPCK GENES IN THE PATHOGENESIS OF THE PANTOTHENATE KINASE-ASSOCIATED NEURODEGENERATION. *P. Tarantino[1], E.V. De Marco[1], D. Civitelli[1], F. Annesi[1], F. Cavalcanti[1], M. Gagliardi[1,2], A. Quattrone[3,4], A. Gambardella[1,3], G. Annesi[1].* 1) Institute of Neurological Sciences, National Research Council, Mangone (CS), Italy; 2) University of Magna Graecia, Catanzaro (CZ), Italy; 3) Institute of Neurology, Department of Medical Sciences, University of Magna Graecia, Catanzaro (CZ), Italy; 4) Neuroimaging Research Unit, Institute of Neurological Sciences, National Research Council, Catanzaro (CZ), Italy.

Mutations in the PANK2 gene, coding for an enzyme involved in the biosynthesis of coenzyme A (CoA), cause a rare autosomal recessive disorder known as PKAN (Pantothenate Kinase-Associated Neurodegeneration), characterized by progressive dystonia and iron accumulation in the brain. A small number of cases may be due to mutations in the PLA2G6 gene. In a recent study performed on the CoA pathway in Drosophila melanogaster, it has been shown that mutations in the PPCS, PPCDC, PPAT, and DPCK genes, orthologs of the corresponding human genes, cause a phenotype related to phenomena of neurodegeneration similar to those observed in patients with PKAN. On this basis, we performed a sequence analysis of these four genes in patients with PKAN to assess their role in the development of the disease. We analyzed 8 patients (6 females, 2 males) including 5 with the classic form (earlier onset and more rapid progression) and 3 with the atypical form, coming from Italy and France. In these patients was previously excluded the presence of PANK2 and PLA2G6 mutations, including exonic rearrangements. Molecular analysis of the PPCS, PPCDC, PPAT, and DPCK genes was performed by direct sequencing of the PCR products of all the exons. We did not find any pathogenic mutation in the PPCS, PPCDC, PPAT, and DPCK genes in the patients with classical and atypical PKAN analyzed. No polymorphic variations were found in the PPCS gene. One polymorphism was identified in the PPCDC gene, whereas many polymorphic variations were identified in the PPAT and DPCK genes. Recently, an increase in reactive oxygen species as well as in sensitivity to DNA damage was detected in a model of mutant Drosophila, together with lipid homeostasis alteration and formation of vacuoles in the brain, resulting in damage of the motor function. These results prompted us to verify the role played by the PPCS, PPCDC, PPAT, and DPCK genes in PKAN. Our results indicate that mutations in these genes do not cause PKAN in our patients. It would also be important to search for new genes, responsible for the cases not linked to PANK2 and PLA2G6. However, due to the small number of patients analyzed, the involvement of the genes studied cannot be definitely excluded. Further studies carried on a more extended number of patients and in other populations are required to confirm our data. This work was granted by AISNAF ONLUS.

505F

***BIN1* and *PICALM* association with Alzheimer disease risk and CSF tau levels in a Flanders-Belgian cohort.** *C. Van Cauwenberghe[1,2], N. Brouwers[1,2], K. Bettens[1,2], S. Engelborghs[2,3], N. Le Bastard[2], R. Vandenberghe[4], P.P. De Deyn[2,3], C. Van Broeckhoven[1,2], K. Sleegers[1,2].* 1) Neurodegenerative Brain Diseases Group, Department of Molecular Genetics, VIB, Antwerpen, Belgium; 2) Institute Born-Bunge, University of Antwerp, Antwerpen, Belgium; 3) Department of Neurology and Memory Clinic, Hospital Network Antwerp Middelheim and Hoge Beuken, Antwerpen, Belgium; 4) Department of Neurology, University Hospitals Leuven and University of Leuven, Leuven, Belgium.

Recent genome-wide association studies (GWA) studies have led to the identification of putative novel risk factors for Alzheimer disease (AD). Genome-wide significant association was obtained with single nucleotide polymorphisms (SNPs) located 5' upstream of bridging integrator 1 (*BIN1*) and the phosphatidylinositol-binding clathrin assembly protein gene (*PICALM*). Both genes appear to be implicated in clathrin-mediated endocytosis (CME), a process responsible for the intracellular trafficking of proteins and endocytosis of synaptic vesicles in neurons. The aim of this project was to fine-map the *BIN1* and *PICALM* association signal in a Flanders-Belgian AD cohort (N = 1905). Further, the effect of SNP genotypes on cerebrospinal fluid (CSF) biomarker profiles was analyzed. We genotyped 1047 patients and 858 healthy control individuals for 24 SNPs in the *BIN1* and 19 in the *PICALM* locus and up- and downstream regions. CSF level of) -amyloid peptide (A) $_{1-42}$), total tau protein (T-tau) and tau phosphorylated at threonine 181 (P-tau$_{181P}$) were available for 342 of the patients. We observed significant genotypic associations for 6 *BIN1* SNPs (p-values ranging from 0.004 to 0.042) of which 2 are located 5' of the gene. Interestingly, 5 *BIN1* variants associated with T-tau levels in CSF of AD patients (p-values ranging from 0.008 to 0.044, of which 3 are located 5' of *BIN1*). No association for *BIN1* was found with A) $_{1-42}$ and P-tau$_{181P}$ CSF levels. One *PICALM* SNP, located 5' of the gene, showed nominal association with AD. 2 SNPs of *PICALM* were significantly associated with T-tau (p-values 0.014 and 0.038) and an association with P-tau$_{181P}$ was found for 2 variants. No association was identified with A) $_{1-42}$. In this study we were able to fine-map the reported *BIN1* and *PICALM* association signals in a Flanders-Belgian cohort. This lends further support to the notion of genetic variations in *BIN1/PICALM* affecting the susceptibility of late-onset AD and representing genuine AD susceptibility factors. Our study extends this genetic association by showing a correlation with tau levels in CSF, suggesting that *BIN1* and *PICALM* might affect AD risk through an effect on tau biology.

506F

Pursuit of the biological variant underlying the association of TMEM106B with frontotemporal lobar degeneration. *J. van der Zee[1,2], S. Engelborghs[2,3], R. Vandenberghe[4], P.P. De Deyn[2,3], M. Cruts[1,2], C. Van Broeckhoven[1,2].* 1) Neurodegenerative Brain Diseases Group, Department of Molecular Genetics, VIB, Antwerpen, Belgium; 2) Institute Born-Bunge, University of Antwerp, Antwerpen, Belgium; 3) Department of Neurology and Memory Clinic, Hospital Network Antwerp Middelheim and Hoge Beuken, Antwerpen, Belgium; 4) Department of Neurology, University Hospitals Leuven and University of Leuven, Leuven, Belgium.

Background: The first genome-wide association (GWA) study in frontotemporal lobar degeneration (FTLD) identified genetic variability at the TMEM106B gene on chromosome 7p21.3 as a potential common risk modifying factor for FTLD. We replicated this association in a Flanders-Belgian patient/control cohort, providing additional evidence for the implication of TMEM106B in FTLD risk. High-density single nucleotide polymorphism (SNP) mapping suggested that the association was solely driven by the gene TMEM106B and that homozygous carriers of the TMEM106B protective alleles had a 50% reduced risk of developing FTLD. Aim: To pursue the biological variant underlying the risk-modifying effect of TMEM106B on FTLD we performed targeted resequencing of the 36 kb minimal associated region containing TMEM106B plus additionally 2.5 kb flanking region to capture the conserved regulatory regions of the promoter. Results: Targeted resequencing of the candidate region was accomplished by Sanger sequencing in 11 patients homozygous for the associated protective haplotype and 10 patients homozygous for the common haplotype. This effort identified a total of 253 single base changes or small insertion/deletions (indels). These sequence variations included 38 rare variants (observed in 1 or 2 patients) and 215 more frequent variants. Of the common variants, 100 were in perfect linkage disequilibrium (LD) with rs1990622, the strongest associated SNP of GWA studies. Further 115 variants were counted that were not in LD with rs1990622. Interpretation: Targeted resequencing captured the complete genetic variability of the associated protective haplotype at the TMEM106B locus with FTLD, identifying 215 candidate variants. Following-up on these results, we will investigate which one of the 215 SNPs shows the strongest genetic association or is the best functional candidate to explain the biology of the disease-modifying effect of TMEM106B on FTLD.

507F

Association of IGF-1 promoter polymorphism (-383C>T) with the susceptibility to multiple sclerosis. *R. Abdolmohammadi[1], H. Ebadi[2], M. Mansoori[1], S. Bakhshandeh[3], A. Tahmasebifar[4], M. Shahbazi[5].* 1) Golestan University of Medical Sciences, Cellular and Molecular Medicine Research Center Gorgan, Iran, MSc; 2) Golestan University of Medical Sciences, Gorgan, Iran, Neurologist; 3) Cellular and Molecular Medicine Research Center Gorgan, Iran, BSc; 4) Golestan University of Medical Sciences, Cellular and Molecular Medicine Research Center Gorgan, Iran, MD-PhD; 5) Golestan University of Medical Sciences, Cellular and Molecular Medicine Research Center Gorgan, Iran, MSc-PhD.

Association of IGF-1 promoter polymorphism (-383C>T) with the susceptibility to multiple sclerosis Abdolmohammadi R, Ebadi H, Bakhshandeh S, Mansoori M , Tahmasebifar A., Shahbazi M. Medical Cellular & Molecular Research Center, Talghani Children hospital, Golestan University of Medical Sciences, Gorgan, Iran Background and aim Insulin-like growth factors (IGFs) play an important role in proper development and myelination in the CNS. Because IGF-1 exhibits neuroprotective and myelinogenetic effects, it possesses therapeutic potential in treating neurodegenerative demyelinating diseases such as multiple sclerosis (MS). This study was undertaken to investigate the association between susceptibility to MS and IGF-1 polymorphisms in MS patients and healthy indvuduals. Methods DNA samples were prepared from the whole blood of 245 patients with MS and 373 healthy controls. We amplified the fragment including the IGF-1 polymorphism at position -383 and visualized the products in a documentation system after agarose gel electrophoresis. Data were analysed using Fisher's exact tests with SPSS-v16 software. Results The T/T genotype was found in 149 individuals (60.8%) 0f MS cases and 236 individuals (63.29%) 0f controls and 78 (31.8%) of MS and 135 (36.21%) of controls carried the C/T heterozygous genotype. only 2 (0.5%) of controls carried the C/C homozygous genotype while 18 (7.4%) of MS case carried the C/C homozygous genotype (P value<0.0001).The frequency of C allele was higher in MS patients than controls (OR=1.35, P<0.0001). Also, we have found a significant difference in the frequency of the C/C genotype among MS patients and controls (OR= 14.25, P value< 0.0001). Conclusion This is first report that, IGF-1 -383 C/C genotype was associated with risk of Ms (OR=14.25, P value< 0.0001).according to main role of IGF-1 in CNS and our finding, IGF-1suggest be, play crucial role in not only MS outcome but also CNS diseases.

508F

Expression profile of microRNAs in focal cortical dysplasia: possible clues into etiology. *S.H. Avansini[1], F.R. Torres[1], F. Rogério[3], D.B. Dogini[1], A.C. Coan[2], R. Secolin[1], C.S. Rocha[1], A.F. Costa[3], A.L.F. Costa[2], L.S. Queiroz[3], F. Cendes[2], I. Lopes-Cendes[1].* 1) Department of Medical Genetics, Faculty of Medical Sciences - State University of Campinas, Campinas, SP., Brazil; 2) Department of Neurology, Faculty of Medical Sciences - State University of Campinas, Campinas, SP., Brazil; 3) Department of Anatomical Pathology, Faculty of Medical Sciences - State University of Campinas, Campinas, SP., Brazil.

MicroRNAs (miRNAs) are small noncoding RNAs which regulate post-transcriptional gene expression. Focal cortical dysplasia (FCD) is a malformation of cortical development which affects up to 36% of patients with drug-resistant epilepsy. The objective of the present work is to investigate a possible role of miRNA regulation in the etiology of FCD. We used brain tissue obtained after surgery for the treatment of medically refractory seizures from seven patients with FCD (three patients with FCD type IIa and four patients with FCD type IIb). In addition, we used brain cortical tissue from autopsy as control. Total RNA was isolated with RecoverAllTM kit (Ambion) and RNA integrity was assessed by Agilent RNA Pico Chip Kit and Bio-Analyzer 2100 (Agilent Technologies, Boeblingen, Germany). MiRNA expression profile was assessed by Affymetrix GeneChip platform miRNA array. Background correction, summarization and normalization were performed by RMA function. Differential miRNA expression was analyzed using RankProd (FDR p < 0.05). We identified 44 human differently expressed miRNAs when comparing patients and controls. Among them we observed a significant down-regulation of several elements belonging to the miR-17~92 cluster. This cluster is known to contribute to transcriptional regulation of cell cycle, aging, as well as fine-tuning of pathways involved in neuronal differentiation. However, we found no difference in miRNA expression profile between FCD types IIa and IIb.

509F

Identifying Rare Variants in Parkinson Disease via Whole Exome Sequencing. *G. Bademci[1,2], K. Nuytemans[1,2], A. Mehta[1], C. Jauregui[1], A.G Martinez[1], G. Beecham[1,2], Y. Edwards[1,2], C. Singer[3], F. Nahab[3], S. Rhodes[4], B. Ritz[4], S. Zuchner[1,2], W.K Scott[1,2], J.M Vance[1,2].* 1) John P. Hussman Institute for Human Genomics, Miller School of Medicine, University of Miami, Miami, FL, 33136, United States; 2) Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami, Miami FL, 33136, United States; 3) Department of Neurology, Miller School of Medicine, University of Miami, Miami, FL, 33136, United States; 4) Department of Epidemiology, UCLA School of Public Health, Los Angeles, CA, 951772, United States.

Despite multiple GWAS studies, the common disease-common variant hypothesis has provided only a few replicated variations associated with Parkinson disease (PD) on a genome-wide significant level. Rare Variants (RV) have a population frequency less than 1-2%, and have been postulated to be part of the “missing” heritability in complex diseases such as PD. Further, these RV may contribute to large differences in age-at-onset (AAO) and progression of symptom severity between PD patients. To investigate the contribution of exonic RV in non-Mendelian PD, we have currently performed whole exome sequencing (WES) on 103 PD cases and 50 controls as part of an initial discovery dataset. These include 7 extended families, 51 unrelated patients whose AAO <40 years, 8 relatives with PD who have a >27 years difference in AAO, and 20 patients who have either very fast or slow disease progression. Agilent SureSelect whole exome kits were used for capture and samples were sequenced on an Illumina HiSEQ 2000. MAQ was used for sequence alignment and variant determination; all variant calls with a minimum depth of ⁄ 8x and a Phred-like consensus quality of ⁄ 20 were considered for bioinformatics and statistical analysis. SeattleSeq was used to annotate RV into functional categories such as missense, nonsense, splice sites, coding, non-coding, UTRs and other novel variants. The average coverage at ⁄ 8X was 81%, average depth was 51X. The first step of our analysis is to screen known Mendelian genes and genes reported with genome wide significance from PD GWAS. Of the known Mendelian PD genes, RV with high Phred-like consensus quality were only seen in *GBA* (I158T, E365K, T408M, R502C) and *LRRK2* (L1795F, in one extended family), while RV were more common in the genes identified by GWAS. Further analysis will determine if exonic RV contribute to earlier AAO, disease progression and/or are risk factors for PD.

510F

Identification of coding variants and gene interactions associated with neural tube defects using microdroplet-based targeted sequencing. *R. Blekhman[1,2], R.H. Finnell[3], G.M. Shaw[4], J.M. Musser[5], A.G. Clark[2], M.E. Ross[1].* 1) Neurogenetics and Development, Weill Medical College of Cornell University; 2) Molecular Biology and Genetics, Cornell University, Ithaca, NY; 3) Dell Pediatric Research Institute, University of Texas at Austin, Austin, TX; 4) Pediatrics, Stanford University, Stanford, CA; 5) The Methodist Hospital Research Institute, Houston, TX.

Neural tube defects (NTDs) are among the most common debilitating birth defects, with a worldwide prevalence of 0.5 to 1 per thousand. Although there are many environmental factors influencing disease risk, NTD has a strong genetic component, and many known gene mutations have been shown to produce NTD phenotypes in the mouse. However, attempts to link human mutations with NTD risk have not been as successful, mainly due to the complex nature of the disease, likely to be caused by interaction of mutations in many genes. Furthermore, the amount of DNA available in NTD patient repositories is limited and does not allow whole-genome or exome sequencing strategies. To overcome these limitations, we used microdroplet-based PCR enrichment of targeted regions followed by massively parallel sequencing, using only 50 ng of DNA extracted from newborn blood spots on Guthrie cards of 24 NTD patients. This allowed us to investigate the full coding sequences of 150 genes in which mutations are known to lead to NTD phenotypes in the mouse. By multiplexing three samples per lane of an Illumina GAII machine, we achieved a mean depth of 100X per sample in the targeted coding regions, with 95% of sites covered by at least 50 reads. We identified ~1000 SNPs and Indels on average in each sample, and ~4000 SNPs overall, of which 852 are novel. Specifically, we discovered 86 previously uncharacterized, likely damaging SNPs that could contribute to neurulation failure. Using these SNPs and data from the 1000 genomes project, we applied a gene-level weighted aggregated association test, and identified multiple genes that are significantly enriched with likely functional rare variants. In addition, we identified multi-SNP interactions that are over-represented in NTD patients compared to the general population, and so may play a role in risk of NTD. We are currently re-sequencing the full coding regions of several candidate genes in a larger NTD cohort to identify additional variants, and the functional effect of several mutations in these genes is being tested.

511F

Two Filipino families with Pelizaeus-Merzbacher Disease (PMD). *B.V. Cavan[1,2], M.S. Modequillo[2], J.R. de la Calzada[1,2].* 1) Pediatrics, Cebu Institute of Medicine, Cebu, Cebu, Philippines; 2) Pediatrics, Perpetual Succour Hospital, Gorordo Ave., Cebu City, Cebu, Philippines.

Pelizaeus-Merzbacher disease (PMD) is an X-linked genetic condition affecting the central nervous system's white matter, specifically formation of myelin. Clinical manifestations include rotatory eye movements (nystagmus), developmental delay, hypotonia, spasticity, titubation, and cognitive problems. The condition has been attributed to mutations on the proteolipid protein 1 (PLP1) gene. This paper presents 2 Filipino families with classical PMD. Clinical presentation, MRI, and mutational studies (c.125G>A) confirmed the diagnosis in the first family. In the second family, clinical presentation, a strongly X-linked recessive pattern of inheritance and PLP1 gene analysis showing sequence variations supported the diagnosis. None of the patients tested showed gene duplication/deletion. It may be then prudent to consider gene sequence analysis, aside from duplication/deletion of the PLP1 gene, in Filipino patients suspected of having PMD due to the genetic heterogeneity noted in these families.

512F

Candidate gene analysis for rare variants in the serotonin system in autism. *E. Crawford[1], A. McGrew[1], E.H. Cook[2], J.S. Sutcliffe[2].* 1) Mol Physiology & Biophysics, Vanderbilt Univ, Nashville, TN; 2) Dept of Psychiatry, Univ Illinois-Chicago, Chicago, IL.

Rare variants in the serotonin transporter (SERT; gene symbol: SLC6A4) have been observed in multiple pedigrees with obsessive compulsive disorder (OCD). Selective serotonin reuptake inhibitors (SSRIs) that selectively target SERT are forefront in therapy for OCD. Serotonin dysregulation has long been implicated in autism (OMIM 209850), a common neurodevelopmental disorder. Patterns of repetitive behaviors and restricted interests are characteristic of the autism phenotype. MZ twin studies indicate high heritability, but evidence supports a multilocus etiology. Thorough investigation of highly complex genetic disorders such as autism should include several genes that encode proteins in the pathway of interest. Our hypotheses aims to discover genes and/or pathways related to SERT regulation and OCD behaviors that harbor autism susceptibility alleles. In this study, we tested these hypotheses following a rare variant susceptibility model. We resequenced exons for four loci, SLC6A4, HTR2A, HTR2B, and ADAMTS6 in unrelated autism probands. 5-hydroxytryptamine (serotonin) receptor 2B and 2A are 5-HT2 receptors encoded by genes HTR2B and HTR2A, respectively. ADAMTS6 encodes a disintegrin and metalloproteinase with thrombospondin motifs 6, and this locus was identified using the SCAN database (www.scandb.org) based on SNPs within this locus that significantly predict variation of SLC6A4 gene expression in lymphoblastoid cell lines. Exonic sequences for these four genes were sequenced in a pilot sample of 150 cases. Preliminary sequence analysis for HTR2A, HTR2B, and SERT has only been preformed in cases, therefore we cannot form a conclusion whether these genes show a greater mutation burden in autism vs. controls. However, multiple synonymous and nonsynonymous variants have been detected in these loci. In contrast, analysis of ADAMTS6, after an additional 130 cases and 200 controls, for rare variants revealed 10 nonsynonymous and 4 synonymous variants in cases vs 3 nonsynonymous and 1 synonymous variants in controls, suggesting a greater burden of rare, transcribed variants in ADAMTS6 in individuals with autism. The need for further resequencing of all four genes was apparent after more ADAMTS6 sequence data was collected. We conclude that further study is warranted for all four of these genes and is currently underway.

513F

Functional assessment of a promoter polymorphism in S100B, a putative risk variant for bipolar disorder. *E. Dagdan[1,2], DW. Morris[1], M. Campbell[2], M. Hill[1], M. Rothermundt[3], F. Kästner[3], C. Hohoff[3], C. von Eiff[4], P. Krakowitzky[5], M. Gill[2,6], P. McKeon[6,7], S. Roche[2].* 1) Trinity College Dublin, Neuropsychiatric Genetics Research Group, Institute of Molecular Medicine, Ireland; 2) Smurfit Institute of Genetics, Trinity College Dublin, Ireland; 3) Department of Psychiatry, University of Münster, Germany; 4) Institute of Medical Microbiology, University of Münster, Germany; 5) Institute of Transfusion Medicine, University of Münster, Germany; 6) Department of Psychiatry, Trinity College Dublin, Ireland; 7) St. Patrick's University Hospital, Dublin, Ireland.

Background: Calcium-binding protein S100B has been implicated in the pathology of bipolar affective disorder (BPAD) and schizophrenia (SZ). S100B protein levels are elevated in serum of patients with both disorders compared to controls. We previously reported genetic association of a SNP in the promoter of S100B, rs3788266, with a psychotic form of BPAD. Methods: To test for genotypic effects of rs3788266 in vivo, S100B serum protein levels were measured in 350 Irish and German subjects of known S100B genotype. The functional effect of rs3788266 on S100B promoter activity was studied using the luciferase reporter system in U373MG glioblastoma and SH-SY5Y neuroblastoma cell lines. Allelic effects of rs3788266 on protein complex formation at the S100B promoter were investigated by an electrophoretic mobility shift assay. Results: Higher mean serum S100B levels were associated with the risk G allele of rs3788266 in BPAD cases (P=0.0001), unaffected relatives of BPAD cases (P<0.0001) and unrelated controls (P<0.0001). Consistent with the in vivo findings, luciferase gene expression was significantly increased in the presence of the G allele compared to the A allele in SH-SY5Y (P=<0.0001), and in U373MG (P=<0.0008) cell lines. The binding affinity of both SH-SY5Y and U373MG protein complexes for the S100B promoter was significantly stronger in the presence of G allele compared to the A allele promoter fragments. Conclusions: These data support rs3788266 as a functional promoter variant in the S100B gene where the presence of the G allele promotes increased gene expression and is associated with increased serum levels of the protein.

514F

A genome-wide association study identifies loci associated with aging-related decline in episodic memory. *P. De Jager[1], J. Shulman[1], L. Chibnik[1], J. Corneveaux[3], A. Myer[4], M. Huentelman[3], J. Hardy[5], R. Wilson[2], E. Reiman[3], D. Evans[2], D. Bennett[2].* 1) Neurology, Brigham & Women's Hospital, Boston, MA; 2) RUSH University, Chicago IL; 3) Neurogenomics Division, Translational Genomics Research Institute and Arizona Alzheimer's Consortium, Phoenix, AZ; 4) Department of Psychiatry and Behavioral Sciences, University of Miami, Miller School of Medicine, Miami, FL; 5) Reta Lila Weston Laboratories, Department of Molecular Neuroscience, Institute of Neurology, Queen Square, London, England.

BACKGROUND: Gene discovery in Alzheimer's disease (AD) has largely relied on the case/control study design; however, this approach is limited by heterogeneity of dementia in cases and subclinical disease in controls. We have implemented a complementary strategy using episodic memory decline, a key feature of AD, as a continuous outcome for a genome-wide association scan (GWAS). METHODS: A GWAS was performed using 6.5 million imputed single nucleotide polymorphism (SNP) genotypes and up to 15 years of prospective clinical data from the Religious Orders Study and Rush Memory and Aging Project (n=1,593). The outcome phenotype was a composite quantitative measure of episodic memory decline slope, based on longitudinal assessment of 7 cognitive tests, and adjusted for age, gender, and education as well as the top 3 principal components calculated from the genetic data. Replication of top results using a similar memory decline trait in the Chicago Health and Aging Project (CHAP) (n=588) is ongoing. RESULTS: In addition to the APOE locus, 5 loci exceed a threshold of genome-wide significance (p<5x10^-8). The SNPs tagging each locus are: rs2411399 (ADAMST12), rs2768668 (LYZL1), rs2159085 (ZNF286A), rs2377342 (intergenic), and rs2628983 (ZNF775). Replication in the CHAP cohort is ongoing and will be presented at the conference. Genomic inflation factor for this study of subjects of European ancestry: 0.998. Nominal associations of episodic memory decline with the known AD susceptibility alleles in the CR1 (p=0.048) and ABCA7 (p=0.044) were noted; in addition, SNPs other than the index SNP in the PICALM (p=2x10^-4) and BIN1 (p=3x10^-4) AD susceptibility loci demonstrated association with decline in episodic memory. CONCLUSIONS: Our strategy identifies several novel loci as being associated with susceptibility for decline in episodic memory. Such decline can be caused by multiple different processes, including AD, and therefore these loci not only offer insights into the dysfunction of a specific cognitive circuit with aging but are also excellent candidates for AD susceptibility loci.

515F

LINGO1 gene and Parkinson's disease: an association study in the Italian Population. *E.V. De Marco[1], F. Annesi[1], F.E. Rocca[1], A. Nicoletti[2], P. Pugliese[3], G. Nicoletti[1,3], G. Arabia[3], P. Tarantino[1], M. De Mari[4], P. Lamberti[4], S. Gallerini[5], R. Marconi[5], A. Epifanio[6], L. Morgante[6], A. Cozzolino[7], P. Barone[7], G. Torchia[3], M. Zappia[2], G. Annesi[1], A. Quattrone[3,8].* 1) Institute of Neurological Sciences, National Research Council, Cosenza, Italy; 2) Department of Neurosciences, University of Catania, Italy; 3) Neurologic Clinic, University Magna Graecia, Catanzaro, Italy; 4) Neurologic Clinic , University of Bari, Bari, Italy; 5) Division of Neurology, Ospedale Misericordia, Grosseto, Italy; 6) Department of Neurosciences, University of Messina, Messina, Italy; 7) Department of Neurological Sciences, University FedericoII, Naples, Italy; 8) Neuroimaging Research Unit, National Research Council, Catanzaro, Italy.

A genome wide association study in European and American patients with Essential Tremor(TE) showed a significant association with the rs9652490 SNP of the leucine rich repeat and Ig domain containing 1 (LINGO1) gene. LINGO1 gene codes for a nervous system-specific transmembrane protein. Its expression is increased in the substantia nigra of some PD patients, thus suggesting that LINGO1 may be involved in PD pathophysiology. In this study, we performed a case-control analysis in the Italian population to assess the role of the LINGO1 gene in PD patients. A total of 567 patients with PD and 468 control subjects were enrolled in Central-Southern Italy. Genotyping of LINGO1 SNPs (rs9652490 and rs11856808) was performed by TaqMan pre-designed assays using an ABI 7900 HT-SDS system. Both variants were significantly associated with PD under a recessive model of inheritance before applying the Bonferroni correction. The GG genotype of rs9652490 and the TT genotype of rs11856808 were less frequent in patients than in controls, suggesting a protective effect against the disease. However, after stringent correction, only the P-values obtained from allele and genotype comparisons of the rs11856808 SNP remained significant. Our findings suggest that LINGO1 might play some role in the development of PD in the Italian population and represents an interesting candidate gene responsible for PD, due to its involvement in neurological processes.

516F

Next-generation sequencing coupled to functional genomics implicates FOXP pathways in autism and language impairment. *P. Derizioti[1,2], B.J. O'Roak[3], L. Vives[3], J. Shendure[3], E.E. Eichler[3,4], S.E. Fisher[1,2].* 1) Wellcome Trust Centre for Human Genetics, Roosevelt Drive, University of Oxford, Oxford, UK; 2) Language and Genetics Department, Max Planck Institute for Psycholinguistics, Nijmegen, The Netherlands; 3) Department of Genome Sciences, University of Washington School of Medicine, Seattle, WA, USA; 4) Howard Hughes Medical Institute, Seattle, WA, USA.

Autism spectrum disorders (ASD) comprise a range of neurodevelopmental syndromes characterized by extensive abnormalities of social interactions and communication, including language impairment and stereotyped behaviours. ASD aetiology has a strong genetic basis, but in most cases the specific molecular risk factors remain unknown. Recent sequencing of exomes in 20 parent-child trios with idiopathic autism uncovered 21 de novo mutations, 11 of which were protein-altering. One involved a single base insertion in FOXP1, introducing a frameshift and premature stop codon (A339SfsX4), in a severely affected ASD proband with regression and language delay. This is of particular interest since FOXP1 belongs to the same group of transcription factors as FOXP2, a gene implicated in rare monogenic speech and language disorders. The proteins encoded by FOXP1 and FOXP2 can directly interact with each other, with the potential to co-regulate downstream targets in neural circuits where they are co-expressed, including those involved in language function. Analyses of proband-derived lymphoblasts indicated that the majority of FOXP1-A339SfsX4 transcripts undergo nonsense-mediated RNA decay. Moreover, in functional cell-based assays we found that transcripts which escape this process yield a truncated protein that is mislocalised from the nucleus to the cytoplasm. Intriguingly, in addition to the FOXP1-A339SfsX4 mutation, this same ASD proband also carries a rare inherited missense variant of CNTNAP2, a gene that has been independently associated with ASD and common language impairments. We found that wildtype FOXP1 protein (like FOXP2) acts as a repressor of CNTNAP2 expression, while the truncated FOXP1 mutant protein leads to misregulation of this downstream target. Overall the functional data suggest that the FOXP1 mutation may amplify deleterious effects of the CNTNAP2 variant in the proband, consistent with a multi-hit model for disease risk. Building on this work, we have now performed similar functional characterisations for novel risk variants identified in targeted high-throughput sequencing of FOXP1 and FOXP2 in a large cohort of 1700 idiopathic ASD cases. Our unpublished findings demonstrate how the coupling of next generation sequencing with functional genomic assays can shed important new light on pathways underlying complex neurodevelopmental phenotypes like autism and language impairment.

517F

Analysis of transcriptional profiles in schizophrenia. *J. Duan[1], H.H Göring[2], E.I. Drigalenko[2], J. Shi[3], W. Moy[1], J. Freda[1], J. Jacobi[1], D. He[1], . MGS[4], A.R. Sanders[1], P.V. Gejman[1].* 1) Dept Psychiatry, NorthShore University/University of Chicago, Evanston, IL; 2) Department of Genetics, Texas Biomedical Research Institute, San Antonio, TX; 3) Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD; 4) Molecular Genetics of Schizophrenia (MGS) Collaboration.

Introduction. We have tested in a large clinical sample the hypothesis that there are transcriptional profiles characteristic of schizophrenia. **Methods.** Using Illumina HT-12v4 microarrays, we have assayed mRNA from EBV-transformed LCLs with pre-existing GWAS data for 446 schizophrenia cases and 457 controls of European ancestry. We have studied and controlled for known confounding effects, both epidemiological (age, sex, ancestry) and cellular (growth rate, energy status, EBV load, and LCL transformation site) characteristics. We used regression analysis to identify transcripts differentially expressed by affection status. In an effort to control for the analytical strategy, we also performed all the aforementioned analyses adding expression principle components (that also detect unknown/unmeasured confounders), with largely consistent results. **Results.** After stringent quality control, we retained 27,118 transcripts and all samples (no QC outliers). We first studied the effects of CNVs associated with schizophrenia (1q21.1, 15q13.3, 16p11.2, and 22q11.21) on local gene expression profiles. Samples with a deletion showed lowered expression for 78%, and those with duplications showed increased expression for 49% of the regional (CNV bounded) transcripts, compared to remaining samples. After regressing out known confounders, we identified 83 transcripts representing 76 genes differentially expressed by affection status at an FDR of 0.05. Histone genes from 1q21.2 and the 6p22.1 MHC region (*HIST1H4K, HIST1H2BC, HIST1H2BD, HIST1H3C, HIST1H2BG*, and *HIST1H2BH*) showed notable enrichment among these 76 genes. Eight transcripts (*CPNE5, BRWD1, ITGA1, MOXD1, THAP10, EHBP1, SV2A,* and *IFITM3*) survived Bonferroni correction for all retained transcripts, of which *CPNE5, BRWD1, ITGA1, MOXD1, THAP10*, and *EHBP1* are brain-expressed, and C*PNE5, BRWD1, SV2A,* and *MOXD1* have functions involving neurotransmitters or intercellular communication. C**onclusions.** The observed differential expression of 6p22.1 histone genes in the MHC region converges with the genetic evidence from schizophrenia GWAS, where this same region is the most significant schizophrenia susceptibility locus. Our results thus provide a plausible functional interpretation of the GWAS results. In addition, we systematically detected newly implicated individual genes with our expression profiling experiment - genes that were undetectable by pure GWAS approaches, thus providing new and functional leads.

518F

Metal content and oxidative stress in autistic Egyptian children. *H.T. El-Bassyouni[1], S.I. Ismail[2], G.S.M. El-Saeed[3], E.A. Alghroury[4].* 1) Clinical Genetics , National Research Centre, Cairo, Egypt; 2) Clinical Genetics , National Research Centre; 3) Medical Biochemistry Department, National Research Center; 4) Clinical Pathology Department, National Research Center.

Background: Many factors have been implicated in autism onset, including excess or deficiency in toxic or essential metals and impaired antioxidant systems. Exposure to metals is a putative risk factor for autism. Many metals could be implicated in autism since they typically disrupt enzyme functions and cell signalling processes and generate oxygen free radicals (ROS). Methods: Sixteen autistic patients whose age ranged from 2.6-12.7years (mean age 5.6 years) were clinically examined. Twenty healthy children matching age and gender were taken as a control group. Serum mercury, lead and nitric oxide were estimated in all patients. Results: Nine presented with seizures and epileptogenic focus in electro encephalogram (EEG). The IQ assessment showed mental retardation in all patients: 9 were moderate, 1 was severe and 6 were profound. One had microcephaly (+2.8). Assessments of the severity of autistic symptoms using childhood autism rating scale (CARS), 3 patients were mild, 6 were moderate and 7 were severe. The estimation of the level of mercury and lead in blood showed significant increase compared to control group (P value 0.0001). Furthermore, nitric oxide was significantly increased than the control group (P value 0.0001). These findings demonstrate a significant increase in both metal content and an unbalance in the oxidative status in the blood of autistic children. Conclusion: Our findings suggest the hypothesis that exposure to metals and oxidative stress may be biomarkers of toxicity in autism. We recommend that patients diagnosed with autism should be given chelators for mercury and lead to improve their condition.

519F

From missing heritability to missing specificity: genomic convergence and pleiotropy in neurodevelopmental disorders. *B. Franke[1], G. Poelmans[2], D. Pauls[3], J. Glennon[2], J.K. Buitelaar[2].* 1) Departments of Human Genetics & Psychiatry, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 2) Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 3) Psychiatric and Neurodevelopmental Genetics Unit, Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston, USA.

Background: The neurodevelopmental disorders attention-deficit/hyperactivity disorder (ADHD), developmental dyslexia and autism-spectrum disorders (ASDs) often co-occur. All are highly heritable and they share heritability. Objective: In the current study, we aimed at integrating current knowledge of the molecular genetics of the three disorders to identify biological processes enriched in the findings and overlapping between disorders. Methods: Bioinformatics and systematic literature analyses were conducted based on the findings of published genome-wide studies of common and rare genetic risk factors for the three disorders. In addition, data from candidate gene association studies, cytogenetic and animal studies as well as studies of miRNAs and pharmacological gene expression were used to confirm and extend observed biological networks. Results: For ADHD, 85 genes were included in the analysis; for ASDs, a total of 200 genes were analysed. Data for developmental dyslexia were most scarce, and only 14 genes could be considered. Analysing the data for ADHD, we found the biological process of neurite outgrowth significantly enriched in the findings; this process was also found enriched in the data for developmental dyslexia, as was neuronal migration. In ASDs, we observed signalling networks regulating steroidogenesis, synaptic function and also neurite outgrowth to be overrepresented. In this disorder, for which most data were available, we also identified new drugable targets, the AKAP (A-kinase anchor protein) genes, using this approach to data integration. In all disorders, common and rare variants were found to `hit' the same networks and even the same genes. Conclusion: Genomic convergence is observed in the findings from genome-wide molecular genetic studies of all three disorders, and biological processes involved in disease etiology but also novel targets for treatment can be identified using data integration. Distinctions between multifactorial and monogenic/oligogenic causes of neurodevelopmental disorders appear artificial as both common and rare variants implicate the same signalling networks and genes. Neurite outgrowth networks are pleiotropic in that they are implicated in all three neurodevelopmental disorders and may explain the findings of overlapping heritability.

520F

Early onset Huntington disease presenting as a Neuroferritinopathy. *M.T. Gabbett[1 2], C.S. Manning[1 2], J.C. MacMillan[1 2].* 1) Genetic Health Queensland, Royal Brisbane & Women's Hospital, Brisbane, Queensland, Australia; 2) The University of Queensland, School of Medicine, Brisbane, Queensland, Australia.

Neuroferritinopathy caused by mutations in the *FTL* gene causes gradual accumulation of iron in the brain. Clinically, this results in a progressive adult-onset chorea or dystonia and subtle cognitive defects. The majority of individuals develop a characteristic orofacial action-specific dystonia that leads to dysarthrophonia. Cognitive deficits and behavioural issues become significant with time. Magnetic resonance imaging (MRI) typically demonstrates hypointensity of the basal ganglia on T2-weighted imaging. Huntington disease (HD) is a progressive disorder of motor, cognitive, and psychiatric disturbances and is caused by an expanded polyglutamine tract in the huntingtin protein. MRI typically demonstrates basal ganglia atrophy. We present the case of a 27 year old Vietnamese woman with a three year history of symptoms including dysphagia, imbalance and low vision. Her father and her brother were said to be similarly affected. MRI demonstrated decreased T2 signal within the basal ganglia, dentate cerebellar nuclei and the substantia nigra, consistent with iron disposition. The clinical presentation and MRI findings suggested an autosomal dominant neuroferrintinopathy. Testing of the *FTL* gene yielded no mutations. Testing of *HTT* demonstrated CAG trinucleotide repeats of 16 and 57, consistent with the diagnosis of HD. Hypointensity of the basal ganglia represents iron deposition on T2-weighted MRI, but has rarely been reported in HD. This case illustrates that HD should be considered as a differential diagnosis for neuroferrtinopathy.

521F

A Rare Genomic Duplication Creates Novel Transcripts from DNA Of a Patient with Schizophrenia. *M. Gasperini[1], C. Rippey[2], T. Walsh[3], A. Nord[2], C. Spurrell[2], MK. Lee[3], MC. King[3,2].* 1) University of Washington, Seattle, WA; 2) Department of Genome Sciences, University of Washington, Seattle, WA; 3) Department of Medical Genetics, University of Washington, Seattle, WA.

Rare, gene-disrupting genomic deletions and duplications - called copy number variants (CNVs) - have been implicated in schizophrenia; however, much remains to be understood about which genes are causative, as well as the cellular mechanisms involved. Biological characterization of individual CNVs has the potential to provide insight into the origin of schizophrenia. We used array Comparative Genomic Hybridization to screen DNA from individuals with schizophrenia for such CNVs, and focused on one found in a patient with early onset. This CNV duplicates the 5' ends of two genes that lie head-to-head on chromosome 11q22: DCUN1D5, a previously uncharacterized gene predicted to be involved in cullin neddylation of ubiquitin ligase complexes, and DYNC2H1, a dynein active in cilia. Both are expressed in brain and are plausible candidate genes for schizophrenia. In order to detect and characterize aberrant transcripts resulting from this duplication, we performed next generation sequencing (RNAseq) of total mRNA from lymphoblasts of the patient. We found multiple different transcripts unique to this patient within the duplicated region, indicating novel expression and splicing of the genomic sequence now adjacent to the 5' end of DCUN1D5. PCR and Sanger sequencing confirmed that these novel exons constitute a new 3' end of the mutant, truncated DCUN1D5. These data support the role of this mutation in schizophrenia by confirming the presence of aberrant transcripts in the patient's RNA. The data also suggest an intriguing mechanism by which a genomic rearrangement can result in a novel, stable transcript via “exonification” of otherwise non-coding sequence.

522F

Role of ANKK1 AND DRD2 gene polymorphisms in genetic susceptibility to migraine in Northern Indian population. *J. Ghosh[1], S. Pradhan[2], B. Mittal[1].* 1) Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow, India; 2) Neurology, Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow, India.

The dopaminergic system plays an important role in migraine and its clinical subtypes: migraine with aura (MA) and migraine without aura (MO). D2 receptor antagonists have been proposed in antimigraine therapy. We aimed to look for association of polymorphism in the ANKK1 [TaqI-A(rs1800497)] and DRD2 [-141Cdel (rs1799732) and NcoI(rs6275)] gene polymorphisms with migraine susceptibility. The migraine patients were recruited in two cohorts - primary (n=213) and replicative (n=128). Simultaneously, 210 healthy age-sex matched free from any neurological diseases were also enrolled. The results of primary cohort were validated in the replicative cohort. On obtaining significant associations in both the cohorts, the results were pooled by meta analysis using Fisher's method (2 and p value) and Mantel-Haenszel test for odds ratio calculation. Logistic regression analysis was applied for statistical analysis and Bonferroni correction for multiple comparisons in subgroup analysis. The study was approved by ethics committee of the Institute. For ANKK1 TaqIA polymorphism, variant genotype and allele (A1) showed highly significant ($p<0.001$) association with migraine and its subgroups in the primary cohort. The results were replicated in overall migraine and MO. Similarly, pooling data from two cohorts also confirmed the significant association in migraine susceptibility. For DRD2 NcoI polymorphism, a significant protective effect of variant (T) allele was found in both primary and replicative cohorts. Mantel - Haenszel test also revealed protective role of this allele (ORMH= 0.7678, 95%CI= 0.6249-0.9434). No significant association for migraine or its clinical subgroups were observed for DRD2 -141C del polymorphism. In conclusion, the present study suggests a significant risk of ANKK1 TaqIA polymorphism with migraine, especially in migraine without aura, and a protective role of DRD2 NcoI polymorphism. Acknowledgements: Financial support from Indian Council of Medical Research (ICMR), Department of Biotechnology (DBT) and Department of Science and Technology (DST), New Delhi, and fellowship JG from Council of Scientific and Industrial Research (CSIR), New Delhi.

523F

Genetic variation influencing memory ability in a population-based sample. *L. Gomez[1], J.B. Rich[2], M.L. Smith[3], C. Barr[1, 3].* 1) Toronto Western Research Institute, Toronto, ON, Canada; 2) York University, Toronto, ON, Canada; 3) Hospital for Sick Children, Toronto, ON, Canada.

Genes involved in the molecular pathways of visual-spatial learning and memory have been identified in animal models and some of these have been implicated in human memory ability. In addition a number of genes contributing to memory ability have been identified in association studies in humans. In this study, we tested three genes (BDNF, CREB1 and CAMTA1) for their contribution to visual-spatial and verbal memory ability in a sample of 100 male university students in Toronto. Quantitative methods were used to examine measures of learning and memory performance to identify genetic factors involved in visual-spatial and verbal measures of immediate, delayed and working memory, and in phonological processing. We found a single marker (rs11030108) in the BDNF gene and a single marker (rs7549382) in the CAMTA1 gene that showed significant p-value when comparing the performance on the Wechsler Memory Scale-III tests for each genotype. Lower scores for the memory tests were associated with the GG genotype for the BDNF marker and higher scores were associated with the CC genotype for the CAMTA1 marker. These preliminary results in this sample support previous findings implicating these two genes in human memory ability.

524F

Improving molecular diagnosis of Dravet syndrome by using multiple prediction algorithms. *M.C. Gonsales[1], P.M. Preto[2], M.A. Montenegro[2], M.M. Guerreiro[2], I. Lopes-Cendes[1].* 1) Department of Medical Genetics, University of Campinas, Campinas, Brazil; 2) Department of Neurology, University of Campinas, Campinas, Brazil.

Mutations in the neuronal voltage-gated sodium channel (1-subunit gene (*SCN1A*) have been identified in several patients with phenotypes within the clinical spectrum of generalized epilepsy with febrile seizures plus (GEFS+). It is now accepted that genetic testing for mutations in *SCN1A* is clinically useful. However, the prognostic value of these mutations and a possible correlation with the different clinical subtypes remain unclear. Therefore, the aim of this study was to search for mutations in *SCN1A* in patients with Doose and Dravet syndromes, both severe forms of epilepsy included in the GEFS+ spectrum. We performed *SCN1A* mutation screening in 13 patients with Doose syndrome and 15 with Dravet syndrome. We used eight in silico prediction algorithms to analyze the possible impact of the amino-acid changes in protein function (SIFT, Polyphen 1 and 2, Pmut, MutPred, PhD-SNP, SNAP e SNP&GO). In addition, multiplex ligation-dependent probe amplification (MLPA) was used to detect copy number variations within *SCN1A*. Patients with Doose syndrome showed no mutations, whereas twelve mutations were identified in 80% of the patients with Dravet syndrome: six missense mutations, three splice-site mutations, two deletions and one insertion. All mutations that lead to amino-acid residue substitutions are predicted to affect protein function according to the prediction algorithms employed. The mutations are mostly located in the pore region and the C-terminal of the protein. No copy number variants were identified. Patients with Dravet syndrome showed a high frequency of *SCN1A* mutations (80%), confirming that molecular testing would be highly recommended in individuals with this phenotype. In addition, our strategy for predicting deleterious effect of mutations using multiple prediction algorithms was able to provide valuable information, helping clinicians with decision making. Moreover, we found that Doose syndrome does not seem to share the same genetic basis as Dravet syndrome. Furthermore, our results confirm that missense mutations can cause severe phenotypes depending on its location and the type of amino-acid substitution. Financial Support: CNPq/FAPESP.

525F

Evidence for Association between L-Type Voltage-Gated Calcium Channel (CACNA1C) Gene Haplotypes and Bipolar Disorder in Latinos: a Family-Based Association Study. *S. Gonzalez[1], C. Xu[1], M. Ramirez[1], J. Zavala[1], R. Armas[2], S.A. Contreras[3], J. Contreras-Rojas[4], A. Dassori[3, 5], R.J. Leach[6], D. Flores[7], A. Jerez[8], H. Raventós[4], A. Ontiveros[9], H. Nicolini[10], M. Escamilla[1].* 1) Center of Excellence for Neurosciences, Texas Tech University Health Sciences Center Paul L. Foster School of Medicine, El Paso, TX; 2) Langley Porter Psychiatric Institute, University of California, San Francisco, San Francisco, CA; 3) Department of Psychiatry, University of Texas Health Science Center at San Antonio, San Antonio, TX; 4) Centro de Investigación en Biología Celular y Molecular y Escuela de Biologia, Universidad de Costa Rica, San Jose, Costa Rica; 5) South Texas Veterans Health Care System, San Antonio, TX; 6) Department of Cellular and Structural Genetics, University of Texas Health Science Center at San Antonio, San Antonio, TX; 7) Los Angeles Biomedical Research Center at Harbor, University of California Los Angeles Medical Center, Torrance, CA; 8) Centro Internacional de Trastornos Afectivos y de la Conducta Adictiva, Guatemala, Guatemala; 9) Instituto de Información e Investigación en Salud Mental AC, Monterrey, N.L., México; 10) Grupo de Estudios Médicos y Familiares Carracci, S.C., México, D.F., México.

Background: Over the past several years, genome-wide association studies (GWAS) have identified genetic variants that contribute to complex human disorders. However, GWAS have been centered on populations of European ancestry, and the degree to which the findings are applicable to other populations has not been extensively investigated. Through recent GWAS studies, several groups have reported significant association between variants in the alpha 1C subunit of the L-type voltage-gated calcium channel (CACNA1C) and bipolar disorder (BP) in Caucasian cohorts. To determine whether CACNA1C is associated with BP in the Latino population, we performed a family-based association study between CACNA1C polymorphisms and BP. Methods: This study consisted of 250 Latino families (913 individuals) recruited from the US, Mexico, Guatemala, and Costa Rica. A total of 482 participants were diagnosed with BP (263 BP Type I with Psychosis, 202 BP Type I without Psychosis, and 17 Schizoaffective BP Type). The Illumina GoldenGate Genotyping Assay was used to genotype two SNPs (rs7297582 and rs1006737) in the CACNA1C gene previously shown to be associated with BP and an additional 55 SNPs that spanned a 603 kb region encompassing the CACNA1C gene. Markers were selected using a Tag SNP method and supplemented with additional SNPs in potential linkage disequilibrium (LD) with the two SNPs previously associated with BP. Individual SNP and haplotype association analyses were performed using the family-based association test (FBAT, version 2.0.3) and Haploview software (version 4.2). Results: We found no association between the two individual SNPs and BP (rs7297582, p= 0.760; rs1006737, p= 0.469) in the Latino population studied. An 8-locus haplotype block was identified to be in high LD with these two SNPs. A positive association between this 8-locus haplotype and BP was observed (permuted P=0.005; global marker permuted P=0.002), which included the two markers previously associated with BP. Conclusions: Although we were not able to replicate findings of association between individual CACNA1C SNPs rs7297582 and rs1006737 and BP, we were able to replicate the GWA signal reported for CACNA1C with a haplotype analysis which encompassed these SNPs. These results provide additional evidence that CACNA1C is associated with BP and provides the first evidence that variations in this gene might play a role in the pathogenesis of this disorder in the Latino population.

526F

Intracranial Tumours in a Family with CADASIL - A Case Report. *B.D. Henderson[1], E. Janse van Rensburg[2].* 1) Division Human Genetics, UFS, Bloemfontein, Free State, South Africa; 2) Department Neurology, UFS, Bloemfontein, Free State, South Africa.

BACKGROUND: Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is an autosomal dominant, mid-adult onset cerebrovascular disorder. It is characterized by migraine with an aura, diffuse white matter changes and infarctions progressing to dementia. It is often confused with multiple sclerosis. It has a highly variable presentation and course. Mutations in *NOTCH3* have been associated with this disorder. OBJECTIVE: A family, in which two affected persons have been proven to have CADASIL, is presented. There are two members of this family who have intracranial tumours. RESULTS: The clinical presentation and MRI findings are presented together with a discussion of the differential diagnosis and the mechanism of the disorder. To our knowledge, tumours have not previously been described in persons proven to have CADASIL. The index case has a novel mutation in exon 6 resulting in a loss of a cysteine residue. He also has a novel mutation in exon 5 of which the implication is uncertain. Studies are planned to determine if these mutations are in cis or in trans. CONCLUSION: This family adds additional information to the clinical spectrum of problems associated with CADASIL. Additional information about effects of *NOTCH3* mutations not involving a cysteine residue may be revealed by further evaluation of this family.

527F

Evidence for more than one Parkinson disease associated variant within the HLA region. *E.M. Hill-Burns[1], S.A. Factor[2], C.P. Zabetian[3], G. Thomson[4], H. Payami[1].* 1) New York State Department of Health Wadsworth Center, Albany, NY; 2) Department of Neurology, Emory University School of Medicine, Atlanta, GA; 3) VA Puget Sound Health Care System and Department of Neurology, University of Washington, Seattle, WA; 4) Department of Integrative Biology, University of California, Berkeley, CA.

In a recent GWAS conducted on the NeuroGenetics Research Consortium (NGRC) dataset, *HLA* was found to significantly associate with risk of Parkinson's disease (PD) [PMID:20711177]. The top hit was rs3129882 ($P=3x10^{-8}$), which is in intron 1 of *HLA-DRA* and is an eQTL influencing expression of *HLA-DR* and *HLA-DQ*. While the association of rs3129882 with PD has been replicated in independent datasets, some GWAS find different SNPs in the *HLA* class II region as the top hit. Most notably, a SNP near *HLA-DRB5* also reached genome-wide significance in a meta analysis [PMID:21292315]. Our aim with this investigation was to determine if different SNPs from various GWAS tag the same locus or if there could be more than one variant in *HLA* associating with PD susceptibility. We performed step-wise conditional analysis on the NGRC dataset (2000 cases, 1986 controls) and included 107 SNPs in the *HLA* region that achieved $P < 0.001$ for association with PD risk. Conditioned on the top SNP (rs3129882), 90 of 106 SNPs lost significance. We repeated the analysis, step-wise, conditioning on all SNPs identified as the most significant in the prior rounds. This yielded four SNPs which were seemingly independent in association with PD. The four SNPs were not in linkage disequilibrium (LD) with each other ($0 \le r^2 < 0.17$). Notably, SNP4, the last SNP remaining significant in NGRC, was the only one of the four SNPs that showed some LD ($r^2=0.6$) with the top hit near *HLA-DRB5* reported by the meta analysis. Haplotype analysis revealed increasing risks with the number of risk alleles at each SNP (from OR=1.3, $P=5x10^{-3}$ to OR=1.7, $P=4x10^{-8}$). To replicate, we used a peer reviewed [PMID:18985386] and publicly available GWA dataset from dbGaP (843 cases and 856 controls). We were able to replicate the seemingly independent effects of SNP1 (rs3129882, main effect P=0.006, conditioned on other significant SNPs P=0.04) and SNP4 (rs9268515, main effect P=0.004, conditioned on other significant SNPs P=0.03). We also replicated the haplotype results for SNPs 1 and 4 which yielded OR=1.3, P=0.01 for a haplotype with one risk allele and OR=1.4, $P=2x10^{-4}$ for the haplotype with both risk alleles (haplotype analysis for SNPs 1 and 4 from NGRC gave OR=1.2, P=0.003 with one risk allele and OR=1.5, $P=1x10^{-9}$ with both risk alleles). Data suggest there may be at least two associations between PD and *HLA*, one with a regulatory element in *HLA-DRA* and one with a classical *HLA-DRB*. Study was funded NIH R01 NS36960.

528F

Mapping diagnostic heterogeneity in an animal model of Fetal Alcohol Spectrum Disorders (FASD). *K. Janus[1], M. Kleiber[1], A. Chokroborty-Hoque[1], E. Wright[2], B. Laufer[1], S. Singh[1].* 1) Department of Biology, University of Western Ontario, London, Ontario, Canada; 2) Department of Neuroscience, University of Western Ontario, London, Ontario, Canada.

Fetal Alcohol Spectrum Disorders (FASD) result from prenatal alcohol exposure and are characterized by neurobehavioral abnormalities including developmental milestone defects, altered activity levels, anxiety-related behaviors, and learning and memory deficits. The phenotypic heterogeneity behind FASD is not well understood. We have modeled acute ethanol exposure in pregnant C57BL/6J (B6) mice using ethanol injections at each of the three trimesters. Our treatment protocol also includes a continuous-preference-drinking (CPD) paradigm, as B6 mice prefer (70%) to drink 10% alcohol when given a free choice between water and ethanol solutions during pregnancy. Progeny resulting from the four treatment groups are followed and assessed for a battery of FASD-related behavioral tests during periods of growth and development until early adulthood (postnatal day 70) in order to assess long-term consequences of FASD. Relevant behavioral measures include developmental milestones, locomotor activity and anxiety, and learning and memory (using Barnes Maze). Progeny from ethanol-treated groups lag behind in achieving some, but not all, developmental milestones. Activity levels are increased (p=0.005-0.001) following ethanol exposure in trimesters one and two, but reduced (p<0.001) under CPD and unchanged when treated at trimester three. Anxiety-related behaviors are increased (p=0.008-0.001) following ethanol exposure in trimester three and CPD, but decreased (p<0.001) when treated at trimester two and unchanged in trimester one. Finally, learning and memory is decreased (p=0.05-0.001) in the progeny of all treatment groups, but to varying degrees. Results demonstrate that the major determinants of phenotypic heterogeneity for FASD are explained by the neurodevelopmental timing of ethanol exposure. All treatments result in developmental milestone delays and cognitive deficits, suggesting that no time during pregnancy is safe from alcohol's teratogenic effects. We have also observed that the behavioral deficits are related to permanent alterations in the “transcriptome”, and are potentially related by epigenetic mechanisms. Some of the behavioral changes may be reflected in high resolution Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS). Additional data included in this presentation will assess any rehabilitative effects of environmental enrichment on neurodevelopment using MRI and MRS.

529F

A variant in the 3'UTR of (-endomannosidase (*MANEA*) mRNA is associated with panic disorder diagnosis and cognitive flexibility. *K.P. Jensen[1], H.R. Kranzler[2], B.Z. Yang[1], L.A. Farrer[3], J. Gelernter[1].* 1) Department of Psychiatry, Yale University School of Medicine, New Haven, CT and VA CT Health Care Center, West Haven, CT, USA; 2) Department of Psychiatry, University of Pennsylvania and the Philadelphia VA Medical Center, Philadelphia, PA, USA; 3) Departments of Medicine, Neurology, Ophthalmology, Genetics and Genomics, Epidemiology, and Biostatistics, Boston University School of Medicine and Public Health, Boston, MA, US.

Genes in the mannosidase family encode proteins that hydrolyze mannose residues from glycoproteins. The mannosidase gene family has been implicated in a range of human disorders from severe developmental mannosidosis to drug use behaviors. In prior family-based studies, common variants in the (-endomannosidase gene (*MANEA*) have been associated with cocaine dependence and cocaine-induced paranoia. We hypothesized that variants within this gene may be associated with other psychiatric phenotypes. Here we report that a common variant in the 3'UTR of the *MANEA* mRNA is associated with panic disorder diagnosis ($\chi^2 = 9.66$, p=0.008) in a European American case (N=199) versus control (N=1982) sample initially recruited as part of a study on the genetics of drug and alcohol dependence. Cognitive function differences often represent endophenotypes for psychiatric disorder risk; therefore we explored whether the *MANEA* 3'UTR variant was associated with cognitive test performance. We found that in non-drug dependent subjects the 3'UTR *MANEA* genotype was associated with Wisconsin Card Sorting Test scores. We investigated the potential functional effect of *MANEA* gene variants on mRNA levels and observed a significant difference in the expression of *MANEA* alleles in human blood cell RNA. These findings suggest that the 3'UTR variant may be in LD with a functional variant that affects *MANEA* gene expression. In sum, functional variants at the *MANEA* loci may underlie interindividual differences in cognitive function and psychiatric disorder risk. Future studies will refine the psychiatric phenotype associated with *MANEA* gene variants and elucidate the mechanisms by which these traits manifest.

530F

Knockdown of PBRM1, a putative risk gene for mood disorder and schizophrenia, induces widespread alterations in gene expression,cellular morphology, and proliferation. *X. Jiang, S. Detera-Wadleigh, F. McMahon.* Human Genetics Branch, NIMH, Bethesda, MD., US.

A meta-analysis of genome-wide association studies (GWAS) in mood disorders found the strongest evidence of association at a synonymous SNP in PBRM1 (McMahon et al 2010). The same SNP has been shown to be associated with schizophrenia (Williams et al. 2011). Although several genes map to the same ~350 kb linkage disequilibrium region on chromosome 3p21.1, in GWAS the causal gene will often be located near the most strongly associated SNP (Allen et al. 2010), so we have undertaken initial functional studies of PBRM1. We employed a loss-of-function paradigm by generating a stable knockdown of PBRM1in both HeLa and SHSY5Y neuroblastoma cells via lentiviral-mediated transduction of short hairpin (sh) RNA. Differential gene expression profiles examined using the Illumina Human HT 12v3 Expression Bead Array revealed 129 overexpressed and 70 underexpressed genes. Notably, PBRM1 knockdown induced a significant downregulation of PDE4B, which encodes cAMP-specific 3'5' phosphodiesterase 4B. This protein is known to interact with DISC1 (Millar et al. 2005), and altered phosphodiesterase signaling has been reported in both mood disorders and schizophrenia. We further found that the underexpression of PDE4B after PBRM1 knockdown was partially reversed by valproate, consistent with its therapeutic role in bipolar disorder. PBRM1 encodes polybromo1 (aka BAF180), which is the chromatin binding component of the Brahma-associated factor (BAF) nucleosome remodeling complex (Ho & Crabtree 2010). It is therefore interesting that 27 of the genes that were differentially expressed after PBRM1 knockdown were histone-related; 9 of these were upregulated at least 1.8-fold. HeLa and SHSY5Y cells with stable PBRM1 knockdown displayed strikingly slower proliferation and altered cellular morphology, suggesting a possible role for this gene in cell cycle control and apoptosis. If PBRM1 plays a causal role in mood disorders and schizophrenia, this role may be mediated by widespread changes in the expression of genes involved in signal transduction, chromatin remodeling, and cellular development. This study demonstrates the value of GWAS findings in directing studies to diverse biological processes potentially involved in disease etiology.

531F

Acute and long-term changes to transcriptome profiles in a mouse model of fetal alcohol spectrum disorder: evidence for the ethanol sensitivity of neurodevelopmental genetic pathways and permanent consequences of exposure. *M.L. Kleiber[1], K. Janus[1], B. Laufer[1], E. Wright[2], S.M. Singh[1,2].* 1) Molecular Genetics Unit, Dept. of Biology, University of Western Ontario, London, Canada; 2) Neuroscience, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Cananda.

Maternal alcohol consumption during pregnancy results in fetal alcohol spectrum disorders (FASD), the leading preventable cause of cognitive and behavioural abnormalities in North America. Despite our increasing knowledge of the magnitude and breadth of abnormalities associated with FASD, little is known about the underlying biological mechanisms. We have established a mouse model (C57BL/6J) of FASD that uses four paradigms of ethanol exposure; binge-like doses during the first, second, and third human trimester equivalent, and voluntary maternal consumption throughout pregnancy. These paradigms result in progeny that show variable yet consistent FASD-related phenotypes. Here, we report changes in the neuro-transcriptome resulting from ethanol exposure, both acutely (4 hours post-treatment) and during young adult (postnatal day 60) for each of the four experimental paradigms. Using Affymetrix Mouse Gene 1.0 ST arrays, differentially expressed genes meeting 1.2-fold up or down-regulation (minimum) and p-value=0.05 with false discovery rate (FDR) cutoffs were subjected to gene set enrichment analysis using Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations, using the web-based analysis tools PANTHER and DAVID. Consistently-affected pathways included those involved in cell survival, calcium signaling and adhesion, extra-cellular matrix remodeling, and neuroactive ligands and receptors. Acute (4h) effects were characterized by pronounced alterations in genes associated with cell survival (Jun, Bcl2-related genes, Dusp gene family) and growth factor signaling (Gdnf, Etv5). In contrast, long-term residual (day 60) analysis showed subtle changes in genes involved in a wide range of biological processes, notably cell adhesion (Ncam, cadherin gene family) and neuroactive signaling (Gabra6, Grin2d, Ht5ra). While this represents a general pattern for all four paradigms, specific differentially expressed gene sets were dependent on developmental timing of exposure. These data represent the most comprehensive examination of transcriptome disruption in current FASD literature. They may provide a foundation for the discovery of effective pharmacological targets towards the amelioration of phenotypes associated with prenatal alcohol exposure.

532F

Molecular Epidemiological Study of *FIG4* mutations in Familial and Sporadic Amyotrophic Lateral Sclerosis in the Japanese population. *F. Kusunoki, Y. Takahashi, T. Matsukawa, J. Goto, S. Tsuji.* Neurology, Univ Tokyo, Tokyo, Tokyo, Japan.

BACKGROUND: Amyotrophic lateral sclerosis (ALS) is a severe neurological disorder, characterized by selective neurodegeneration of lower and upper motor neurons in the spinal cord, brainstem, and cortex. Approximately 5-10 % of cases are familial ALS (FALS). A number of causative genes for FALS have been identified, including *SOD1*, *FUS*, *TARDBP*, *ALS2*, *SETX*, *VCP*, *DCTN*, *ANG*, *VAPB* and *OPTN*. These genes account for approximately 30% of FALS pedigrees. The remaining 90-95% of ALS cases are sporadic ALS (SALS). A small number of SALS cases harbor causative mutations for FALS genes. Recently, mutational analysis for *FIG4*, initially reported as a causative gene for autosomal recessive Charcot-Marie-Tooth disease type 4J (CMT4J), revealed novel nonsynonymous variants in 3 out of 109 (2.8%) FALS patients and 6 out of 364 (1.6%) SALS patients of European ancestry, all of which were not present in controls. This report concluded that heterozygosity for a deleterious allele of *FIG4* appears to be a risk factor for ALS, designated as ALS11. However, frequencies of *FIG4* mutations in ALS in other ethnic populations have been unknown. The purpose of this study was to establish molecular epidemiology of *FIG4* mutations in ALS Japanese population. **METHODS:** 41 FALS patients were enrolled in this study. Previous mutational analysis for FALS causative genes identified 19 pedigrees with *SOD1* mutations, 3 with *FUS* and 1 with *TARDBP*. The remaining 18 pedigrees were subjected to mutational analysis for *FIG4*. In addition, 192 SALS patients were also enrolled. SALS patients had an average age at onset of 58.9 years. All the 23 coding exons and splice junctions of *FIG4* were amplified with genomic PCR using specific primers, and further subjected to direct nucleotide sequence analysis. **RESULTS:** 11 variants were identified, including two known nonsynonymous SNPs, rs2295837 and rs9885672 substituting methionine for leucine and valine for alanine, respectively, and a known synonymous SNP (rs9398218) in coding exons. Seven known SNPs were identified in introns, and a known SNP was identified in 3'UTR. Neither novel variants nor known mutations for ALS were identified in this cohort. **CONCLUSION:** This study suggests that mutations in *FIG4* were not the frequent cause for ALS in the Japanese population. Further investigation in large-scale cohorts or in cohorts with different ethnic backgrounds should be necessary to establish genetic epidemiology of ALS with *FIG4* mutations.

533F

Genetic and Functional Analyses of SHANK2 Mutations Support a Multiple Hit Model of Autism Spectrum Disorders. *C.S. Leblond[1], J. Heinrich[2], R. Delorme[1,3], C. Betancur[4,5,6], G. Huguet[1], M. Rastam[7], J. Melke[8], B. Regnault[9], D. Skuse[10], M. Poot[11], A.P. Monaco[12], I. Jarvela[13], S. Curran[14], S.M. Klauck[15], C.M. Freitag[16], E. Bacchelli[17], A. Battaglia[18], L. Mazzone[19], A. Vicente[20], G. Oliveira[21], S.W. Scherer[22], M. Lathrop[23], F. Devillard[24], B. Assouline[25], M.C. Mouren[3], M. Leboyer[26,27], C. Gillberg[28,29], T.M. Boeckers[2], T. Bourgeron[1].* 1) Human Genetics and Cognitive Functions, Institut Pasteur, Paris, France; 2) Institute of Anatomy and Cell Biology, Ulm University, 89081 Ulm, Germany; 3) Assistance Publique-Hôpitaux de Paris, Robert Debré Hospital, Department of Child and Adolescent Psychiatry, Paris, France; 4) INSERM, U952, Paris, France; 5) CNRS, UMR 7224, Paris, France; 6) UPMC Univ Paris 06, Paris, France; 7) Department of Clinical Sciences in Lund, Lund University, Lund, Sweden; 8) Institute of Neuroscience and Physiology, Department of Pharmacology, Gothenburg University, Sweden; 9) Eukaryote Genotyping Platform. Genopole. Institut Pasteur, Paris, France; 10) Behavioural and Brain Sciences Unit, Institute of Child Health, University College London, UK; 11) Department of Medical Genetics, University Medical Center Utrecht, Utrecht, NL; 12) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; 13) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 14) Academic Department of Child & Adolescent Psychiatry, Institute of Psychiatry, King's College London, UK; 15) Division of Molecular Genome Analysis, German Cancer Research Center (DKFZ), Heidelberg, Germany; 16) Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Goethe University, Frankfurt am Main, Germany; 17) Department of Biology, University of Bologna, Italy; 18) Stella Maris Clinical Research Institute for Child and Adolescent Neuropsychiatry, Calambrone (Pisa), Italy; 19) Division of Child Neurology and Psychiatry, Department of Pediatrics, University of Catania, Catania, Italy; 20) Instituto Nacional de Saude Dr Ricardo Jorge, Lisbon, Portugal; 21) Unidade Neurodesenvolvimento e Autismo, Hospital Pediátrico Coimbra e Faculdade Medicina, Universidade Coimbra, Portugal; 22) The Centre for Applied Genomics and Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Canada; 23) Centre National de Génotypage, Evry, France; 24) Genetics Department, Hôpital Couple-Enfant, Grenoble, France; 25) CADIPA - Centre de Ressources Autisme Rhône-Alpes, Saint Egrève, France; 26) INSERM, U955, Psychiatry Genetic team, Creteil, France; 27) Fondation FondaMental, France; 28) Institute of Child Health, University College London, London, UK; 29) Gillberg Neuropsychiatry Centre. University of Gothenburg, Göteborg, Sweden.

Autism spectrum disorders (ASD) are a heterogeneous group of neurodevelopmental disorders with a complex inheritance pattern. While many rare variants in synaptic proteins have been identified in patients with ASD, little is known about their effects at the synapse and their interactions with other genetic variations. Here, following the discovery of two *de novo SHANK2* deletions by the Autism Genome Project, we screened *ProSAP1/SHANK2* for copy number variants (CNVs) and coding mutations in patients with ASD (n>230) and controls (n>230). We detected a novel 421 kb *de novo SHANK2* deletion in a patient with autism and 19 non-synonymous SHANK2 variants (ASD n= 12, controls n=5, shared by both groups n=2). Unexpectedly, in an additional screen of 1590 controls, we identified a subject carrying a SHANK2 splice site mutation. In neuronal cell cultures, a majority of the variants identified in this study, even those shared by patients and controls, reduced synapse density at dendrites. Interestingly, the three patients with *de novo SHANK2 deletions* also carried inherited CNVs at 15q11-q13 previously associated with neuropsychiatric disorders. In two cases, the nicotinic receptor *CHRNA7* was duplicated and in one case the synaptic translation repressor *CYFIP1* was deleted. The control subject with the SHANK2 splice site mutation carried CNVs in different genes coding for the NLGN3/4X binding partner SNTG2 and the myelin transcription factor MYT1L. These results strengthen the role of synaptic gene dysfunction in ASD, but also highlight the presence of putative modifier genes, which is in keeping with the “multiple hit model” for ASD. A better knowledge of these genetic interactions will be necessary to understand the complex inheritance pattern of ASD.

534F

Genetic variation in dopaminergic and cholinergic signaling moderates smoking-induced dopamine release. *T.M. Levin[1], E.L. Nurmi[1], K.S. Mallya[1], K. Ta[2], J. La Charite[2], J.T. McCracken[1], A.L. Brody[1,2].* 1) Department of Psychiatry, University of California at Los Angeles, Los Angeles, CA; 2) Dept of Psychiatry, Greater Los Angeles VA Healthcare System, Los Angeles, CA.

Objective: Genetic variation in smoking-induced dopamine release may be relevant to clinical aspects of smoking, including success with smoking cessation. We previously reported genetic variants in dopaminergic systems that predict interindividual variation in smoking-induced dopamine release measured by positron emission tomography (PET); nevertheless, variation in synaptic dopamine remains only partially explained. We hypothesized that more direct moderators of dopamine signaling in the striatum would include genetic polymorphisms in the downstream target of synaptic dopamine, the dopamine D2 receptor (DRD2), and the upstream site of nicotine action in the brain, nicotinic cholinergic receptors. **Methods:** In 102 tobacco-dependent smokers, we measured the association of smoking-induced change in 11C-raclopride binding in the ventral striatum with comprehensively tagged common genetic variation at DRD2 and six cholinergic receptor subunit genes (CHRNA3, CHRNA4, CHRNA5, CHRNA7, CHRNB2, and CHRNB4). **Results:** In addition to evidence for dopaminergic markers in smoking-induced dopamine release, our results support a role for genetic variation in the cholinergic system. Homozygotes for the common allele (GG) at an intron 2 variant in the (7 cholinergic receptor subunit (CHRNA7, rs12915695) had a greater than 3-fold reduction in radiotracer binding, indicating significantly greater smoking-induced dopamine release, compared to carriers of the minor A-allele (-10.8% vs. -3.0% respectively, p= 0.002). This result remains significant after correction for multiple comparisons. Similarly, CC homozygotes at a variant in the promoter of the (4 subunit (CHRNA4, rs755203) had almost twice the reduction in radiotracer binding (-10.7% vs. -5.7%) as carriers of the minor T-allele (p=0.047). This risk allele is in modest linkage disequilibrium with a published variant associated with nicotine dependence. We further correlated genetic variants and imaging data with behavioral measures of craving. **Conclusions:** Findings at nicotinic (4 and (7 cholinergic receptor subunits are consistent with animal and in vitro studies, implicating these subunits in dopamine-mediated reinforcement associated with smoking. Common human variants appear to exert significant effects on dopamine release. A detailed understanding of genetic moderators of nicotine reward and risk for dependence may facilitate the development and individualization of successful treatment strategies.

535F

Calcium Channel Genes are Associated with Autism Spectrum Disorders and Their Defining Features. *A. Lu[1], R.M. Cantor[1,2].* 1) Dept Human Genetics, David Geffen School of Medicine, Univ California, Los Angeles, Los Angeles, CA; 2) Department of Psychiatry, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles.

Autism Spectrum disorders (ASD), characterized by (1) deficits in communication skills, (2) deficits in social skills and (3) the presence of restricted and repetitive behaviors, are genetically complex with few established causal genes. Evidence supporting the role of calcium channel genes (CCG) in ASD has been accumulating. CCG are suggested by 1) Timothy Syndrome (TS), a Mendelian disorder that features ASD and is caused by a missense mutation in the 8th exon of the CACNA1C gene that encodes the alpha 1C subunit protein of L-type calcium channel, and 2) independent ASD associations that implicate CACNA1G and RyR2, both CCG. We conducted association analyses on a panel of 10 of these genes to better assess the potential role of CCG in ASD. The genes selected encode the 10 (1 subunits of the CCG. First, a combined reference panel from the 1000 Genomes Project and HapMap3 was used to impute 5,658 SNPs in the 10 CCG in 543 multiplex Caucasian families (1103 trios) from the Autism Genetics Resource Exchange (AGRE), using the IMPUTE2 software and 1,389 SNPs that were genotyped as part of a prior GWAS . Because the sex ratios in ASD and TS are skewed (4:1) and the published CCG associations included sex differences in the analyses, these 7,047 SNPs were assessed using joint tests of each CCG SNP and its interaction with sex. A Bonferroni correction was applied (p<7.0e-6), and 38 SNPs in 8 CCG were associated with ASD, with 10 of these also exceeding genomewide significance (p<5.0e-08). Similar association analyses were conducted to assess whether the 3 ASD defining features , language deficits (L), social deficits (S) and repetitive behaviors (R), quantified by heritable scores derived from items on the Autism Diagnostic Instrument Revised, were also associated with CCG. Most of the ASD associated SNPs were associated (10 for L, 27 for R and 28 for S), and a ninth CCG was associated with 1 additional SNP for R and 2 additional SNPs for S. These associations provide support for the role of CCG in both ASD and the features that define it. They also provide a concrete example that SNPs in a gene for a Mendelian disorder that includes ASD, such as CACNA1C for TS, may also predispose to the genetically complex trait of ASD. A significant role for Mendelian disorder genes in complex traits is very likely.

536F

Comprehensive resequencing of *PEX5*, *PEX13* and *PEX14* gene in patients with X-linked adrenoleukodystrophy (ALD) and association studies with the phenotypes of ALD. *T. Matsukawa[1], Y. Takahashi[1], J. Goto[1], Y. Suzuki[2], N. Shimozawa[2], H. Takano[3], O. Onodera[3], M. Nishizawa[3], S. Tsuji[1].* 1) Dept Neurology, Univ Tokyo, Tokyo, Japan; 2) Gifu University, Gifu, Japan; 3) Niigata University, Niigata, Japan.

Background & Purpose: Adrenoleukodystrophy (ALD) is an X-linked disorder affecting primarily the white matter of the central nervous system occasionally accompanied with adrenal insufficiency. The ages at onset and clinical presentations of ALD are substantially broad. There are various kinds of phenotypes, like Childhood cerebral ALD (CCALD), Adult cerebral ALD (AdultCer), Adrenomyeloneuropathy (AMN). Despite the discovery of the causative gene, *ABCD1*, clear genotype-phenotype correlations have not been established. *ABCD1* gene encodes adrenoleukodystrophy protein (ALDP), which is localized to the peroxisomal membrane. *ABCD1* mutant mice show a milder AMN phenotype. On the other hand, mice lacking *PEX5* gene expression, which is related to peroxisomal protein import, in their oligodendrocytes develop cerebral demyelination involving the corpus callosum as occurs in CCALD. When the *PEX5* product (Pex5p) is transported to the peroxisome, it interacts with the *PEX14* product (Pex14p) and the *PEX13* product (Pex13p). Association studies based on SNPs identified by comprehensive resequencing of gene related to *ABCD1* may reveal genes modifying the phenotypes of ALD. The purpose of this study is to investigate genotype-phenotype correlations and to explore the possibility of *PEX5*, *PEX13*, and *PEX14* as the disease modifying gene. **Methods:** We analyzed 55 Japanese patients with ALD (CCALD 16, Adlescent Cerebral ALD 3, AdultCer ALD 6, AMN with cerebral (AMN-Cer) ALD 5, Cerebello-brainstem ALD 2, AMN 20, Addison disease 1, and asymptomatic 2). Sequences of all of the exons, introns around the exon-intron boundary, 5'UTR and 3'UTR of *PEX5*, *PEX13*, and *PEX14* were analyzed by direct nucleotide sequence analysis. **Result:** 2 novel non-synonymous SNPs in exon, 1 novel SNP in intron, 2 novel SNPs in 3'UTR and 2 known SNPs in 3'UTR were detected in *PEX5*. 2 known SNPs in 3'UTR were detected in *PEX13*. 1 novel and 1 known non-synonymous SNP and 1 novel and 3 known synonymous SNP in exon, 2 novel SNP in 3'UTR, and 1 known SNPs in intron were detected in *PEX14*. SNPs identified by comprehensive resequencing of *PEX5*, *PEX13*, and *PEX14* were used for association studies. There were no significant associations between these SNPs of *PEX5*, *PEX13* and *PEX14*, and ALD phenotypes in the Japanese population. **Conclusion:** The present study indicates that *PEX5*, *PEX13*, and *PEX14* are less likely the disease modifying genes, necessitating further studies to identify genes modifying the phenotypes of ALD.

537F

The application of whole-exome resequencing to identify the genetic basis of a novel autosomal recessive syndrome characterized by epilepsy, microcephaly and cognitive impairment. *L.M. McDonell[1,3,4], D. Foster[2], F.C.C. FORGE Canada Consortium[3], D.E. Bulman[1,4], K.M. Boycott[3,4].* 1) Regenerative Medicine Program, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada; 2) Algoma Public Health Unit, Sault Ste Marie, Ontario; 3) Children's Hospital of Eastern Ontario Research Institute, Ottawa, Ontario; 4) Department of Biochemistry Microbiology and Immunology, University of Ottawa, Ottawa, Ontario.

Homozygosity mapping in consanguineous families coupled with whole-exome resequencing has been successful in identifying the causal variant for more than nine Mendelian disorders including Ochoa syndrome and van Den Ende-Gupta syndrome. Exome resequencing facilitates the rapid and efficient discovery of novel mutations by revealing variants in regions which are identical-by-descent (IBD) amongst affected individuals. This approach enables the study of rare disorders in small families where the region(s) of interest is potentially large and/or gene-rich. Here we report a consanguineous Franco-Ontarian family with four affected siblings; two males and two females. The parents are first cousins. The disorder in this family is characterized by early-onset severe epilepsy that is difficult to control requiring the use of multiple anticonvulsants, microcephaly with occipital-parietal circumference of -3SD and severe cognitive impairment. The siblings are now adults and all live in a supported care setting. Language is limited to short phrases in the two female siblings and is essentially absent in the male siblings. Dysmorphic features, minor anomalies or other neurological features are absent. Fragile X testing, MECP2 and metabolic investigations were unrevealing. The four affected family members were genotyped using SNP 6.0 arrays (Affymetrix). Homozygosity mapping using Homozygosity Mapper lead to the identification of two regions IBD on chromosomes 13 and 19 involving 2 and 265 genes, respectively. Neither of these regions contains a known phenotype associated with microcephaly or epilepsy, suggesting that this is a novel autosomal recessive disorder. Exome resequencing of one affected family member using the Agilent Sureselect 50Mb target enrichment method coupled with the Illumina Hi-Seq platform is underway and analysis of the data will be presented.

538F

Spastic paraplegia due to CYP7B1 mutations (SPG5) : what can we learn about 27-hydroxycholesterol metabolism? *F. Mochel[1,2,3], F. Lamari[3,4], D. Rinaldi[3], C. Goizet[5,6], D. Rainteau[7], V. Ratziu[8], A. Durr[1,2].* 1) INSERM UMR S975, Institut du Cerveau et de la Moelle, Hôpital de La Salpêtrière, Paris, France; 2) AP-HP, Département de Génétique, Hôpital de La Salpêtrière, Paris, France; 3) AP-HP, Unité Fonctionnelle de Biochimie Métabolique, Hôpital de La Salpêtrière, Paris, France; 4) Unité Fonctionnelle Neurométabolique, Hôpital de La Salpêtrière, Paris, France; 5) Département de Génétique Médicale, CHU de Bordeaux, Hôpital Pellegrin, Bordeaux, France; 6) Université Victor Segalen Bordeaux 2, CHU de Bordeaux, Hôpital Pellegrin, Bordeaux, France; 7) AP-HP, Laboratoire de Biochimie Métabolique, Hôpital Saint-Antoine, Paris, France; 8) AP-HP, Service d'Hépato-Gastroentérologie, Hôpital de La Salpêtrière, Paris, France.

Background: CYP7B1 encodes the cytochrome P450 7alpha-hydroxylase and plays a role in the alternate/acidic pathway for primary bile acid production. Mutations in CYP7B1 were identified in children with severe liver disease and, recently, in adult SPG5 patients presenting with hereditary spastic paraplegias (HSP) (Tsaousidou, 2008). Increased levels of 27-hydroxycholesterol (27-OHC) were reported in the plasma of 4 SPG5 patients (Schule, 2010). Besides its role in hepatobiliary metabolism, in vitro studies suggest that 27-OHC may decrease bone mineral density, inhibit the cardiovascular protective effects of estrogens and increase oxidative stress in retinal pigment epithelial cells. Methods: We investigated 9 SPG5 patients from 6 families in order (i) to determine whether plasma 27-OHC can be used as a biomarker to screen HSP patients, and (ii) to explore the non-neurological manifestations that may result from the altered oxysterols metabolism in SPG5 patients - i.e. hepatobiliary and cardiovascular functions, bone homeostasis and retina. Results: The marked elevation of plasma 27-OHC was associated with altered liver functions, reduced bone density and optic atrophy in SPG5 patients. Conclusion: Following these investigations, we are now designing a clinical trial to determine the best candidate drugs - and doses - to lower 27-OHC in SPG5 patients.

539F

Challenges in Interpreting Copy Number Alterations in Regions Associated with Autism: Deletions and Duplications within the AUTS2 Gene. *R.E. Pyatt[1,2], A. Bailes[1], A. McKinney[1], A. Brock[1], C. Deeg[1], L. Erdman[1], C. Weber[1], J. Weslow-Schmidt[1], L.M. Lehwald[3], L. Konczal[4], P. Nash[5], E. de Los Reyes[6], M. Miller[7], R.B. Pfau[7], J. Atkin[8,9], G. Herman[8,9], D.L. Thrush[1,9], S. Hashimoto[1], S. Reshmi[1,2], C. Astbury[1,2], J.M. Gastier-Foster[1,2,9].* 1) Pathology and Laboratory Medicine, Nationwide Childrens Hosp, Columbus, OH; 2) Department of Pathology, The Ohio State University, Columbus, OH; 3) Pediatric Neurology, Nationwide Children's Hospital, Columbus, OH; 4) University Hospitals, Case Western Reserve University; 5) Department of Behavioral Pediatrics, Nationwide Children's Hospital, Columbus, OH; 6) Department of Neurology, Nationwide Children's Hospital, Columbus, OH; 7) Dayton Children's Medical Center, Dayton, OH; 8) Molecular and Human Genetics, Nationwide Children's Hospital, Columbus, OH; 9) Department of Pediatrics, The Ohio State University, Columbus, OH.

Disruptions in the *AUTS2* gene have been associated with severe mental retardation and autism through a series of individuals with balanced translocations and breakpoints within the gene. The *AUTS2* gene located at 7q11.22 encodes for multiple transcripts including a full length isoform containing 19 exons, a slightly smaller isoform containing 18 exons, and a truncated isoform containing the first 4 exons and an alternatively spliced fifth exon. Benign copy number alterations are rare within the *AUTS2* gene region. We present here a series of alterations in the *AUTS2* gene identified through clinical array-comparative genomic hybridization (array-CGH) using custom designed BAC and oligonucleotide platforms. This series includes 6 deletions and 1 duplication with a likely benign loss at 9p24.3 as the only co-identified copy number alteration in one case. Deletions within the gene ranged in size from 31 to 220 kb and included single exon deletions involving the long transcript isoforms (exon 6), single exon deletions involving only the short isoform (alternate exon 5), and multiple exonic deletions (exons 3, 4, and alternate exon 5 from all isoforms, exons 6 through 19 of the long isoforms). These alterations were identified in individuals presenting with developmental delay, autism, microcephaly, and ataxia, and also as an incidental finding during parental analysis to determine inheritance of a separate array-CGH finding in a reportedly phenotypically normal mother. Parental samples were only available for one deletion proband which was determined to be de novo. Deletions restricted to intronic, non-coding regions were also found (regions within introns 1 and 6) in individuals presenting with attention deficit hyperactivity disorder, oppositional defiant disorder, ataxia, migraine episodes, and tonic paroxysmal upgaze. The 538 kb duplication identified in this series contained exons 7 through 19 of the *AUTS2* gene and a portion of the *WBSCR17* gene. To our knowledge, this is the first series of alterations identified in the *AUTS2* gene suggesting a pathogenic role or ascertainment bias due to the cohort screened. Interpretation of alterations involving regions predisposing to neurocognitive phenotypes can be challenging due to variable expressivity and incomplete penetrance, and is often not aided by parental analysis. These cases additionally underscore the need to extend the understanding of clinical array-CGH findings to the transcript level.

540F

Temporal gene expression of *bdnf* and *ntrk2b* in adult zebrafish after Pentilenotetrazole-evoked seizure. *F.C. Reis-Pinto, R.F.P. Mangolin, P.G. Barbalho, C.V. Maurer-Morelli.* Medical Genetics, Faculty of Medical Sciences-UNICAMP, Campinas, Brazil.

Animal models have been contributing to a better understanding of human diseases. In this context, *Danio rerio*, popular named as zebrafish, has been recognized for epilepsy studies. Epilepsy is a severe neurological disorder affecting a large number of individuals in the world. Besides to trophic properties, brain-derived neurotrophic factor (BDNF) appears to be associated with epileptogenesis by modifying synaptic transmission. However, there are still controversies about the main role of BDNF in epilepsy. The aim of this study was to determine temporal gene expression of *bdnf* and its receptor, *ntrk2b* after Pentilenotetrazole (PTZ) evoked seizure in zebrafish. Adults zebrafish were separated in control (n=3) and epileptic (n=3) groups (a pool of 2 brains was used to compose one sample). Animals from epileptic group were individually exposed to PTZ 15mM for 2-3 minutes and observed the three phases of epileptic behavior as previous described. Control animals were submitted to the same condition and time as epileptic group but in PTZ free water. After that, animals from both groups were placed into aquariums with PTZ free water in accordance to their groups. Twenty-four and 48h after seizure animals were anesthetized and their brains removed for RNA extraction. Quantitative Real Time PCR with TaqMan™ assays was performed to evaluate the gene expression of *bdnf* and *ntrk2b*. Runs were carried out in triplicate using 18S as endogenous control. This study was approved by animal ethical committee. Comparisons between control and epileptic groups for *bdnf* and *ntrk2b* genes showed evident up-regulation after 48h, ΔRQ= 2.18 and ΔRQ= 2.49, respectively. However, only a slightly difference was found in 24h (ΔRQ= 0.28 and ΔRQ= 0.36). Gene expression profile in the epileptic group, from 24h to 48h after seizure, found both genes highly increased (*bdnf*: ΔRQ= 2.55 and *ntrk2b*: ΔRQ= 2.74). It has been reported that both BDNF transcript and protein levels are increased after seizures in epileptic patients and in experimental models. However, in rats the transcript levels were reported to return to control levels 12h after seizure. Our results have yielded insights into a different pattern of gene expression of *bdnf* and *ntrk2b* genes after seizure insult and can contribute to the knowledge of the role of BDNF in human epilepsies. Additional studies are underway in order to point out the role of neurotrophins in epileptogenesis using zebrafish as an animal model.Support:CNPq.

541F

Evaluation of the predisposition to autism using a combination of risk-associated common variants. *T. Rio Frio, F. Rousseau, K. Fontaine, C. Vazart, C. Amiet, F. Liebaert, J. Carayol.* IntegraGen, Evry, France.

BACKGROUND: Autism spectrum disorders (ASDs) are highly heritable complex neurodevelopmental disorders with a 4:1 male: female ratio. Early identification of children at-risk of an ASD is a key challenge given that multiple studies have shown that early intervention leads to significant improved outcome in children with an ASD. Currently, there is no clinically validated tool to identify at-risk siblings before 18 months of age. In some complex diseases, the utility of genetic scores which combine associated common variants has been demonstrated to improve risk prediction. AIM: To propose a method to identify common variants associated with autism and to explore the predictive ability of derived gender-specific genetic scores to evaluate the risk of autism. MEHODS: Gender-based genome-wide association studies (GWAS) were performed on 544 families from the Autism Genetic Resource Exchange including 967 children with autistic disorder and 304 unaffected siblings. To improve the power of detection of common variants associated with an increased risk of autism we prioritized SNPs based on GWAS results, relevant biological data, and internal validation. We then assessed the ability of gender-specific genetic scores (GS), the sum of individual risk-associated alleles of highly ranked and reproducible SNPs, to discriminate between individuals with or without autism. RESULTS: A total of 88 SNPs associated with an increased risk of autism were selected. Of these, 52 SNPs were associated with autism in both males and females, 17 SNPs were associated with autism in males only and 19 SNPs in females only. Gender-specific GSs were constructed for fully genotyped 1,034 children with ASDs and 300 unaffected siblings. For males, the area under the receiver operating characteristic curve (AUC) was 0.83 (95%CI: 0.78-0.87); a GS of 68 was associated to an 86% specificity (95%CI: 79-92) and a 56% sensitivity (95%CI: 52-59). In females, the AUC was 0.84 (95%CI: 0.80-0.88); a GS of 66 was associated to an 86% specificity (95%CI: 80-92) and a 60% sensitivity (95%CI: 52-66). Furthermore, in both male and female samples, lowest and highest genetic scores were observed only in unaffected individuals and in children with ASDs, respectively. CONCLUSIONS: Our findings demonstrate that a gender-specific genetic score based on the presence of multiple risk-associated common variants allows for the identification of children who have a significantly higher risk to develop autism.

542F

Rare de novo and transmitted mutations in autistic spectrum disorders. *M. Ronemus[1], D. Levy[1], I. Iossifov[1], Y.-H. Lee[1], S. Gilman[2], R. Demeter[3], V. Magrini[3], A. Leotta[1], S. Marks[1], J. Kendall[1], K. Ye[4], A. Buja[5], A. Krieger[5], B. Lakshmi[1,6], S. Yoon[1,7], J. Troge[1], L. Rodgers[1], P. Andrews[1], I. Hakker[1], J. Rosenbaum[1], B. Yamrom[1], E. Mardis[3], R. Wilson[3], M. Schatz[1], D. Vitkup[2], R. McCombie[1], M. Wigler[1].* 1) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 2) Center for Computational Biology and Bioinformatics and Department of Biomedical Informatics, Columbia University, New York, NY; 3) The Genome Institute at Washington University, St. Louis, MO; 4) Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY; 5) Department of Statistics, the Wharton School, University of Pennsylvania, Philadelphia, PA; 6) Ontario Institute for Cancer Research, Toronto, Ontario, Canada; 7) Department of Psychiatry, Mount Sinai School of Medicine, New York, NY.

To explore the genetic contribution to autistic spectrum disorders (ASDs), we have studied rare de novo and inherited mutation in the Simons Simplex Collection (SSC). The SSC represents a large cohort of families with a single affected child and at least one unaffected sibling. The first stage of the analysis was performed by comparative genomic hybridization (CGH) of 1000 SSC families. Based on these data, we confirm a major contribution from de novo deletions and duplications, and also find evidence of a role for inherited `ultra-rare' duplications. De novo CNVs were found in 7.9% of probands and 2.0% of unaffected siblings. Relative to males, females appear to have greater resistance to autism from genetic causes, which raises the question of the fate of female carriers. By analysis of the proportion and number of recurrent loci, we set a lower bound for distinct target loci at several hundred. Because CGH-detectable de novo CNVs account for a relatively small portion of the pool of genetic variation underlying ASDs, we have initiated a finer-scale analysis by whole-exome sequencing of the SSC. From the combined CGH and sequencing analyses, we find many new candidate regions, adding substantially to the list of potential gene targets, and confirm a number of loci previously observed. The overall results point to a great diversity of genetic causes, but also suggest functional convergence upon synaptogenesis as a primary cause of ASDs.

543F

Genetic predictors of knee pain in persons with mild to moderate osteoarthritis. *D. Schutte[1], N. Mukhopadhyay[2], C. Vance[4], R. Walder[4], K. Sluka[4], B. Rakel[3], M. Govil[2].* 1) College of Nursing, Michigan State University, East Lansing, MI; 2) Center for Craniofacial and Dental Genetics, Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA; 3) College of Nursing, The University of Iowa, Iowa City, IA; 4) Physical Therapy and Rehabilitation Graduate Program, College of Medicine, The University of Iowa, Iowa City, IA.

Osteoarthritis (OA) affects 10 million adults in the US and is associated with significant negative sequela, such as functional losses, disability, and risks associated with polypharmacy. Little is known about factors, including genetic variation, that influence the pain experience in this population. The purpose of this study was to examine the extent to which genetic variability influences knee pain in persons with mild to moderate OA. Seventy-five subjects diagnosed with medial compartment knee OA were recruited from the Orthopedic and Sports Medicine Department of a large Midwestern tertiary care center. Subjects exhibited a mean age of 56.3 years; females comprised 61% of the sample. Measures of pain included subjective pain intensity (VAS) at rest and with movement, cutaneous mechanical pain testing using von Frey filaments, heat pain threshold, and pressure pain threshold using a digital pressure algometer. Pain measures were obtained at the knee and anterior tibialis regions on both the ipsilateral and contralateral side. Each measurement type consists of pain response along multiple dimensions, e.g subjective pain at rest is characterized by intensity and distress values. The mean self-reported pain intensity at rest and mean distress were 22.82 and 23.03 respectively on a 0-100 scale with higher scores meaning more severe pain. Subjective measures were positively correlated; correlation between intensity and distress at rest was 0.85, whereas measures provided by instruments showed varying degrees of correlation ranging from -0.41 to 0.84. Seventy-four of the seventy-five subjects were successfully genotyped for DNA variants in 11 candidate genes with a known or hypothesized role in central or peripheral pain pathways: *NGFB*, *NTRK1*, *EDNRA*, *EDNRB*, *EDN1*, *OPRM1*, *TAC1*, *BDNF*, *BDKRB1*, *5HTT*, and *COMT*. Linear regression modeling was conducted, using PLINK, to examine genotype effect on each pain outcome on the ipsilateral side. Marker rs5333 in the *EDNRA* gene had an association p-value of 0.0019 for heat pain threshold, below the bonferroni-corrected value of 0.002. The results from this study will help guide the development and evaluation of tailored strategies to decrease pain, improve function, and prevent the development of new chronic pain syndromes in adults experiencing OA. NIH grant # (K99, R00) DE018085, ARRA suppl DE018085-01A2S1, R03 NR010405-01.

544F

An investigation of neurogenetic correlates of early-age physiological stress response: A prospective study. *H. Sheikh, E. Hayden, K. Kryski, H. Smith, S. Singh.* Department of Biology, Molecular Genetics Unit, University of Western Ontario, London, ON, Canada.

Psychiatric disorders such as anxiety and depression place an enormous burden on individuals and society. The etiology of these disorders is unclear but risk of developing these disorders is polygenic in nature and show a strong heritable component. Specific genes that may be involved include genes that regulate neuronal neurotransmission and their plasticity but also genes that may shape physiological correlates of psychopathology development, such as the human physiological stress response termed the hypothalamic-pituitary-adrenal (HPA) axis. Activation of the HPA axis culminates in the release of cortisol, a glucocorticoid hormone with long-lasting biological implications. Excessive and sustained cortisol reactivity (glucocorticoid exposure) could be maladaptive and reported in many individuals with depression and anxiety and posited as an important marker of early vulnerability to mood disorders. Etiological data from twin-studies show that cortisol reactivity is also highly heritable but only a handful of studies have looked at genetic variants associated with excessive childhood HPA axis reactivity. We investigated whether functional polymorphisms of genes involved in neuronal plasticity such as BDNF, GLP-1R, and neurotransmission such as SLC6A3, SLC6A4, DRD2, DRD4, COMT and MAOA are associated with child cortisol reactivity in a community sample of 410 preschoolers. Preliminary data shows that functional polymorphism of the BDNF, GLP-1R genes, are not associated with child cortisol reactivity ($p>0.05$), a finding previously unreported in this population. Additionally, we investigated genes of the HPA axis pathway, the NR3C1, CRH, CRHR1and MC2R genes, using a haplotype based association analyses. Haplotypes were generated using tag-SNPs spanning ± 10kb upstream and downstream of the key HPA axis pathway gene coding regions. Chromosomal phase was determined using expectation-maximization algorithm and the tag-SNP tests covered 100% of the genetic variance reported by HapMap for the CEPH (Caucasian) population. To our knowledge, this study is the first of its kind to investigate the early age genetic underpinnings of the human HPA axis.

545F

Exome sequencing approach to identify the responsible variant for a novel type of hereditary motor and sensory neuropathy with proximal dominancy in the lower extremities found in a Japanese descent. *H. Shibata[1], S. Miura[2], H. Kida[2], K. Noda[2], Y. Kaku[2], A. Iwaki[1], M. Ayabe[2], T. Taniwaki[2], Y. Fukumaki[1].* 1) Div Human Molecular Genetics, Dept Molecular Genetics, Medical Inst Bioregulation, Kyushu Univ, Fukuoka, Japan; 2) Div Respirology, Neurology, and Rheumatology, Dept Medicine, Kurume Univ Sc Med, Kurume, Fukuoka, Japan.

We previously reported a clinically new type of autosomal dominant disorders of motor and sensory neuropathy with proximal dominancy in the lower extremities, urinary disturbance, and paroxysmal dry cough (Miura et al. J Neurol Sci 2008). On the multipoint linkage analysis using 15 family member including six patients, we obtained the logarithm of odds (LOD) scores of > 0.7 on 1p31.1-q23.3 (LOD = 1.704) and 4pter-p15.2 (LOD = 1.421). The linkage regions included the *MPZ* gene at 1q22 of which mutations have been reported in a autosomal dominant hereditary motor and sensory neuropathy. However, we observed no mutations in the entire coding sequences of *MPZ* as well as no aberrant copy number in the *MPZ* exons. Since further genetic dissection by linkage analysis is not possible to pursue due to the limitation of the size of the family, we performed exome sequencing in seven individuals including six patients and one healthy relative. We identified 16 novel heterozygous NS mutations specifically shared by patients in 12 genes on 1p31.1-q23.3 and in 4 genes on 4pter-p15.2. None of the 16 genes have been reported to be responsible for any type of neuropathy. Although the validation by Sanger sequencing is under way, the current results suggest this disorder is not only clinically but also genetically a novel type of hereditary motor and sensory neuropathy.

546F

Absolute quantification of Survival Motor Neuron (SMN) transcripts in whole blood samples from controls, carriers, Spinal Muscular Atrophy (SMA) patients and post-treatment Carnival Type 1 patients. *L.R. Simard[1], F.D. Tiziano[2], E. Slominski[1], J.T. Kissel[3], C.B. Scott[4], S.P. Reyna[5], B. Elsheik[3], T.O. Crawford[6], K.J. Krosschell[7], G. Ascadi[8], M.K. Schroth[9], G. D'Anjou[10], B. LaSalle[11], T.W. Prior[12], S. Sorenson[13], J. Maczulski[14], M.B. Bromberg[5], G.M. Chan[15], K.J. Swoboda[5], Project Cure SMA Investigators Network.* 1) Dept. of Biochemistry & Medical Genetics, University of Manitoba, Winnipeg, MB, Canada; 2) Istituto di Genetica Medica, Università Cattolica, Rome, Italy; 3) Dept. of Neurology, The Ohio State University Medical Center; 4) CBS Squared, Inc., Fort Washington, Pennsylvania; 5) Dept. of Neurology, University of Utah School of Medicine, Salt Lake City; 6) Depts. of Neurology and Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland; 7) Dept. of Physical Therapy and Human Movement Sciences, Feinberg School of Medicine, Northwestern University, Chicago, Illinois; 8) Dept. of Neurology and Pediatrics, Wayne State University School of Medicine, Detroit, Michigan; 9) Dept. of Pediatrics, University of Wisconsin School of Medicine, Madison; 10) Division of Pediatric Neurology, Hôpital Sainte-Justine, Montréal, Québec, Canada; 11) General Clinical Research Center, University of Utah School of Medicine, Salt Lake City; 12) Dept. of Molecular Pathology, The Ohio State University Medical Center; 13) Primary Children's Medical Center, Salt Lake City, Utah; 14) Pediatric Occupational Therapy Services, Chicago, Illinois; 15) Dept. of Pediatric Neonatology, University of Utah, Salt Lake City.

SMA, a lower motor neuron disease, is caused by mutations in the *SMN1* gene. The highly homologous *SMN2* copy gene appears to produce an excess of an alternatively spliced transcript (SMN-del7) that is translated into a very unstable protein. In therapeutic strategies targeting the *SMN2* gene, measures of SMN mRNA can be used to test the effectiveness of a drug. To determine the relationship between disease status or valproic acid (VPA) treatment and SMN expression, absolute values of full-length (fl) and del7 SMN transcripts in whole blood samples was determined for 22 controls, 26 carriers, 19 type 1, 10 type 2, 18 type 3 and 13 Project Cure Carnival type 1 SMA patients. SMN1-fl transcripts were not detected in SMA patients and SMN2-fl/del7 transcripts were lacking in individuals with homozygous absence of *SMN2* exon 7. As expected, controls produced over twice the amount of SMN1-fl transcripts compared to carriers (284 vs. 107 molecules/ng RNA) whereas the levels of SMN2-fl transcripts were similar. *SMN1* and *SMN2* genes produced the same amount of total SMN transcripts in unaffected individuals. Surprisingly, type 1 patients had the highest SMN2-fl levels. Type 1 and type 3 patients had the highest level of SMN2-del7 transcripts. SMN2fl:SMNdel7 mRNA ratios ranged from 0.8 to 1.3 in SMA patients compared to 2.2 in controls suggesting that gene expression varied between *SMN2* genes and may have been affected by gene conversion events. There was no correlation between SMN expression and *SMN2* copy number. Project Cure Carnival type 1 patients fell into 3 groups regarding VPA response: 5 Type 1 patients displayed increased levels of SMN2-fl transcripts post-treatment (~38%) and 4 of these also had increased SMN2-del7 transcripts. SMN2-fl and del7 transcripts were stable or lower in group 2. Large fluctuations were detected in Group 3 patients. **Conclusions:** The highest *SMN2* expression was observed in the youngest patients consistent with down-regulation of *SMN* genes during development. Differences in SMN2fl:SMNdel7 transcripts in unaffected and affected individuals suggested that *SMN2* gene expression may be affected by gene conversion events. While VPA was likely acting to enhance *SMN2* gene expression in a subset of SMA type 1 patients, it will be important to correlate any changes in SMN expression levels with a clinically meaningful outcome measures before assessing whether SMN mRNA is a valid biomarker.

547F

Genetic influence on anti-JC-virus antibody seropositivity in MS patients and controls. *E. Sundqvist[1], C. Warnke[1], M. Khademi[1], L. Alfredsson[2], J. Hillert[1], S. Goelz[3], I. Kockum[1], T. Olsson[1].* 1) Dept. Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden; 2) Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; 3) Biogen Idec Inc., Weston, MA, USA.

Lytic infection of oligodentrocytes by JC-virus (JCV) is considered as the cause of progressive multifocal leukoencephalopathy (PML) a condition occurring as a severe and potentially fatal complication in patients with multiple sclerosis (MS) treated with natalizumab. As there is no treatment directed towards the cause of PML, it is important to devise methods by which patients at risk can be identified, decreasing PML incidence and increasing patient safety. Ascertainment of the presence or absence of anti-JCV antibodies using the recently refined assay has been suggested as one such method in which 40-50 % of adults remain anti-JCV negative, indicating a potential genetic background to JCV susceptibility. The aim was to analyse the influence of HLA-A and DRB1 genotype on anti-JCV antibody status in incident MS cases, the majority of which were not treated with natalizumab, and matched (age, sex, and area of residence) population based controls, recruited throughout Sweden. Anti-JCV antibodies were measured in plasma, and status was determined to be either seropositive or -negative by applying a method published by Gorelik et al, 2010. HLA-A and DRB1 genotyping was performed using low resolution SSP-kits. The influence of HLA-alleles was analysed using logistic regression on 999 controls and 961 MS cases, separately and combined. Alleles with an allele frequency of over 10% in JCV-negative individuals were grouped into HLA-A*X and DRB1*X, respectively. A stepwise selection was applied, adjusting for age at sampling, sex and area of residence, and for cases, also for natalizumab treatment. For controls, DRB1*13 and 15 were associated to JCV seropositivity, OR 1.7 (1.2-2.3 95%CI) p=0.003, and 0.7 (0.6-1.0 95%CI) p=0.03, respectively. For MS cases DRB1*13 (OR 1.5, 1.1-2.2 95%CI, p=0.02), DRB1*15 (0.7, 0.6-0.9 95%CI, p=0.004) and DRB1*X (1.3, 1.0-1.6 95%CI, p=0.03) were associated to JCV-positivity. Both DRB1*15 and 13 were associated in the combined analysis (p=4x10^-6 and 0.002, respectively) with similar ORs as in the subgroups analyses. We have shown that there is a genetic influence on JCV-seropositivity and that the major MS risk allele, DRB1*15, has protective effect. Further study is needed to fully elucidate the genetic regulation of JCV seropositivity, and host genetic factors relevant to the risk for developing PML. Thus, as a first step, we are currently assessing the influence of several non-HLA genes on JCV seropositivity in the same cohort.

548F

Resequencing-based case control study of *SQSTM1* for sporadic amyotrophic lateral sclerosis based on common disease-multiple rare variant hypothesis. *Y. Takahashi, J. Goto, S. Tsuji.* Dept Neurology, Univ Tokyo, Tokyo, Japan.

[Background]Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by progressive loss of upper and lower motor neurons. Approximately 5~10% of patients were with familial ALS (FALS), about 30% of which are accounted for by known causative genes, and the remaining 90~95% of patients were with sporadic ALS (SALS). Molecular backgrounds of SALS have been largely unknown, except for small number of patients harboring causative mutations of FALS genes. Resequencing studies based on common disease-multiple rare variants hypothesis have been increasingly conducted to identify genetic factors for *SALS*. Very recently, rare nonsynonymous variants in *SQSTM1* have been reported to be associated with FALS and SALS (Fecto, F. et al. Neurology 76, 2011 (Suppl 4)). *SQSTM1* is one of promising candidates because it is suggested to be involved in the regulation of TDP-43 aggregation, one of the pathological hallmarks of SALS. [Objectives] To test the hypothesis that rare nonsynonymous variants in *SQSTM1* are associated with SALS in the Japanese population. [Subjects and Methods] Two hundred and fourteen SALS patients and 276 controls were enrolled in this study. Specific primers for genomic PCR were designed for whole exons of *SQSTM1* using ExonPrimer website. Mutational analysis for whole exons was conducted in SALS patients as well as 96 controls employing direct nucleotide sequencing method. Novel nonsynonymous variants identified in SALS patients were further analyzed in the additional 180 controls. The effects of amino acid substitutions were predicted using PolyPhen-2 website. [Results]Two novel nonsynonymous variants, R50Q and P273R, were identified in two patients (1%) with SALS. R50Q was predicted as probably damaging with a score of 0,957 and P273R as benign with a score of 0.383. These variants were not identified in the 276 controls. Resequencing of 96 controls revealed a novel nonsynonymous variant V144I in one subject, predicted as benign with a score of 0.038. [Discussion and Conclusion] The interpretation of the novel nonsynonymous variants in SALS was elusive, because such a variant was also observed in controls. This study emphasizes the importance of the resequencing of controls as well as patients to investigate the significance of rare variants. Further mutational analysis in different cohorts as well as functional studies should be essential to establish *SQSTM1* as a gene associated with SALS.

549F

PLA2G6 in patients with Parkinson's disease / frontotemporal type of dementia. *H. Tomiyama[1,2], H. Yoshino[3], K. Ogaki[1], L. Li[1], C. Yamashita[1], Y. Li[3], M. Funayama[1,3], R. Sasaki[4], Y. Kokubo[4], S. Kuzuhara[5], N. Hattori[1,2,3].* 1) Dept Neurology, Juntendo Univ Sch Medicine, Tokyo, Japan; 2) Dept Neuroscience for Neurodegenerative Disorders, Juntendo Univ Sch Medicine, Tokyo, Japan; 3) Research Inst for Diseases of Old Age, Graduate Sch Medicine, Juntendo Univ, Tokyo, Japan; 4) Dept Neurology, Mie Univ Sch Medicine, Tsu, Japan; 5) Dept Medical Welfare, the Faculty of Health Science, Suzuka Univ of Medical Science, Suzuka, Japan.

PLA2G6 is the causative gene for heterogeneous disorders such as infantile neuroaxonal dystrophy (INAD), neurodegeneration associated with brain iron accumulation (NBIA), Karak syndrome, and recently reported PARK14-linked autosomal recessive early-onset dystonia-parkinsonism. Very recently, heterozygous PLA2G6 p.P806R (c.2417C>G) mutation in exon 17 was reported to be a possible Parkinson's disease (PD)-related mutation in Singapore. To determine the significance of the PLA2G6 mutation, we conducted an association study by performing direct sequencing of PLA2G6 exon 17 in 379 Japanese sporadic PD patients and 310 controls in the Japanese general population. In this group, we found 12 patients (12/379= 3.16%) and 10 controls (10/310=3.23%) with a heterozygous p.P806R mutation (P=0.96, $\chi 2$=0.0019), suggesting that PLA2G6 p.P806R was not a disease-associated polymorphism in PD. Moreover, we performed direct sequencing of all exons and exon-intron boundaries of PLA2G6 in 116 Japanese patients with sporadic PD. Two novel single heterozygous rare variants (p.R301C or p.D331N) were found (both frequencies: 1/379 patients vs 0/310 controls), arising the possible roles for the pathogenicity or risk factors. Subsequently, we reported three Japanese patients (two families) with PARK14-linked parkinsonism. Intriguingly, our two probands with compound heterozygous mutations (age/age at onset = 35/20 and 33/25 years old) and the previously reported patients with PARK14-linked parkinsonism had dementia with frontal lobar dysfunction such as frontal executive dysfunction, frontal lobar atrophy, or frontal lobar hypoperfusion on neuroimaging. Thereafter, to determine the significance of the PLA2G6 mutation, we conducted extended mutation analysis in 23 Japanese patients (male 12, female 11) with frontotemporal type of dementia. Among them, 11 patients had family histories of frontotemporal type of dementia. As a result, direct sequencing of all the PLA2G6 exons revealed no pathogenic mutations or significant SNPs. Thus, combined with the previous report, our findings suggest that PLA2G6 mutations are unlikely to be the major causes or risk factors of PD and frontotemporal type of dementia at least in Asian populations. To clarify the possible roles of PLA2G6 for the pathogenicity or risk factors, further large studies in various populations are needed because patients with PLA2G6 mutations can show heterogeneous clinical features.

550F

The pervasive neurologic phenotype of mild Chediak-Higashi Syndrome. *C. Toro[1], A. Cullinane[2], W. Westbroek[2], M. Huizing[2], C. Groden[1], W. Gahl[1,2,3], W. Introne[3].* 1) Undiagnosed Diseases Program, National Institutes of Health, Bethesda, MD; 2) Metabolic Genetics Branch, NHGRI, National Institutes of Health, Bethesda, MD; 3) Office of the Clinical Director (OCD), NHGRI, National Institutes of Health, Bethesda, MD.

Chediak-Higashi syndrome (CHS) is a very rare autosomal recessive condition due to mutations in lysosomal trafficking regulator gene (*LYST/CHS1* gene, OMIM #606897). The CHS1 protein is thought to function in the formation and trafficking of lysosome-related organelles. Clinically, CHS is characterized by partial albinism, a bleeding diathesis, recurrent infections that are often ultimately fatal, and hemophagocytic lymphohistiocytosis, or the accelerated phase. Giant inclusions within white blood cells are diagnostic. Mortality is high in the first decade of life, and the only available treatment is bone marrow transplantation (BMT). Some CHS patients present with mild findings, i.e., inconspicuous hair and eye hypopigmentation and subtle cognitive difficulties. By their early 20s, these patients manifest slowly progressive neurological deterioration, resembling adults with typical CHS who underwent successful BMT as children. 8 young adults with confirmed CHS, but without BMT, were evaluated at the National Institutes of Health Clinical Center. Testing included neurologic examination, neuropsychiatric testing, EMG, and MRI of the brain. The CHS neurological phenotype involves a mixture progressive length-dependent peripheral neuropathy with foot drop, distal atrophy, weakness, sensory loss and Parkinsonism. Cognitive function, only mildly impaired in childhood, declines progressively with worsening executive dysfunction. Cerebellar signs, basal ganglia dysfunction, upper motor neuron and posterior column dysfunction develop progressively. By their 3rd decade, most patients have diminished capacity for activities of daily living. The pervasive, late neurologic features of CHS, which occur despite BMT, are under-recognized and can appear without the classical features of the disease. The length dependent nature of several neurological features in CHS suggests a possible disruption of axonal transport within neurons. CHS should be considered in the differential diagnosis of patients with unexplained peripheral neuropathy, cognitive decline, cerebellar symptoms, Parkinsonism or long tract findings.

551F

Combined linkage and genome-wide association analysis of multiple schizophrenia (SZ) and bipolar data (BP) sets from public repositories reveals striking new evidence of distinct and overlapping genes. *V.J. Vieland[1], K.A. Walters[1], K. Tobin[2], M. Azaro[2], T. Lehner[3], L.M. Brzustowicz[2].* 1) Battelle Ctr Math Med, Res Inst Nationwide Child Hosp, Columbus, OH; 2) Dept of Genetics, Rutgers Univ, Piscataway, NJ; 3) NIMH, Bethesda, MD.

We have revisited all of the available SZ and BP multiplex family data with available genome scans from the NIMH Human Genetics Initiative (HGI) (N = 1,341 families), together with SZ and BP GWAS data from the GAIN (N = 1,984 cases, 2,393 controls), using the PPL statistical framework for the accumulation of linkage and/or association (LD) evidence across multiple potentially heterogeneous data sets. Extensive cleaning of the family data and phenotypes led to substantial changes in linkage results relative to previous publications on these data (see Walters et al., this meeting) and PPL results differed both from original publications and results of meta-analysis. Additionally the statistical framework allows us to combine linkage information from pedigrees (PPL) with association evidence from case-control data (PPLD) in a mathematically rigorous way. We have therefore undertaken combined linkage and LD mapping separately for SZ, for BP, and also across SZ and BP data looking for common genes for both disorders. We find 5 regions in which the “omnibus” linkage signal is substantially higher for SZ-BP combined than for SZ or BP alone: 5p13.3; 6q21-q22.2; 7q36.3; 10q26.2-q26.3; 14q21.3. Within each region multiple SNPs showed evidence of LD; peak PPLDs (posterior probability of LD, or trait-marker association) per region were respectively 44% (rs376538), 99% (rs9480825, rs7770621), 99% (rs10949808), 97% (rs12245508), 46% (rs12432423). Because the PPLD can show evidence against LD as well as in favor, by comparing these results with associations occurring for SZ alone and BP alone, we are able to construct a set of loci and genes influencing one disorder but not the other as well as genes increasing the risk of both disorders.

552F

***IGF2* variants are related to human episodic memory performance.** *C. Vogler[1,2,3], A. Papassotiropoulos[1,2,3], D.J.F. de Quervain[4,5].* 1) Psychiatric University Clinic, Division of Molecular Neuroscience, University of Basel, Basel, Switzerland; 2) Department of Psychology, Division of Molecular Neuroscience, University of Basel, Basel, Switzerland; 3) Department Biozentrum, Life Sciences Training Facility, University of Basel, Basel, Switzerland; 4) Department of Psychology, Division of Cognitive Neuroscience, University of Basel, Basel, Switzerland; 5) Psychiatric University Clinic, Division of Cognitive Neuroscience, University of Basel, Basel, Switzerland.

Interindividual differences in memory performance can be partially explained by genetic variation. Therefore, behavioral genetic studies are an important tool for understanding the molecular pathways that underlie human memory. Recent findings in rodent experiments indicate that hippocampal administration of insulin-like growth factor II enhances memory retention and prevents memory loss. To investigate the association of naturally occurring genetic variation in *IGF2* with memory performance in a healthy human population, we studied a cohort of N=701 young individuals that underwent a verbal episodic memory task. The discovery cohort was instructed to learn 30 words that differ in their emotional valence for immediate recall. Five minutes and 24h after presentation of these words, subjects underwent an unexpected delayed free-recall test. Genetic variation was assessed by analyzing 21 single nucleotide polymorphisms (SNPs) that cover *IGF2* as well as 50 kbp up- and downstream of *IGF2*. SNP rs11564708 was significantly associated with memory performance for neutral words in the 24 hours delayed recall task (Bonferroni corrected p-value < 0.049, unadjusted p-value = 0.0023). The association of this SNP with memory performance for neutral stimulus material could be replicated in an independent replication cohort of N=900 individuals that underwent a similar memory performance task with emotional and neutral pictures and 10 minutes and 24 delayed free recall (p-value: 0.044). Interestingly the association was only found for neutral stimuli, which is in accordance with the experimental findings in rodents. eQTL analysis in fibroblasts showed significant association with mRNA levels of *IGF2* and SNP rs3922756, which resides in close proximity to rs11564708 (intermarker distance: 13,6 kbp, linkage disequilibrium in HapMap CEU population: r2=0.94). We conclude that *IGF2* variants are associated with human memory performance, possibly through regulation of gene expression levels.

553F

Mosaic Deletion of Both Filamin A and Emerin in a Female with Periventricular Nodular Heterotopia. *J.P. Warman[1], S. Aradhya[2], K. Boycott[3].* 1) Division of Neurology, The Ottawa Hospital, Ottawa, Ontario, Canada; 2) GeneDx, Gaithersburg, MD, USA; 3) Neurogenetics Program Children's Hospital of Eastern Ontario Ottawa, Ontario, Canada.

ABSTRACT Periventricular nodular heterotopia is a rare neuronal migrational disorder with a wide clinical spectrum, ranging from clinically asymptomatic to severe cognitive impairment with seizures. The vast majority of x-linked dominant periventicular nodular heterotopia is caused by Filamin A mutations at Xq28 locus. Centromeric to Filamin A at Xq28 is the Emerin gene. Emerin gene mutations cause Emery-Dreyfuss Muscular Dystrophy and present with humoperoneal weakness, cardiac involvement and early contractures. We describe the clinical manifestations and report the results of a patient presenting with a mosaic pattern of a complete deletion of Filamn A and Emerin genes. DNA analysis revealed a deletion with a minimum size of 34.5 kb, extending from chrX:153576890-153611431, and the maximum deleted region was 67.6 kb extending from chrX:153560505-153628073. The log2 ratios for probes in Filamin A demonstrated a mosaic deletion, and the deleted interval included both the complete Filamin A and Emerin genes. This is the first report describing mosaic deletions of both Filamin A and Emerin genes in a patient with periventricular nodular heterotopia.

554F

The Expanding Role of the Methyl-CpG-binding Domain Family in Autism Etiology. *P. Whitehead[1], H. Cukier[1], B. Butler[1], V. Mayo[1], H. Wright[2], R. Abramson[2], J. Haines[3], M. Cuccaro[1], J. Gilbert[1], M.A. Pericak-Vance[1].* 1) John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL. USA; 2) University of South Carolina School of Medicine, Columbia, SC, USA; 3) Center for Human Genetics Research, Vanderbilt University, Nashville, TN, USA.

The methyl-CpG-binding domain (MBD) genes have been linked to autism for over a decade ever since Rett syndrome, which falls under the umbrella of autism spectrum disorders (ASDs), was revealed to be caused by mutations in the MECP2 gene. Since that time, a few classic autistic patients have carried alterations in MECP2. Recent reports also demonstrate that MBD5 deletion patients present with intellectual difficulties, impaired speech, repetitive behaviors and seizures, all features commonly found in ASD patients. We now describe the first study evaluating ASD individuals in four of the remaining MBD family members, MBD5, MBD6, SETDB1 and SETDB2. All coding exons of these genes were Sanger sequenced in 576 samples (288 ASD individuals and 288 controls). We identified a total of 180 alterations, the vast majority of which were novel (129 variants, 71.7%). These novel changes were comprised of 122 SNPs and 7 insertions/deletions. The overall mutational load between cases and controls was the same; however, we identified some potentially pathogenic alterations. Fifty-two of the alterations change an amino acid sequence, including fifty-one missense variations and a three base pair deletion predicted to remove a single amino acid. Most alterations occurred in only a single individual. To determine which alterations were most likely to contribute to ASD susceptibility, we prioritized changes that were either unique to affected individuals or that had an increased frequency in cases when compared to controls. For example, we identified a MBD6 c.-2C>A change predicted to affect the Kozak consensus sequence in sixteen cases, but only five control individuals. We also identified sixteen ASD specific novel, nonsynonymous alterations. These included a MBD5 Tyr1269Cys change transmitted from a father to all three affected children. A second alteration, SETDB1 del1067Pro, was passed maternally to both affected sons. A third ASD specific change in SETDB1 Pro529Leu was identified in five singleton families, four of which were inherited maternally and one which was de novo. In two of the families with maternal transmission, the mother presented with anxiety/panic disorder. From our studies, we provide the first examples of autistic patients carrying potentially detrimental alterations in MBD6 and SETDB1, thereby demonstrating that the MBD gene family potentially plays a larger role in rare and private genetic causes of autism than was initially believed.

555F

GRIN2B Genetic Variants Associated with Comorbid Anxiety Disorders in Women with Bulimia Nervosa. *Z. Yilmaz[1, 2], A.S. Kaplan[1, 2, 3], C.C. Zai[1, 2], R.D. Levitan[1, 2], J.L. Kennedy[1, 2].* 1) University of Toronto, Toronto, ON, Canada; 2) Centre for Addiction and Mental Health, Toronto, ON, Canada; 3) Toronto General Hospital, Toronto, ON, Canada.

Rationale: GRIN2B gene regulates the activity of NMDA receptor NR2 subunit, which acts as the agonist binding site for glutamate and has been associated with anxiety and impulse control-related disorders. Majority of patients with bulimia nervosa (BN) also report a history of anxiety disorders and a significant proportion have impulse control problems, suggesting that BN may also be associated with glutamatergic abnormalities. The purpose of this study is to (1) examine the frequency of GRIN2B genetic variants in BN and healthy controls and (2) explore the role of the GRIN2B gene in comorbid psychiatric disorders among bulimic women. **Methodology:** For the first part of the study, we genotyped 243 women with BN and equal number of ethnicity-matched female controls for GRIN2B rs2284411, rs1806201, rs1019385 and rs890, markers previously associated with psychiatric disorders. We then performed genetic analyses on the BN probands to investigate if the GRIN2B variants and haplotypes were associated with comorbid psychiatric diagnoses and severity of eating disorder symptoms. **Results:** The data analysis for the first part of the study is currently underway. Within the BN group, we found a significant association of GRIN2B markers and haplotypes with a lifetime history of anxiety disorders ($p = .015$). GRIN2B genotypes or haplotypes were not associated with childhood ADHD or history of substance use in BN probands. **Conclusions:** To our knowledge, this is the first study to look at the role of GRIN2B gene in BN. The pathophysiology of BN with comorbid anxiety disorders may be a distinct subphenotype related to underlying glutamatergic abnormalities, and these finding may have implications for treatment for BN patients with comorbid anxiety disorders.

556F

Autosomal recessive hereditary spastic paraplegia (ARHSP) - clinical and genetic characteristics. *G. Yoon[1,6], B. Baskin[2], M. Tarnopolsky[3], K.M. Boycott[4], S. Goobie[5], B. Banwell[6], P.N. Ray[2].* 1) Div Clinical & Met Gen, Hosp Sick Children, Toronto, ON, Canada; 2) Division of Molecular Genetics, The Hospital for Sick Children, Toronto, Canada; 3) Neuromuscular and Neurometabolic Clinic, McMaster University, Hamilton, Canada; 4) Children's Hospital of Eastern Ontario, Department of Genetics, Ottawa, ON, Canada; 5) Division of Genetics, University of Western Ontario, London, Canada; 6) Division of Neurology, The Hospital for Sick Children, Toronto, Canada.

Hereditary spastic paraplegias are a heterogeneous group of disorders and may be inherited as an autosomal dominant, autosomal recessive or X-linked trait. We describe the features of 16 patients who were screened by whole gene sequencing and multiplex ligation probe amplification (MLPA) for mutations in nine genes known to cause autosomal recessive hereditary spastic paraplegia (ARHSP). Mutations were most frequent in KIAA1840 (SPG11, 8 patients) followed by SPG7 (5 patients), PNPLA6 (SPG39, 2 patients) and ZFYVE26 (SPG15, 1 patient). The types of mutations detected included missense, nonsense, splicing, frameshift and exonic deletions. Four of the patients met the clinical criteria for ARHSP but had only one confirmed mutation. Mutations predicted to cause splice site alterations were further investigated with mRNA analyses. Onset of symptoms occurred in childhood or adolescence in the majority (12/16) patients, with the predominant symptom being gait disturbance. Motor delay was not a common feature (2/16 patients), but the majority (14/16) reported significant difficulties with ambulation and falling by adulthood. Learning disability was a feature in 6/16 patients, all of whom required a modified educational program. As expected, all patients demonstrated abnormalities in corticospinal tract function (spasticity, hyperreflexia, extensor plantar response) and 14/16 also had lower extremity weakness. Speech abnormalities were present in 9/16 patients, and 4/16 had evidence of muscle atrophy. Cerebellar involvement and neuropathy were each present in 4/16 patients. Frank dementia/cognitive decline was present in 3 patients. Whole gene sequencing of nine ARHSP genes enabled the identification of mutations in both alleles for 12/16 patients and in one allele for 4/16 patients in whom the diagnosis of ARHSP was clinically confirmed. The majority of mutations were “private”, which also supports whole gene sequencing as the preferred diagnostic option for these disorders. Although there are published data suggesting an association between some clinical manifestations of ARHSP and specific genes, most patients are tested at an early stage of the disease when phenotype/genotype correlations are not obvious. Further studies of well-characterized cohorts of patients will be essential to establishing phenotype/genotype correlations that can be used to guide genetic testing.

557F

Modeling functional significance of *NRXN1* in neurodevelopment using human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs). *L. Zeng[1], P. Zhang[2], W. Lu[2], K. Wang[1].* 1) Zilkha Neurogenetic Institute, University of Southern California, 1501 San Pablo Street, Los Angeles, CA, USA 90089; 2) Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research, University of Southern California, 1425 San Pablo Street, Los Angeles, CA, USA 90089.

NRXN1 functions as a presynaptic neuronal adhesion molecule and its genetic abnormality has been linked to complex neurodevelopmental and neuropsychiatric disorders, such as Autism Spectrum Disorders (ASDs) and Schizophrenia. There are two predominant *NRXN1* isoforms, including (-*NRXN1* and the shorter) -*NRXN1*, and we are trying to understand how (-*NRXN1* deletion influences neuron development using human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) as model. We selected the Open Biosystems TRIPZ lentivirus inducible shRNAmir knockdown system to knockdown *NRXN1* expression in hESCs and hiPSCs derived neural stem cells (NSCs). The knockdown efficiency was first tested in HEK 293T cells as they have native *NRXN1* expression. qPCR data showed that, all the three shRNAmir clones led to 85%-95% knockdown of (-*NRXN1*, and the knockdown effect was subsequently confirmed by Western blot. We then differentiated hESCs cell line H9 and hiPSCs generated from human dermal fibroblasts to NSCs *in vitro*, and infected them with lentivirus shRNAmir to knockdown the expression of endogenous (-*NRXN1*. The identity of hESCs and hiPSCs was verified by pluripotency markers *Oct4*, *Nanog*, *SSEA-4*, and *Tra-1-60/81*, using immunocytochemistry and qPCR. qPCR and Western blot indicated that more than 80% of (-*NRXN1* has been knocked down in hESCs derived NSCs, and we are currently testing whether (-*NRXN1* shRNAmir produces a similar knockdown effect in hiPSCs derived NSCs. Neurons were differentiated from NSCs by removing the growth factor from conditional media, and the identity of NSCs and neurons was verified by tissue-specific markers *PAX6*, *NESTIN* and *TUJ-1* using immunocytochemistry and qPCR. We are planning to monitor the morphological (dendrite length and synaptic connection *etc.*) and electrophysiological changes in (-*NRXN1* knockdown samples during differentiation. In addition, we will collect RNA samples at 3-4 time points during neuron differentiation stage from cells with *NRXN1* knockdown and control cells. High-throughput RNA-Seq will be performed, and sequence data will be analyzed using well-established bioinformatics analysis pipelines at the Wang Lab. Our results present strong evidence that hESCs and hiPSCs are similar in terms of differentiation potential, and we will investigate how *NRXN1* plays a crucial role in neuron development.

558F

Sleep deprivation affect adult hippocampal NG2 cells and induces depression-like and anxiogenic-like behaviors in Balb-C male mice. *R. Gonzalez Castaneda[1,2], C. Martinez-Quezada[1], A. Galvez Contreras[1], O. Gonzalez-Perez[3], F. Jauregui-Huerta[1], S. Luquin de Anda[1], J. Garcia-Estrada[1], R. Ramos-Zuñiga[1].* 1) Dept Neurosciencias, Univ de Guad, CUCS, Guadalajara, Mexico; 2) Instituto Tecnológico de Estudios Superiores de Monterrey. División de Ciencias de la Salud; Escuela de Medicina. Campus Guadalajara. Guadalajara, Jalisco. México; 3) Facultad de Psicología, Universidad de Colima. Colima,Col. México.

Background: Insomnia is one of the most common sleep disorders in humans. Due to ethical and methodological difficulties involved in human research have created mouse models to evaluate what has been called Sleep Deprivation (DS). Currently there are several investigations about possible effects, but has not been able to clarify fully the implications of owning the etiology of depressive-like (DB) and anxiogenic-like (BA) behaviors. Objective: To analyze the effect of DS model on the development of anxiogenic and / or depressed behaviors in Balb-C male. Material and Methods: We used 30 Balb/C male mice divided in three groups: controls, 48-h DS group and 96-h DS group. After DS, three behavioral analyses were conducted: Forced Swimming (SF), Open Field activity (OF) and sucrose consumption (SC). Results: DS modified the behavior of exposed animals. SC quantification showed statistically significant difference among controls (17.70 ± 1.22) as compared to the 48-h SD group (13.90 ± 4.15) and the 96-h group (12.00 ± 1.88 96; P <0.05). SF assays indicates an anhedonic immobility behavior of experimental groups: 11.38 ± 5.58 (in the 48-h SD group) and 17.75 ± 3.99 (in the 96-h SD group) as compared to controls (0.00 ± .00; P< .01) and disorganized swimming behavior: 50.25 ± 12.74 (in the 48-h SD group) and 85.50 ± 14.94 (in the 96-h SD group) as compared to controls (34.0 ± 12.54; P <0.01). The OF showed a decrease in the exploratory activity in the periphery and an increase in the core areas. Conclusions: The DS promotes and maintains depressive behavior and generates anxiety.

559F

Deficits in eye movement control in children diagnosed with 22q11.2 deletion syndrome. *S.M. Nikkel[1,2], S.A. Kalwarowsky[3], S.C. Kimmett[3], J.N. Reynolds[3].* 1) Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada; 2) Department of Pediatrics, University of Ottawa, Ottawa, ON, Canada; 3) Department of Pharmacology and Toxicology, Centre for Neuroscience Studies, Queen's University, Kingston, ON, Canada.

The 22q11.2 deletion syndrome is associated with both cardiac and craniofacial anomalies, but it is the central nervous system dysfunction that is most likely to affect child development and day-to-day life. The measurement of eye movement control is a powerful tool for assessing brain function in different clinical populations. Saccadic eye movement behaviours reflect the integrity of multiple brain structures and oculomotor tasks have been designed to probe different aspects of behavioural and cognitive function. This study tested the hypothesis that children with the 22q11.2 deletion syndrome would have deficits in eye movement control in comparison to typically developing children. 16 children with the deletion and 32 age and sex matched controls completed prosaccade, antisaccade, delayed memory-guided sequence and predictive eye movement tasks. The circuitry controlling visually-guided saccades was found to be largely intact. However, in the tasks requiring higher cognitive control of eye movements, regulated by the prefrontal cortex and the basal ganglia, children with the syndrome exhibited deficits. These impairments are consistent with behavioural studies that demonstrate deficits in response inhibition/suppression and working memory. Deficits in saccade metrics (e.g., accuracy, amplitude, velocity) in children with the syndrome also suggested cerebellar dysfunction, which is supported by previous studies that showed reduced volume of the cerebellum, especially the vermis and other midline structures. These volumetric reductions overlap with the structures involved in cerebellar oculomotor control. This battery of eye movement tasks may allow for the assessment of frontostriatal and cerebellar function, the areas most often atypical in this population. Further work needs to be done to determine how these deficits correlate to the development of psychiatric diagnoses in individuals with the 22q11.2 deletion.

560F

GWAS Analysis across five psychiatric illnesses - Results of a combined dataset power of 56,867 individuals. *S. Ripke[1,2], Psychiatric GWAS Consortium - Cross Disorder Group.* 1) Ctr Human Gen Researc, Mass Gen Hosp, Boston, MA; 2) Broadinstitute, 7 Cambridge Center, Cambridge, MA.

A major aim of the Psychiatric GWAS Consortium was to quantify the extent of common genetic variation across several distinct psychiatric phenotypes. This was done by analyzing a combined GWAS of unprecedented size, consisting of 46 case-control-studies, for a combined study size of 56,867 independent individuals of European ancestry (N = 31,035 cases / 25,832 controls), derived from five distinct diseases: MDD (9,229 / 7,347), BIP (6,988 / 4,859), SCZ (9,372 / 7,815), AUT (3,499 / 3,864), ADD (1,947 / 1,947), where the latter two comprise mostly family-based datasets. Access to raw genotypes was crucial for several analysis steps, e.g. creating a non-overlapping collection of samples, common imputation quality/format, polygenic analysis. Initially, we show highly significant genetic overlap across studies with polygene-analysis, as described by Purcell 2009 (Nature). To determine specific SNPs contributing to this overlap, our primary analysis performed a SE (standard error) - weighted meta-analysis of the estimates of genetic effect from the five disease-specific analyses. We obtained genome-wide significant p-values for markers in ITIH3/4 (Chr. 3), MIR-137 (Chr. 1), MHC (Chr. 6), CACNA1C (Chr. 12), CACNB2 (Chr. 10), CNNM2 (Chr. 10) and TCF4 (Chr. 18), confirming multiple genes previously implicated in either single-disease studies or previously performed cross-disorder studies. Many genes within regions with suggestive p-values are undergoing further investigation. Following this primary analysis, several follow-up analyses were performed in order to statistically assess the relative contributions of the single disease samples to the top signals from the meta-analysis. Efforts to replicate the main findings will also be discussed, along with the difficulties inherent in assembling a similar sized replication set and creating a similar weighting scheme across phenotypes. In conclusion, while these large cross-disorder meta-analyses have provided some intriguing preliminary results, they also present many challenges. We will discuss steps that the Cross-Disorder-Subgroup of the PGC has taken to address some of these issues and an outlook into future analysis plans.

MDD - major depressive disorder **BIP** - bipolar disorder **SCZ** - schizophrenia **AUT** - autism spectrum disorder **ADD** - attention deficit and hyperactivity disorder.

561F

Three mutations in two causal genes (EGR2 p.Glu412Gly and MPZ p.Asp246Asn + p.Ala15Ala) in a family with dominant type of hereditary motor and sensory neuropathy type I (HMSN I). *P. Seeman[1], D. Brožková[1], R. Mazanec[2], S. Nevšímalová[3], B. Rautenstrauss[4,5].* 1) Child Neurology, DNA Lab, Charles University, 2nd School of Medicine and University Hospital Motol Prague, Praha 5, V uvalu 84, Czech Republic; 2) Neurology, Charles University, 2nd School of Medicine and University Hospital Motol Prague, Praha 5, V uvalu 84, Czech Republic; 3) Neurology Clinic, 1st Medical School, Charles University Prague and General University Hospital, Czech Republic; 4) Medical Genetics Center Munich, Bayer Str. 3-5, Munich, Germany; 5) Ludwig Maximilian University, Friedrich Baur Institute, Munich, Germany.

Hereditary motor and sensory neuropathies (HMSN) or Charcot-Marie-Tooth disorders (CMT) are clinically and genetically heterogeneous peripheral neuropathies. The demyelinating type HMSN I may be caused by mutations in more than 11 genes. CMT1D is a rare, usually severe, autosomal dominant form caused by mutations in the EGR2 gene. Mutations in the MPZ gene cause various CMT forms ranging from early onset, demyelinating to late onset, axonal. EGR2 encodes a transcription factor regulating expression of myelin genes and Schwann cell development. We report a Czech family with four members in three generations affected by HMSN I where mutations in two genes, causal for CMT are present. The proband - is now 37 years old, he was first examined at the age of 7 years due to his gait problems and distal weakness. Subsequent neurological and electrophysiological examination in his mother and older sister revealed a demyelinating neuropathy in all of them. The proband is the most severely affected in the family with CMT Neuropathy Score (CMTNS) of 21, whereas in his mother the CMTNS is only 11 and in the sister 7. Nerve conduction study showed more pronounced abnormality in the proband (MNCV 27 m/s) compared to his mother, sister and nephew (MNCV 35-39 m/s). Most common causes of dominant HMSN I were initially excluded (no CMT1A/HNPP rearrangement and no causal mutation in GJB1, PMP22 and MPZ genes). However two variants were detected in MPZ gene: the p.Asp246Asn not segregating with the disease and present in the probands healthy father and the p.Ala15Ala - probably a rare polymorfism not likely to influence splicing. Subsequent linkage analysis using SNP chips revealed 35 regions with positive linkage including 7 regions previously associated with autosomal dominant CMT. Five of these regions comprised genes associated with CMT (NEFL, YARS, EGR2, HSPB1 and already examined MPZ gene). No causal mutations were detected by sequencing of NEFL, YARS and HSPB1 genes. In EGR2 gene we detected a novel heterozygous mutation p.Glu412Gly (c.1235 A>G) segregating with HMSN I in the family. The codon p.Glu412 is highly conserved among species and another mutation was already reported at this codon. The most severely affected proband carries two missense mutations in two CMT causal genes and we propose, his earlier and severe CMT symptoms may be caused by the negative influence of the EGR2 mutation on the otherwise asymptomatic heterozygous MPZ missense mutation. IGANS10552.

562F

Functional NPY gene polymorphisms - association with antipsychotic-induced weight gain. *E.J. Brandl[1], A.K. Tiwari[1], O. Likhodi[1], H.Y. Meltzer[2], J.L. Kennedy[1], D.J. Müller[1].* 1) Neurogenetics Section, Centre for Addiction and Mental Health, Toronto, ON , Canada; 2) Department of Psychiatry, Vanderbilt University, Nashville, TN, USA.

Objective: Significant body weight gain (BWG) is a serious side-effect of a number of antipsychotic drugs. Previous studies have demonstrated an influence of clozapine, but not haloperidol on Neuropeptide Y (NPY) expression in the brain. Since NPY is a potent orexigenic peptide stimulating food intake and genetic variation of the gene has been shown to influence development of obesity, we investigated the impact of NPY polymorphisms on antipsychotic-induced BWG. Methods: We analyzed four polymorphisms in the NPY gene (rs16147, rs16475, rs5573 and rs5574) in schizophrenia subjects (n=80), undergoing first exposure to clozapine for 6 weeks. Association was tested using analysis of covariance with change (%) from baseline weight as the dependent variable and baseline body weight as covariate. Results: A significant association of rs16147 genotype with weight change was observed (p=.002) in patients of European ancestry. Carriers of the C-allele gained significantly more weight compared to individuals with TT-genotype (TC+CC vs. TT; 5.61±5.4 vs. 0.32±4.8). Similarly, two other polymorphisms (rs5573 and rs5574) were also significantly associated with weight change (p=.009 and p=.022). The three associated polymorphisms are in high linkage disequilibrium with each other. There was no association of rs16475 (p=.654). Conclusions: Our observation of association of NPY polymorphisms gives further evidence for a genetic influence on antipsychotic-induced BWG. The polymorphism rs16147, present in the promoter region, has been previously described to affect NPY expression both in vivo and in vitro. However, these results warrant replication in independent and larger samples.

563F

Glial cell line-derived neurotrophic factor (GDNF) variations in Parkinson's disease. *D. Tegay[1], J. Gemmill[1], E. Hatchwell[2, 3].* 1) Medicine Dept, New York College of Osteopathic Medicine of NYIT, Old Westbury, NY; 2) Pathology Dept, Stony Brook University Medical Center, Stony Brook, NY; 3) Population Diagnostics, Melville, NY.

Glial cell line-derived neurotrophic factor (GDNF) is a potent survival factor for nigrostriatal dopaminergic (DA) neurons, which are known to degenerate in Parkinson's disease (PD), and altered striatal GDNF expression levels are believed to be involved in the pathophysiology of PD. Additionally, GDNF mutations have been reported to modify risk for Hirschsprung disease and severe constipation, a significant early feature of PD. Consequently, the neuroprotective properties of GDNF have been widely studied as a potential therapeutic tool to treat PD, however, GDNF variations as a contributing factor in PD pathogenesis have been inadequately explored. We employed a retrospective case-control methodology to explore the hypothesis that deleterious GDNF variations may be associated with increased PD risk by comparing mutation frequencies in subjects with PD (cases) against population frequencies (controls).

DNA from 95 anonymous PD subjects was obtained from the Coriell Cell Repositories (Plate NDPT-089). GDNF copy number variations (CNV) were detected by analysis of data obtained on a custom designed genome-wide and PD candidate gene targeted oligonucleotide multiplex aCGH platform with Agilent Feature Extraction (v10.1) and DNA Analytics (v4.0) software. PCR primers for all GDNF isoform coding regions were designed using Primer 3 (http://frodo.wi.mit.edu/primer3) with amplification followed by standard Sanger sequencing. Sequencing data was analyzed using CodonCode sequence assembly and alignment software. Novel variations and polymorphism frequencies were compared with population frequencies ascertained through online databases (TCAG DGV [http://tcag.ca] and dbSNP [http://www.ncbi.nlm.nih.gov/projects/SNP]).

Preliminary data analysis revealed that CNV's involving GDNF were present in 2 PD subjects, while studies in TCAG indicated a frequency of GDNF CNV's of 1 in 450 HapMap control samples. Additionally, differences in GDNF polymorphism frequencies in PD can be detected in comparison to reported normal control frequencies in dbSNP. This pilot data indicates the need for further studies of GDNF variation in larger cohorts to provide increased power to further elucidate statistically significant associations with PD.

564F

Co-Occurrence of Behavioral, Psychiatric, and Medical Issues in Families Ascertained for Autism and Language Learning Impairment. *Z. Fermano[1], J. Flax[1], A. Hare[1], B. Zimmerman-Bier[4], L. Hou[3], S. Yeon[3], C. Bartlett[3], S. Buyske[1, 2], L. Brzustowicz[1].* 1) Department of Genetics, Rutgers University, Piscataway , NJ; 2) Department of Statistics, Rutgers University, Piscataway, NJ; 3) The Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Children's Hospital and Department of Pediatrics, The Ohio State University, Columbus, OH; 4) Saint Peter's University Hospital, New Brunswick, NJ.

Converging evidence suggests that when compared to control families, relatives of individuals with autism exhibit behavioral, medical, and/or psychiatric characteristics that are milder but qualitatively similar to the defining features of autism. Shared etiology is of particular interest to scientists who study the genetic basis of autism. Thus, studying family members who exhibit elevated scores on ratings of (1) language deficits, (2) rigid personality traits/social aloofness, and (3) medical and psychiatric inventories, yet, do not meet the criteria for autism, allows for more specific phenotypic characterization. Over the past seven years, The New Jersey Language and Autism Genetics Study (NJLAGS) has studied families with at least one proband with autism and a second proband with a significant language learning impairment (LLI), while each family member has also been directly assessed for measures from the three domains addressed above with the goal of identifying phenotypes and behavioral biomarkers related to language and other conditions associated with autism. Since families were preselected for having at least one family member with a history of language learning problems, the rates of language, reading, and spelling are significantly higher than what would be expected in the general population. However, one-third of the families in our sample have at least one other family member, independent of the autism proband and language learning impaired proband, with a language-based learning disability. The rates of several other behavioral and psychiatric disorders (i.e., obsessive compulsive disorder, social impairment) were greater in family members (including and excluding the autism probands) than rates of these disorders in the general population. Certain medical conditions such as gastrointestinal problems and allergies were reported at higher rates based on family history questionnaire data. Moreover, there were a number of non-spectrum family members who exhibited multiple behavioral, psychiatric, or medical conditions. This study suggests that in families who were collected for a history of autism and language disorders, there is a greater risk for other behavioral, psychiatric, and medical disorders in comparison to the general population. These findings support the theory of a broader view of autism-related behaviors in families and are an essential component of phenotypic characterization for genetic linkage and association analyses.

565F

Profiles of Language and Reading Impairment in a Family Study of Autism Spectrum Disorders and Specific Language Impairment. *A. Hare[1], J. Flax[1], Z. Fermano[1], S. Buyske[2], L. Hou[3], SY. Cheong[3], B. Zimmerman[4], C. Bartlett[3], L. Brzustowicz[1].* 1) Department of Genetics, Rutgers University, Piscataway, NJ; 2) Department of Statistics, Rutgers University, Piscataway, NJ; 3) The Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Children's Hospital and Department of Pediatrics, The Ohio State University, Columbus, OH; 4) Saint Peter's University Hospital, New Brunswick, NJ.

Over the past decade, comparing the language profiles of individuals with Autism Spectrum Disorders (ASD) to individuals with Specific Language Impairment (SLI) has been one approach to investigating shared genetic etiology in the language domain for autism. While ASD may include individuals who have significant dysfunction in communication, social interactions, and repetitive and restrictive behaviors, SLI refers to individuals who have no other obvious behavioral, cognitive, or neurological issues other than deficient language. Conflicting results from previous studies either supporting or negating the genetic link, suggest that perhaps previous language phenotypes were not refined enough to address the more subtle constructs that the two disorders may share. The New Jersey Language and Autism Genetics Study (NJLAGS) has been studying families who have at least one individual with a diagnosis of Autistic Disorder and at least one other individual with SLI, with the goal of identifying language phenotypes to serve as behavioral biomarkers for linkage and association studies. This study is unique in that in addition to segmental aspects of oral language, reading and higher order language constructs are also examined. All individuals received a comprehensive neuropsychological battery including standardized measures of oral and written language. Each family has contributed a blood or saliva sample for genetic analyses. Individual family members were categorized in terms of language impairment (LI) and reading impairment (RI) based upon their scores on each respective assessment. ASD probands were compared to other sample individuals (LI, RI, and LI+RI). ASD probands who were verbal did not significantly differ from the LI group on any language and reading tasks. ASD probands scored significantly lower than the RI group on assessments that address overall spoken language ability and metalinguistic language abilities. Also, ASD probands scored significantly higher than the LI+RI group on measures of phonological awareness and word identification. The results of this study suggest that in addition to segmental language constructs such as structure and phonology, shared language behaviors might occur on the suprasegmental level and written language level and should be considered when addressing phenotypic characterizations for genetic analyses.

566F

The use of mixed effects modelling to identify heritable autism endophenotypes for linkage and association studies. *M. Woodbury-Smith, P. Szatmari.* Department of Psychiatry & Behavioural Neurosciences, McMaster University, ON, Canada.

Autism is a neurodevelopmental disorder of early childhood onset, characterized by specific social, communicative and behavioural abnormalities. It is one of the more common childhood onset disorders, and evidence for its genetic basis has accumulated over the years. However, despite several decades of research, familial susceptibility genes have not yet been identified. This is believed to be partly due to the genetic and phenotypic heterogeneity that underlie the disorder. Supporting this, studies that have examined genetic linkage in more homogeneous phenotypic subgroups have shown improved signals. In the study described in this presentation, a more specific empirically driven method of subgrouping is proposed, in which groups are formed according to intermediate phenotypes with a priori evidence of significant heritability. Such phenotypes represent neurobiological characteristics associated with the disorder, but which, being located upstream of its clinical expression, and thereby more proximal its genetic causality, are associated with a more simple genetic architecture. In this way samples that are not only more homogeneous but also genetically enriched can be identified, and can then form the basis of subsequent genetic linkage and association studies. This presentation describes a study investigating phonological processing as a possible endophenotype of autism. Data on a measure of phonological processing, the CTOPP, have been collected on nuclear autism families recruited across Canada as part of ongoing autism genetic studies. After demonstrating the familiality of performance on the CTOPP, a mixed effects model is used to calculate the heritability of its subdomains. The mixed effects method allows for the decomposition of phenotypic variance into genetic and environmental components according to biometrical models of familial covariance. We calculated heritabilities of between 24% and 35% for the CTOPP subdomains, lending support to phonological processing as an autism endophenotype, and its potential usefulness as a measure by which families can be stratified for further linkage analysis.

567F

Influence of the normal allele CAG expansion at age of onset in Peruvian population with Huntington's disease. *M. Cornejo[1,2,3], R. Badillo[5], N. Mori[2,3], R. Chacon[1,3], S. Lindo[1,3], V. Marca[1,3], O. Ortega[1], C. Cosentino[3,4], L. Torres[3,4], P. Mazzetti[1,3].* 1) Neurogenetics Division, Instituto Nacional de Ciencias Neurológicas , Lima, Lima 1, Perú; 2) Fogarty International Clinical Research Scholar, University of Washington; 3) Universidad Nacional Mayor de San Marcos, Lima, Perú; 4) Movement Disorders Unit, Instituto Nacional de Ciencias Neurologicas, Lima, Peru; 5) Biomedical Research Comitee, Insituto Nacional de Ciencias Neurológicas, Lima, Perú.

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by movement disorders, cognitive impairment and abnormal behavior with progressive and fatal course. It is caused by an unstable trinucleotide CAG expansion in exon 1 of the Huntingtin gene (HTT) in chromosome 4p16.3. The CAG expansion of the abnormal allele explains around 60% of variation in clinical presentation, particularly age of onset. Other associated factors include modulating effects of other genes and environmental conditions. Many studies mostly developed in Europe and the USA have been suggested that normal Huntingtin could modulate the effects of mutant Huntingtin and influence age of onset and also severity and progression of the disease. In Peru, we have one of the biggest focuses of HD of South America, after Venezuela. We assessed the influence of the normal over the mutant one and its effect on age of onset in Peruvian Huntington's disease population. DESIGN/METHODS We used data from the National Registry for HD at the Neurogenetics Division at the “Instituto Nacional de Ciencias Neurológicas” (INCN). All DNA samples were extracted from peripheral blood leukocytes using the standardized proteinase K method, amplification of the HTT gene were made by PCR. All patients gave written informed consent. We included all participants with clinical diagnosis of HD whose ages at onset and CAG repeat numbers in both alleles (mutant allele／ 36 repeats) were available (n= 195). Using bivariate analysis we assessed the influence of the normal allele on age of onset and severity of the disease (n= with 41 UHDRS score) stratified by two different categories of the abnormal allele expansion. RESULTS In subjects with mutant CAG expansions in low range, increasing size of the normal repeat did not correlate significantly with earlier age of onset of the disease(p=0.78), while for subjects with abnormal expansions in the high range, increasing size of the normal allele repeat correlated with later age of onset and less severe symptoms (p=0.04). CONCLUSIONS In Huntington's disease, increasing number of CAG repeats in the normal allele of HTT gene seems to influence the mutant allele to delay age of onset in affected individuals in a high expansion range. This feature could be helpful for genetic counseling of pre-symptomatic individuals for HD.

568F

Parkinsonism in Machado Joseph disease/ SCA 3: possible role of the GBA gene. *L.B. Jardim[1 3 4], M. Siebert[1 6], K.C. Donis[4], M. Socal[2], C.R.M. Rieder[2], V.E. Emmel[3], F. Vairo[1], K. Michelin-Tirelli[1], J.A.M. Saute[2], M. França Junior[7], A. D'Abreu[7], C. Bettencourt[9], M. Lima[9 10], I. Lopes-Cendes[8], M.L. Saraiva-Pereira[1 3 5].* 1) Medical Genetics Service, Hospital de Clinicas de Porto Alegre RS, Brazil; 2) Neurology Service, Hospital de Clinicas de Porto Alegre RS, Brazil; 3) Genetic Identification Laboratory, Hospital de Clínicas de Porto Alegre RS, Brazil; 4) Department of Internal Medicine, Universidade Federal do Rio Grande do Sul, Brazil; 5) Department of Biochemistry, Universidade Federal do Rio Grande do Sul, Brazil; 6) Biotechnology Centre, Universidade Federal do Rio Grande do Sul, Brazil; 7) Department of Neurology, Faculty of Medical Sciences, University of Campinas (UNICAMP), Campinas, SP, Brazil; 8) Department of Medical Genetics, Faculty of Medical Sciences, University of Campinas (UNICAMP), Campinas, SP, Brazil; 9) Center of Research in Natural Resources (CIRN) and Department of Biology, University of the Azores, Ponta Delgada, Portugal; 10) Molecular and Cellular Biology Institute (IBMC), University of Porto, Porto, Portugal.

Machado-Joseph disease/spinocerebellar ataxia type 3 (MJD/SCA3) may rarely presents a parkinsonian phenotype. Since mutations in the glucocerebrosidase (GBA) gene have been associated with Parkinson disease, we investigated whether these would be more prevalent in MJD/SCA3 patients with parkinsonian manifestations than in those without them. **Methods**: MJD/SCA3 patients with parkinsonian features were identified and compared with relatives and with a MJD/SCA3 control group without Parkinson. The GBA gene was sequenced and, in a subset of patients and in normal volunteers, the enzymatic activity of GBA was measured. **Results**: we identified nine index MJD/SCA3 patients with parkinsonian manifestations. Overall, GBA sequence variations were found in 3/9 MJD/SCA3 index-cases with parkinsonian manifestations (33%) and in 0/40 MJD/SCA3 controls without parkinsonism ($p = 0.03$, Fisher exact test). The GBA sequence variations found were p.K(-27)R, p.E326K, and p.T369M. The last two sequence variations were also found in two symptomatic relatives without parkinsonian manifestations. A MJD/SCA3 relative belonging to the first positive pedigree, and carrier of the p.K(-27)R mutation, started with ataxic symptoms two years ago and presented, in the last follow-up visit, with parkinsonian manifestations. Enzymatic activities of GBA in MJD/SCA3 patients were similar to those found in the normal control group. **Conclusion**: sequence variations at GBA gene may play a role as a minor, modifying gene of the MJD/SCA3 phenotype. This hypothetical role was not related to changes in GBA activity in peripheral leukocytes.

569F

A polyglutamine expansion in (1A calcium channel C-terminal exerts toxicity in the cytoplasm with CREB transcriptional activation. *M. Takahashi[1], M. Obayashi[1], T. Ishigurop[1], N. Sato[1], Y. Niimi[1], K. Mogushi[2], Y. Mahmut[2], H. Tanaka[2], K. Ishikawa[1], H. Mizusawa[1].* 1) Department of Neurology and Neurolgical science, Tokyo Medical and Dental University, Tokyo, Japan; 2) Information Center for Medical Sciences, Tokyo Medical and Dental University, Tokyo, Japan.

Spinocerebellar ataxia type 6 (SCA6), caused by a small polyQ expansion in the cytoplasmic carboxy(C)-tail of (1A voltage-dependent calcium channel (Cav2.1), is characterized by Purkinje cell degeneration with microscopic Cav2.1 aggregations. Recent advances in understanding SCA6 pathophysiology have shown that a 75-kDa C-terminal fragment (CTF) containing polyQ, which remains soluble in normal brains, becomes insoluble in the cytoplasm with additional localization to the nuclei in SCA6 Purkinje cells. However, the mechanism by which CTF aggregation leads to neurodegeneration is completely elusive. We here show that the CTF with a small polyQ expansion (28Qs) of a length corresponding to the normal-repeat size for other polyQ diseases causes cell death when localized in the cytoplasm rather than in the nucleus of cultured cells. By using rat pheochromocytoma (PC12) cell lines, in which expression of CTF could be induced by doxycyclin removal, we show that cytoplasmic CTF aggregation precedes cell death and induces cAMP response element-binding protein (CREB)-dependent transcription. In addition, microarray analysis revealed that expression of hypoxia-related genes Egln3 and S100a11 were consistently altered as cell death approached. In human SCA6 Purkinje cells, microscopic polyQ aggregates co-localized with CREB as in cultured cells, and staining with other antibody was coincided with the results of microarray analysis. Taken together, the present study suggests that neurodegeneration caused by CTF with a small polyQ expansion resides in the cytoplasm of Purkinje cells, and that unique changes in gene transcription associated with CREB activation may underlie SCA6 pathogenesis.

570F

Modifier genes of age of onset in SCA diseases. *S. Tezenas du Montcel[1,2], A. Durr[3,4,5,6], G. Stevanin[4,5,6], S. Forlani[4,5,6], P. Bauer[7], O. Riess[7], C. Cazeneuve[3], A. Camuzat[4,5], A. Brice[3,4,5,6,8] for the EUROSCA group.* 1) AP-HP, Department of Biostatistics and Medical Informatics and Pitié-Salpêtrière Charles-Foix Clinical Research Unit, Paris, France; 2) University Pierre et Marie Curie, ER4, Modelling in Clinical Research, Paris, France; 3) AP-HP, Pitié-Salpêtrière Hospital, Department of Genetics and Cytogenetics, Paris, France; 4) Université Pierre et Marie Curie-Paris6, Centre de Recherche de l'Institut du Cerveau et de la Moelle épinière, UMR-S975, Paris, France; 5) Inserm, U975, Paris, France; 6) Cnrs, UMR 7225, Paris, France; 7) University of Tubingen, Tubingen, Germany; 8) AP-HP, Pitié-Salpêtrière Hospital, Federation of Neurology, F-75013 Paris, France.

Major advances have been made in the understanding of autosomal dominant cerebellar ataxias since the 1980s. A polyQ-coding (CAG)n repeat expansion has been identified as responsible for the disease in six genes: SCA1-3, SCA6-7 and SCA17. The clinical symptoms of these SCA subtypes appear above a threshold number of CAG repeats with a negative correlation between the number of CAG repeats and the age at onset. However, the correlation factor ranges from 0.5 to 0.7 in most studies suggesting that other genetic factors contribute to the variability.

Regression analysis was used to test the influence of the size of the expanded and the normal alleles of 8 polyQ genes (SCA1-3, SCA6-7, SCA17, DRPLA, HD, SBMA) on the age at onset in 1267 patients with known SCA types (SCA1: 319, SCA2: 316, SCA3: 405, SCA6: 167, SCA7: 60), recruited through the EUROSCA consortium.

We evidenced an interaction between the expanded and the normal alleles in trans in the SCA1 and SCA7 subtypes.

In addition, we found an influence of an additional polyQ gene for several SCA subtypes. For SCA2 patients, the effect of the SCA2 expanded allele was significant only for the patients with intermediate SCA7 ($p = 0.0005$). In addition, for the patients with moderately expanded SCA2 alleles, DRPLA gene protected against effect of the SCA2 gene ($p = 0.03$) ($R^2 = 53$ %). SCA3 patients had younger age at onset with smaller SCA1 alleles ($p = 0.0008$) and SCA2 intermediate alleles ($p = 0.007$) ($R^2 = 53$ %). SCA6 patients had younger age at onset with smaller SCA3 gene ($p = 0.03$), longer SCA7 gene ($p = 0.02$) and smaller DRPLA gene ($p = 0.04$) ($R^2 = 61$%). SCA7 patients had younger age at onset with an allele of SCA2 gene longer than 22 ($p = 0.02$), and smallest number of repeats for the SCA3 gene ($p = 0.03$). For SCA1 patients, none of the PolyQ genes tested influenced the age at onset.

We have shown that the polyQ genes interacts between each other in SCA diseases modifying the age at onset even with a number of repeat considered as normal. However the variances remaining after taking into account both the major gene and, when appropriate, the additional gene are still small suggesting the role of other genetic factors in these diseases.

571F

Complete genome sequence based genetic analysis of monozygotic twins discordant (MZD) for schizophrenia. *C. Castellani[1], S. Maiti[1], R. O'Reilly[2], S. Singh[1,2].* 1) Molecular Genetics Unit, Department of Biology, The University of Western Ontario, London, Ontario, Canada; 2) Department of Psychiatry and London Health Sciences Centre, London, Ontario, Canada.

The reality of individual genome sequencing now offers a new hope in search of the cause(s) of complex diseases. When combined with genetic relationships, individual sequences add an unrivaled proficiency. Given the near identical genetic structure of monozygotic twins, any difference between monozygotic twins discordant (MZD) for a disease will have a high likelihood of being causal. With this in mind we have sequenced the DNA of six individuals, which includes two pairs of MZ twins discordant for schizophrenia and one set of parents. Sequencing was carried out using the Complete Genomics Analysis system (Drmanac *et al*, 2010). The Complete Genomics platform has a 99.999% sequencing accuracy, 47.27-fold coverage and >99% reference genome coverage. The sequences were further assessed for accuracy in relation to SNP Array 6.0 results.

Genome wide variations including SNPs, indels, CNVs and miRNAs were assessed. It has allowed us to evaluate the similarities and differences across unrelated individuals, parents and children, as well as between MZ twins. The results show that an individual carries approximately 3.7 million SNPs, 150 CNVs, 400,000 indels and 220 miRNA variants. Also, two unrelated individuals differed for 1.5-1.8 million SNPs (~45%), a parent and child differed for 0.9-1.0 million SNPs (30%) and a pair of MZ twins differed for 100,000 (~3%) SNPs. Differences in the identity of CNVs for the three comparisons were 45%, 30% and 4%, respectively. Interestingly, CNV and SNP differences between MZD twins affect a set of genes enriched in neurodevelopment, as well as genes that have been already implicated in Schizophrenia. Potentially deleterious mutations (a nonsense mutation in PDE4DIP and a nonstop mutation in LOC339742) were found in both affected patients but not in their unaffected co-twins or parental samples.

The results support our strategy and identify patient specific genetic changes that may lead to schizophrenia. The novel results re-enforce that individual genomes harbor extensive variability, some inherited and others acquired during parental meiosis and/or mitosis. There is no single human genome sequence. Even monozygotic twins are not identical and each individual may be a mosaic, potentially carrying different sequence variations in different cells.

572F

Association between the *CLOCK* gene and autism symptoms in a community-based twin cohort. *L. Jonsson[1], H. Larsson[2], L. Westberg[1], H. Anckarsäter[3], P. Lichtenstein[2], J. Melke[1].* 1) Department of pharmacology, Neuroscience and physiology, Univ. of Gothenburg, Gothenburg, Sweden; 2) Department of medical epidemiology and biostatistics, Karolinska institute, Stockholm, Sweden; 3) Department of forensic psychiatry, Neuroscience and physiology, Univ. of Gothenburg, Gothenburg, Sweden.

Autism spectrum disorders (ASDs) are pervasive developmental disorders that include Autistic disorder, Asperger syndrome, and pervasive developmental disorder-not otherwise specified (PDD-NOS). Many patients with ASD have sleep impairments and timing problems, suggesting disturbances in the regulation of circadian rhythm as causative factors for these disorders. Indeed, low levels of melatonin are recurrent biological findings and we have previously found association between genes in the melatonin pathway and ASDs. Melatonin is closely related to the circadian rhythms, which is mainly regulated in the suprachiasmatic nucleus (SCN) by a set of clock genes. Genetic variation in the clock genes have previously been investigated in autism patients showing an association with the clock genes *PER1* and *NPAS2*. In this study, we have investigated the possible association of five core circadian clock genes on autism symptoms in a community based twin cohort. The subjects (N=1771, 9-12 years old) in this study are a subset of the "The Child and Adolescent Twin Study in Sweden" (CATSS), identified through the Swedish Twin Registry. Autism symptoms flexibility, language and social interaction were measured by the Autism-Tics, AD/HD, and other Comorbidities inventory (A-TAC), a telephone interview designed for large-scale epidemiological research in neuropsychiatry. To cover most of the genetic variability in the investigated genes, a total of 26 SNPs were genotyped in the clock genes *CLOCK*, *CRY1*, *CRY2*, *NPAS2*, and *PER1*. Our results show a significant association in girls between rs1801260, in the 3'-UTR, of the *CLOCK* gene, and the symptom flexibility ($p = 10^{-5}$), but not with the symptoms language and social interaction. Carriers of the CC-genotype had significantly higher means on the symptom flexibility. Thus, our results support the hypothesis that clock genes may be involved in autism related disorders. Moreover, since not all symptoms of autism showed similar association with the investigated genes in this study, our findings also emphasizes that genetic research may benefit from taking a symptom-specific approach to finding genes associated with autism.

573F

Genetic exploration of genome-wide variation in monozygotic twins discordant (MZD) for schizophrenia. *S. Maiti[1], K. Kumar HB[1], C. Castellani[1], R. O'Reilly[2], S. Singh[1,2].* 1) Molecular Genetics Unit, Department of Biology, The University of Western Ontario, London, Ontario, Canada; 2) Department of Psychiatry and London Health Sciences Centre, London, Ontario, Canada.

We have assessed genome-wide copy number variations (CNVs) and single nucleotide polymorphism (SNPs) in eight individuals representing two nuclear families, each with a pair of monozygotic twins discordant (MZD) for schizophrenia, using Affymetrix Human SNP array 6.0. This presentation will deal with the distribution of CNVs and SNPs across each individual. The results have allowed us to conclude the following:

Each individual carried 35 to 65 CNVs, majority (~80%) represented gains. Also, ~10 % of the CNVs were *de novo* (not present in parents). Of these, 30% arose during parental meiosis (and are present in both twins) and 70% arose during developmental mitosis (not present in parents and different between twins). We also observed novel SNPs (not present in parents) in the twins, which constituted 0.12% of all SNPs seen in the twins. In 65% of cases these SNPs arose during parental meiosis compared to 35% during development (mitosis). The best explanation for the observed differences in CNVs and SNPs between MZ twins - that started life as a single zygote - is *de novo* mutations, probably occurring during early embryogenesis. The results show that (unlike the assumption of 100% genetic identity), monozygotic twins are genetically different.

These results have major implications in the understanding of development including disease discordance. CNV and SNP differences between two MZD pairs used have led to the identification of potential genes that may explain their discordance for schizophrenia. They include three genes (PSMC1, C14orf102, KIAA0146) for family 1 and two different genes (DPRX1 and ZNF331) for family 2. Interestingly, a number of these genes have already been implicated in schizophrenia. They provide a foundation for observed genetic heterogeneity and individual specific genomic changes implicated in this complex disease. We conclude that enduring genome-wide changes, often ignored in genetic analyses involving large number of patients, if incorporated with selected patients will reinforce the search for disease related genetic changes, begin to interrogate the mechanisms involved and offer novel strategies for diagnosis and treatment.

574F

Adenosine deaminase G22A polymorphism and sleep electroencephalogram spectral power in a large population sample from Sao Paulo, Brazil. *D.R. Mazzotti, A.A.L. Souza, C. Guindalini, R. Santos-Silva, L.R.A. Bittencourt, S. Tufik.* Universidade Federal de São Paulo, São Paulo, SP, Brazil.

Background: Sleep is a complex phenomenon and certain aspects of sleep electroencephalogram (EEG) indicate that it is a heritable trait. Slow wave oscillations in EEG during sleep may reflect both sleep need and intensity, which implies in a homeostatic regulation. Adenosine is strongly implicated in sleep homeostasis and a single nucleotide polymorphism in adenosine deaminase gene (*ADA* G22A) has been associated with deeper and more efficient sleep. Objectives: The present study aimed to verify the association between sleep EEG spectral power and *ADA* G22A polymorphism in a large population sample from Sao Paulo, Brazil. Methods: Nine-hundred and fifty eight individuals of both genders participating in the Epidemiologic Sleep Study of Sao Paulo city, Brazil with age range from 20 to 80 years were subjected to an extensive sleep survey followed by full-night polysomnography. *ADA* G22A genotyping was conducted by allele-specific polymerase chain reaction. Spectral analysis of the EEG was carried out in all individuals using the Fast Fourier Transformation of the oscillatory signals for each sleep stage and for each EEG electrode. Results are represented by mean spectral power ($\mu V^2/Hz$) ± standard error of the mean, and analyses were controlled by age, body mass index and apnea/hypopnea index. Results: When compared to GG genotype carriers, A allele carriers (GA and AA genotypes) showed higher spectral power of theta bandwidth in rapid eye movement (REM) sleep (3.01±0.03 vs. 1.98±0.07; p=0.01) and in Stage 2 of non-REM sleep (2.85±0.03 vs. 1.77±0.06; p<0.01). In addition, A allele carriers also showed higher spectral power of delta bandwidth in Stage 1 (16.92±0.08 vs. 15.01±0.21; p=0.04), Stage 2 (18.20±0.09 vs. 17.21±0.22; p=0.03) and Stages 3 and 4 of non-REM sleep (18.10±0.08 vs. 16.99±0.24; p=0.03). Conclusions: The present findings suggest that the sleep of individuals carrying the A allele might be more intense, as evidenced by higher spectral power of waves related to deeper sleep. Therefore, *ADA* G22A polymorphism may exert a more refined effect on sleep by modifying specific spectral power of the sleep EEG, thus being an important source of variation in response to sleep homeostasis in humans. Acknowledgements: This work was supported by grants from AFIP, FAPESP and CNPq.

575F

***De novo* CNVs affecting multiple genes/pathways may explain discordance of monozygotic twins for schizophrenia.** *R. O'Reilly[1], C. Castellani[2], S. Maiti[2], S. Singh[1,2].* 1) Department of Psychiatry and London Health Sciences Centre, London, Ontario, Canada; 2) Molecular Genetics Unit, Department of Biology, The University of Western Ontario, London, Ontario, Canada.

Schizophrenia is a common (1%) psychiatric disorder of high heritability (80%) and low concordance (48%) between monozygotic twins. Such epidemiological features are best explained by involvement of genetic, epigenetic and environmental interactions. Given an extensive genetic heterogeneity implicated in this disease we feel that an explanation for the discordance of MZ twins (MZD) using genome-wide analysis of rare twins may offer an insight into the causation of this disease. Specifically, we report on genome-wide copy number changes (CNCs) between six MZD twin pairs that allow identification of pair specific *de novo* CNCs that may explain their discordance.

We (O'Reilly, Psychiatrist) identified six pairs of monozygotic twins discordant for schizophrenia and collected their leukocyte DNA. The DNA was hybridized to Affymetrix Human SNP Array 6.0 that includes 1.8 million markers. CNVs were called by Affymetrix Genotyping Console 4.0 as well as Partek Genotyping Suite software. Twin pairs differed for one to thirteen CNVs. The best explanation for the observed differences in CNVs between MZ twins is *de novo* mutation (DNM) occurring during early embryogenesis. This explanation was confirmed in two of the six pairs using parental genotypes. Our analysis shows that each MZD pair differs for a number of SNPs and CNVs. More important, the SNP and CNV differences between ill and well twin invariably affect a set of genes that are implicated in neurodevelopment as identified by Ingenuity Pathways Analysis and GENEMANIA. In addition, they include already reported potential contributors to schizophrenia by family and population studies. Further, each of the six MZD pairs is different with respect to the schizophrenia related gene(s) affected. The only common pathway affected in more than one pair involves olfactory receptors that have been implicated in the development of schizophrenia.

The results show that genome wide *de novo* changes are common during ontogeny. Depending on the nature of changes and the genes involved they may contribute to discordance of MZD twins for any feature including disease. The fact that different MZD pairs have identified different genes offers support for the extensive genetic heterogeneity in schizophrenia and may argue for the need for “patient specific” etiology and treatment strategies for schizophrenia.

576F

Gene environment interactions with a novel polymorphic Monoamine Oxidase A transcriptional enhancer element. *J.M. Plume[1], P. Wernett[1], H. Packer[1], S. Beach[2], C. Cutrona[3], G. Brody[4], R. Philibert[1].* 1) Department of Psychiatry, University of Iowa, Iowa City, IA., USA; 2) Department of Psychology, University of Georgia, Athens, GA., USA; 3) Department of Psychology, Iowa State University, Ames, IA., USA; 4) Department of Child and Family Development and Center for Family Research, The University of Georgia, Athens, GA., USA.

Monoamine Oxidase A (MAOA) is a key regulator of monoaminergic neurotransmission. Traditionally, activity of this gene has thought to be regulated by a 22 bp variable nucleotide repeat (VNTR) that is approximately 600 bp upstream of the translational start site. Unfortunately, clinical studies utilizing genetic data from this VNTR system have been not been routinely reproducible. One of the reasons for this lack of reproducibility may be the VNTR itself may be in tight linkage equilibrium with a second more functional motif. Recently, using data from the Iowa Adoption Studies, we demonstrated the existence of a 10 bp VNTR system, termed MAOA P2, that is approximately 600 bp from the first VNTR and which has both markedly greater enhancer effects and better predicts clinical outcomes. In this communication, we extend these studies to other ethnic groups and show that African Americans have considerably greater genetic diversity at this locus with at least two additional alleles. We compare and contrast the relative enhancer capacities of each of these alleles and provide an improved nosology for the classification of genetic variation in the MAOA promoter region. We conclude that the genetic regulation of the MAOA locus is more complex than previously thought and suggest that improved power for genotype/phenotype studies of this locus can obtained through the genotyping of the MAOA P2 locus.

577F

Serotonin Transporter Gene in Bulimia Nervosa: A Multiple Model Meta-Analysis. *G.N. Polsinelli, V. De Luca.* Psychiatry, Center for Addition and Mental Health (CAMH), Toronto, Ont, Canada.

Objective: Several lines of research have found that genes in the serotonergic system may cause susceptibility to eating disorders (EDs). In particular, functional polymorphisms of the serotonin transporter gene 5-HTT have been suspected to play a role in the pathogenesis of EDs. Several previous studies have investigated the association between the 5-HTTLPR polymorphism and bulimia nervosa (BN), though the results of these investigations have been unclear. The aims of this meta-analysis were (1) to investigate the association between 5-HTTLPR and BN under different models (i.e. dominant, recessive and additive); (2) extend upon previous meta-analyses by including recently published studies. Method: PsychINFO, ISI and PubMed databases were searched for studies published up until May 2011. Ultimately, eight articles were included. Data was pooled using both random and fixed effects models. Results: Using a fixed effects model, a trend was observed for the short allele conferring risk for BN (p = 0.066). However, this trend was lost once data was analyzed using a random effects model (p = 0.183). Hence, there was no association found between the 5- HTTLPR polymorphism and BN. Discussion: Considering a dominant effect, the short allele showed a slight trend in conferring risk for BN although no statistical significance was observed. However, this does not detract from recent research suggesting that the 5-HTTLPR polymorphism is one contributor within the serotonergic system that may be responsible for the phenotypic variability of psychopathological symptoms observed in patients diagnosed with BN. This should continue to be an area of further research.

578F

An association study of the serotonin 1B receptor gene with suicidal behavior in an population isolate from Colombia. *M. Cuartas[1,2], C. Palacio[2], J. Garcia[2], M. Duque[2], G. Montoya[2], G. Bedoya[2], C. Lopez[2], CODI Grant. University of Antioquia.* 1) Psychology , University of SanBuenaventura , Medellin, Colombia, san Benito 56C Nro. 51 - 90; 2) University of Antioquia, Medellin, Colombia. calle 67 No. 53 - 108.

Background: 5-HT1B Receptor Gene interacts with inhibitory G-proteins to decrease adenylyl cyclase (AC) activity. Polymorphisms in the gene for 5-HT1B, including a common G861C marker (rs6296)has been associated with several psychiatric phenotypes;and postmortem brain studies suggest that abnormalities in serotonin 1B may be associated with suicide. Methods: in a case- control study, we tested for genetic association between the 5-HT1B G861C polymorphism and suicide associate with major depression, using a logistic regression and analyzed multiple inheritance models (co-dominant, dominant, recessive, over-dominant and log-additive), and analysis of interactions (gene-gene or gene-environment). Results: The “G” allele of a polymorphic gene at the HTR1B locus was associated with suicide behavior in patients with major depression personality disorders[(OR: 1.63 P-value: 0.03) n = 372]. Conclusions: This finding suggests that allelic variability at the HTR1B locus may be associated with suicide assocaited to major depression. This analysis was applied to historically related local population which currently are considered to be genetically isolated.

579F

Gene Regulation by Drugs Used to Treat Mood Disorders. *X.Y. Deng[1], K. Doudney[1], P.C. McHugh[1], P.R. Joyce[2], M.A. Kennedy[1].* 1) Department of Pathology, University of Otago, Christchurch, New Zealand; 2) Department of Psychology and Psychological Medicine, University of Otago, Christchurch, New Zealand.

Antidepressant and mood stabilizer drugs are the main treatments for mood disorders including major depressive disorder and bipolar disorder.Although widely used, the mechanisms of action of these drugs are not well understood. This project seeks to establish a model system in which we can explore specific effects of such drugs on gene regulation. Findings in this area using animal models of mood are often conflicting and confusing, and we sought to develop a simple, easily manipulated model system with a relevant cellular context. Initial work focused upon promoter regions of candidate genes chosen from the literature and previous results from our laboratory. Nineteen constructs were investigated in the rat serotonergic cell line RN46A, using a luciferase reporter assay and a commonly prescribed SSRI antidepressant paroxetine. Although significant differences in expression were noted between drug-exposed and control cell cultures, the system proved inconsistent,despite considerable care in experimental design and execution. For a broader, more reliable approach, real-time quantitative PCR was utilised to detect drug-induced gene expression differences in RN46A cells. Five drugs were used: the antidepressants paroxetine, citalopram and nortriptyline; the antipsychotic haloperidol; and the mood stabilizer sodium valproate(VPA). Of five potential reference genes, Actb, G6pd and Rnf4 were stably expressed over differing treatments. We screened 39 candidate genes for expression changes attributable to drug exposure. The most striking results included a reproducible up-regulation of two genes in the biopterin pathway by the drug VPA. Members of this pathway are involved in the biosynthesis of neurotransmitters,and have previously been implicated in the biology of depression. Another gene, the serotonin receptor 2A (Htr2a) was significantly down-regulated by the SSRIs paroxetine and citalopram. Subsequently, western blotting confirmed increased protein expression of one of the upregulated biopterin pathway genes in treated cells, and detected at least three isoforms recognised by a commercially sourced antibody. Collectively, these data established the biopterin biosynthesis protein and a number of other proteins in the aetiology of mood disorders and their treatment, and provide a basis for further study of the effects of valproate and antidepressants in a mammalian setting.

580F

Evolution of Cognition in Humans is Linked with Down regulation of the Calreticulin Gene. *E. ESMAEILZADEH GHAREHDAGHI[1], M. Ohadi[1], E. ESMAEILZADEH GHAREHDAGHI[2], A. HEIDARI[1].* 1) Genetics Research Center, University of Social Welfare , Tehran, Tehran, Iran; 2) Tehran University of Medical Sciences.

Tissue-specific expression of the CALR gene in the brain gray matter in late-adolescence and early adulthood coincides with the expression of the psychoses phenotypes. Indeed, increased expression of the chaperone genes in the prefrontal cortex has recently been reported in patients affected by schizophrenia. We have recently reported cases of psychosis-associated mutations in the CALR gene promoter. One of those mutations at -48 was found to increase the expression of the gene in comparison with the wild type sequence. A recently identified mutation at -220 reverts the conserved block harboring nucleotide -220 to the ancestral type, and has an approximate prevalence of 0.7% in psychoses. In this study, we analyzed the functional implication of this mutation in the human neuroblastoma cell line BE(2)-C, and non-neural Human Embryonic Kidney 293 (HEK-293), and show that the -220A mutation results in a constitutive increase in the expression of the CALR gene($p<0.0003$). We checked homology of the first 1000-bp CALR promoter sequence across species, and found that nucleotide -220C is the only human-unique nucleotide in that stretch. The -220A mutation ,on the other hand, co-occurs with severe cognition deficit in humans, and is the rule across the species except humans. To our knowledge, the -220A mutation is the first reported instance of a cognition deficit-associated mutation which reverses the human promoter to the primitive type. It may be speculated that, at least the basal transcription of the CALR gene, relating to the proximal promoterregion, has been decreased during the process of evolution to humans.

581F

Examination of Serotonin Transporter Sequence Variation and Gene Expression in African-Americans. *M. Vijayendran[1], C. Cutrona[4], S.R.H. Beach[3], G.H. Brody[3], D. Russell[4], R.A. Philibert[1,2].* 1) Psychiatry, University of Iowa, Iowa City, IA; 2) Neuroscience and Genetics Programs, University of Iowa, Iowa City, IA; 3) Institute for Behavioral Research, The University of Georgia, Athens, GA; 4) Iowa State University, Ames, IA.

Although sequence variation in the coding sections of genes typically garners the most attention, other forms of genetic variation, in the form of both genetic and epigenetic variation may have greater impact on gene expression and cellular behavior. The serotonin transporter (SLC6A4) is a critical regulator of serotonergic neurotransmission and its protein product is the main target for the vast majority of anti-depressants on the market. Unfortunately, despite repeated examinations, the mechanisms underlying the regulatory control of the serotonin transporter and their relationship to major depression remain inadequately characterized, in particular with respect to depression in populations containing the Very Long (VL) gene variant. In order to better determine the relationship between the serotonin transporter linked polymorphic region (5HTTLPR) variation and gene transcription, we conducted genotyping and splice variant expression analyses using biomaterial from 180 lymphoblast cell lines contributed by African-American subjects who participated in the Family and Community Health Studies. For females, the expression levels of both exons one and eight were more statistically significant ($p<0.007$ and $p<0.051$ respectively) as compared to the expression levels of males ($p<0.17$). Also, for females, there is a significant relationship between genotype and SLC6A4 expression as measured by the exon one probe ($p<0.001$, ANOVA), but not the exon eight probe ($p<0.051$, ANOVA). Consistent with prior analyses, there was only a modestly strong correlation between exon one and exon eight transcript levels which taken together with the prior results just that the exon one assay more accurately captures the effects of the 5HTTLPR variation and that several splice variants may be expressed inside the lymphoblast cell lines. Finally, despite suggestions that the VL allele may result in greater transcriptional activation than the more common long (L) allele, we found no difference in gene expression between those cell lines possessing the VL or just the L allele. We conclude that variation in the serotonin transporter is an important regulator of transcription and that a fuller understanding of that regulation necessitates measuring more than one splice variant.

582F

Brain transcriptome analysis by RNA-Seq reveals novel genes differentially expressed in bipolar disorder. *N. Akula[1], J.R. Wendland[1], G. Laje[1], K. Choi[2], S.K. Sen[3], H.C. Bravo[4], S.D.D. Wadleigh[1], F.J. McMahon[1].* 1) Mood and Anxiety Disorders Section, Human Genetics Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA; 2) Department of Psychiatry and Program in Neuroscience, Uniformed Services University of the Health Sciences, Center for the Study of Traumatic Stress, Bethesda, MD, USA; 3) Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA; 4) Center for Bioinformatics and Computational Biology, University of Maryland, College Park, MD, USA.

RNA-Seq is a relatively new technique that leverages high-throughput sequencing technologies to provide estimates of transcript abundance at a precision level not accomplished by previous hybridization-based microarray assays. RNA-Seq also enables the detection of novel, low abundant transcripts, allele-specific expression, alternative splicing, and post-transcriptional modifications, such as RNA editing. In this preliminary study, we used RNA-Seq to characterize the brain transcriptome in individuals with bipolar disorder. We performed deep-sequencing (~130M paired-end reads) of high quality total RNA (RNA-Integrity Number ⁄ 7) extracted from the prefrontal cortex (PFC) of post-mortem brains from 4 bipolar I disorder cases and 5 age- and sex-matched, psychiatrically-healthy controls, obtained from the Stanley Medical Research Institute. Fragmentation, cDNA library preparation, and PCR enrichment of the target RNA was followed by paired-end sequencing on the Illumina GA-IIx system. The resulting reads were mapped and aligned to the reference genome (hg18) using TopHat (Trapnell et al. 2009). Preliminary analysis of differential expression of known (NCBI-RefSeq and Ensemble) and alternate transcripts was performed using Cuffflinks (Trapnell et al. 2010), in parallel with HTSeq (http://www-huber.embl.de/users/anders/HTSeq/) and DESeq (Anders and Huber 2010) analyses. The transcripts with raw counts ≤ 50 were excluded from downstream analysis. Differentially expressed transcripts were identified based on a p-value ≤ 0.05, adjusted for multiple testing. These transcripts were further analyzed using DAVID (Dennis et al. 2003; Huang da et al. 2009) in order to detect enriched functional groups of genes. A total of 172 transcripts were differentially expressed by as much as tenfold; of these 154 transcripts were down-regulated in the PFC of individuals with bipolar disorder. Differentially-expressed transcripts showed significant enrichment of the Gene Ontology terms such as “neuronal development,” “apoptosis,” “G-protein coupled receptors,” and “calcium ion homeostasis.” Further analyses to identify novel alternate transcripts that are differentially expressed in the PFC of individuals with bipolar disorder are underway. These preliminary results demonstrate that RNA-Seq may reveal gene expression changes in biologically-relevant pathways that were not detected consistently in previous microarray-based expression profiling studies.

583F

Gene RNA expression study and eQTL analysis for genes located at 13q13-q14 that showed reproductible linkage with schizophrenia and bipolar disorder in multigenerational families from Eastern Quebec. *Y.C. Chagnon, A. Bureau, J. Croteau, C. Mérette, M-A. Roy, M. Maziade.* Dept Molec Psychiatry, Laval University Robert-Giffard Res Ctr, Quebec, PQ, Canada.

BACKGROUND. We observed in our multigenerational pedigrees from the Eastern Quebec region different significant linkages with schizophrenia (SZ) and bipolar disorder (BP) in 11 chromosomal regions. Among these, a linkage at 13q13-q14 (NPLpair=2.62) proved to be replicated in a second sample of the same origin (NPLpair =3.36; combined NPLpair=5.21). OBJECTIVE. To identify possible candidate genes for this linkage at 13q13-q14 by analysing gene RNA expression for subjects from the family showing the strongest linkage at 13q13-q14. SAMPLES. 21 subjects including 4 BP, 2 SZ and 15 unaffected relatives were selected from one large pedigree showing the strongest linkage at 13q13-q14. METHODS. We evaluated RNA expression in the immortalized lymphocytes using Nimblegen whole-genome expression microarray (12 x 135K) with a MAUI hybridisation and washing system, and a Tecan 2 uM scanner. Microarray scan results were interpreted using the NimbleScan software (Roche). RESULTS. Suggestive differences between cases and non-affected relative subjects were observed for several genes on chromosome 13 including the already known candidate gene DAOA/G72 at 13q34. A variance component linkage analysis was performed on the log normalized RNA expression values of the genes showing suggestive differences between cases and non-affected relative subjects using 44 microsatellite markers covering the entire chromosome 13. The strongest linkage signals we observed ($0.0006<p<0.004$) were between the expression level of predicted gene AK095260, located at 110 Mb (5 Mb telomeric to DAOA) and D13S1493, D13S1248 and D13S1312 located respectively at 32.9, 39.7, and 44.8 Mb, the later 2 within our 13q13 linkage region, suggesting a possible interaction between the two linked regions 13q13 and 13q34 that we observed in our families. In summary, this preliminary analysis confirmed linkage results at 13q13 and provide promising leads for candidate genes selection and analysis at 13q13-q14 in our population.

584F

Comparative co-expression network analysis of RNA-sequencing data from post-mortem brain tissue of patients of schizophrenia, bipolar disorder and healthy controls. *S. Hong[1], J. Chen[2], J. Xu[3], F. Xu[3], Y. Zhang[3], Z. Peng[3], M. Xiong[1], X. Chen[2].* 1) Department of Biostatistics, University of Texas Health Science Center, Houston, TX.,; 2) Department of Psychiatry and Virginia Institute for Psychiatric and Behavioral Genetic, Virginia Commonwealth University, Richmond, VA 23219; 3) BGI-Shenzhen, Shenzhen, China.

We have recently finished mRNA sequencing for 82 post-mortem brain samples from the collection from Stanley Medical Research Institute, of them 31 were from schizophrenia patients, 25 were from bipolar disorder patients and 26 were from healthy controls. In this study, we take advantage of the rich RNA-seq data to explore the co-expression networks amongst the 3 groups. After base-calling assessment, qualified reads are aligned to human genome reference, and mapped to known genes and transcripts. The levels of gene expression are calculated with the RPKM algorithm and normalized by sequencing batch, total number of reads per sample, brain pH, sex and age. Differential expressions amongst the groups are evaluated by t test. We find that there are 405 differential expressed genes between cases (schizophrenia and bipolar) and controls. We then use sparse graphical model to construct co-expression networks of these 405 differentially expressed genes for schizophrenia, bipolar disorder and normal tissues samples, respectively. The co-expression networks for schizophrenia, bipolar disorder and control samples share 405 common nodes, but they have considerably different numbers of edges (378, 214 and 228 respectively). We then construct differentially expressed networks (DENs) amongst the groups by subtracting the common nodes and edges between the comparing groups. The DEN between schizophrenia and control consists of 271 nodes and 340 genes and the DEN between bipolar disorder and control has 166 nodes and 180 edges. From the DENs, we have identified multiple modules that are involved in neuron development and differentiation, synaptic transmission, cell projection organization and neuronal cell morphogenesis. A total of 11 genes playing key roles in the DENs are reported to be associated with schizophrenia and bipolar disorder in the literatures.

585F

The analysis of two SNPs of the reelin gene in a South African Autistic and Control population. *Z. Arieff[1], J. Rajan Sharma[1], F. October[1], M. Davids[1], M. Kaur[2], L. van der Merwe[1,3].* 1) Biotechnology, University of the Western Cape, Bellville, Western Cape, South Africa; 2) Computational Bioscience Research CenterTechnology, King Abdullah University of Science and Technology, Thuwal 23355-6900, Kingdom of Saudia Arabia; 3) Biostatics Unit, Medical Research Council, PO Box 17090, Tygerberg, 7505, South Africa.

Autism is a disorder characterized by a triad of impairments; namely impairment in social interaction, impaired communication skills and restrictive and repetitive behavior. Magnetic resonance imaging studies show that autism is a neurological disorder affecting the development of the brain. The reelin gene (RELN) has been identified as necessary for proper formation of brain, which indicates that RELN abnormalities could contribute to the etiology of several neurogenetic diseases such as autism. The gene is located on chromosome 7, a chromosome that has demonstrated suggestive association to autism spectrum disorders (ASD). Two SNPs on RELN, intronic rs736707 and exonic rs3622691, were investigated for evidence of association in ASD in South African autistic and healthy population. We have followed DSM-IV criteria and the screening for autism was carried out using CARS. Genomic DNA was isolated from cheek cell swabs from three distinctive South African ethnic groups (Black, Caucasian and Mixed ancestry). The Taqman ®Real-Time PCR and Genotyping assay was utilized to ascertain the distribution of alleles and genotypes. Our study group consisted of 141 unrelated autistic children and 217 unrelated control children. More than 90% were successfully typed for each SNP. The genetic association with autism was tested with logistic regression models, adjusting for gender and ethnicity. We tested association of autism with genotypes, alleles and haplotypes, inferred from the genotypes. We observed a significant additive (G) allelic association with autism (OR=0.67; 95% CI=0.45-0.98; p=0.0413, after adjusting for race and gender) for intronic SNP rs736707 of RELN in the total SA group. The G allele of SNP rs736707 is under-represented in the autistic group, indicating a possible protective effect. In addition, haplotype analysis showed that the A-G haplotype (rs736707-rs3622691) is significantly (p =0.0388; 64% versus 54%, respectively in autistic and control group after adjusting for ethnicity and gender) more prevalent in the autistic population than in control population. In conclusion, these results are interesting but require further investigation.

586F

Brain-derived neurotrophic factor gene in Thai major depressive patients. *D. Buntup[1], S. Nuengkeaw[2], N. Sitdhiraksa[3], V. Praphanphoj[4], P. Govitrapong[2,5].* 1) Addiction Studies Program, ASEAN Institute for Health Development, Mahidol University, Nakhon Pathom, Phuthamonthon, Thailand; 2) Research Center for Neuroscience, Institute of Molecular Biosciences, Mahidol University; 3) Department of Psychiatry, Faculty of Medicine Siriraj Hospital, Mahidol University; 4) Center for Medical genetics research, Rajanukul Institute, Bangkok, Thailand; 5) Center for Neuroscience and Department of Pharmacology Faculty of Science, Mahidol University, Bangkok, Thailand.

The results from Thai national survey of epidemiology in 2003 found that major depressive disorder (MDD) is the major population among psychiatric disorders. However, the factors that influence of this disease remains unclear. Many researchers have focused on the study of the genetic risk factors. Brain-derived neurotrophic factor (BDNF) is a member of the nerve growth factor family and found in the brain of mammalian. Previous studies indicated that functional polymorphism of the BDNF gene plays an important role in development of MDD. We examined whether genetic variations in the BDNF (rs6265, Val66Met) could be associated with increased susceptibility for MDD by using a PCR-RFLP and gel electrophoresis. Blood samples were collected from 189 of patients and 184 of controls. The data showed that no significant differences were found in the frequencies of the alleles and genotypes of tested BDNF gene between patients and normal control group. This study suggests that polymorphisms in the BDNF gene may not play a major role in the genetic vulnerability for MDD in our studied population. **Acknowledgements:** This study was supported by a Mahidol University Research Grant to PG and NS and a TRF-senior Research Scholar Fellowship from the Thailand Research Fund to PG.

587F

Neurexin 3 is associated with nicotine dependence. *E. Docampo[1], M. Ribases[2], M. Gratacos[1], R. Rabionet[1], C. Sanchez-Mora[2], C. Arribas[3], S. Moran[3], M. Montfort[3], G. Nieva[4], E. Bruguera[4], M. Casas[4], X. Estivill[1].* 1) Genes and Disease Program, Center for Genomic Regulation (CRG-UPF), CIBER en Epidemiología y Salud Publica (CIBERESP), Barcelona 08003, Catalonia, Spain; 2) Psychiatric Genetics Unit, Vall d'Hebron Research Institute (VHIR), Barcelona, Spain; 3) Barcelona Genotyping Node, CeGen-ISCIII, Barcelona, Catalonia, Spain; 4) Department of Psychiatry, Hospital Universitari Vall d'Hebron, Barcelona, Spain.

Nicotine dependence has been previously associated with Neurexin 1 gene, and prior studies on Neurexin 3 point towards its pivotal role in alcohol dependence, illegal substance abuse as well as in obesity. In order to evaluate the possible role of neurexin 3 in nicotine dependence susceptibility, we evaluated tagSNPs and candidate SNPs located along the genomic region that contains the neurexin 3 gene (NRXN3). The study included 157 samples from smoker individuals and 1,439 controls. Nicotine dependence was assessed by Fagerström index and the number of cigarettes smoked per day. All participants were of Caucasian origin. Based on the CEU HapMap genotyped SNPs, we selected Tag SNPs covering the genomic region of NRXN3 (1,170 Kb), and we forced Haploview software to include potential functional variants located near splice sites as well as SNPs that had been previously associated with disease. Case-control association analyses were performed using PLINK software. Haplotype analysis was performed with SNPassoc package of R software. After quality control, 35 SNPs and 153 cases and 1,425 controls were included for the analyses. Allelic and genotypic (log-additive model) association test showed that four SNPs were nominally associated with a lower risk of being a smoker. Based on the linkage equilibrium (LD) of the region, we defined 10 blocks. The haplotype analysis showed that one block of 35 Kb, and consisting of three of the significant SNPs in the single-SNP analysis, was also associated with lower risk of being a smoker (OR = 0.50 [0.31-0.80]; permuted P = 0.037). These preliminary results show the potential involvement of genetic variants in NRXN3 with nicotine dependence. All the significant tagSNPs and are located in introns, and although their potential biological effect is unknown, we cannot discard that they in are in LD with a true causing functional unknown variant. Finally, the replication of these results in a larger cohort of nicotine dependence is warranted.

588F

Characterization of three intronic functional polymorphisms conferring risk of schizophrenia. *A.M. Dulencin[1,2,3], N.S. Wratten[4], P.G. Matteson[1], E. DiCicco-Bloom[5], L.M. Brzustowicz[4], J.H. Millonig[1,4,5].* 1) Center for Advanced Biotechnology and Medicine, University of Medicine and Dentistry of New Jersey, Piscataway, New Jersey 08854, USA; 2) Graduate School of Biomedical Sciences, University of Medicine and Dentistry of New Jersey, Piscataway, New Jersey 08854, USA; 3) Biotechnology Training Program, Rutgers The State University of New Jersey, Piscataway, New Jersey 08854, USA; 4) Rutgers University Department of Genetics, 145 Bevier Road, Piscataway, New Jersey 08854, USA; 5) Department of Neuroscience and Cell Biology, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, New Jersey 08854, USA.

Schizophrenia is a common and a serious neuropsychiatric disorder with a strong genetic basis. Previous reports demonstrated significant linkage for schizophrenia to chromosome 1q22 in a sample of 22 Canadian medium-sized and multi-generational pedigrees. Further mapping and association studies identified Nitric Oxide Synthase 1 Adaptor Protein 1 (NOS1AP) as a likely susceptibility gene. NOS1AP is involved in NOS, PSD95 and NMDAR signaling which have been implicated to play role in schizophrenia pathology.

Recent experiments have identified three SNPs in intron 2 associated with schizophrenia: rs12742393 (A and C), rs1415263 (C and T), and rs4145621 (C and T). Alleles A, T, and C, respectively, are overtransmitted to affected individuals, and are also likely to be inherited together as they are in strong LD with each other (r2>0.73). Luciferase assays determined that all three intronic SNPs are functional, and that they are functional in the same way. Transfection constructs used in the assays contained 850bp of a conserved intronic sequence encompassing each allele followed by a NOS1AP promoter with a luciferase reporter gene. The schizophrenia-associated alleles increased levels of a luciferase reporter expression in rat primary cortical neurons ($p<0.01$) and in human neuronal cells (rs12742393 only, $p<0.05$). Increased expression of the schizophrenia-associated alleles for each SNP was especially noteworthy since the pairs of plasmids for each SNP differed by only a single nucleotide. Furthermore, electrophoretic mobility shift assays demonstrated that over-transmitted alleles of all three SNPs bind nuclear factors more avidly than the non-associated ones, providing a molecular explanation for the increase in expression. Moreover, bioinformatic analysis and mass spectrometry identified candidate proteins that bind better to the schizophrenia-associated alleles. Considering the unbiased approach of identification, it is compelling that many of these proteins are transcription factors and mRNA regulators known to directly participate in pathways involved in schizophrenia risk.

Identification and validation of these factors will not only link NOS1AP with pathology and phenotypic manifestations of schizophrenia, but will also suggest potential drug targets for personalized therapies.

589F

Genetic analysis of GABRB3 in autism, schizophrenia, and heroin addiction. *C-C. Huang[1,2], SS-F. Gau[3,4], D-L. Liao[5], Y-Y. Wu[6], H-M. Tsai[7], C-H. Chen[1,7].* 1) Institute of Medical Sciences, Tzu-Chi University, Hualien, Taiwan; 2) Department of Anesthesiology, Buddhist Tzu-Chi General Hospital, Hualien, Taiwan; 3) Department of Psychiatry, National Taiwan University College of Medicine, Taipei, Taiwan; 4) Department of Psychiatry, National Taiwan University Hospital, Taipei, Taiwan; 5) Bali Psychiatric Center, Department of Health, Executive Yuan, Taipei, Taiwan; 6) Department of Psychiatry, Chang Gung Memorial Hospital- Linkou Medical Center, Chang Gung University College of Medicine, Tao-Yuan, Taiwan; 7) Division of Mental Health and Addiction Medicine, Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Taiwan.

Objectives: Genetic variants of the GABRB3 gene have been shown to be associated with psychiatric diseases such as autism, schizophrenia, and drug addiction. The aim of this study was to search for functional genetic variants at the promoter of the GABRB3 gene and investigate their association with autism, schizophrenia, and heroin addiction in Taiwanese patients. Methods: We resequenced the 5' region of the GABRB3 gene in our subjects and carried out reporter gene activity assay of the haplotypes derived from genetic variants identified in this study. In addition, we conducted genetic association of a tag SNP (rs4906902) at the promoter region among autism, schizophrenia, and heroin addiction, and control subjects. Results: We identified three SNPs (rs4906902, rs8179184, and rs20317) at the 5' region of the GABRB3 gene which formed strong linkage disequilibrium in our population. Reporter gene activity assay revealed that two major haplotypes derived from these three SNPs had significant differential reporter activities. We observed significant differences in genotype and allele frequency distributions of the tag SNP rs4906902 between heroin addicts and control subjects, but no association with autism and schizophrenia. However, further analysis showed a significant heterozygous advantage in male control subjects compared to male autism ($p=0.01$). Conclusions: Our data indicated that SNPs at the 5' region of the GABRB3 gene may confer differential expression of GABRB3 gene, and associate with subgroups of heroin addicts and autism, given the clinical and genetic heterogeneity in autism patients. Further investigation will be of great value to explore the nature of the gene.

590F

Genetic Examination in a Subset of Autism Spectrum Disorders with Aggression. *J. Lee Robinson[1], D. Ma[1], A. Griswold[1], P. Whitehead[1], L. Nations[1], J. Haines[2], M. Cuccaro[1], M. Pericak-Vance[1].* 1) University of Miami, Miami, FL; 2) Vanderbilt University, Nashville, TN.

Genetic and phenotypic heterogeneity has been a widely recognized obstacle to understand the genetics of autism spectrum disorders (ASDs). It is estimated that up to 30 percent of individuals with ASDs display some form of aggression, potentially representing a unique subset of ASDs. Twin and adoption studies have demonstrated that genetic and environmental factors play a role in aggression. In this study, we targeted a subset of ASD families where the affected individuals display moderate to severe aggression. A genetic examination was conducted at both SNP-based association level on aggression-related genes and copy number variation (CNVs) level to see if aggression could serve as an endophenotype to dissect the heterogeneity of ASDs. A literature review of previously identified genes linked to aggression either through twin, association, or animal model studies revealed 21 potential candidate genes for analysis. Using ratings on the Autism Diagnostic Inventory-Revised (ADI-R) self-injury, aggression to caregivers and aggression to non-caregivers questions, we identified a total of 468 unrelated affected individuals with idiopathic ASD who displayed either moderate to severe aggression from 468 families. Families were ascertained through University of Miami, Vanderbilt University and the Autism Genetic Resource Exchange. Using data from high density SNP arrays, family-based association analysis was carried out using Combined Association in the Presence of Linkage test (CAPL). CNV detection was carried out on samples genotyped by Illumina 1M chip. High confidence CNVs were detected by two distinct algorithms. 199 SNPs fell into the 21 candidate gene regions. Only six SNPs presented a p-value of less than 0.05 [0.014, 0.043]. None of these survived the multiple testing corrections. A total of 327 case-unique CNVs were identified among the 129 patients who were genotyped using illumina 1M chip. However, none of the 21 candidate genes appear to be disrupted by these CNVs. In addition, a search of disrupted genes within a subset of 66 patients who had the most severe form of self-injurious behavior provided no evidence for common genetic changes. Based on these results, the subset of ASDs with aggressive behavior does not present its genetic independence indicating it may not serve as a good endophenotype to assist in dissecting the genetic heterogeneity in ASDs and suggests the potential genetic complexity of aggression itself in ASDs.

591F

***CNR1* and *DGCR2* genes interaction may have an effect upon pathogenesis and treatment response in schizophrenia.** *V.C. Mrad[1,2], A.F.S. Filho[2,3,4], A. Gadelha[2,3,4], V.K. Ota[1,2], M.L. Santoro[1,2], F.T.S. Bellucco[1], L.M.N. Spíndola[1,2], D.M.L.V. Rocha[2,3,4], D.M. Christofolini[1], J.J. Marí[4], M.I. Melaragno[1], M.A.C. Smith[1], R.A. Bressan[2,3,4], S.I.N. Belangero[1,2].* 1) Genetics Division, Department of Morphology and Genetics, Federal University of Sao Paulo (UNIFESP), Sao Paulo, Brazil; 2) Interdisciplinary Laboratory of Clinical Neurosciences (LiNC), Sao Paulo, Brazil; 3) Schizophrenia Program (PROESQ), Department of Psychiatry, Federal University of Sao Paulo (UNIFESP), Sao Paulo, Brazil; 4) Department of Psychiatry, Federal University of Sao Paulo (UNIFESP), Sao Paulo, Brazil.

The pathogenesis of schizophrenia involves a complex interaction between genetic vulnerabilities and environmental risk factors, like cannabis use. The endocannabinoid system is involved in this process and has implications not only in its psychopathology, but also in the pharmacological response to antipsychotic drugs. The *CNR1* gene, that encodes the cannabinoid receptor 1, is on chromosome 6q14, a susceptibility locus for schizophrenia, as well 22q11.2 region, that includes several genes as the *DGCR2* (DiGeorge syndrome critical region gene 2), an adherence receptor protein coding. We aimed to investigate the interaction between *CNR1* and *DGCR2* and its influence on treatment response and on schizophrenia etiology. We recruited 143 schizophrenia patients (71 refractory and 72 non refractory). They were assessed and diagnosed according to DSM-IV and genotyped for the polymorphisms *CNR1* rs806380 and *DGCR2* rs807759 and rs2073776 by real time PCR. It was correlated to schizophrenia diagnosis, treatment refractoriness and cannabis use. Genetic stratification analysis was made in order to correct statistical confounding ethnic factors. We found a significant association between the interaction of the *CNR1* and *DGCR2* polymorphisms and the treatment refractoriness. Carriers of AA(rs807759) and AG(rs806380) genotypic combination were more frequent than the GG (rs807759)/GG (rs806380) carriers in non-refractory patients (p=0.018; OR= 0.249). Moreover, there was a notable association between the interactions of these two genes with schizophrenia etiology, showing that the AG (rs806380)/TT (rs2073776) carriers were more frequent than the CNR1 GG (rs806380)/CC (rs2073776) carriers in schizophrenia patients (p=0.011; OR=3.365). We found no kind of association with the cannabis use or the polymorphisms individually. These results showed an interaction between *CNR1* and *DGCR2* genes, where AG-genotype associated with AA-genotype, respectively, seem as a protection factor for antipsychotics refractoriness. Our data suggest these genes seem to play a role on treatment response and they could be prediction factors in the pharmacogenomics. The association with the etiology of schizophrenia reinforces the literature, suggesting a role of *CNR1* and *DGCR2* in the pathophysiology of schizophrenia. Thus, it would be interest to investigate these two genes even more in order to unravel the action mechanism of antipsychotics in the brain and validate this data in different populations.

592F

Results from dense SNP genotyping and CNV analysis of the 9q22-q31 CRI in ASP from the NIMH and NCRAD cohorts. *R.T. Perry, H. Wiener, R.C.P. Go.* Dept Epidemiology, Assistant Professor, Univ Alabama at Birmingham, Birmingham, AL.

Background: Our original linkage scan of Alzheimer's Disease (AD) families followed by additional microsatellite genotyping confirmed and narrowed a 9q22 signal to 11.5 cM. Four candidate genes located between 2-6 Mb proximal to this peak have been reported from candidate gene and genome wide association studies to be significantly associated with AD and we have confirmed the association of one of these genes, *NTRK2*, in a separate set of families from NCRAD. Methods: We performed dense genotyping of this 18 Mb region using haplotag SNPs and unblocked SNPs from HapMap. Additional SNPs located in reported CNVs from this region were also identified and chosen. Finally, SNPs from *TOMM40*, *PICALM*, *CR1*, and *CLU* were similarly identified and genotyped. A total of ~ 5700 SNPs in 4500 individuals were genotyped using the iSelect platform from Illumina. Our preliminary genotyping results from family based association testing suggested several signals and CNVs from the 9q22 region to follow-up. Results: Using Family Based Association Testing, several signals from the 9q22 region were just below the level of significance (10-4) under an additive model after multiple testing. The only signal that reached significance was from *TOMM40* (10-13). CNV analysis was performed using PennCNV and ~15 % of the individuals were identified with at least one CNV or indel in the 9q22 region. Approximately a dozen “hot spots” appeared to be present in most of these individuals. Initial association testing of one of these “hot spots” was marginally significant (p=0.06). Conclusion: Dense SNP genotyping of the 9q22 candidate region and six Mb of genome proximal to the candidate region identified several signals that are just below the threshold of significance to AD in ASP from NIMH and NCRAD cohorts. There are also several CNVs present and preliminary testing indicates there is suggestive association with AD. Using the CNV information and the presence of functionally relevant genes and/or regulatory elements, we have begun selecting the most promising signals from the 9q22 region to follow-up by sequencing in selected families.

593F

Paraxial mesoderm developmental genes in Chiari type I malformation: A genetic case-control association study in the Spanish population. *A. Urbizu Serrano[1], MA. Poca[2], C. Toma[3], A. Berlanga[1], J. Sahuquillo[2], B. Cormand[3], A. Macaya[1].* 1) Pediatric Neurology, Institut de Recerca Hospital Vall d'Hebron, Barcelona, Barcelona, Spain; 2) Neurosurgery, Hospital Vall d'Hebron, Barcelona, Barcelona, Spain; 3) Department of Genetics, Faculty of Biology, University of Barcelona, Barcelona, Barcelona, Spain.

Chiari type I malformation (CMI) is a disorder characterized by hindbrain overcrowding into an underdeveloped posterior cranial fossa, often causing progressive neurological symptoms. The estimated prevalence is 1/1200, with a male to female sex ratio of approximately 1:2. Diagnosis relies upon radiological evidence of downward herniation of the cerebellar tonsils, least 3-5 mm through the foramen magnum. The etiology of CMI remains unclear and is most likely multifactorial. A genetic contribution to CMI is suggested by familial aggregation and twin studies; however, the genes involved are presently unknown. Experimental models and human morphometric studies have provided evidence of occipital bone underdevelopment in CMI, suggesting an underlying paraxial mesoderm insufficiency. We performed a case-control association study of 49 candidate genes involved in early paraxial mesoderm development in a sample of 415 patients and 524 sex-matched controls. According to genetic coverage parameters we selected 384 tagSNPs from genes that participate in the signaling gradients that occur during segmental patterning of the vertebrae and included genes from the FGF8/Wnt/retinoic acid pathways and from BMP, Notch, Cdx and Hox pathways. In addition, genes from the VEFG family, critical for placental development in the 4th week embryo and genes responsible for two conditions frequently associated with CMI, NF1 and STAT3, were also included. Single-marker analysis identified nominal associations of the phenotype with eighteen SNPs in fourteen genes (CDX1, FLT1, RARG, NKD2, MSGN1, RBPJ1, FGFR1, RDH10, NOG, RARA, LFNG, KDR, ALDH1A2, BMPR1A). None of these overcame corrections for multiple comparisons, although we obtained p-values <0.002 in three genes. In contrast with previous reports in Chiari malformation type II, we did not find evidence that common variants in some of the genes involved in paraxial mesoderm or placental development in the human embryo may confer genetic susceptibility to develop the complex disorder CMI.

594F

Association analysis of Casein kinase2 with Schizophrenia in the Japanese Population. *A. Yoshikawa[1,2], F. Nishimura[1,3], M. Tochigi[1], Y. Kawamura[4], T. Umekage[5], T. Sasaki[6], K. Kasai[1], C. Kakiuchi[1].* 1) Department Neuropsychiatry, Medicine, University of Tokyo, Japan; 2) Department of Psychiatry, Fukui Memorial Hospital; 3) Department of Psychiatry, Saitama Kounan Hospital; 4) Department of Psychiatry, Yokohama Clinic; 5) Division for Environment. Health and Safety, University of Tokyo; 6) Department of Halth Education, Graduate School of Education, University of Tokyo.

Background: Schizophrenia is a devastating mental illness with worldwide lifetime risk of approximately 1%. The onset of the disease starts in young adolescence. The pathophysiological mechanisms underlying schizophrenia have not been fully elucidated. However, according to the previous family and twin studies, there is a strong evidence for a genetic contribution to the etiology. The NMDA receptor (NMDAR) is involved in the glutamatergic synatptic transmission and plays a critical role in synaptic plasticity, learnign and memory. A hypofunction of NMDA receptor has been implicated in the pathophysiology of schizophrenia. By the postmortem studies, reduced NR2B mRNA expression in hippocampus has been reported. NMDARs are composed of two NR1 subunits and two NR2A or NR2B subunits in the brain of mammals. During the critical period, the composition of the NMDAR subunit changes dramatically from NR2B to NR2A, and it may contribute to neuronal maturation process. Recently Casein kinase 2 (CK2) is reported to regulate the `switching' of NMDAR subunit via endocytosis of NR2B, and the activity of CK2 reaches their highest level during the critical period. The reduced CK2 levels in the postmortem brain of the patients and the increased levels of CK2 by antipsychotic drugs were reported. Thus, we conducted the genetic association study between CK2 and schizophrenia. **Aim of the study:**To investigate the genetic association of Casein kinase2 with schizophrenia. **Methods:**We performed a case-control association study between CK2 and schizophrenia in 384 healthy controls and 384 age and sex matched patients with schizophrenia diagnosed by DSM-IV criteria. All the subjects were ethnically Japanese. Written informed consent was obtained from all the subjects. The Ethics Committees of the Faculty of Medicine, University of Tokyo approved the study. We selected the 19 tag SNPs from the JSNP database. Genotyping was performed using the fluorogenic 5' nuclease method (Taqman, ABI7900HT). We analyzed the association by using the Haploview programs. **Results:**We detected no evidence of significant association with any tested SNPs and haplotypes of CK2. **Discussion:**No significant association was found between CK2 and schizophrenia in the Japanese population. Assessing the association with well-phenotyped schizophrenic samples such as the onset age will be needed. It will be also interesting to examine the association of other genes regulating the NMDAR switching with schizophrenia.

595F

Rare and common CLU variants and susceptibility to Alzheimer disease. *K. Bettens[1,2], N. Brouwers[1,2], S. Engelborghs[2,3], J-C. Lambert[4], R. Vandenberghe[5], F. Pasquier[6], E. Rogaeva[7], P. St. George-Hyslop[7], P.P. De Deyn[2,3], P. Amouyel[4,6], C. Van Broeckhoven[1,2], K. Sleegers[1,2].* 1) Neurodegenerative Brain Diseases Group, Department of Molecular Genetics, VIB, Antwerpen, Belgium; 2) Institute Born-Bunge, University of Antwerp, Antwerpen, Belgium; 3) Department of Neurology and Memory Clinic, Hospital Network Antwerp Middelheim and Hoge Beuken, Antwerpen, Belgium; 4) INSERM U744, Institut Pasteur de Lille, Université Lille Nord de France, Lille, France; 5) Department of Neurology, University Hospitals Leuven and University of Leuven, Leuven, Belgium; 6) Université Lille Nord de France, CHR&U de Lille, Lille, France; 7) Centre for Research in Neurodegenerative Diseases, Departments of Medicine (Neurology) and Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada.

Background: Recent GWA studies provided compelling evidence for association of the clusterin gene (CLU) and Alzheimer disease (AD) susceptibility. In this study an in-depth genetic CLU analysis was performed on a Flanders-Belgian cohort. Secondly, findings were replicated in French and Canadian samples and meta-analyses were performed on rare and common CLU variants. Methods: Additional common variants at the CLU locus were genotyped. CLU was sequenced in Flanders-Belgian individuals (849 AD patients, 659 control individuals). Significant findings were followed up sequencing exons encoding the CLU beta-chain in French (1465 AD,716 controls) and Canadian samples (525 AD,520 controls). Allele frequencies of rare variants were collapsed across the beta-chain. All possible pathogenic variants in the beta-chain were meta-analyzed combining information on 6,740 AD patients and controls. Results: Five common CLU SNPs were nominally associated with AD in the Belgian cohort ($p<0.05$). Meta-analyses combining the Belgian, French and Canadian sample confirmed association for these variants (fixed-effect p-values from 0.001 to 0.045). Additionally, patient specific rare variants (MAF<1%) were found clustering in the CLU beta-chain in the Flanders-Belgian cohort. Interestingly, five were predicted to have possible and probable damaging effects on CLU protein function. Furthermore, we observed an almost four times increase of rare protein-affecting mutations in the CLU beta-chain in AD patients compared to variants found in controls only ($p=0.02$). Sequencing the CLU beta-chain coding exons in the French and Canadian samples identified 4 patient-specific predicted pathogenic missense mutations and one deletion previously identified in Flanders-Belgian patients, and one novel mutation in a French patient. Meta-analyzing our novel sequence information with previously published information on rare predicted pathogenic variants in the CLU beta-chain, strengthened the increased presence of rare pathogenic variants in the beta-chain in AD patients compared to healthy control persons (ORMH= 1.50 [95%CI 1.12-2.01], pMH=0.005). Conclusions: Fine-mapping previous CLU association signals, our findings suggest that both common and rare CLU variants are independently contributing to AD susceptibility in populations of different ethnicity. Our results call attention to the CLU beta-subunit and warrant further functional elucidation of rare CLU variants in disease.

596F

Genetic Association of Antidepressant Response and Meta-analysis of the Brain-Derived Neurotrophic Factor (BDNF) Val-66-Met Polymorphism in Obsessive-Compulsive Disorder (OCD). *G. Zai[1,2], C. Zai[1], E. Burroughs[3], J.L. Kennedy[1,2], M.A. Richter[1,2,3].* 1) Neurogenetics Section, CAMH, Toronto, ON, Canada; 2) Department of Psychiatry, Toronto, ON, Canada; 3) Department of Psychiatry, Sunnybrook Health Science Centre, Toronto, ON, Canada.

Background: Brain-derived neurotrophic factor (BDNF) has extensive effects on the nervous system including cell survival, differentiation, growth, and maintenance. It promotes synaptic plasticity and interacts with serotonergic neurons. The BDNF Val66Met polymorphism has been studied in obsessive-compulsive disorder (OCD) with mixed results. Other BDNF gene variants could play an important role in the etiology of OCD and could lead to changes in brain circuitry that would in turn cause variable response to antidepressants. Hypothesis: We hypothesized that genetic variation in BDNF may be associated with OCD and may predict serotonin reuptake inhibitor (SRI) response in OCD patients. Method: Meta-analysis of nine different association studies including our own between Val66Met and OCD was performed using CATMAP (R version 2.11.0). We have performed Family-Based Association Test to analyze association between BDNF and OCD, Yale-Brown Obsessive-Compulsive Scale score, and age at onset, in 150 families with an OCD proband, in addition to case-control analyses using Pearson χ^2 in 83 case-control pairs. Retrospective response data on SRI trials was collected in 107 OCD patients. Individuals were grouped into those who improved following an adequate trial of ⁄ 1 SRI(s) as compared with those who reported “minimal”, “no change”, or “worsening”. We examined four BDNF polymorphisms in addition to Val66Met. Results: Meta-analysis was negative (OR-Val=1.061, 95% CI 0.844-1.336, P=0.610). We did not detect family association between BDNF and OCD. We found nominally significant results in case-control analyses with OCD for single polymorphisms (P=0.015-0.033) including the Val allele of Val66Met (P=0.051; males: P=0.035), and a four-marker haplotype (P=0.039). C270T was associated with response to fluvoxamine treatment (genotype: P=0.025; allele: P=0.035), any SRI response (genotype: P=0.028; allele: P=0.034), and any SSRI response (genotype: P=0.028; allele: P=0.034). Conclusion: The BDNF gene may play an important role in OCD. Replication in larger and independent samples is required.

597F

A Metallothionein Gene Family Co-expression Module Is Differentially Expressed in Multiple Studies of Schizophrenia Postmortem Brains. *C. Chen[1], L. Cheng[1], F. Pibiri[1], C. Zhang[1], J. Badner[2], E. Gershon[2], C. Liu[1].* 1) Department of Psychiatry, University of Illinois at Chicago, CHICAGO, IL; 2) Department of Psychiatry and Behavioral Neuroscience, The University of Chicago, CHICAGO, IL.

Studies of differential gene expression in the brains of schizophrenia(SZ) patients have each yielded a long list of interesting candidate genes, but very few findings are consistent and replicated among studies. This may be largely due to strong heterogeneity of samples and relatively small changes in patients. We hypothesized that coordinated gene expression changes in patient brains are more robust than individual gene expression change is. We used weighted co-expression network analysis(WGCNA) to detect such changes. Five different data sets were studied, including three different brain regions (cerebellum[CB], parietal cortex [PC] and prefrontal cortex [PFC]) of the same 45 SZ and 46 normal samples from the Stanley Medical Research Institute (SMRI) collection, and two independent replicates of PFC data from the Charing Cross Hospital prospective collection (CCHPC)(28 SZ and 23 controls) and the Victorian Brain Bank Network(VBBN)(30 SZ and 29 controls). After strict quality control measures, we performed two different disease correlation tests for detected modules. First, we ran multiple linear regression models for each gene individually, with all covariates controlled, including age, pH, sex and postmortem interval (PMI). The Wilcoxon rank-sum test was applied to the correlation coefficients of cases and controls from each module, to test whether that module was enriched in disease-associated genes. Second,we calculated singular value decomposition (SVD) for each module. The top module eigengene, which is equivalent to the first principal component in principal component (PCA), was referred to as the module eigengene (ME). We tested correlation between ME and disease after adjusting all covariates by linear regression model.We found that one module, containing MT1X, MT1E, MT1F, MT1G and MT2A from the metallothionein(MT) gene family, was significantly correlated with schizophrenia in SMRI PC samples (FDR q<0.05, by both Wilcoxon rank-sum test and ME-based correlation test). This disease correlation was replicated in all the other four CB and PFC data (nominal p value<0.05) with various sample sources and microarray platforms.The MT gene family proteins can be regulated by metals and stress,are related with neuroprotection against central nervous system inflammation, neurodegeneration and cell death.It suggestedthat MT gene family is involved in schizophrenia pathogenesis.

598F

Translational profiling of serotonergic neurons identifies BRUNOL6 as a candidate gene for autism. *J.D. Dougherty[1], L. Sonnenblick[2], G. Coppola[2], A.G. Ercan-Sencicek[3], B.S. Abrahams[4], D.H. Geschwind[2], N. Heintz[5].* 1) Genetics and Psychiatry, Washington University School of Medicine, St. Louis, MO; 2) UCLA Center for Autism Research and Treatment, Semel Institute of Neuroscience, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA; 3) Yale University School of Medicine, New Haven, CT, USA; 4) Depts. of Genetics & Neuroscience, Albert Einstein College of Medicine, New York, NY, USA; 5) Laboratory of Molecular Biology, The Rockefeller University, New York, NY, USA.

The immense molecular diversity of neurons challenges our ability to deconvolute the relationship between the genetic and the cellular underpinnings of neuropsychiatric disorders. We suspected that comprehensive approaches to parsing this complexity may inform human genetics studies. The serotonergic system has long been suspected in a variety of disorders, including autism. We generated a bacTRAP mouse line to permit the in vivo profiling of all ongoing translation in these cells. From this, we identified 174 serotonergic-cell enriched and specific genes, including all known markers of these cells. Analysis of common variants in these genes in human families with autism implicated two genes, C1QTNF2 and the RNA-binding protein BRUNOL6. Screening for rare variants in BRUNOL6 identified a premature stop codon found only in a family with autism. This work provides a reproducible and accurate method to assess the translational profiles of serotonergic neurons under a variety of conditions in vivo, and suggests cell-specific information may provide some insight into the genetic etiology of complex psychiatric disorders.

599F

Investigation into the cause of Myoclonus Dystonia. *A. Smith[1,2], DA. Grimes[1,2,3], DE. Bulman[1,2].* 1) Ottawa Hospital Research Institute, Ottawa, Ontario, Canada; 2) University of Ottawa, Ottawa, Ontario, Canada; 3) The Ottawa Hospital, Ottawa, Ontario, Canada.

Myoclonus Dystonia (MD) (DYT11) is classified as a dystonia plus-syndrome. MD is a neurological disorder characterized by rapid muscle contractions (myoclonus) and sustained twisting movements resulting in abnormal and painful postures (dystonia). Mutations in epsilon-sarcoglycan (SGCE) have been found in approximately 40% of patients with MD. In muscle, members of the sarcoglycan (SG) family are known to form a complex. This muscle SG complex is part of the dystrophin-glycoprotein complex (DGC), which acts as a scaffolding complex. The importance of the SG complex in muscle is evident as mutations in (,) , . and +-SGs cause different forms of autosomal recessive limb girdle muscular dystrophy (LGMD). Conversely little is currently known about the DGC and the function of SGCE in the central nervous system (CNS). To determine the role of SGCE in the CNS, we have set out to characterize both the known and unknown members of SG complex members present within neurons using a mouse neuroblastoma cell line, N1E-115. First to identify the known complex members we used both RT-PCR and immunoprecipitations and found that) , + and , SG's are not only expressed in neurons but also form a complex, whereas (and . were not detected. Expanding on this we have been investigating the presence of other known members of the muscle DGC complex within our neuronal cell line using RT-PCR. We detected sarcospan, caveolin, (and) -dystroglycan, whereas dysferlin was not detected. Next, to identify the yet uncharacterized binding partners of SGCE within neurons, we are using tandem affinity purification-mass spectrometry (TAP-MS), which is an effective approach for identifying novel protein complexes, as no prior knowledge of complex members is necessary. The TAP-MS is a two-step purification process which is meant to minimize non-specific interactions. With a N1E cell line stably expressing our SGCE-CTAP fusion protein we have isolated the SG-complex present in neurons using the TAP-tag method. After purification the collected complexes have been sent for mass spectrometry. The SGCE interacting proteins will then validated and functional assessment of the interacting proteins will be performed. Elucidation of the SG complex in the brain will provide us with an understanding of how mutations in SGCE cause MD. Furthermore, the interacting proteins will be candidates for other forms of dystonia, just as the binding partners in muscle cause other forms of muscular dystrophy.

600F

Correlation between ATRA-induced and basal *CD38* expression: Clinical implications of basal expression as a response indicator to treatment of autism spectrum disorder (ASD) with retinoids. *P.S. Lai[1], O.S. Yim[2], S. Chhabra[1], H. Muralidharan[1], M. Monakhov[3], R.P. Ebstein[2].* 1) Department of Pediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119228; 2) Department of Psychology, Faculty of Arts and Social Sciences, National University of Singapore, Singapore 117570; 3) Department of Economics, Faculty of Arts and Social Sciences, National University of Singapore, Singapore 117570.

CD38 is a transmembrane glycoprotein with ADP-ribosyl cyclase activity expressed in lymphocytes, neurons and glial cells. Recently, CD38 has been shown to be essential for secretion in the brain of oxytocin (OT), a nonapeptide hormone that mediates social relationships in mice and men. Two independent studies have shown genetic association between CD38 SNPs and autism spectrum disorder (ASD). We have also shown reduced transcription of this gene in lymphoblastoid cells derived from autism subjects. Since CD38 can be upregulated in lymphocytes cultured in the presence of all-trans-retinoic acid (ATRA), an isomer of Vitamin A, we suggest the notion that retinoids may be novel therapeutic agents in ASD. Notably, basal CD38 and ATRA-modulated expression of this gene show marked individual differences prompting us to investigate whether this variability is in part due to genetic factors. To clarify the molecular basis for this variability in expression, two CD38 SNPs, rs6842880 and rs13137313, were investigated for their relationship with ATRA-induced expression. METHODS: Fresh lymphocytes from 21 subjects were cultured with and without 0.1 μM ATRA. Quantitative assay of CD38 expression was carried out using SYBR Green and normalized to) -actin mRNA. Genotyping was carried out using Taqman assays. Psychological questionnaires measuring autism, empathizing and systemizing quotients were administered to all subjects. Statistical tests were carried out using MINITAB for Windows (v.15). RESULTS: There was no significant association between the two SNPs and induced fold increase of CD38 expression. However, there was a trend of association between SNP rs6841880 and CD38 basal level (p=0.0062) with the homozygote common allele having a lower basal level. Interestingly, this genotype was associated with a higher systemizing ability (p=0.0040), a common personality trait in ASD subjects. Significant negative correlation was observed between CD38 fold increase and basal level of CD38 expression (p=0.001), suggesting that subjects with lower basal level might be more sensitive to ATRA exposure. CONCLUSION: This is the first report showing a link between basal CD38 basal levels and CD38 fold increase following ATRA treatment, suggesting that the former can be used an indicator of responsiveness to ATRA. The possible clinical implications of this study will be discussed.

601F

Increased incidence of Attention Deficit Hyperactivity Disorder (ADHD) in children with Nail Patella Syndrome (NPS). *I. McIntosh[1,2], C. López-Arvizu[3,4], A. Diaz-Stansky[3,4], E.P. Sparrow[3,7], M.J. Strube[5], C. Slavin[2,3], J.E. Hoover-Fong[2,6], E. Tierney[3,4].* 1) Dept Molecular & Cell Biology, American University of the Caribbean, Cupecoy, St. Maarten; 2) Mckusick-Nathans Inst Genetic Medicine, Johns Hopkins University, Baltimore, MD; 3) Kennedy Krieger Institute, Baltimore, MD; 4) Dept Psychiatry & Behavioral Sciences, Johns Hopkins University, Baltimore, MD; 5) Dept Psychology, Washington University, St. Louis, MO; 6) Greenberg Center for Skeletal Dysplasias & Dept Pediatrics Johns Hopkins University, Baltimore, MD; 7) Sparrow Neuropsychology, P.A., Raleigh, NC.

Nail Patella Syndrome (NPS, OMIM 161200), is an autosomal dominant disorder that is the result of loss-of-function mutations in LMX1B, a LIM-HD transcription factor. Ablation of Lmx1b has been shown to result in abnormalities in the development of the dopaminergic and serotinergic pathways. We hypothesized that subjects with NPS would present with ADHD symptoms and have shown previously that adults with NPS exhibit increased incidence of both ADHD and Major Depression. The Conners' Parent Rating Scales, Revised: Long version (CPRS-R:L) were completed for 18 children with NPS. All subjects were determined to have NPS by clinical exam. LMX1B mutation detection was carried out in subjects' genomic DNA. Elevation was reported for the following scales: Cognitive Problems/Inattention, Psychosomatic, ADHD Index, DSM-IV ADHD Inattentive, and DSM-IV ADHD Total. Overall, ADHD symptoms were present in 40% of children (vs. 2-7% in general North American population). LMX1B mutations were found in 14 of 18 subjects. There was no association between type of LMX1B mutation and elevation in any of the scales, consistent with the concept that LMX1B haploinsufficiency underlies NPS. In conclusion, inattention may comprise part of the neurobehavioral phenotype of NPS.

Summary of Elevations (T⁄ 60) on CPRS-R:L Scales of interest for 18 Boys & Girls

	DSM-IV ADHD Inattent	Cognitive Prob/ Inatt	Psycho-somatic	ADHD Index	DSM-IV ADHD Total
F	4 of 10 (40%)	5 of 10 (50%)	3 of 10 (30%)	4 of 10	3 of 10 (30%)
M	4 of 8 (50%)	4 of 8 (50%)	3 of 8 (38%)	4 of 8 (50%)	4 of 8 (50%)
Total	8 of 18 (44%)	9 of 18 (50%)	6 of 18 (33%)	8 of 18 (44%)	7 of 18 (39%)

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602F

Generation and characterization of a WNK1/HSN2-/- mouse model of hereditary sensory autonomous neuropathy type II (HSAN2). *V. Lavastre[1,2], S. Ramalingan[1,2], G. Houle[1,2], J. Laganiere[1,2], S. Laurent[1,2], R. Gaudet[1,2], P. Hince[1,2], D. Rochefort[1,2], G. Castonguay[1,2], K. Huot[1,2], P.A. Dion[1,2,3], G.A. Rouleau[1,2,4].* 1) Medicine, CHUM Research Center, Montreal, Canada; 2) Centre of Excellence in Neurosciences, Montreal, Canada; 3) Department of Pathology and Cellular Biology, Universite de Montreal, Montréal, Canada; 4) Research Center, CHU Ste-Justine, and Department of Pediatrics and Biochemistry, Universite de Montreal, Montréal, Canada.

Hereditary sensory and autonomic neuropathy type II (HSAN2) is a rare recessive and progressive disease typically diagnosed in the first decade of life. This autosomal disorder is characterized by variable sensory and autonomic dysfunction such as distal loss of sensitivity to thermal and painful stimuli leading to infections, fractures, and lacerations. Although recessive mutations in the nervous system-specific HSN2 exon of WNK1 (refers as WNK1/HSN2 isoform) and in the FAMB134B gene have been discovered, the cause of HSAN2 remains unknown. Moreover, WNK1/HSN2 is principally expressed in sensory components of the peripheral nervous system and the CNS, associated with sensory and nociceptive signals. The aim of this study is to elaborate and characterized a knock-out mouse model that reproduce part or all of the features of HSAN2 with the purpose to evaluate if the WNK1/HSN2 is required in the animal for normal behavioral responsiveness. Consequently, we generated a WNK1/HSN2 conditional ko model which targets the HSN2 exon by using the Cre/Lox conditional ko system to inactivated this gene in a temporal and spatial manner (by crossing the mouse with different Cre recombinase line). We designed a targeting vector corresponding to the WNK1/HSN2 flanked by loxP site (Flox). This construct was electroporated into embryonic stem cells. Once identified, the positive clone was injected into blastocysts and implanted in the uterus of a foster mother. The transgenic offsprings were identified by standard method and termed HSNloxP. These animals were absolutely normal, as expected, and there expression of the HSAN protein was normal. To remove the WNK1/HSN2 exon, we then crossed those mice with PCX-NLS general deletor Cre recombinase mice. Following proper recombination, this mating removed the DNA between the two loxP sites resulting in the loss of the WNK1/HSANII expression and was revealed by RT-PCR, Northern and Western blot. We are monitoring the animals on a monthly basis to test for the apparition of any disease-relevant behavioral and/or histological phenotype.

603F

A novel transgenic mouse model of Machado-Joseph Disease: genetic, phenotypic and pathologic similarities with the human disease. *S.D. Silva, A.S. Fernandes, A.N. Carvalho, A.T. Castro, C.S. Cunha, P. Maciel.* Health Sciences School, Life and Health Sciences Research Institute/3B's, Braga, Portugal.

Machado-Joseph disease (MJD), also known as Spinocerebellar Ataxia type 3 (SCA3), is an autosomal dominant neurodegenerative disorder caused by the expansion of a polyglutamine tract (polyQ) in the C-terminus of the ATXN3 gene product, ataxin-3. Although ataxin-3 is ubiquitously expressed, only restricted neuronal populations of the central nervous system are affected in the disease. We have generated three lineages of transgenic mice expressing human ataxin-3 with an expanded CAG tract under the control of the CMV promoter: CMVMJD83, CMVMJD94 and CMVMJD135, carrying Q83, Q94 and Q135 polyQ stretches, respectively. Behavioral analysis revealed that transgenic CMVMJD83 mice did not manifest motor deficits during their lifetime (84 weeks), whereas CMVMJD94 animals developed a mild motor uncoordination phenotype starting at 16 weeks of age. Interestingly, CMVMJD135 transgenic animals developed a more aggressive motor uncoordination phenotype along with other neurological features such us gait impairment, body balance deficit, loss of limb clasping, grasping and muscular tonus. Immunohistochemistry revealed that ataxin-3 is located mainly in the perinuclear regions of neurons forming small-punctate aggregates in CMVMJD94 and CMVMJD135 mice brains. In contrast, in the CMVMJD135 model we observed large intranuclear inclusions, although not exclusively in the affected areas. In summary, we have created two mouse models for MJD with different pathological and phenotypical features that may model different stages of the disease, which may contribute substantially for the understanding of MJD pathogenesis. In addition, these models are currently being used for therapeutical trials targeting different molecular pathways that might be involved in the disease such as autophagy, transcription deregulation, oxidative stress and protein aggregation/misfolding.

604F

Somatic mutations mediated by Alu in schizophrenia. *M. Ueno, A. Akahane, T. Hata, S. Nanko.* Department of Psychiatry, Teikyo University School of Medicine, Tokyo, Japan.

Recently, the dynamics of mobile elements L1 have been reported to contribute to the neuroplasticity in adult brain. The dynamics of mobile elements is not fully fixed, and might be progressively in human genome. In previous reports, we have identified the de novo mutations mediated by mobile elements in the patients with schizophrenia. Herein, we have attempted the screening of somatic mutations mediated by mobile elements Alu to clarify the dynamics of genome in schizophrenia.

DNA samples were obtained from a monozygotic twin discordant for schizophrenia and the sequential samples collected for every 5 years from a patient and a control. We performed to screen the mutations by the microarray assay using Alu-PCR products from each samples. Thereafter, Fluorescent in situ hybridization (FISH) analysis was performed on combed DNA using Alu probe and BAC probes which were containing the mutated genes regions.

Consequently, we have identified the somatic mutations mediated by Alu. The microarray assay reveals that the somatic mutations in the sequential samples and unaffected twin showed a tendency for Alu to delete. Moreover, FISH analysis on combed DNA allows us to detect the progressive deletions of Alu in the latest sample from the patient. Our results provided the evidence in support of one possible role of dynamics of Alu in schizophrenia for recovery and protection. Further research using the screenings of progressive somatic mutations is needed to clarify the etiology in schizophrenia.

This study was done under the approval of the Ethical Committee for Genetic Research, Teikyo University School of Medicine.

605F

The effect of TOMM40 poly-T length and APOE genotype on age of onset of neurodegenerative diseases: a pilot study of frontotemporal lobe dementia. *I. Grossman[1], A.M. Saunders[2], M.W. Lutz[2], O. Chiba-Falek[3, 4], K.M. Hayden[5, 6], D.G. Crenshaw[2], C.M. Hulette[7], B.L. Plassman[6], K.A. Welsh-Bohmer[5, 6], J.R. Burke[4, 5], A.D. Roses[1-4].* 1) Cabernet Pharmaceuticals, Chapel Hill, NC, USA; 2) Deane Drug Discovery Institute, Duke University, Durham, NC, USA; 3) Institute for Genome Sciences & Policy, Duke University, Durham, NC, USA; 4) Division of Neurology, Department of Medicine, Duke University, Durham, NC, USA; 5) Joseph and Kathleen Bryan ADRC, Duke University, Durham, NC, NC, USA; 6) Department of Psychiatry and Behavioral Sciences, Duke University, Durham, NC, USA; 7) Department of Pathology, Duke University, Durham, NC, USA.

Background: Frontotemporal lobe dementia (FTLD) is the 4th most common form of dementia. Clinically, late onset FTLD can resemble Alzheimer's disease (AD) and often cases are misdiagnosed as AD until postmortem examination confirms FTLD pathology. Subtypes of FTLD can be characterized based on histopathological brain protein deposits, as well as presence of mutations in several genes, e.g. microtubule-associated protein tau (*MAPT*) and progranulin (*PGRN*). However, only a minority of FTLD cases carries these mutations and the high heritability attributed to FTLD (~42%) remains largely unexplained. A meta-analysis (2002) reported a trend association between the *APOE*, 2 allele and earlier onset of FTLD, an unexpected finding as *APOE*, 2 alleles are infrequent in late-onset AD and are associated with later disease onset. Additionally, we recently discovered a variable length poly-T tract in the *TOMM40* gene (rs10524523), which, together with *APOE* genotype, plays a fundamental role in age of onset (AOO) of late-onset AD. Hypothesis: *TOMM40* rs10524523 interacts with *APOE* genotype, and both influence onset age of FTLD. Methods: *TOMM40* rs10524523, *APOE* rs429358 and *APOE* rs7412 were genotyped in autopsy-confirmed, unrelated FTLD cases from the Duke ADRC and the Duke Twins Study cohorts. Genetic data were analyzed in association with AOO of first symptoms and diagnosis. Results: An initial cohort of 32 cases had an age of disease onset ranging from 42-84 (median=62). *APOE*, 2 carriers (, 2/2, , 2/3) developed FTLD significantly earlier (average AOO=51.1, N=9) than non-carriers (, 3/3, , 3/4, average AOO=65.6, p=0.0006). Stratifying each *APOE* genotype group by rs10524523 length identified a trend for the shorter poly-T to be associated with earlier FTLD AOO, particularly for *APOE*, 3 carriers. Additional samples (total N=52) are currently being analyzed. Conclusions: *APOE-TOMM40* haplotypes influence susceptibility and possibly onset age of FTLD. Our data suggest that FTLD and AD share a complex locus that affects the rate of progression to disease onset. This suggests two hypotheses: 1. there may be a common mitochondrial mechanism for dementia, despite pathologically distinct diagnoses, and 2- AD and FTLD are distinguished by a difference in amyloid pathology, but share an important genetic mechanism of differential intraneuronal cleavage of apoE2 compared to apoE3 and apoE4 proteins. Follow-up in independent, larger cohorts of FTLD and related disorders is warranted.

606F

SCA7: clinical and genetic analysis of an Italian family. *D. Italiano[1], P. Tarantino[2], C. Lombardo[1], E.V. De Marco[2], C. Pastura[1], P. Bramanti[1], A. Quattrone[3,4], G. Annesi[2]*. 1) IRCCS Centro Neurolesi "Bonino-Pulejo", Messina, Italy; 2) Institute of Neurological Sciences, National Research Council, Cosenza, Italy; 3) Institute of Neurology, University Magna Graecia, Catanzaro, Italy; 4) Neuroimaging Research Unit, National Research Council, Catanzaro, Italy.

Spinocerebellar ataxia type 7 (SCA7) is a rare form of autosomal dominant cerebellar ataxia (ADCA)clinically characterized by progressive ataxic syndrome and retinal degeneration. In several studies it represents about 2% of all ADCA. The disease is caused by the expansion of a CAG trinucleotide repeat within the SCA7 gene, encoding for a protein termed ataxin-7, of unknown function. Normal SCA7 alleles harbor from 4 to 35 CAG repeats. De novo expansions occur on intermediate alleles carrying from 28 to 35 CAG repeats. SCA7 appears to be very rare in Italy (~1%), with only four families diagnosed so far. Here we describe a family from Sicily whose proband was clinically and genetically diagnosed as SCA7. Complete neurological and ophthalmic examinations were performed to evaluate the clinical features of the disease. Molecular tests for SCA expansions were performed by polymerase chain reaction (PCR). PCR products were separated onto a capillary sequencer (3130Xl genetic analyzer Applied Biosystems) and the length of specific SCA fragments was calculated referring to a size standard and to related SCA controls. The proband was a 49-year-old man presenting progressive gait disturbances, clumsiness and visual impairment for three years. Neurological examination revealed bilateral and symmetrical gait and limb ataxia, dysarthria, slight solid dysphagia and dysmetria. Brain MRI showed vermian and hemispheric cerebellar atrophy. Ophthalmologic work-up revealed markedly decreased visual acuity, hypermetric saccadic eye movements and pigmentary changes in macular and peripheral retinal regions. Molecular analysis identified an expanded allele with 38 CAG repeats within the SCA7 gene. The 25-year-old proband's son was normal at neurological examination, but he had complained of visual impairment for two years. Ophthalmologic examination revealed reduced visual acuity and early macular pigmentary disturbances. He refused to undergo genetic analysis. In this family the proband presented a low number of pathologic GAC repeats (38) and his parents were reported as not exhibiting any neurological disturbance, so we can argue that the disease is probably caused by a de novo expansion of a premutated intermediate allele carried by one parent. The proband's son can be considered as probably affected since his retinal changes were strongly suggestive of cone-rod dystrophy. This would confirm the extreme anticipation (20 years /generation) previously observed in SCA7.

607F

Two new Rett syndrome families: expanding the knowledge of *MECP2* frameshift mutations. *K. Ravn[1,2], G. Roende[1], M. Duno[3], K. Fuglsang[1], KL. Eiklid[4], Z. Tümer[2], JB. Nielsen[1], OH. Skjeldal[5]*. 1) Center for Rett syndrome, Kennedy Center, Glostrup, Denmark; 2) Center for Applied Human Molecular Genetics, Kennedy Center, Glostrup, Denmark; 3) Department of Clinical Genetics, University of Copenhagen, Rigshospitalet, Denmark; 4) Department of Medical Genetics, Oslo University Hospital HF, Ullevål Hospital, Oslo, Norway; 5) Women and Children's Clinic, Vestre Viken Hospital Thrust, Norway.

Background: Rett syndrome (RTT) is an X-linked dominant neurodevelopmental disorder, which is usually caused by *de novo* mutations in the *MECP2* gene. More than 70 % of the disease causing *MECP2* mutations are eight recurrent C to T transitions, which almost exclusively arise on the paternally derived X chromosome. About 10 % of the RTT cases have a C-terminal frameshift deletion in *MECP2*. Only few RTT families with a segregating *MECP2* mutation, which affects female carriers with a phenotype of mental retardation or RTT, have been reported in the literature. In this study we describe two new unrelated families with three and four individuals, respectively. Furthermore, we compare the type of mutations observed in RTT families to those of the sporadic cases and we present our study on the parental origin of *de novo MECP2* frameshift mutations in our RTT cohort. **Results**: In both families a C-terminal frameshift mutation segregates. Clinical features of the mutation carriers vary from classical RTT to mild mental retardation. XCI profiles of the female carriers correlate to their respective geno-/phenotypes. The majority of the de novo frameshift mutations occur on the paternally derived X chromosome (7/9 cases), without a paternal age effect. **Conclusions**: The present study suggests a correlation between the intrafamilial phenotypic differences observed in RTT families and their respective XCI pattern in blood, in contrast to sporadic RTT cases where a similar correlation has not been demonstrated. Furthermore, we found *de novo MECP2* frameshift mutations frequently to be of paternal origin, although not with the same high paternal occurrence as in sporadic cases with C to T transitions. This suggests further investigations of more families. This study emphasizes the need for thorough genetic counselling of families with a newly diagnosed RTT patient.

608F

Analysis of modulation of FXTAS rCGG-mediated neurodegeneration by CUGBP1 in Mus musculus. *Z.A. Zalewski[1], P. Jin[2], L.T. Timchenko[3], D.L. Nelson[1]*. 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA; 3) Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX.

Fragile X-associated Tremor/Ataxia Syndrome, or FXTAS, has been identified as a late-onset neurodegenerative disorder in Fmr1 premutation carriers, principally in older males. The neuropathological hallmarks of FXTAS include ubiquitin-positive intrauclear inclusion bodies throughout the brain and marked dropout of cerebellar Purkinje neurons. We hypothesize that FXTAS arises though an RNA-mediated gain-of-function toxicity model. In this model, the ribo-CGG (rCGG) repeat acts to functionally limit rCGG repeat-binding proteins. Previous work in Drosophila demonstrated the singular sufficiency of rCGG to exert a toxic gain-of-function. The Drosophila ortholog of hnRNP A2/B1 was found to be capable of suppressing phenotype and binding rCGG. A subsequent genetic screen identified CUGBP1 as a specific interacting partner of hnRNP A2/B1, and also as a modifier of rCGG-mediated toxicity. We report our recent work in furthering these earlier results using mouse models. We previously reported two Purkinje neuron -specific transgenic models in which a CGG premutation is fused to a reporter (FMR1 or EGFP), and are also making use of the "knock-in" model developed by the Oostra group, which replaces the endogenous 5 CGG repeats with a >100 CGG-repeat fragment within the Fmr1 locus. We predict that reduced levels of CUGBP1 will exacerbate rCGG phenotypes and that CUGBP1 overexpression will rescue the FXTAS phenotype. To this end, we have developed two mouse models to examine the role of Cugbp1 in FXTAS pathogenesis: a gene trap loss-of-function, in which vector pGT1Lxf has been inserted into the second intron within the coding sequence of Cugbp1, and mice with tetracycline-controlled overexpression of Cugbp1 in Purkinje neurons. We have crossed these mice to our FXTAS mouse models and are in the process of determining the resulting phenotypes. These results should significantly advance our understanding of FXTAS pathogenesis, and provide potential targets for therapeutic intervention.

609F

Bayesian SNP-set Analysis for Case-control Genome-wide Association Studies. *X. Wang[1], D. Qian[2].* 1) Department of Population Science, City of Hope, Duarte, CA 91010; 2) Department of Biostatistics, City of Hope, Duarte, CA 91010.

Despite the success in genome-wide association studies (GAWS), the single marker association tests suffer from the limited power to identify disease genes. Some genes may be genuinely associated with disease trait, but each marker within a gene may contain little variation and not satisfy a stringent genome-wide significance threshold in any GWAS. Therefore, association analysis requires statistical methods that can effectively combine the information across markers within a gene or several genes and estimate their overall effect. In this study, we extend a Bayesian SNP set analysis method using Bayesian generalized linear model to test the joint effect of each SNP set while adjusting for additional covariate effects including demographic and environmental variables. Our method jointly models the overall effect of a SNP set and the weights of multiple markers within the SNP set. Rather than predetermining the weights of markers in the existing methods, our method estimates them based on their contributions to disease trait. Using simulated data, we have shown our method can control type I error rates very well. We will conduct more extensive simulation studies to evaluate the performance of our method by comparing with the existing methods.

610F

Multivariate quality control improves genotyping accuracy and call rate. *B.T. Webb[1], J.E. Dellava[1], L.M. Hack[1], M.C. Neale[1], A. Corvin[2], M. Gill[2], F.A. O'Neill[3], D. Walsh[4], K.S. Kendler[1], B.P. Riley[1].* 1) Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, VA; 2) Department of Psychiatry, Trinity College Dublin, Dublin, Ireland; 3) Department of Psychiatry, Queens University, Belfast, Northern Ireland, United Kingdom; 4) Health Research Board, Dublin, Ireland.

Genotype calling using SNP arrays can be biased by inclusion of poor quality samples in a data set. Identifying and removing problematic samples can be time consuming and subjective but keeping poor quality samples can lead to spurious results. Ideally, an iterative process of identification and exclusion followed by recalling should be used. Although many quality control (QC) measures are routinely generated as part of genotyping pipelines, few variables are commonly used to filter samples (e.g., call rate and contrast QC for Affymetrix arrays). This study aimed to develop and evaluate a multivariate approach to identifying poor quality samples in order to maximize genotyping calling and accuracy. Using 4548 Affymetrix 6.0 SNP arrays genotyped as part of the Welcome Trust Irish Schizophrenia Case Control Sample (ISCCS), factor analyses were performed on QC variables generated pre and post genotyping. Using 19 measures, a four factor solution fit best and accounted for 0.758 of the cumulative variance. Since loadings differed by sex, a three factor model fit best for sex specific analysis. Factor scores were then used to calculate a median Mahalanobis distance score. Replicate arrays were used to evaluate the utility of the multivariate Mahalanobis Score (MVMS) based sample filtering in improving genotype call quality. The MVMS, call rate, and contrast QC were correlated with discordance. Removing the worst 5% of arrays by MVMS resulted in a substantial reduction of discordant genotypes than using a single QC measure. To further test the MVMS, a subset of 162 replicate arrays representing 73 samples were recalled (Birdseed v2) under three conditions. The 162 replicates were paired with an additional 162 arrays representing the best, random, and worst arrays by MVMS. Discordance and fail rate was examined in high and low quality SNPs. The fail rate was highest and lowest in the replicates called in the presence of the worst and best MVMS arrays, respectively. Little difference was observed between the best and random array sets. The effect of external array quality on replicate array fail rate and discordance was increased for poor SNPs. In conclusion, the MVMS has the advantage of identifying samples that are lower quality across different domains but would fail to be excluded using only univariate thresholds. Therefore, using the MVMS potentially reduces the number of samples needed to be excluded to improve genotype calling.

611F

Characterisation of genome-wide association epistasis signals for serum uric acid in human population isolates. *W. Wei[1], G. Hemani[2], A. Hicks[3], V. Vitart[1], C. Cabrera-Cardenas[1], P. Navarro[1], J. Huffman[1], C. Hayward[1], S. Knott[4], I. Rudan[5,6], P. Pramstaller[3,7,8], S. Wild[5], J. Wilson[5], H. Campbell[5], N. Hastie[1], A. Wright[1], C. Haley[1,2].* 1) Biomedical System Analysis, MRC Human Genetics Unit, Edinburgh, Scotland, United Kingdom; 2) The Roslin Institute and R(D)SVS, University of Edinburgh, Roslin, Midlothian, UK; 3) Institute of Genetic Medicine, European Academy Bozen/Bolzano (EURAC), Bolzano, Italy. Affiliated Institute of the University of Lübeck, Germany; 4) Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, UK; 5) Centre for Population Health Sciences, University of Edinburgh, Edinburgh, UK; 6) Croatian Centre for Global Health, University of Split, Split, Croatia; 7) Department of Neurology, General Central Hospital, Bolzano, Italy; 8) Department of Neurology, University of Lübeck, Lübeck, Germany.

Genome-wide association (GWA) studies so far have identified nine loci underlying human serum uric acid (SUA) levels including the *SLC2A9* gene. Gene-gene interactions (epistasis) are largely unexplored in these GWA studies. We performed a full pair-wise genome scan in the Italian MICROS population (n = 1201) to characterise epistasis signals in SUA levels. In the resultant epistasis profile, no SNP pairs reached the Bonferroni adjusted threshold for the pair-wise genome-wide significance. However, *SLC2A9* was found interacting with multiple loci across the genome, with *NFIA - SLC2A9* and *SLC2A9 - ESRRAP2* being significant based on a threshold derived for candidate gene interactions and jointly explaining 8.0% of the phenotypic variance in SUA levels (3.4% by interaction components). Epistasis signal replication in a CROATIAN population (n = 1772) was limited at the SNP level but improved dramatically at the gene ontology level. In addition, gene ontology terms enriched by the epistasis signals in each population support links between SUA levels and neurological disorders such as cognitive aging. We conclude that GWA epistasis analysis is valuable in small isolated populations.

612F

Analyze X: A Comparison of X Chromosome and Autosomal Results in GWAS. *A. Wise, L. Gyi, T. Manolio.* National Human Genome Research Institute (NHGRI), Bethesda, MD.

Genome-wide association studies (GWAS) have identified over 1,200 associations with p<5x10-8 for over 200 traits, yet only 7 such associations have been reported on the X chromosome (chr). This raises the question: why is the X chr so poorly represented in GWAS results? A review of over 300 GWAS suggests that this is largely due to exclusion of X chr variants from analyses as one of the first steps of quality control, despite these regions being assayed on many current microarray platforms. Reviewing the NHGRI Catalog of Published GWAS (www.genome.gov/gwastudies) showed that only 121 of 374 (32%) of GWAS published from Jan 2010 through Mar 2011 reported analyzing the X chr in their Methods sections. Accounting for the number of genes on the X chr also does not eliminate the gap in GWAS associations; as the X chr (1,669 genes) has only 7 reported associations at 5 distinct loci, while chr 7 (1,880 genes) has 48 reported associations at more than 20 distinct loci. Interrogating all of the variants found in papers that identified X chr hits at p<1x10-5 in the NHGRI Catalog, to account for sample size and genotyping platform variations, X chr variants have similar minor allele frequencies (MAFs) as autosomal variants (X chr avg MAF=0.34 and autosomal avg MAF=0.39, p=0.27). Median p-values for the hits found on the X chr were higher, however, by approximately 1 order of magnitude than those seen for autosomal variants in these same studies (p=0.04), indicating that power may indeed be an issue perhaps due to smaller effective chr numbers. Comparing the functional classes assigned to these variants by NCBI, none (0/51) of the variants on the X chr were missense or synonymous variants, as compared to 5% (27/582) of the autosomal variants. Intronic variants were found in similar proportions in the X chr and autosomal hits, 31% and 37% respectively; while intergenic variants, on the other hand, were more common amongst the X chr hits, 67% versus 49%, p=0.02. Given the similarity found between the X chr and other autosomes in variant MAFs, and overall number of genes, these factors cannot explain why the X chr is so poorly represented in GWAS results. However, the higher p-values found on the X chr and lack of missense or synonymous variants point towards potential issues with power. Improvements in genotype calling accuracy and methods developed specifically for the X chr may thus improve power to detect important associations on the X chr in future GWAS.

613F

Detecting Pleiotropy via Bayesian Latent Variable Modeling. *L. Xu[1], R. Craiu[1], A.D. Paterson[2,3], L. Sun[2,1].* 1) statistics, University of Toronto, Toronto, Ontario, Canada; 2) Division of Biostatistics, Dalla Lana School of Public Health, University of Toronto,Toronto, Ontario, Canada; 3) Genetics and Genomic Biology, Hospital for Sick Children, Toronto, Ontario, Canada.

Pleiotropy occurs when a single gene influences multiple quantitative or qualitative phenotypes and is present in many loci for complex diseases or traits. For example, in genetic studies of complications or secondary manifestations of a disease, it is often believed that there are common genetic risk factors for the different phenotypes. In other cases, the primary and often conceptual phenotype (e.g. disease severity) may not be directly measured and a set of surrogate response variables must instead be used. Robust and powerful methods for the study of pleiotropy are underdeveloped in the literature due to data complexities, which include a) the phenotypes of interest can be continuous or discrete or combinations, b) the joint effect of the covariates on the multiple phenotypes is difficult to specify, and c) the familial, temporal/longitudinal and other correlations are often present in the data. To overcome these challenges, we propose to use the latent variable (LV) methodology. LV has been widely used in many scientific fields including economics, psychology and social sciences but yet to be exploited in the context of genetic studies. The LV approach links the multiple observed phenotypes by introducing an unobserved latent random variable. The effect of pleiotropic locus on the multiple phenotypes is indirect and induced via the LV, i.e. the locus (and relevant covariates) has a direct effect on LV, and in turn LV (and relevant covariates) influences the phenotypes. We base our LV inference on a Bayesian model that provides useful statistics such as mean, variance and percentiles of the posterior distribution of the unknown population parameters. Samplings from the posterior distribution are obtained using Markov Chain Monte Carlo (MCMC) algorithms. The parameter expansion technique is used to improve the mixing of chains and the Bayesian deviance information criteria and the posterior predictive discrepancy measures are used for model selection. Extensive simulations show that our method performs well in capturing the correlations among the multiple traits due to pleiotropy and other common covariates and in selecting the correct model. Application to a genome-wide association study of type 1 diabetes complications also demonstrates the utilities of the proposal method.

614F

Quantitative trait prediction based on SNP-array data, a simulation study. *W. Yip, C. lange.* Dept Biostatistics, HSPH, Boston, MA.

Using simulation studies for quantitative trait loci, we evaluate the prediction quality of regression models that include covariates SNPs which did not achieve genome wide significance. We compare the results of such regression models to the standard approach which is to include only SNPs that are significant. Using MSPE as the model metric, our simulation results suggest that by using the coefficient of determination value as a guideline to increase or reduce the number of SNPs included in the regression model, we can achieve better prediction quality. However, important parameters such as trait heritability, the approx. no. of QTLs have to be determined from other studies.

615F

Common Variants near *FRK/COL10A1* and *VEGFA* are Associated with Advanced Age-Related Macular Degeneration. *Y. Yu[1], T.R. Bhangale[2], J. Fagerness[3,4], S. Ripke[3,4], G. Thorleifsson[5], P.L. Tan[6], E.H. Souied[7], A.J. Richardson[8], J.E. Merriam[9], G.H.S. Buitendijk[10], S. Raychaudhuri[3,4,11], L. Sobrin[12], E. Evangelou[13], A.Y. Lee[14], R.H. Guymer[8], U. Chakravarthy[15], T.W. Behrens[16], K. Stefansson[5,17], C.M. van Duijn[18], J.R. Vingerling[10], C.C.W. Klaver[10], R. Allikmets[9,19], M.A. Brantley, Jr[14], P.N. Baird[8], N. Katsanis[6], U. Thorsteinsdottir[5,17], J.P.A. Ioannidis[13,20,21], M.J. Daly[3,4], R.R. Graham[16], J.M. Seddon[1,22].* 1) Ophthalmic Epidemiology and Genetics Service, Department of Ophthalmology, Tufts Medical Center, Boston, MA; 2) Department of Bioinformatics and Computational Biology, Genentech Inc, South San Francisco, CA; 3) Center for Human Genetic Research, Massachusetts General Hospital, Boston,MA; 4) Program in Medical and Population Genetics, the Broad Institute of Harvard and MIT, Cambridge, MA; 5) deCODE genetics, Reykjavik, Iceland; 6) Center for Human Disease Modeling and Departments of Cell Biology and Pediatrics, Duke University, Durham, NC; 7) Department of Ophthalmology, University Paris Est Creteil, Hopital Intercommunal de Creteil, Creteil, France; 8) Centre for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital, East Melbourne, Victoria, Australia; 9) Department of Ophthalmology, Columbia University, New York, NY; 10) Department Ophthalmology, Department Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands; 11) Divisions of Genetics and Rheumatology, Brigham and Women's Hospital, Boston, MA; 12) Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, Boston, Harvard Medical School, Boston, MA; 13) Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece; 14) Ophthalmology & Visual Sciences,Barnes Retina Institute, Washington University School of Medicine, St Louis, MO; 15) Center for Vision and Vascular Science,The Queen's University, Belfast, Northern Ireland; 16) ITGR Human Genetics Group, Genentech Inc, South San Francisco, CA; 17) Faculty of Medicine, University of Iceland, Reykjavik, Iceland; 18) Department Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands; 19) Department of Pathology and Cell Biology, Columbia University, New York, NY; 20) Stanford Prevention Research Center, Department of Medicine, and Department of Health Research and Policy, Stanford University School of Medicine, Stanford, CA; 21) Center for Genetic Epidemiology and Modeling, ICRHPS, and Tufts Clinical and Translational Science Institute, Tufts Medical Center, Tufts University School of Medicine, Boston,MA; 22) Department of Ophthalmology, Tufts University School of Medicine, Boston, MA.

Advanced age-related macular degeneration (AMD) is a leading cause of visual loss in people older than 60 years. In the last 6 years, common risk variants for AMD have been identified in several genes by genome-wide association studies (GWAS) or candidate gene studies. Despite significant progress in the identification of genetic loci for AMD, not all of the heritability has been explained. To identify additional variants that might account for the remaining genetic susceptibility, we performed a large meta-analysis of GWAS for advanced AMD. We imputed >6 million SNPs in 2594 cases and 4134 controls using the 1000-Genomes reference genotypes, followed by replication genotyping to confirm association of top signals in 5640 cases and 52,174 controls (total combined effective sample size of 8639 cases and 8639 controls). We identified two new common susceptibility alleles, rs1999930 (T) on 6q21-q22.3 near *FRK/COL10A1* (odds ratio 0.87; P= 1.1×10^{-8}) and rs4711751 (T) on 6p12 downstream of *VEGFA* (odds ratio 1.15, P=8.7×10^{-9}). In additional to the two novel loci, ten previously reported loci in *ARMS2/HTRA1* (rs10490924, P=3.6×10^{-322}) , *CFH* (rs1061170, P = 1.3×10^{-261} and rs1410996, P=7.4×10^{-235}), *CFB* (rs641153, P=5.5×10^{-31}), *C3* (rs2230199, P=4.6×10^{-29}), *C2* (rs9332739, P=2.4×10^{-23}), *CFI* (rs10033900, P=4.1×10^{-10}), *LIPC* (rs10468017, P=2.7×10^{-12}), *TIMP3*(rs9621532, P= 2.2×10^{-15}) and *CETP* (rs3764261, P=6.9×10^{-9}) were confirmed with genome-wide significant signals in this large study. Loci in the recently reported genes *ABCA1* (rs1883025, P=1.2×10^{-7}) and *COL8A1* (rs13095226, P=9.7×10^{-7}) were also detected with suggestive evidence of association with advanced AMD. Using a standard liability threshold model, we estimated that all of the above loci combined explain approximately 39% of the total variance (or 55% of the heritability) of advanced AMD. Based on these associated variants, we generated a genetic risk score that can be used to predict advanced AMD risk for individuals with different genetic profiles. This study identified two novel variants and suggested that angiogenesis (*VEGFA*) and extracellular collagen matrix (*FRK/COL10A1*) pathways, in addition to the complement and HDL cholesterol pathways, contribute to the development of advanced AMD. This study increases our understanding of common variants that influence individual risks of AMD and provides new clues for developing effective therapies.

616F

The Kaiser Permanente/UCSF Genetic Epidemiology Research Study on Adult Health and Aging: Design of genotyping arrays using imputation and a hybrid SNP selection algorithm. *Y. Zhan[1], T. Hoffmann[2], J. Gollub[1], M. Kvale[2], Y. Lu[1], G. Mei[1], M. Shen[1], T. Webster[1], P. Kwok[2], C. Schaefer[3], N. Risch[2,3].* 1) Affymetrix Inc, Santa Clara, CA; 2) UCSF Institute for Human Genetics, San Francisco, CA; 3) Kaiser Permanente Northern California Division of Research, Oakland, CA.

Four custom Axiom™ genotyping arrays were designed for genome-wide genotyping of 100,000 participants from the Kaiser Permanente/UCSF Genetic Epidemiology Research Study on Adult Health and Aging. The detailed design strategy of the first array (Axiom™ Genome-Wide EUR Array), specifically for European ancestry populations, has been reported. A similar strategy was used to design the second array (Axiom™ Genome-Wide EAS Array), which focuses on populations of East Asian ancestry. For both designs, most SNPs were picked by a greedy SNP selection algorithm, which selects SNPs to efficiently tag, through pairwise r^2, a large set of target SNPs in one or more populations while also considering factors such as genotyping performance. When imputation is a planned step of the GWAS data analysis, imputation-based coverage, as well as coverage based on pairwise tagging, could be considered in the SNP selection process. To this end, we developed a hybrid SNP selection method, which is based on the greedy SNP selection method, but also considers imputation-based coverage. SNPs are selected in consecutive rounds of greedy tag SNP selection; after each round, SNPs are considered covered and removed from the target set if they can be imputed with an $r^2>0.8$. The hybrid SNP selection method produces enhanced imputation-based coverage compared to results from simple greedy SNP selection in tests where SNPs were selected based on YRI genotype data and coverage tested in HapMap ASW samples.

For the design of the last two custom arrays (Axiom™ Genome-Wide AFR and LAT Arrays, focusing on subjects with African and Latino ancestries, respectively), we first pre-selected about 198K SNPs considered to have high value, including SNPs with known associations with diseases/traits from earlier studies, SNPs from gene regions with different levels of special interest, and SNPs used to tag these important SNPs in YRI, similar to the process for designing the Axiom EUR and EAS Arrays. We then applied the hybrid SNP selection algorithm to achieve genome-wide coverage in populations of interest. Coverage was also enhanced by tiling of high performing SNPs with a single representation on the arrays, which yielded a total of 894K SNPs for the AFR array and 817K SNPs for the LAT array. The resulting arrays achieve high coverage for the targeted populations with SNPs that each can fit into a single Axiom Array.

617F

Imputation reference panel selection using maximal phylogenetic diversity. *P. Zhang[1,2], N.A. Rosenberg[1], S. Z&oumlllner[1,2,3].* 1) Center for Computational and Medicine and Bioinformatics, Univ Michigan, Ann Arbor, MI; 2) Center for Statistical Genetics, Univ Michigan, Ann Arbor, MI; 3) Psychiatry, Univ Michigan, Ann Arbor, MI.

Genotype imputation has become an important component of genome-wide association studies (GWAS). Imputation methods typically rely on a reference panel of densely genotyped individuals to predict missing genotypes at untyped markers in a GWAS sample. Until recently, reference panels in most GWAS have been constructed from existing resources such as the HapMap project. The selection of reference data affects the quality of genotype imputation. For study populations that differ substantially from the HapMap populations in ancestral background, the HapMap data may poorly represent the study populations in their patterns of genetic variation, resulting in reduced imputation accuracy in the study populations. With sequencing costs continuing to drop, it is becoming feasible to sequence a subset of a study sample, and to then treat the sequences as reference data for imputation in the remaining sample. A natural challenge that arises is the selection of the optimal subset for sequencing. Here, we propose a novel algorithm that selects for sequencing the subset of a sample with maximal phylogenetic diversity. Intuitively, the most diverse reference panel can benefit the imputation of less common variants, because it is designed to capture higher diversity in a sample than randomly selected subsets of study individuals. Such a reference panel is therefore more likely to include individuals that carry rarer variants. Our algorithm first constructs a neighbor-joining tree of individuals using pairwise genotype differences between study individuals in pilot genotyping data. It then applies a greedy algorithm to select a reference panel with maximal phylogenetic diversity. To evaluate the proposed algorithm, we simulate sequences of 100kb in length under different scenarios. We then perform imputations on the simulated datasets, separately using reference panels constructed by our phylogenetic diversity algorithm and reference panels consisting of randomly selected individuals. We summarize the performance of our algorithm using several metrics, including the discordance rates between imputed and simulated genotypes and imputation accuracy as measured by estimated r^2. We identify scenarios where the proposed algorithm for selecting sequenced individuals improves imputation quality. Thus we provide a novel tool of leveraging existing genotype data when planning sequencing studies.

618F

A High-Performance Computing Package for Relatedness and Principal Component Analysis in GWAS. *X. Zheng, D. Levine, J. Shen, C. Laurie, B. Weir.* Department of Biostatistics, University of Washington, Seattle, WA.

Genome-wide association studies (GWAS) are widely used to understand the genetic basis of diseases and traits, but pose many computational challenges. We developed SNPRelate, a high-performance parallel computing R package to accelerate two key computations in GWAS: principal component analysis (PCA) and relatedness analysis using identity-by-descent (IBD) measures. R is the most popular statistical environment, but by default it is not optimized, nor does it perform calculations in parallel. The kernels of our algorithms are written in C/C++ and have been highly optimized by blocking the computations to exploit the high-speed cache memory available on modern computer architectures. Benchmarks show the uniprocessor implementations of PCA and IBD in SNPRelate are ~10 to 45 times faster than the implementations provided in the popular EIGENSTRAT and PLINK programs, respectively, and can be sped up further by utilizing multiple cores. SNPRelate can analyze tens of thousands of samples with millions of SNPs. As an example, our package was used to perform PCA on 55,000 subjects from the “Gene-Environment Association Studies” (GENEVA) consortium studies. Our study is supported by National Institutes of Health (HG 004446).

619F

The Effect of Genome-Wide Autozygosity on Cognitive Ability. *D.P. Howrigan[1], G. Davies[2], B.M. Neale[3], A.F. McRae[4], S.E. Harris[2], N.G. Martin[4], M.J. Wright[4], N. Pendleton[5], A. Payton[5], M. Horan[5], W.E. Ollier[5], A. Tenesa[6], J.M. Starr[7], I.J. Deary[2], P.M. Visscher[4], M.C. Keller[1].* 1) Dept. of Psychology and Neuroscience, University of Colorado at Boulder, Boulder, CO; 2) Department of Psychology, University of Edinburgh, Edinburgh, UK; 3) Broad Institute, Harvard University and MIT, Boston, MA; 4) Genetic Epidemiology, Molecular Epidemiology and Queensland Statistical Genetics Laboratories, Queensland Institute of Medical Research, Brisbane, Australia; 5) School of Translational Medicine, University of Manchester, Manchester, UK; 6) Colon Cancer Genetics Group, Institute of Genetics and Molecular Medicine, University of Edinburgh and MRC Human Genetics Unit, Edinburgh, UK; 7) Geriatric Medicine, University of Edinburgh, Royal Victoria Hospital, Edinburgh EH4 2DN, UK.

Hundreds of scientific investigations on plants and animals have found that offspring of genetically related individuals tend to have weaker constitutions and lower fertility, a phenomenon called “inbreeding depression.” Previous studies based on known pedigrees have found small but reliable inbreeding depression effects for the intelligence quotient (IQ) in humans. An alternative to the pedigree approach of studying inbreeding depression of IQ is to use genome-wide SNP data to detect interpersonal differences in the levels of autozygosity (stretches of the two homologous chromosomes within the same individual that are identical by descent). Autozygosity is a genetic signature of inbreeding, even at low levels, and can reveal the full effects of rare, partially recessive, deleterious alleles that negatively impact cognitive ability. We hypothesize that individuals with a larger burden of autozygous segments in their genome will express more cognitive-impairing, recessive, deleterious alleles, which will manifest as a negative relationship between autozygosity burden and IQ. To test this hypothesis, we examined genome-wide SNP data and measures of general cognitive ability from 4,489 Caucasian individuals from five geographic regions: British Isles (n=3,229), Australia (n=537), Northern Europe (n=365), North America (n=290), and Spain (n=68). We scanned genomes for runs of homozygosity (ROH), and used thresholds tuned specifically to capture autozygosity, both within and across the five geographic regions. After controlling for possible confounds such as sex, age when cognitive test was taken, year born, income, and education, we test the association of genome-wide ROH burden on general cognitive ability. Preliminary analysis on ~1,000 subjects in a combined sample found higher ROH burden to be significantly and negatively associated with general cognitive ability (beta=-3.27, p=0.002). Results from the entire sample set, to be completed in summer of 2011, will determine the whether these initial findings are reliable.

620F

Loss of accuracy when imputing genotypes in previously phased individuals. *P.H. Keung[1], B.L. Browning[2].* 1) Department of Biostatistics, University of Washington, Seattle, WA; 2) Department of Medicine, Division of Medical Genetics, University of Washington, Seattle, WA.

It is now possible to impute genotypes for >10 million markers using data from the 1000 Genomes project as a reference panel. Imputation of such large numbers of genotypes is computationally expensive, and one strategy for reducing computation time is to phase the to-be-imputed samples prior to imputation. However, pre-phasing the to-be-imputed individuals may cause significant losses in imputation accuracy since imputation algorithms can no longer take into account haplotype phase uncertainty in the to-be-imputed data. We investigate the differences in imputed genotype accuracy between phased and unphased to-be-imputed panels from the 1958 UK birth cohort and the GAIN African-American Schizophrenia datasets using 3 popular imputation software packages: Beagle, Impute and Mach/Minimac.

We masked every 10th marker in the to-be-imputed data and evaluated imputation accuracy using two metrics: genotype discordance between imputed and masked genotypes and squared correlation between imputed and masked minor allele dosage. We used two reference panels for each data set: a perfectly-matched reference panel from the same data set and a 1000 Genomes Project reference panel.

Our results demonstrate that pre-phasing the to-be-imputed data can result in a noticeable loss in imputation accuracy. Thus, one has to consider whether the loss in accuracy is worth the savings in computation effort obtained by pre-phasing.

621F

"Next Generation" Imputation: Assessing the 1000 Genomes Project reference panel and imputation into multi-ethnic cohorts. *S.C. Nelson[1], C.C. Laurie[1], B.L. Browning[2], S.M. Gogarten[1], J. Li[3], M. Sale[4], B.B. Worrall[5], K. Doheny[6], E. Bookman[7], GARNET Collaborative Research Group.* 1) Dept of Biostatistics, University of Washington, Seattle, WA; 2) Dept of Medicine, Division of Medical Genetics, University of Washington, Seattle, WA; 3) Dept of Human Genetics, University of Michigan, Ann Arbor, MI; 4) Center for Public Health Genomics, University of Virginia, Charlottesville; 5) Dept of Neurology, University of Virginia, Charlottesville, VA; 6) Center for Inherited Disease Research, Johns Hopkins University, Baltimore, MD; 7) National Human Genome Research Institute, Bethesda, MD.

The 1000 Genomes (1000G) Project has increased the potential for genotype imputation to enhance genome-wide association studies. In particular, 1000G panels will enable the imputation of many more and rarer variants. We also expect imputation accuracy to improve as the number and genetic diversity of samples sequenced increases. Using BEAGLE software, we assessed the robustness of imputation in two domains: (1) accuracy of 1000G imputation for variants that are not on standard SNP microarrays and (2) effect of imputing multiple ethnic groups together using a cosmopolitan reference panel. For aim (1), we compared imputed genotypes for two unrelated CEU samples to those observed in the 1000G pilot 2 deep sequencing project. HapMap3 SNPs served as the imputation input, and all CEU and TSI HapMap 3 samples not in the 1000G EUR reference panel were included in the to-be-imputed sample. Our results show that imputation accuracy using the EUR reference is consistently high (mean per-SNP concordance rates of 96%) at SNPs with minor allele frequency (MAF) ⁄ 0.1 in the reference, but decreases rapidly as MAF falls below 0.05. While indicating that 1000G imputation accuracy at very rare variants is currently limited, these results also suggest that remaining variants are imputed as well as if not better than when using a HapMap3 reference. Taking imputation quality into account during downstream analysis is advisable to compensate for this range in accuracy. As the Project matures, yielding larger and higher quality datasets, we expect imputation at these rarer variants to improve. To address aim (2), we compared two separate imputations of 2,069 participants from the Vitamin Intervention for Stroke Prevention (VISP) trial, using HapMap 3 references. While the majority of VISP participants have European genetic ancestry (EA), 18% show significant Asian and African components. We compared the correlation between results for the EA participants from imputing (a) EA alone using a CEU and TSI HapMap 3 reference versus (b) all VISP together using a combination of 10 HapMap 3 populations. We show that expected allele dosages for EA participants in the two scenarios are highly correlated (mean=0.994), indicating that imputation need not be restricted to a genetically homogenous set of study samples. Our findings from both aims (1) and (2) support a broader goal for genetic studies to include more genetically diverse samples and interrogate rarer variants.

622F

Challenges and future directions for causal inference from multiple omics datasets using Structural Equation Modeling. *S-Y. Shin[1], A-K. Petersen[2], K. Small[3], E. Grundberg[1,3], P. Deloukas[1], T.D. Spector[3], K. Suhre[4,5], C. Giegar[2], N. Soranzo[1,3].* 1) Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, United Kingdom; 2) Institute of Epidemiology, Helmholtz Zentrum München, Neuherberg, Germany; 3) Department of Twin Research & Genetic Epidemiology, King's College London, London, United Kingdom; 4) Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, Neuherberg, Germany; 5) Faculty of Biology, Ludwig-Maximilians-Universität, München, Germany.

Recent developments in technology allow us to collect multiple highly-dimensional `omics' datasets from thousands of individuals in a highly standardized and unbiased manner. For instance, the characterization of such datasets, including genome-wide SNP data, transcriptomics, proteomics, metabolomics and others, collected in deeply-phenotyped population-based cohorts, can be applied to search for new biomarkers and to characterize pathways underlying disease risk. Open questions remain how best to integrate or bridge the multiple omics datasets to understand underlying biological mechanisms and infer causal pathways. We have begun exploring causal relationships between genetic variants, clinically-relevant quantitative phenotypes and metabolomics datasets using Structural Equation Modeling (SEM), applied to a subset of the common disease loci identified from genome-wide association studies. We provide proof-of-principle evidence that SEM analysis is able to identify reproducible path models supporting association of SNPs to intermediate phenotypes through metabolomics intermediates. In this study, we aim to address further challenges arising from the analysis of multiple omics datasets and suggest future directions for causal inference using SEM and other statistical inference methods. Firstly, complex biological systems tend to be nonlinear in nature. We seek here to detect and incorporate nonlinearity into our model. Another further concern is how to deal with the correlated structure of the data when seeking to extend these analyses to more complex data structures including multiple genetic variants and traits. While Principal Component Analysis (PCA) can be applied to extract useful information out of a high dimensional highly correlated dataset, it is hard to interpret and not applicable to multiple omics datasets. Performing dimensionality reduction on the complete dataset simultaneously, preserving the important links between the data types would be more desirable. We explore other projection based and machine-learning techniques to overcome these limitations and extend our original analysis.

623F

A comparison of seven statistical methods for detecting rare variant association. *R. Weyant, C. Fuchsberger, T.M. Teslovich, W. Chen, Y. Hu, M. Boehnke.* Biostatistics, University of Michigan, Ann Arbor, MI.

Large-scale sequencing projects such as the 1000 Genomes Project have identified millions of low frequency and rare genetic variants in human populations. Multiple methods have been proposed to analyze genes harboring rare variants to test for disease association and so to better understand how these rare variants contribute to human disease risk. In planning a genetic association study, an investigator might ask: which analysis method is best for my data? Is one method best to address certain genetic hypotheses, but less powerful for others? Is there a single test that is uniformly most powerful under a reasonable set of constraints? We are carrying out a computer simulation study to compare seven prominent methods for rare-variants genetic association tests: C-ALPHA (Neale et al.), the variable threshold method (Price et al.), the weighted sum test (Madsen and Browning), the CMC test (Li and Leal), and SKAT (Wu et al.), in addition to other methods, which use intuitive sum statistics, alternative weightings or find variants unique to cases. We compare the performance of these methods under a variety of disease models. Specifically, we simulate several loci with different fractions of causal variants of various allele frequencies and effect sizes. Additionally, we vary the locus length and recombination patterns to simulate different scenarios, and examine the effect on power when associated loci contain both protective and risk rare variants. Initial simulation results suggest that under the null model, most methods maintain an approximate type I error rate, while the frequency-based weighted sum method appears anti-conservative as locus size increases. We are now carrying out simulations to allow us to compare power of the various methods under biologically plausible scenarios.

624F

Characterisation of the glaucoma phenotype in patients carrying risk alleles at novel open angle glaucoma genes, *TMCO1* and *CDKN2B-AS1*. *K.P. Burdon[1], R. Casson[2], A. Crawford[1], A.W. Hewitt[3], S. Macgregor[4], D.A. Mackey[5], J.E. Craig[1].* 1) Dept Ophthalmology, Flinders University, Adelaide, SA, Australia; 2) South Australian Institute of Ophthalmology, Hanson Institute and Adelaide University, Adelaide, SA, Australia; 3) Centre for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital, Melbourne, VIC, Australia; 4) Genetics and Population Health, Queensland Institute of Medical Research, Brisbane, QLD, Australia; 5) Lions Eye Institute, University of Western Australia, Centre for Ophthalmology and Visual Science, Perth, WA, Australia.

Open Angle Glaucoma (OAG) is a progressive neuropathy of the optic nerve affecting 3% of the population over age 50 years, and may lead to severe visual loss. We recently identified SNPs in *TMCO1* and *CDKN2B-AS1* that are associated with OAG at genome-wide significance with subsequent replication (Burdon et al. *Nat Genet.* 2011 43(6):574-8). Here we describe the clinical characteristics of OAG patients with glaucoma risk alleles at these loci compared to patients without these risk alleles in order to better characterise the phenotype associated with these loci. We obtained data from 1432 glaucoma patients with genotype data available at the most significantly associated SNPs at both loci from the published GWAS (rs4656461 and rs7518099 at *TMCO1* as well as rs1012688 and rs4977756 at *CDKN2B-AS1*). Data was available for a range of OAG risk factors including intraocular pressure (IOP), cup:disc ratio and central corneal thickness as well as age at diagnosis and sex. Linear regression was used to detect associations between the SNPs of interest and these traits. The risk alleles at the *TMCO1* locus result in younger age at diagnosis (p=0.003) which remains significant after adjustment for all other variables (p=0.020). The risk alleles at *CDKN2B-AS1* are associated with lower peak intraocular pressure in this cohort, independently of other covariates (p=0.004). To investigate the combined effect of the two loci, the number of risk alleles at SNPs rs4656461 and rs4977756 were added to generate a total number of risk alleles (ranging from 0 to 4). An increasing number of glaucoma risk alleles is associated with a younger age of onset (p=0.005) and larger cup:disc ratio (p=0.001), however only age of onset remained significant under multivariate analysis (p=0.022). The CDKN2B-AS1 locus is associated with cup:disc ratio in the normal population, consistent with that observed here. In conclusion, glaucoma risk alleles at the *TMCO1* gene are associated with a younger age of glaucoma diagnosis while a relationship was observed between the *CDKN2B-AS1* locus and IOP. Carriers of 4 risk alleles are diagnosed around 6 years younger than carriers of 0 or 1 allele. These results suggest that carriers of these risk alleles are more likely to develop glaucoma at a younger age and without displaying elevated IOP. These genetic variants may be useful for identification of patients at high risk of progression in the absence of the traditional risk factors, elevated IOP and age.

625F

A phenome-wide analysis of SNPs in the National Human Genome Research Institute genome-wide association catalog. *J. Denny[1,2], L. Bastarache[1], M. Ritchie[1,3,4], M. Basford[6], J. Pulley[6], R. Zuvich[3,4], P. Peissig[8], D. Carrell[9], J. Pathak[7], L. Rasmussen[8], J. Pacheco[16], A. Kho[10], N. Weston[9], S. Pendergrass[4], H. Xu[1], R. Li[11], T. Manolio[11], I. Kullo[12], C. Chute[7], R. Chisolm[13], E. Larson[9], C. McCarty[14], D. Masys[1], D. Roden[2,15], D. Crawford[3,4].* 1) Department of Biomedical Informatics, Vanderbilt University, Nashville, TN; 2) Department of Medicine, Vanderbilt University, Nashville, TN; 3) Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN; 4) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 5) Division of Epidemiology, Mayo Clinic, Rochester, MN; 6) Office of Research, Vanderbilt University, Nashville, TN; 7) Department of Biomedical Statistics & Informatics, Mayo Clinic, Rochester, MN; 8) Biomedical Informatics Research Center, Marshfield Clinic Research Foundation, Marshfield, WI; 9) Group Health Research Institute, Seattle WA; 10) Department of Medicine, Northwestern University, Chicago, IL; 11) Office of Population Genomics, National Human Genome Research Institute, Bethesda, MD; 12) Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN; 13) Department of Cell & Molecular Biology, Northwestern University, Chicago, IL; 14) Center for Human Genetics, Marshfield Clinic Research Foundation, Marshfield, WI; 15) Department of Pharmacology, Vanderbilt University, Nashville, TN; 16) Center for Genetic Medicine, Northwestern University, Chicago, IL.

Candidate gene and genome-wide association studies (GWAS) have provided greater understanding of the genetic underpinnings of many traits. Genetic data coupled to electronic medical records (EMR) offer the possibility of the inverse experiment, a “hypothesis-free” analysis of many phenotypes, or phenome-wide association scan (PheWAS). In this study, we used a cohort of 13,859 European-ancestry individuals genotyped in the Electronic Medical Records and Genomics (eMERGE) network. We performed PheWAS on 945 SNPs significantly associated ($p<5x10^{-8}$) with traits in the NHGRI GWAS catalog. We examined each SNP for association with 947 phenotypes algorithmically defined using billing codes derived from normal clinical processes. We used logistic regression models for each phenotype adjusted for age, gender, and principal components. PheWAS replicated 42%; (226/545) of GWAS catalog SNP-trait associations in 57 phenotypes, and 63% (82/130) of associations sufficiently powered for replication given our case size. A total of 46 SNP-phenotype associations in 24 phenotypes achieved genome-wide significance in PheWAS; 34 of these were previously known. Replications included Alzheimer's disease (*APOE*/*TOMM40*, $p=1x10^{-25}$), hemochromatosis (*HFE*, $p=2x10^{-25}$), type 2 diabetes (*TCF7L2*, $p=3\ x10^{-16}$), ischemic heart disease (9p21.3, $p=1.10^{-12}$), and prostate cancer (8q24.21, $p=9x10^{-8}$). New associations included acquired hypothyroidism ($p=4x10^{-12}$) with *FOXE1* variants previously associated with thyroid cancer; skin neoplasms ($p=4x10^{-17}$) and actinic keratosis ($p=8x10^{-11}$) with *IRF4* variants previously associated with skin color; and aplastic anemia ($p=3x10^{-6}$) with *WDR66* variants previously associated with mean platelet volume. Known pleiotropy at the 9p21.3 region (myocardial infarction, abdominal aortic aneurysm, and cerebrovascular disease) was replicated, and new associations were suggested (carotid stenosis, asthma, atherosclerosis, and other aneurysms, all $p<10^{-5}$). *IRF4* variants were the most pleiotropic, with 14 phenotypes associated at $p<10^{-5}$. EMR-based PheWAS replicated many SNP-disease associations in a real-world cohort, and thus can serve as a tool to improve confidence in GWAS associations. This analysis also suggested new, biologically plausible associations (“hypothesis-generating”) for further study. Future use of PheWAS in tandem with traditional genetic analysis may help define pleiotropy and improve understanding of the genetic architecture of complex diseases.

626F

Sub-clinical Phenotypes used to Dissect Genetic Heterogeneity in Cleft Lip with/without Cleft Palate. *M.L. Marazita[1], M.E. Cooper[1], M. Govil[1], R.S. DeSensi[1], T. McHenry[1], S. Weinberg[1], J.C. Murray[2], K. Neiswanger[1], C. Brandon[1], A.E. Czeizel[3], A.R. Vieira[1], E.E. Castilla[4], F. Poletta[5], J.M. Resick[1], S. Daack-Hirsch[2].* 1) Center for Craniofacial and Dental Genetics, Univ Pittsburgh SDM, Pittsburgh, PA; 2) Pediatrics, University of Iowa, Iowa CIty, IA; 3) Foundation for the Community Control of Hereditary Diseases, Budapest, Hungary; 4) Department of Genetics, FIOCRUZ, Rio de Janeiro, RJ, Brazil; 5) CEMIC, Buenos Aires, Argentina.

Orofacial clefts (OFCs), particularly cleft lip with/without cleft palate (CL/P), are the most common craniofacial birth defects in every population worldwide. Our group and others have made major strides in recent years in identifying genes and genomic locations that increase the risk of OFCs, notably IRF6, 8q24 (a gene desert region), FOXE1, MAFB, ABCA4-ARGHAP29 and others. Sequencing studies are underway in the genes/regions identified in order to identify etiologic variants. Thus, a current challenge for studies of OFCs is to understand the genetic heterogeneity implicit in our results to date. We hypothesize that evaluation of sub-clinical phenotypes (physical features with no major health consequences) is a valuable tool to dissect the observed genetic heterogeneity. In the current study we performed genetic studies incorporating three phenotypes (lip muscle discontinuities--OOM, lip print whorls---LIP, and velopharyngeal insufficiency--VPI). The study sample is 364 families (1,814 individuals—529 cleft affected, 1285 unaffected) ascertained and phenotyped as part of a large international collaboration, with genotypes including a genome-wide panel of 5,755 SNPs plus SNPs in the genes/regions above. Association analyses using FBAT were done: (1) with OFC as affected (baseline), (2) adding OOM, LIP or VPI as affected, (3) in groupings of families based on phenotypes; e.g., OOM-Yes families had at least one family member with OOM, and OOM-No did not. RESULTS: (1) adding sub-clinical phenotypes increased statistical significance compared to baseline association analyses for multiple SNPs; e.g. from p=0.002 to 0.00008 for rs1002122 in CNTN5. (2) notably, there were also significant differences between the pattern of results in OOM-Y vs. OOM-N, LIP-Y vs. LIP-N, and VPI-Y vs. VPI-N. For example, at baseline and in OOM-Y there was no significant association with rs12697439 (near ZNF131), but in OOM-N there was (p=0.0002). Further, the difference between the results for OOM-Y vs. OOM-N was significant (p=0.00004). In summary, these results indicate that sub-clinical phenotypes have the potential to be a powerful tool for understanding genetic heterogeneity in CL/P. Understanding the genetic architecture of CL/P via phenotyping could also lead to important clinical implications since such phenotypes can be viewed as “rescue” of the birth defect in some family members carrying CL/P risk factors. Support: DE016148, DE008559, DE018085.

627F

Rare variant association methods and their performance when confounded by population substructure. *T.D. O'Connor[1], G. Wang[2], E. Kenny[3], C.D. Bustamante[3], S.M. Leal[2], M.J. Bamshad[1,4], J.M. Akey[1] on behalf of the NHLBI Exome Sequencing Project.* 1) Genome Sciences, University of Washington, Seattle, WA; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Department of Genetics, Stanford University, Palo Alto, CA; 4) Department of Pediatrics, University of Washington, Seattle, WA.

With advances in next-generation sequencing technology it is now possible to systematically test the contribution of rare variation to the burden of Mendelian and complex disease. It is well known that association tests of common alleles can lead to false positives in the presence of population stratification. However, the influence of population structure on association methods for rare variants remains poorly defined. To address this critical gap in knowledge, we performed exhaustive coalescent simulations, whose parameter values were carefully calibrated with over 300 European-American exomes, to evaluate the performance of ten rare variant association tests. We find that all methods have an inflated type I error rate for parameter values consistent with levels of differentiation and differences in disease prevalence among European populations, and in some cases dramatically so. For example, at a nominal significance level of 5%, some test statistics have a false positive rate as high as 40%. We investigated two approaches for mitigating spurious associations in the presence of population structure; the incorporation of covariates, such as eigenvectors from principle component analysis (for methods that allow covariates) and a matching strategy where cases and controls are matched based on population origin. In both cases, the type I error rate is reduced to nominal, or near nominal, levels. In conclusion, population stratification is a confounding factor that can lead to spurious associations in rare variation association methods, but relatively simple approaches can ameliorate such issues, though at a slight cost to power. Thus, our results have important implications for the design, analysis, and interpretation of genome-wide association studies of rare variants.

628F

Genome-wide scan in Italian isolated populations identify loci for food preferences. *N. Pirastu[1], C. Lanzara[1], A. Robino[1], G. Pistis[2], M. Traglia[2], C. Sala[2], D. Toniolo[2], B. Tepper[3], Y. Koelliker[3], P. Gasparini[1].* 1) Medical Genetics, IRCCS Burlo Garofolo/Università degli Studi di Trieste, Trieste, TS, Italy; 2) Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milano, Italy; 3) Department of Food Science, School of Environmental and Biological Sciences, Rutgers University, New Brunswick, NJ.

Taste is the most important factor guiding food choices, and the knowledge of the genetic basis of taste perception is increasing year by year. However these efforts focused mainly on taste receptor genes and not on food preferences themselves. In this study we present the first genome wide scan looking for genes that underlie food preferences in 3 isolated Italian populations coming from the Italian Network of Genetic Isolates (INGI): Carlantino (CARL, Southern Italy), Friuli Venezia Giulia (FVG, North-Eastern Italy) and Val Borbera (VB, North-Eastern Italy). In particular we administered, during a wider screening, a questionnaire on 55 food preferences (such as ham, eggplant, ice cream, etc.) in a total of 2769 people. Each person had to score liking for each food from 1 to 9 where 1 was “I don't like it at all” and 9 was “I like it a lot”. For all people information on health parameters genealogy and life styles were available. Moreover all of the samples were genotyped with the 370k Illumina Chip and subsequently imputed to the 2.5M HapMapII SNP set using MACH. In order to understand the genetic bases of food preferences we defined 2 measures: the first was “absolute preference” based on the liking scores; the second was corrected for each person's median liking score across all the foods, which we called “relative preference”. Relative preference measures liking of individual foods relative to all foods. For each trait we ran a genome-wide scan corrected for kinship using the GenABEL/ProbABEL suite using sex and age as covariates. Each population was run separately and pooled together through inverse variance meta-analysis for a total of 110 total genome wide scans. We identified 4 genome wide significant loci ($p \leq 5x10\text{-}8$) for Whole Milk, Hot Tea, Ice Cream and Chilli Pepper liking, plus 29 genome wide suggestive loci ($p \leq 5x10\text{-}7$). Three out of four significant identified genes which have not been previously described for taste, but that have interesting chemosensory and cell signalling functions while the third one falls close to a gene of unknown function. We believe that this study represents a good starting point for understanding the genetics of food preferences. This knowledge will impact deeply in our views on taste and food choice and will help understand the basis of obesity and other diseases in which food intake is a strong component.

629F

DNA repair genotype-phenotype correlation and interplay between different DNA repair pathways. *F. Voglino[1], A. Allione[1], A. Russo[1,2], F. Ricceri[1,2], S. Guarrera[1], S. Polidoro[1], F. Rosa[1], C. Di Gaetano[2], V. Simonelli[3], P. Porcedda[4], F. Mazzei[3], C. Giachino[4], E. Dogliotti[3], G. Matullo[1,2].* 1) Human Genetics Foundation, Turin, Italy; 2) Department of Genetics, Biology and Biochemistry, University of Turin, Italy; 3) Department of Environment and Primary Prevention, ISS, Rome, Italy; 4) Department of Clinical and Biological Sciences, University of San Luigi Gonzaga, Turin, Italy.

Individual DNA repair capacity (DRC) is modulated by the genetic background to which hundreds of SNPs or haplotype combinations in DNA repair genes are likely to contribute. In order to identify functional SNPs modulating DNA repair phenotypes and to investigate their potential impact on cancer risk, we have performed a genotype-phenotype correlation study evaluating levels of DNA damage and DRC in blood cells treated at low doses of different DNA damaging agents. We investigated the association between genotypes and the DRC measured with the following DNA repair phenotypic assays on cryopreserved lymphocytes from 225 healthy individuals: Comet assay after treatment with BPDE, phosphorylation of histone H2AX after treatment with gamma-rays, OGG1 glycosylase activity, OGG1 and ERCC1 expression levels. 65 DNA repair genes and a total of 768 SNPs have been analysed on the 225 DNA samples by using a custom GoldenGate SNP chip (Illumina). Genotype-phenotype correlation analysis identified some new functional variants and haplotypes. A confirmation of some already described interplays between different DNA repair pathways have been obtained from SNP analysis and a suggestion of new possible cross-talks have been identified that need further experimental validation. Our results can contribute to identify subjects carrying combinations of less efficient DNA repair variants/haplotypes possibly at increased cancer risk or who can better benefit from anti-cancer therapies.

630F

Rare and Common SNPs in DRD5 Have Unique and Independent Effects on Risk for Childhood ADHD. *I. Waldman[1], M. Nikolas[2], K. Jernigan[3], J. Nigg[4], K. Friderici[3].* 1) Dept Psychology, Emory Univ, Atlanta, GA; 2) Dept Psychology, Michigan State University, East Lansing, MI; 3) Dept Micro. and Mol. Gen., Michigan State University, East Lansing MI; 4) Dept Psychiatry, Oregon Health and Science University, Portland OR.

Considerable controversy exists in the recent genetics literature regarding the contributions of common versus rare variants in conferring risk for common disorders and traits. In this study, we resequenced the dopamine receptor D5 gene (DRD5) to search for rare variants that may confer risk for childhood ADHD. DRD5 is an important candidate gene for ADHD, but examination of it as a risk factor has been hampered by its location in a segmentally duplicated chromosomal region. Restriction enzyme digestion of genomic DNA was used to eliminate the pseudogenes prior to PCR amplification of the functional gene. Resequencing of DRD5 was conducted on 319 children with ADHD and 296 control children. The sample was 56% male, aged 6-17 years (M=10.8, SD=2.3), and ethnically diverse, with 20% having some African-American ancestry. Sequencing revealed 13 rare SNPs (6 non-synonymous, 6 synonymous, and 1 frameshift mutation) and 2 common SNPs. Rare variants were found disproportionately on haplotypes containing the minor alleles of the 2 common SNPs. Generalized Linear Models with GEE were used to test for the association of the SNPs with ADHD and its diagnostic subtypes and symptom dimensions. Analyses revealed that neither the genotypes of the 2 common SNPS nor the presence of a rare variant were associated with ADHD, nor with the Combined or Inattentive subtypes. In contrast, levels of hyperactive-impulsive (but not inattentive) symptoms were uniquely and independently associated with both of the 2 common SNPs and the presence of rare variants (p-values ranging from .005 to <.001). The 2 common SNPs explained 2% and 3.7% of the variance in hyperactive-impulsive symptom levels, with the presence of a rare variant contributing an additional 1.3% of the variance. Children with a rare DRD5 variant showed an increase of 2.1 hyperactive-impulsive symptoms as compared with children with no rare variants. Similarly, children with the risk-inducing allele at the 2 common SNPs showed increases of 1.6 and 2.0 hyperactive-impulsive symptoms as compared with children with the low-risk allele at those markers. These results suggest that both common and rare SNPs in or near the coding region of DRD5 make unique and independent contributions to the risk for the hyperactive-impulsive symptoms of ADHD in children. Our results also highlight the signal importance of using genotyping procedures that avoid segmentally duplicated chromosomal regions common to DRD5 and other genes.

631F

HIV-associated nephropathy and focal segmental glomerulosclerosis: APOL1 trypanolytic mutations underpin a major health disparity. *C. Winkler[1], G. Nelson[2], G. Genovese[3], M. Pollak[3], J. Kopp[4].* 1) Basic Research Laboratory, Center for Cancer Research, SAIC-Frederick, NCI, NIH, Frederick, MD; 2) Basic Science Program-Center for Cancer Research Genetics Core, SAIC-Frederick, NCI, NIH, Frederick MD; 3) Beth Israel Deaconess Medical Center, Harvard Medical School, Boston MA; 4) NIDDK, NIH, Bethesda, MD.

HIV-associated nephropathy (HIVAN) occurs in 10% of HIV-infected African Americans (AA) in the absence of antiretroviral treatment, but was rarely observed in non-Africans. We recently showed that two APOL1 alleles, (S342G-I384M (G1) and N388del:Y389del (G2), occurring on mutually exclusive haplotypes, are recessively associated with kidney disease in AA. These mutations also restore APOL1 lysis activity against Trypansoma b. rhodensiense in plasma from carriers of one or two copies of either G1 or G2; thus there appears to be a heterozygous advantage/homozygous disadvantage. The role of these variants in HIVAN has not been investigated. We studied patients with HIVAN and focal segmental glomerulosclerosis (FSGS) to test for interaction between HIV and APOL1 risk alleles, to determine effect sizes, and to determine if APOL1 risk alleles are associated with particular clinical phenotypes. Genotypes were obtained for 439 cases with HIVAN or FSGS and 939 normal controls or HIV-infected subjects with no evidence of kidney disease. In a recessive model, APOL1 variants were strongly associated with HIVAN (OR= 29; 95% CI 13, 68) and FSGS (OR= 17; 95% CI 11, 26) for FSGS. For FSGS associated with two APOL1 risk alleles, compared to other FSGS patients, onset age was earlier (p=0.03), steroid sensitivity was similar (p>0.5), and progression to ESKD was faster (log-rank p<0.01). Two APOL1 risk alleles confer an attributable risk of 67% for FSGS and HIVAN and explain 35% of HIVAN and 18% of FSGS. HIV-infected, untreated individuals with two risk alleles have an estimated 50% lifetime risk for developing HIVAN compared to a 4% risk of FSGS for uninfected individuals with two risk alleles. APOL1 kidney risk alleles are present only on African chromosomes and the risk alleles are most frequent in West Africans (342G-384M~36%; deletion~8%). The finding that individuals carrying two copies of G1 or G2 have a greatly increased risk of HIVAN and FSGS provides a genetic basis for a major global health disparity. These findings have particular relevance for sub-Saharan Africans where the HIV prevalence is high and the frequencies for the risk alleles exceed 40% among some West African populations.

632F

Association Tests for Rare Variants Based on Regularized Regression Methods. *C. Xu[1,2], M. Ladouceur[1,3], J.B. Richards[2,3,4,5], A. Ciampi[2], C. Greenwood[1,2,6].* 1) Lady Davis Institute for Medical Research, Montreal, QC; 2) Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, QC; 3) Department of Human Genetics, McGill University, Montréal, QC; 4) Department of Medicine, Jewish General Hospital, McGill University, Montreal, QC; 5) Twin Research and Genetic Epidemiology, King's College London, London, United Kingdom; 6) Department of Oncology, McGill University, Montreal, QC.

Collapsing or pooling methods are often used to detect association with rare variants while ignoring common variants, even though they may have direct or indirect association with the outcome. We compare a variable selection method (LASSO) and a dimension reduction method (partial least squares regression, PLS) with two commonly used pooling methods (weighted minor allele counts, and variable threshold technique). For LASSO and PLS, the models are fit with and without pooling rare variants. Power of these methods was compared in simulations, where continuous phenotypes were generated but genotypes were generated from Sanger sequencing of one gene in 1998 individuals (provided by GlaxoSmithKline). Permutations ensured valid type 1 error. Simulation results for three scenarios are shown in the following table. In Scenario 1, 30% of the SNPs with 0.001<MAF≤0.01 are causal. In Scenario 2, 10% of SNPs with MAF≤0.01 are causal. Finally, in Scenario 3, there are 4 causal rare SNPs with MAF≤0.01 and 4 causal common SNPs. All rare variant effects acted in the same direction. The rare SNPs effects follow a normal N(-1.64, 0.02) distribution, non carriers follow N(0,1), and the common SNPs effect are simulated from N(-0.07, 0.01). LASSO and PLS outperform simple pooling approaches in some situations. These novel association tests will be evaluated in additional sequenced genes.

Scenario	Count weighted by 1/MAF	Variable threshold	LASSO w/o rare pooling	LASSO with rare pooling	PLS w/o rare pooling	PLS with rare pooling
1	0.78	0.97	1.00	1.00	1.00	1.00
2	0.39	0.58	0.72	0.73	0.68	0.71
3	0.88	0.72	0.87	0.69	0.71	0.65

633F

Identifying rare variants with heterogeneous effects from pooled sequencing data. *Q. Zhang[1], K. Wang[2], M.A. Province[1], I. Borecki[1].* 1) Division of Statistical Genomics, Washington University School of Medicine, St. Louis, MO, USA; 2) Department of Biostatistics and Epidemiology, College of Public Health, East Tennessee State University, Johnson City, TN, USA.

Separately sequencing pooled DNA from two groups of subjects with discrepancy in a target trait and comparing allele frequencies between the groups provide an economical strategy for identifying rare variants (RVs) associated with human complex traits; however, statistical methods for association analysis of pooled sequencing data, are very limited, especially the methods for collectively testing a group of RVs with heterogeneous (i.e. some positive and some negative) effects on a trait. All existing data-driven methods for collective test of heterogeneous effects of multiple RVs were developed for individuals' sequence data, and are inapplicable to the data from pooled experiments because they require permutation test which cannot be performed with no individuals' information. In this study, we propose three methods, a Fisher's combined probability test (FCPT), a likelihood ratio test (LRT) and an exact probability test (EPT), for collectively detecting heterogeneous effects of a group of RVs from pooled sequencing data. Unlike the commonly-used cohort allelic sums test (CAST) method (Morgenthaler and Thilly,2007) that tests the difference in the total RV numbers between two groups of samples, our methods test whether a group of RVs are randomly distributed in samples. Our simulation shows that the three proposed methods substantially increase power in comparison with CAST; the EPT method produces the most unbiased false positive rate (FPR) under the null hypothesis, and overall, demonstrates the best receiver operating characteristic (ROC). The appeal of the proposed approach is illustrated in an application to real sequence data. Finally, we discuss how to use simulation-based approach to appropriately approximate p-values when dataset is too large and the EPT method is too time-consuming to be used.

634F

Next-generation haplotype phasing: combining identity-by-descent with haplotype frequency models. *B.L. Browning[1], S.R. Browning[2].* 1) Department of Medicine, Division of Medical Genetics, University of Washington, Seattle, WA; 2) Department of Biostatistics, University of Washington, Seattle, WA.

Standard methods for inferring haplotype phase for a population sample incorporate sophisticated population haplotype frequency models, but do not explicitly detect or make use of recent shared ancestry between individuals. We present a new haplotype phasing method that uses both sources of information to phase pedigree and population data. Combining phasing constraints imposed by identity-by-descent (IBD) with population haplotype frequencies can provide a substantial improvement in phasing accuracy over the best existing methods. The new method achieves high phase accuracy for an individual in regions where the individual has a detectable IBD tract with another genotyped individual. Our previous work has shown that IBD tracts due to a common ancestor within approximately the last 25 generations are ubiquitous in large samples and can be detected with high power and low false discovery rate [1]. We demonstrate improved genotype phase accuracy using Wellcome Trust Case Control Consortium and HapMap data. This new haplotype phasing method will be incorporated in version 4 of the BEAGLE software package.

[1] B. L. Browning and S. R. Browning (2011) A fast, powerful method for detecting identity-by-descent. American Journal of Human Genetics 88(2):173-182.

635F

Validation of a method for classical HLA allele imputation. *C.S. Franklin[1], M. Hernandez-Fuentes[2], I. Rebollo-Mesa[2], J. Mollon[2,3], F. Delaney[2], E. Perucha[2], P. Conlon[4], M.E. Weale[2,3], N. Soranzo[1], G. Lord[2], United Kingdom and Ireland Renal Transplant Consortium, WTCCC3.* 1) Wellcome Trust Sanger Institute, Cambridge, Cambridgeshire, United Kingdom; 2) MRC Centre for Transplantation, King's College London, London, UK; 3) Department of Medical and Molecular Genetics, King's College London, London, UK; 4) Royal College of Surgeons in Ireland, Dublin, Ireland.

The classical HLA loci play important roles in susceptibility to autoimmune, infectious and a wide range of other diseases. Furthermore, they are known to affect the outcome of organ transplantation, therefore HLA alleles are typically determined prior to transplant. It has been shown that classical HLA alleles can be determined from dense SNP data through imputation (Dilthey et al 2011). However, the accuracy of the HLA imputation software has not been extensively tested. As part of the WTCCC3 renal transplant dysfunction project, we have genotyped 2,500 kidney donor and recipient pairs using Illumina Human660-Quad SNP arrays. Classical HLA alleles were determined prior to transplant. We have used the HLA*IMP program (Dilthey et al 2011) to obtain imputed HLA alleles and tested the concordance of these imputations with the directly typed alleles for a subset of the samples. We show that imputation provides highly accurate results at the 2-digit allele resolution, with concordance exceeding 97% for all alleles tested (HLA-A, HLA-B, HLA-C and HLA-DRB). This independent validation demonstrates that the HLA*IMP program can provide accurate HLA information in datasets with genome-wide SNP data. This suggests that imputation is a viable strategy for studying the contribution of HLA alleles to genetic risk in a range of diseases where genome-wide SNP data are available.

636F

Association of PPARG gene polymorphisms with obesity and insulin resistance syndrome in north India. *J. prakash[1], N. Srivastava[1], S. Awasthi[2], C. G. Agarwal[3], B. Mittal[4].* 1) Physiology, Chatrapati Shahuji Maharaj Medical University, Lucknow, Uttar Pradesh, India; 2) Pediatrics, Chatrapati Shahuji Maharaj Medical University, Lucknow, Uttar Pradesh, India; 3) Medicine, Chatrapati Shahuji Maharaj Medical University, Lucknow, Uttar Pradesh, India; 4) Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, U.P., India.

The worldwide increasing prevalence of obesity is considered as a major health problem, since excess body fat deposition is a strong risk factor for insulin resistance, type 2 diabetes (T2D) and dyslipidemia, leading to premature morbidity and death. Peroxisome proliferator-activated receptor gamma (PPAR-.), a nuclear hormone receptor, controls adipocyte differentiation and regulates a number of genes associated with energy homeostasis. In the present study, we investigated the association of PPAR-. gene Pro12-Ala (rs1801282) and C1431T (rs3856806) polymorphisms with morbid obesity and related phenotypes, in north Indian population. A case control study was conducted with 642 (309 obese, BMI⁄ 30) and 333 non-obese, BMI<30) subjects. Insulin and adiponectin levels were determined by enzyme-linked immunosorbent assay RIA (Linco Research, Inc.). The degree of insulin resistance was calculated according to the homeostasis model assessment (HOMA). The fasting glucose concentration was measured by Glucose oxidase-Peroxidase (GOD-POD) method. Commercial enzymatic test kits were used for determining HDL, total serum cholesterol and triglyceride concentrations, while LDL cholesterol was calculated by the formula of Friedewald. All subjects were genotyped by PCR restriction fragment length polymorphism (PCR-RFLP). All statistical analyses were conducted using SPSS for Windows version 15.0. Informed consent was obtained from each participant and the study was carried out in accordance with the local ethics committee. The ProAla+AlaAla genotypes of PPAR-. Pro12Ala were significantly associated with higher risk of obesity while C1431T polymorphism did not show any significant association. None of the haplotypes defined by both SNPs showed association with morbid obesity. However, a strong association of variant haplotypes was observed with lower levels of insulin, HOMA-IR index, and higher serum adiponectin concentrations. The combined variant haplotypes (Pro-T, Ala-C and Ala-T) also showed association with percentage body fat and fat mass, while these haplotypes did not show any association with the hypertension and lipid profile. PPAR-. polymorphisms influence obesity phenotype in a complex manner, probably involving insulin sensitivity in north Indian population. Acknowledgment: Indian Council of Medical Research, Department of Biotechnology, Department of science and Technology, New Delhi, India.

637F

Fine-mapping of autoimmune susceptibility loci using Immunochip identifies novel susceptibility loci for psoriatic arthritis. *J. Bowes[1], P. Ho[1,2], E. Korendowych[3], N. McHugh[3], H. Marzo-Ortega[4], J. Packham[5], I.N. Bruce[1,2], A. Barton[1,2].* 1) Arthritis Research UK Epidemiology Unit, University of Manchester, Manchester, UK; 2) The Kellgren Centre for Rheumatology, Central Manchester Foundation Trust, NIHR Manchester Biomedical Research Centre, Manchester, UK; 3) Royal National Hospital for Rheumatic Diseases and Dept Pharmacy and Pharmacology, University of Bath, UK; 4) NIHR-Leeds Musculoskeletal Biomedical Research Unit, Leeds Institute of Molecular Medicine, University of Leeds, UK; 5) Haywood Hospital, Stoke on Trent and Arthritis Research UK Primary Care Centre, Keele University, UK.

Psoriatic arthritis (PsA) is a chronic inflammatory joint disease that typically accompanies psoriasis vulgaris (PsV). Both conditions are considered to be complex diseases with both genetic and environmental susceptibility factors. Comparing the results from genome-wide association studies performed across many common autoimmune diseases, including PsA and PsV, has revealed an extensive overlap in the genetic liability between autoimmune diseases in general.

Based on the observed sharing of susceptibility loci across autoimmune diseases, the aim of this project is to identify novel susceptibility loci for PsA by fine-mapping all the currently confirmed susceptibility loci for 12 autoimmune diseases.

Data was available for 929 PsA cases and 4537 healthy controls genotyped using the Immunochip Illumina iSelect array at the Sanger Centre (www.sanger.ac.uk). Control data was sourced from the WTCCC (www.wtccc.org.uk). This custom array was designed to comprehensively fine-map confirmed autoimmune susceptibility loci and contains 196,524 SNPs covering approximately 200 candidate regions. A strict SNP-focused quality control process was applied to the dataset followed by single point analysis using the Armitage test for trend.

Association analysis of 182,883 high quality SNPs in 862 cases and 4306 controls detected robust association to previously confirmed PsA risk loci; *HLA-C* (p_{trend}=8.2x10-35), *IL23R* (p_{trend}=8.4x10-8) and *TRAF3IP2* (p_{trend}= 2.8x10-7), *IL12B* (p_{trend}=6.5x10-6). In addition we find convincing evidence to support association to a number of novel loci not previously reported for PsA, including; 17q21 (p_{trend}=3.3x10-05, *SMARCE1*), 18p11 (p_{trend}=2.0x10-05, *PTPN2*), 11q23 (p_{trend}=1.4x10-05, *TREH*), and 19p13 (p_{trend}=9.8x10-05, *TYK2*). Interestingly, we also find convincing evidence for association to the *CARD15* gene, supported by multiple SNPs.

Validation of these results is currently underway in an independent sample collection. This preliminary single-point analysis will be followed by analyses to identify independent effects at known and newly discovered loci.

638F

Evidence for a role of the familial cylindromatosis tumor suppressor *CYLD* in inflammatory bowel disease. *I. Cleynen[1], E. Vazeille[2], M. Artieda[3], M. Szczypiorska[3], M. Bringer[2], H.W. Verspaget[4], P.L. Lakatos[5], F. Seibold[6], A. Tariq[7], R.K. Weersma[8], I. Arijs[1], S. Müller[6], A. Tordai[9], D.W. Hommes[4], K. Parnell[7], C. Wijmenga[8], P. Rutgeerts[1], D. Lottaz[10], K. Van Steen[11], A. Darfeuille-Michaud[2], S. Vermeire[1], IBDase FP7 European Consortium.* 1) Targid, Gastroenterology, KU Leuven, Belgium; 2) Université d'Auvergne, Clermont-Ferrand, France; 3) Progenika Biopharma, S.A., Derio, Spain; 4) Gastroenterology and Hepatology, LUMC, the Netherlands; 5) Medicine, Semmelweiss University, Hungary; 6) Gastroenterology, Spitalnetz, Switzerland; 7) Peninsula Medical School, UK; 8) Gastroenterology and Hepatology, UMC Groningen, the Netherlands; 9) Molecular Diagnostics, Hungarian National Blood Transfusion Service, Hungary; 10) Rheumatology, Inselspital, Switzerland; 11) Electrical engineering and computer science, Montefiore Institute, ULG, Belgium.

Proteases and protease inhibitors (P/PI) affect several components that contribute to mucosal barrier integrity. We aimed to elucidate whether and if any P/PIs are involved in pathogenesis of Inflammatory Bowel Disease(IBD)trough a genetic association study. Selection of genes was based on a previous performed systematic review of relevant published genetic studies in IBD, in which the comprehensive list of P/PIs was ranked according to available genetic evidence for association with IBD. A total of 185 tagging SNPs in 23 genes were genotyped in an exploratory cohort of 650 Crohn's disease (CD) patients (CD1), and 542 healthy controls (HC1). Replication of SNPs with $p_{uncorrected}$<0.1 was performed in 4 independent cohorts: CD2 (n=634), HC2 (n=900); CD3 (n=377), HC3 (n=354); CD4 (n=432); and CD5 (n=227). Cases and controls were compared based on an additive genetic model (SVS v7.4.0). P-values were corrected for multiple testing using FDR correction. 12 SNPs showed significant association (p_{FDR}<0.05) in the combined cohort [CD1-5 (n=2320) vs HC1-3 (n=1796)]: 5 SNPs in *USP40*, 2 in *APEH*, 1 in *USP3*, and 4 in *CYLD*. Strongest signals were seen in *CYLD*, a cytoplasmic deubiquitinating enzyme located 9kb downstream of *CARD15*. Logistic regression including the *CARD15* and *CYLD* SNPs pointed to independent signals (p*CYLD*rs12324931<0.001, OR=1.8[1.3-2.5]; p*CARD15*rs2066845<0.001, OR=3.1[1.7-5.4]; p*CARD15*rs2066847<0.001, OR=3.4[2.2-5.4]). *CARD15*-negative patients showed significant association with rs12324931 (p=0.008). Interaction analysis showed significant interaction between rs2066844 and rs12324931, also after correction for main effects (p=0.001). CYLD has previously been shown to be highly downregulated in the intestine of IBD patients. Upon transfection of T84 intestinal epithelial cells with siRNA directed against CYLD, the CD-associated adherent-invasive *Escherichia coli* strain LF82 showed significantly increased adhesion (2.3-fold, p<0.01), invasion (2.6-fold, p<0.03) and survival ability (2.1-fold, p<0.05), compared to control siRNA transfected cells. We provide strong evidence for association of *CYLD* on 16q12.1 with CD, which is independent from *CARD15*. CYLD is a known key negative regulator of NF-2B, and as shown in this study, could play a role in CD trough regulation of antibacterial defense. Moreover, we identified several genetic variants in other protease/protease inhibitor genes which are implicated in CD, and which warrant further follow-up.

639F

Novel genes for Crohn's Disease using a genetic-localisation approach: the way forward in Genome-Wide Association mapping. *H. Elding, W. Lau, D.M. Swallow, N. Maniatis.* Research Department of Genetics, Evolution & Environment, University College London, London, United Kingdom.

Despite extensive Genome Wide Association (GWA) studies on Crohn's Disease (CD) only 20-25 percent of the reported genetic risk has been accounted for. With the aim of shedding light on the remaining genetic contribution, we have re-analysed the publicly available Wellcome Trust (WTCCC) data using a powerful multi-marker mapping approach. This model takes directly into account the underlying Linkage Disequilibrium (LD) structure by modelling on genetic distances in Linkage Disequilibrium Units (LDU) rather than physical locations. This method also provides an estimated location of the causal variant. Here we report novel susceptibility genes, which were replicated using an independent GWA dataset. New genes include *LPP*, *IL1R2*, *SOX4*, *MARCKS*, *IFNGR1*, *MTUS1*, *FAS*, *CDH1/CDH3*, *IRF8* as well as several other genes. The function of the genes mentioned here can be classified into 4 different categories: cell-cell adhesion and motility, phagocytosis, apoptosis and immunity and inflammation, all four being of great relevance to the phenotypic characteristics which define CD. Interestingly, some of the above mentioned genes have been reported for other immune-related diseases, which share similar characteristics with CD. *LPP* was previously reported for Celiac Disease and Vitiligo whereas *FAS* was reported for Immunoglobulin A deficiency. *IL1R2* was identified for Ankylosing Spondilitis and for Ulcerative Colitis (UC), which is the other form of Inflammatory Bowel Disease (IBD) together with CD. Remarkably, *SOX4* was identified in a study on Bone Mineral Density, an indirect indicator of Osteoporosis, where the latter also happens to be one of the extra-intestinal manifestations of CD. The method used for this study not only gives novel insights into the genetics underlying CD and evokes the fact that the well-reported missing genetic contribution could be the result of the methods currently in use, but also provides a promising way forward in understanding the genetics of CD and other complex disorders.

640F

Combining family- and population-based genetic association from multiple rare variants. *D. Fardo[1], A. Druen[1], I. Ionita-Laza[2].* 1) Dept. of Biostatistics, University of Kentucky College of Public Health, Lexington, KY; 2) Dept. of Biostatistics, Columbia University Mailman School of Public Health, New York, NY.

Genetic association studies can be broken into two broad categories: population studies (most often of the case-control variety) that collect unrelated individuals, and family studies that recruit related pedigrees. It is not uncommon for studies of both categories to be available for a particular disease. In this situation, it is more powerful to combine the evidence for association rather than to conduct the analyses separately. Various aggregation approaches have been developed, but they have all been designed in the context of common variants.

Recently developed next generation sequencing technologies have made it feasible to assess association between multiple rare variants and disease. This, in combination with motivation to target the so-called `missing heritability' from genome-wide association studies, has made the study of rare variation particularly attractive. Methods to handle the problems due to the sparsity of rare variants generally focus on rules to collapse variation across some genetic unit, e.g., a gene. We present here a unified method to aggregate population and family data using resequencing data and assess its performance using simulation.

641F

Use of 1000 Genomes Project Data to Search for Functional Variants in the GWAS Catalog. *J. Farrell.* Biomedical Genetics L320, Boston Univ Med, Boston, MA.

Genomes-wide association studies have successfully found highly significant associations between SNPs and many diseases. Detecting the functional variants that are driving these signals is more challenging. Often the most significantly associated SNPs in a GWAS are not biologically relevant but are instead in strong linkage disequilibrium (LD) with one or more nearby functional variants. To prioritize the search for functional variants, a bioinformatics tool has been developed to find all non-synonymous SNPs in strong LD with the top results of a GWAS analysis. With the recent availability of the 1000 genomes project data, a comprehensive list of all potential variants in LD with top GWAS results is now feasible. To demonstrate the tool, an LD scan for all non-synonymous SNPs were completed on all SNP-trait associations listed in the May, 2011 version of the NHGRI GWAS Catalog available for download from the UCSC genome browser. At the time of the analysis, the GWAS catalog had 5631 SNPs associated with 419 phenotypic traits. Among these cataloged associations, there were a total of 205 traits with associations with non-synonymous SNPs (3.6%). The European 1000 Genomes Data (August, 2010 release) were scanned for all non-synonymous SNPs in strong LD with the GWAS Catalog SNPs. An LD scan of the 5631 SNPs detected an additional 1306 non-synonymous SNPs in strong LD (r2 > 0.4). Of these, 791 SNPs were in very strong LD (r2> 0.8). A total of 242 of these non-synonymous SNPs were predicted to be possibly or probably damaging based on the Polyphen-2 prediction regarding the impact of the amino acid change. The usefulness of this approach can be seen with a phenotype such as Alzheimer's disease which has a previously well established association (pre-GWAS) with APOE4. In the GWAS Catalog, the most significant p-values reported are for SNP rs2075650 (p-value= 1x10-295) which is found in the intron of the nearby TOMM40 gene and not APOE. The LD scan of non-synonymous SNPs in strong LD with rs2075650 did find SNP rs429358 (r2=0.45). SNP rs429358 is one of the 2 non-synonymous SNPs that define the amino acid changes found in the APOE4 protein. The use LD scans of the 1000 genomes data for non-synonymous SNPS appears to be a computationally efficient method to rapidly detect a comprehensive list of potential functional variants for replication, pathway analysis and follow-up experiments for genome wide association studies.

642F

Dense mapping across a European population shows association with Ikaros family member *Aiolos* in SLE. *S.G. Guerra[1], J. Bentham[1], D.L. Morris[1], V. Anand[1], C.L. Pinder[1], A.M Delago-Vega[2], P.R Fortin[3], J. Wither[3], J. Martin[4], M.E. Alarcón-Riquelme[5], J.D Rioux[6], T.J Vyse[1], D.S. Cunningham-e Graham[1], BIOLUPUS, CaNIOS-GenES.* 1) Department of Medical & Molecular Genetics, King's College london, London, United Kingdom; 2) Department of Genetics and Pathology, University of Uppsala, Dag Hammarsjölds väg 20, 75185 Uppsala, Sweden; 3) Toronto Western Hospital, Main Pavilion, 399 Bathurst St, Toronto, Ontario, M5T 2S8, Canada; 4) Instituto de Parasitología y Biomedicina Lopez-Neyra, CSIC, Calle del Conocimiento, 18100 Armilla, Spain; 5) GENYO, Centro de Genómica e Investigación Oncológica Pfizer, Universidad de Granada, Junta de Andalucía, Avenida de la Ilustración 114, 18007 Granada, Spain; 6) Montreal Heart Institute, Université de Montréal, 5000 rue Belanger, Montreal, Quebec, H1T 1C8, Canada.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by hyperactive T and B cells, autoantibody production and immune complex deposition. The exact aetiology of the disease is not fully understood; however, a strong genetic component has been established. We conducted a genome-wide association study (GWAS), comprising (post QC) 4,254 European SLE cases and 8,578 controls on 750,000 SNPs (Omni1-Quad chip. Analysis of these data suggests *Aiolos* is a novel risk locus for SLE. *Aiolos* (*IKZF3*), a transcription factor of the Ikaros zinc-finger protein family, plays an important role in B cell differentiation and proliferation. Located at 17q21, *Aiolos* can form heterodimers with *Ikaros* (*IKZF1*), located at 7p12, important in B cell differentiation and proliferation. *Aiolos* and *Ikaros* have previously been associated with other autoimmune diseases such as RA and *Ikaros* has been associated with SLE p=2.79x10^{-23}. Previous reports in *Aiolos*$^{-/-}$ mice have shown increased levels of pre-B and immature B cells with a hyperactive BCR signalling leading to spontaneous auto-antibody production. These mice also present an increased rate of apoptosis suggesting a wider role of *Aiolos* in cell survival. The aforementioned are all characteristics seen in SLE.

Our data shows multiple associated SNPs in *Aiolos* after SNPTEST analysis of our European cohort. Our top associated SNPs were rs34758895 and rs9899345 (both intronic), p values of 1.71x10^{-8} and 2.79x10^{-8}, respectively, OR=[1.33-1.82, 1.30-1.75]. Preliminary analysis using SNPTEST, suggests 3 independent associated signals, with 1 signal in the 3'UTR and 2 intronic signals. We will perform a meta analysis combining our data with published GWAS data (1716 cases, 3500 controls) to improve the power of our study. Using qPCR we are currently quantifying levels of *Aiolos* transcript isoforms in B cells of cases and controls. Expression levels will be correlated with genotype and sub-phenotype groups such as nephritis. Our data suggest variants in *Aiolos* increase the susceptibility of SLE. Further work is needed to analyse the function of *Aiolos* in B cell development and the effect that variants have on B cell differentiation and proliferation. Further investigation is also needed into interactions between *Aiolos/Ikaros* and the effect variants have on these heterodimers and their pathway.

643F

Integrated pipeline for copy number variation, linkage and homozygosity, aids interpretation of variants identified through Next-Gen sequencing. *K. Schmitz[1,2], K. Markianos[1,2].* 1) Genetics, Harvard Med, Boston, MA; 2) Genetics, Children's Hospital Boston, MA.

With the wide, low cost availability of Next Generation sequencing, there is a need to combine information from family linkage, copy number variation (CNV) as well as population level homozygosity to aid interpretation of sequencing results. We integrate existing methods into an automated pipeline that will facilitate the search for disease genes. For CNV analysis we use multiple calling algorithms to improve specificity and our own common variation database to improve interpretation of the results. For Affymetrix 6.0 SNP data, we use Birdsuite, Penncnv, Nexus and Affymetrtics Genotyping Console to obtain copy number events. To increase specificity, we use the intersection of two or more algorithms. To eliminate common variation in our results, we have compiled a CNV catalog of 1257 samples from the International HapMap project using the same pipeline. We automatically filter new findings against known variants using locus, matching CNV status (copy number 0, 1 or gain) and an optional minor allele frequency (MFA). Data management has been implemented and extended with links to Genome browsers as UCSC and DVG (Database of Genomic Variants) to visualize all the results in one composite view. Linkage and traditional homozygosity mapping can also be incorporated in the analysis and visualized along CNV results to highlight putative susceptibility loci. We present successful application of the pipeline in real world projects. Currently, we are working with brain malformations as Autism, Walker-Warburg, Microcephaly and mental retardation. For Congenital Sideroblastic Anemia (CSA), we were able to interpret a rare case with a two locus hit. A rare CNV (duplication) in the alpha globin (HBA) locus and a pathogenic mutation in the beta (HBB) locus. In addition to CSA the patient has been already diagnosed with Beta Thalassemia. For Centronuclear myopathy, we are performing functional analysis for a gene identified using linkage, CNV and overlapping mutations from exome sequencing. In our experience automation of the process minimizes the error inherent in manual curation of discrete genetic information and greatly speeds up interpretation of large data sets.

644F

Genetic variation that predicts white blood cell count differential leukocyte types in the eMERGE Network. *D. Crosslin[1,4], A. McDavid[3], N. Weston[2], S. Nelson[4], X. Zheng[4], E. Hart[2], M. de Andrade[5], I. Kullo[6], C. McCarty[7], K. Doheny[8], E. Pugh[8], A. Kho[9], M. Hayes[9], S. Pretel[10], A. Saip[12], M. Ritchie[11], D. Crawford[11,12], P. Crane[13], K. Newton[2], D. Mirel[14], A. Crenshaw[14], E. Larson[2], C. Carlson[3], G. Jarvik[1], The electronic Medical Records and Genomics (eMERGE) Network.* 1) Division of Medical Genetics, University of Washington, Seattle, WA; 2) Group Health Research Institute, Center for Health Studies, Seattle, WA; 3) Fred Hutchinson Cancer Research Center, Public Health Sciences Division, Seattle WA; 4) Department of Bi statistics, University of Washington, Seattle, WA; 5) Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN; 6) Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN; 7) Center for Human Genetics, Marshfield Clinic Research Foundation, Marshfield, WI; 8) Center for Inherited Disease Research, Johns Hopkins University, Baltimore, MD; 9) Division of Endocrinology, Metabolism, and Molecular Medicine, Northwestern University, Chicago, IL; 10) National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD; 11) Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN; 12) Department of Biomedical Informatics, Vanderbilt University, Nashville, TN; 13) Division of General Internal Medicine, University of Washington, Seattle, WA; 14) Program in Medical & Population Genetics, Broad Institute of Harvard & MIT; Cambridge, MA.

We performed a joint and ancestry-stratified genome-wide association analyses to identify variants associated with white blood cell count (WBC) differential leukocyte types in 13,923 subjects in the electronic Medical Records and Genomics (eMERGE) Network. The Network is a consortium of five U.S. cohorts linked to electronic medical record (EMR) data for conducting large-scale, high-throughput genetic research. A majority of the subjects had multiple visits over many years for the duration of the EMR, which presented challenges and opportunities to assess different association methods utilizing repeated measures. The cell types are measured as a proportion of the total WBC. These types, in order of predominance, include neutrophils, which respond to acute infection; lymphocytes, both B and T cells with antigen receptors; monocytes, large, phagocytic cells; eosinophils, antiparasitic markers of allergic disease; and basophils, that release histamine.

Results for neutrophil count and lymphocyte count strongly resembled two WBC-associated regions ($p \leq 5.0e\text{-}8$) that we previously reported. These include the region surrounding the Duffy antigen/chemokine receptor gene (*DARC*) on chromosome 1 in the African ancestry subjects and chromosome 17 in the European ancestry subjects. SNPs in the latter region tag three genes (*GSDMA*, *MED24* and *PSMD3*) that have been reported to be associated with inflammatory diseases including asthma and Crohn's disease.

Variants in two genes were associated with monocyte count; in *ITGA4*, which encodes a receptor for fibronectin in WBC and participates in T-cell interactions with target cells (p for rs2124440 = 5.7e-17); and in *IRF8*, which encodes a transcription factor that specifically binds to the upstream regulatory region of type I IFN and IFN-inducible MHC class I genes (p for rs424971 = 2.8e-16).

The joint sample reveals multiple regions with genome-wide significant associations for eosinophil and a single region for basophil counts. This single region includes *GATA2* on chromosome 3 that has been reported to be associated with coronary heart disease. Assessment of the contribution of top hits to candidate pathways is underway.

645F

A Genome-Wide Meta-Analysis of Serum Total IgE among Racially and Ethnically Diverse Populations. *A.M. Levin[1], R.A. Mathias[2], L. Huang[2], L.K. Williams[3], K.C. Barnes[2], The EVE Consortium.* 1) Public Health Sciences, Henry Ford Hlth System, Detroit, MI; 2) Department of Internal Medicine, The Johns Hopkins University, Baltimore, MD; 3) Center for Health System Research, Henry Ford Hlth System, Detroit, MI.

Immunoglobulin E (IgE) is an important mediator in allergic inflammation and is often elevated in atopic conditions, such as asthma. Recently published genome-wide association studies for asthma and serum total IgE levels suggest that there are some, but not many, overlapping common single nucleotide polymorphisms (SNPs) in genes associated with these phenotypes. Additionally, serum total IgE levels are also known to differ by population group in the U.S. However, African Americans and Latinos have not been well represented in genome-wide association studies of total IgE to date. The EVE consortium comprises nine U.S groups with genome-wide association data on individuals with and without asthma, and includes associated phenotypes, such as total IgE levels. We carried out a genome-wide meta-analysis of serum total IgE levels across this broadly representative patient population. In total, 5,064 individuals (2,456 African Americans, 1,563 European Americans, and 259 Latinos) were available for analysis. Race-ethnic specific association analyses were carried out within each of the EVE study groups stratified by asthma case and control status. The resulting p-values and directions of effect were combined via an inverse normal meta-analysis weighted by the number of individuals contributing to each association model. Eleven unique regions were identified in the combined analysis which met a threshold p-value of $5x10^{-6}$ and were informative in at least two of the three race-ethnic groups. Ten of these regions were novel, and three of these appeared to be unique to populations with a component of African admixture (i.e., African American and Latino participants). One region, HLA-DQB1, which had been previously identified as associated with total IgE among Europeans and European Americans, was associated with IgE in our overall analysis ($p=1.9x10^{-6}$) and within the individual race-ethnic groups (African American p=0.002, European American p= 0.035, and Latino $p=6.4x10^{-6}$). We also validated a number of previously identified genes associated with total serum IgE levels across multiple race-ethnic groups; these genes included FCER1A, RAD50, STAT6, IL13, HLA-DRB1, RORA, IL4R/ IL21R, IL4, FCERB1, and SLC22A5. This study provided new insight into the genetics of total IgE by confirming previously identified loci across multiple race-ethnic groups and by identifying potentially novel race-ethnic specific effects in African American and Latino individuals.

646F

Immunochip-based GWAS follow-up study identifies novel primary biliary cirrhosis risk loci and better defines allelic architecture at known loci. *J.Z. Liu[1], M.A. Almarri[1], G.F. Mells[2,3], H.J. Cordell[4], M.A. Heneghan[5], J.M. Neuberger[6], P.T. Donaldson[7], D.E. Jones[7], G.J. Alexander[3], R.N. Sandford[2], C.A. Anderson[1], The UK PBC Consortium and The Wellcome Trust Case Control Consortium 3.* 1) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 2) Academic Department of Medical Genetics, Cambridge University, Cambridge, UK; 3) Department of Hepatology, Cambridge University Hospitals National Health Service (NHS) Foundation Trust, Cambridge, UK; 4) Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, UK; 5) Institute of Liver Studies, King's College Hospital NHS Foundation Trust, Denmark Hill, London, UK; 6) The Liver Unit, Queen Elizabeth Hospital, Birmingham, UK; 7) Institute of Cellular Medicine, Medical School, Newcastle University, Newcastle upon Tyne, UK.

Primary biliary cirrhosis (PBC) is an autoimmune disorder characterized by immune-mediated destruction of the hepatic bile ducts resulting in cholangitis and, ultimately, cirrhosis of the liver. PBC is a leading cause of liver transplantation and the prevalence in the UK is around 35 per 100,000 adults. Although the etiology is poorly understood, a complex interplay of genes and environment is known to drive disease susceptibility and progression. Genome wide association studies (GWAS) have confirmed 19 loci associated with PBC risk, and highlighted the role of NF-KB signaling, T-cell differentiation and toll-like receptor (TLR) and tumor necrosis factor (TNF) signaling in disease pathogenesis. In an effort to identify further disease associated loci and better define causal variants we performed an association and fine-mapping study of approximately 3000 PBC cases and 7000 population controls genotyped using Immunochip. The Immunochip is an Illumina iSelect HD custom genotyping array that contains SNPs derived from population based sequencing efforts (such as 1000genomes) across confirmed autoimmune disease risk loci. Seventeen previously associated loci achieve genome-wide significance (P<5x10-8) with the remaining two loci showing convincing evidence of replication (P<5x10-3). Through conditional analysis we found evidence for secondary independent genome-wide significant associations at three distinct loci (2q32, 7q32 and 16p13), while an additional locus on chromosome 3q26 contained three such associations. At the previously associated 17q12 locus, we identified a 1000genomes derived SNP within *IKZF3* that was more than four orders of magnitude more significant than the most associated SNP from our recent GWAS. This preliminary finding suggests that *IKZF3*, rather than *ORMDL3*, could harbor the causal variant(s) at this locus. We also discovered five novel PBC regions, thus implicating several functional candidates involved in immune regulation and inflammation such as *CCL20*, *IL12B*, *TYK2*, *ICOSLG* and *IL22RA2*. These loci have previously been reported to be associated with other distinct autoimmune disorders including type 1 diabetes, systemic lupus erythematosus, Crohn's disease and ulcerative colitis. This study further highlights the shared genes and pathways in autoimmunity and illustrates the use of cost-effective custom-made chips in exploring disorders of similar genetic etiology.

647F

Genome-wide genetic investigation into variation of food allergen antibodies in participants of the San Antonio Family Heart Study. *R. Rubicz[1], R. Yolken[2], E. Drigalenko[1], T.D. Dyer[1], J. Kent, Jr.[1], M. Carless[1], J. Curran[1], M. Johnson[1], S.A. Cole[1], L. Almasy[1], E.K. Moses[1], J. Blangero[1], H.H.H. Göring[1].* 1) Dept of Genetics, Texas Biomed, San Antonio, TX; 2) Dept of Pediatrics, Johns Hopkins School of Medicine, Baltimore, MD.

Aberrant responses to dietary antigens affect millions each year in the U.S., with symptoms generally consisting of gastrointestinal dysfunction and autoimmunity. Previous research indicates that such hyperactive immune response to certain food proteins tends to run in families, and although genetic factors have been implicated in some instances (i.e., 27 different loci associated with celiac disease) the underlying disease processes remain largely unknown. Here we quantify the genetic contribution to antibody levels against food antigens, and attempt to localize genetic factors influencing these traits. Participants included >1300 Mexican Americans in the San Antonio Family Heart Study. Antibody titers were determined by ELISA for the following food antigens: bovine casein; two forms of wheat gliadin; two forms of bovine serum albumin (BSA), as well as human serum albumin (HSA). In addition to food allergies, these antibodies have been associated with autoimmune and psychiatric disorders. Additive genetic heritability (h2) was calculated using variance component (VC) pedigree analysis with the computer program SOLAR, and the influence of shared environmental factors was assessed by including a random effects household component in the VC model. Genome-wide variance components linkage and measured genotype association analyses were performed using 931,219 SNPs. Heritability estimates are significant for all traits, ranging from h2=0.15 (BSA-a) to 0.53 (gliadin-b), and shared household is also significant for all traits (0.06-0.33, for BSA-b and gliadin-b, respectively). Although no significant genome-wide linkage or joint linkage and association results were obtained, there are suggestive association results for gliadin-b (p=9.75x10-8) for SNP rs7192 located in the HLA region on chromosome 6, and casein (p=3.33x10-7) for SNP rs11635085 on chromosome 15. We also performed genome-wide expression profile analysis using expression data from peripheral blood lymphocytes (for >16,000 transcripts), as an independent means of prioritizing likely candidate genes and to help interpret the linkage/association results. Significantly correlated transcripts were identified for 3 of 5 food allergens (BSA-a, gliadin-a, and gliadin-b) and for HSA. These results demonstrate that individual genetic differences contribute to food allergen antibody measures in this population, and further investigation may help elucidate the underlying immunological processes involved.

648F

Heritability and genetic mapping of hundreds of immune-related quantitative traits measured in a large Sardinian cohort. *S. Sanna[1], G. Sole[1], M. Steri[1], M. Dei[1], S. Lai[1], F. Virdis[1], D. Schlessinger[2], M. Congia[3,4], S. Naitza[1], V. Orrú[1], E. Fiorillo[1], F. Cucca[1,5].* 1) IRGB-CNR, Monserrato, Italy; 2) Laboratory of Genetics, NIA, Baltimore, MD, USA; 3) Dipartimento di Scienze Biomediche e Biotecnologie, Università degli studi di Cagliari, CA, Italy; 4) Azienda Sanitaria Locale n.8, Cagliari, Italy; 5) Dipartimento di Scienze Biomediche, Università di Sassari, SS, Italy.

Genome-wide association scans (GWAS) have identified over hundreds of regions associated with immune diseases, but identification of the specific causal variants and clarification of the underlying functional mechanisms remain a great challenge. In particular, GWAS discovery of pathogenetic changes in the immune system have been largely limited to major classes of white cells. Here we systematically evaluated quantitative variation of the majority of lymphocyte cell populations (T cells, B cells, Natural Killer (NK) cells, regulatory T cells, dendritic cells, and their subsets) as well as T cell maturation in minimally manipulated fresh blood samples by polychromatic flow cytometry, resulting in 56 immune-related traits. This approach can be informative not only for the understanding of associated loci but also for identification of novel genetic factors in health and disease through the dissection of the endo-phenotypes. Some of the cellular populations were assessed both as absolute count (cell/ul) and as percentage referred to parents and grandparents, leading to a total of 184 quantitative traits for analysis. To avoid confounding effects due to the pathology itself, these phenotypes are being measured in a large cohort of >6,000 healthy individuals enrolled in the SardiNIA project. A preliminary assessment on 946 individuals in 317 families showed that 117 traits are highly heritable (>40%), and for all traits sex and age play an important role in explaining part of the non-genetic variation (up to 50%). Using existing data, we also performed a GWAS for all traits to evaluate the additive effect of ~2.5 Million markers, either genotyped or imputed. Despite the small sample size, we observed 21 loci showing genome-wide significant pvalues for 20 non-redundant traits ($1x10^{-12} < P < 5x10^{-8}$). Notably, in addition to completely novel loci, our results include the previously reported association within the cluster of genes from the Schlafen family and NK cell level variation (rs9916629, $P=3x10^{-8}$). Two other loci have been previously associated with ulcerative colitis, proving the relevance of our approach for the understanding of the biological mechanisms underlying complex immune-related diseases. In conclusion, we have initiated a systematic evaluation of immune-phenotypes on a large, intensively genotyped cohort and established the bases for a biologically informative extensive battery of GWAS for the identification of the associated genetic factors.

649F

Parent-of-Origin Effect of a T2D-associated *GRB10* Polymorphism on Glucose Homeostasis. *J. Liu, C.M. Damcott, J.R. O'Connell, B.D. Mitchell, A.R. Shuldiner, Y. Cheng.* Dept Human Gen, Univ Maryland, Baltimore, MD.

Genome-wide association studies (GWAS) have identified common variants in growth factor receptor-bound protein 10 (*GRB10*), which encodes an adaptor protein regulating insulin receptor signaling, as a strong candidate for glucose homeostasis and type 2 diabetes (T2D). Nevertheless, genetic associations between *GRB10* and T2D have not been consistently replicated. *GRB10* is an imprinted gene that is expressed in an isoform- and tissue-specific manner. Therefore, we hypothesize that the effect of genetic variants of *GRB10* is dependent on the parent-of-origin (POO). In our previous T2D GWAS study, rs2237457 (T/C polymorphism) in *GRB10* showed the strongest association with T2D (OR=1.64, p=1.1x10-5, T allele) and was also associated with glucose area under the curve (GAUC) in response to an oral glucose tolerance test (OGTT) in non-diabetic individuals (p=0.001). To test our hypothesis, we studied an extended Amish cohort (n=745) characterized for 2 hour glucose (2hGlu, mmol/l) and GAUC (mg/dl-hr) in response to OGTT and genotyped for rs2237457. The paternal and maternal alleles of rs2237457 were inferred by breaking the large Amish pedigree into smaller nuclear families and estimating the most likely pattern of gene flow in the families as implemented in MERLIN software. We first confirmed the initial associations of rs2237457 and OGTT traits in this extended cohort: GAUC () =15.04, p=5.24X10-5) and 2hGlu () =0.35, p=1.46X10-4), adjusting for age, sex, BMI, and familial relatedness. To examine the effect of rs2237457 according to their POO, we divided heterozygous individuals (n= 268) into two groups: paternal T/maternal C (TpCm) genotype and maternal T/paternal C (TmCp) genotypes. Compared with CC genotypes (n=307), the paternal T allele (TpCm) was significantly associated with GAUC ($)_{GAUC}$= 16.31, p=0.007) and 2hGlu ($)_{2hGlu}$=0.47, p=0.002), but the maternal T allele (TmCp) was not ($)_{GAUC}$=0.99, p=0.89 and $)_{2hGlu}$=0.12, p=0.49). We further compared glucose levels directly between heterozygous subjects with the paternal T allele transmitted vs. those with the maternal T allele transmitted. These comparisons revealed POO effects to be significant for GAUC (p= 0.01) and marginally significant for 2hGlu (p=0.05). In conclusion, we found that the paternal, but not maternal, allele of rs2237457 was associated with OGTT glucose traits in the Amish, suggesting POO could be one possible mechanism underlying the contribution of GRB10 to the risk of glucose homeostasis and T2D.

650F

A new statistical model for family-based case-control studies of genetic imprinting. *Z. Wang[1], R. Wu[2].* 1) Division of Biostatistics, Yale University, New Haven, CT; 2) Center for Statistical Genetics, The Pennsylvania State University, Hershey, PA.

Genetic imprinting has been recognized to play an important role in the formation and pathogenesis of human diseases. In this study, we present a statistical model for testing the effect of genetic imprinting (or parent-of-origin) on a human disease in case-control studies with family structure. For each subject sampled from a case and control population, we not only genotype its own single nucleotide polymorphisms (SNPs), but also collect its parents' genotypes. By tracing the transmission pattern of alleles from parental to offspring generation, the model allows the characterization of genetic imprinting effects based on χ^2 tests of a contingency table. The model is expanded to test the interactions between imprinting effects and additive, dominant, and epistatic effects in a complex web of genetic interactions. Statistical properties of the model are investigated and its practical usefulness is validated by a real data analysis.

651F

Genetic variations and interactions in anti-inflammatory cytokine pathway genes in the outcome of Leprosy. *S. AGGARWAL[1], S. Ali[1], R. Chopra[1], A. Srivastava[1], K. Arasan[1], D. Malhotra[1], S. Gochhait[1], V.K. Garg[2], S.N. Bhattacharya[3], R.N.K. Bamezai[1].* 1) National Centre of Applied Human Genetics, School of life Sciences, Jawaharlal Nehru University, New Delhi, 110067, India; 2) Department of Dermatology and Sexually Transmitted Diseases, Maulana Azad Medical College, Lok Nayak Jai Prakash Hospital, New Delhi, 110002, India; 3) Department of Dermatology and Venereology, University College of Medical Sciences and GTB Hospital, India, G.T.B. Hospital, Delhi, 110095, India.

Mycobacterium leprae is the etiologic agent of leprosy, an ancient scourge that still causes illness in several regions of the world. The role of host genetic factors in conferring susceptibility to this disease has long been a focus of research, with the discovery of many loci by segregation, twin, case-control and genome-wide studies. To identify, how genes in a pathway control and in unity contribute to disease susceptibility, we genotyped 51 SNPs in anti-inflammatory cytokines (IL10, TGFB1, IL6, IL4 and IL13) and receptors (IL10RA, IL10RB, TGFBR1, TGFBR2, IL6R, IL4R, IL5RA, IL5RB and IL13RA1) using Sequenom Mass Array. We included a total of 2082 individuals, including patients and controls from the North Indian population and replicated our results in an East Indian population, from the geographically distinct state of Orissa. Significant associations (P<0.05) were observed for 8 polymorphisms (rs1800871, rs1800872, rs1554286 of IL10; rs3171425, rs7281762 of IL10RB; rs2228048, rs744751 of TGFBR2 and rs1800797 of IL6) with leprosy. This association was replicated for 4 SNPs (rs1554286 of IL10, rs7281762 of IL10RB, rs2228048 of TGFBR2, rs1800797 of IL6). Beside this, we also evaluated the effect of SNP-SNP interaction in providing risk or protection to leprosy depending upon the combination of genotypes. The interaction study revealed significantly greater effect of leprosy risk than that obtained for any SNP individually. Furthermore, we also established the functional status of the SNPs through in-vitro reporter assays. Our results unravel the role of as yet unknown functional polymorphisms in immunologically important genes, to understand the severity of the disease. In conclusion, this study provides an interesting cue to cumulative polygenic host component which regulates leprosy pathogenesis.

652F

Estimating Heritability of Human Papilloma Virus (HPV) Vaginal Infection in Korean Women. *M. Han[1], D. Lee[1], H. Lee[4], J. Lee[4], K. Go[4], Y. Song[2], K. Lee[3], J. Sung[1].* 1) Complex Disease and Genetic Epidemiology Branch, Department of Epidemiology and Institute of Environment and Health, School of Public Health Seoul National University; 2) Department of Family Medicine, Samsung Medical Center, and Center for Clinical Research, Samsung Biomedical Research Institute, SungKyunKwan University School of Medicine, Seoul, Korea; 3) Department of Family Medicine, Busan Paik Hospital, Inje University College of Medicine, Korea; 4) Department of Environmental Health, Institute of Health and Environment, School of Public Health, Seoul National University, Seoul, Korea.

Cervical cancer is the second most common cancer in women worldwide. Infection with the human papilloma virus (HPV) is regarded as a sexually transmitted disease (STD). Also HPV infection is well-established and the most important causes uterine cervix cancer. Several studies indicate that genetic background of host is important for cervical cancer susceptibility. However, only limited evidence has been reported regarding whether the host genetic susceptibility contributes to the HPV infection. This study aims to assess overall genetic contribution to HPV infection quantitatively in terms of heritability. Vaginal smears during the Papanicolau test were taken from 951 women, (including 327 monozygotic (MZ)) between 2006 and 2009, (mean age 48 ± 11.4, 25~79 year old) counting 663 families, in the Healthy Twin study: a twin-family cohort in Korea. To detect HPV, Polymerase chain reaction (PCR) was executed using two primer sets of GP5+/GP6+ and PGMY09/PGMY11. The identification of HPV was confirmed by nucleic acid sequence analysis. Phylogenetic analysis was performed using the neighbor-joining method to confirm the genotype of HPV. Among HPV genotyping result of the participants 7.3% were positive for HPV, among which HPV 16 type observed in 13.9%, HPV 18 in 11.1%, and high risk genotype including HPV 16, 18 infection in 66.7%. Behavioral, reproductive characteristics, socioeconomic position (SEP), and male factors were assessed by questionnaire. The odds ratio (OR) for taking oral contraceptives and not taking oral contraceptives is 1.87 (95% C.I. 1.08~3.24). Tetrachoric correlations about HPV-positive were 0.39, 0.16, 0.13 and 0.10 for MZ pairs, sisters, any first degree (sisters together with mother-daughter pairs) , and mother-daughter pairs, respectively. Heritability was calculated by Variance component method using Sequential Oligogenic Linkage Analysis Routines (SOLAR). Heritability estimation for HPV infection was 0.24 to 0.33 in AE model, 0.21 to 0.30 in ACE model. In AE model, Additive genetic factors (A) is 0.31, unique environmental factor (E) is 0.69 adjusting for age, oral contraceptives intake, smoke and alcohol. In ACE model, A is 0.27, common environment factor (C) is 0.02 and E is 0.71. The study results indicate that there is a hereditary component of HPV infection. After adjusting to environmental, genetic factors were shown. These findings provide evidence that there is genetic susceptibility of HPV infection.

653F

Detection of Genotyping Errors in Dense Markers on Large Pedigrees. *C.Y.K. Cheung[1], E.A. Thompson[3], E.M. Wijsman[1,2].* 1) Dept. Biostatistics, University of Washington, Box 357232, Seattle, WA. 98195-7232, USA; 2) Dept. of Medicine, University of Washington, Box 359460, Seattle, WA. 98195-9460, USA; 3) Dept. of Statistics, University of Washington, Box 354322, Seattle, WA 98195-4322, USA.

Accurate results in linkage analysis depend on clean genotypes. To detect genotyping errors in pedigrees, Mendelian inconsistent (MI) error checks and multi-point methods, which flag Mendelian consistent (MC) errors with improbable joint genotypes, have been developed. However, detection of MC errors with exact computation for dense markers is restricted to small pedigrees due to computational reasons. In addition, the existence of linkage disequilibrium adds complication to error detection for dense markers. Here we introduce a new computationally-efficient method that allows detection of errors on very dense markers (e.g. SNPs and sequencing data) typed even on large pedigrees. Our method first samples inheritance vectors (IVs) using a moderately sparse but informative set of markers (framework panel) with gl_auto, a program in the MORGAN package that samples IVs either with exact Lander-Green calculations or a Markov Chain Monte Carlo approach. This scheme avoids violation of the necessary assumption of linkage equilibrium between dense markers and allows otherwise intractable computation. Conditional on IVs realized at framework positions, we then efficiently sample IVs at positions of dense markers. For error detection, we calculate either the percentage of IVs inconsistent with jointly observed genotypes (S1) or the posterior probability of error configurations (S2) and flag markers when the chosen statistic exceeds a threshold.

We tested our method on a simulated 5 generation 52-member pedigree, in which 34 subjects in the lower generations were observed. We simulated a clean framework panel of SNPs at 0.5cM density on a 100cM chromosome to infer IVs. We independently simulated 25000 markers with genotyping errors at a fixed rate of ~0.1%. Of 825 markers with at least 1 error, only 12.7% were MI. Applying our method to MC markers, with specificity of 99.9%, S1 and S2 flagged 85.3% and 85.8% of the remaining erroneous markers, respectively, with corresponding positive predictive value of 95.9% and 96.1%. S2 attributed error to the appropriate individual with 84.1% accuracy. The above results suggest that our method is effective in detecting MC genotyping errors. Moreover, the much quicker S1 statistic closely matches the performance of S2 while not requiring knowledge of marker allele frequencies or the use of an error model. Meanwhile, S2 allows us to attribute a detected error to an individual.

654F

Polygenic Modeling of Disease Risk. *J. He, G. Chen, B. Henderson, C. Haiman, D. Stram.* Preventive Medicine, University of Southern California, Los Angeles, CA.

Purpose: Purcell et al (Nature 2009) described a substantial polygenic component to the risk of schizophrenia by constructing a risk score with thousands of SNPs. Here we evaluated the validity of this method in the African American Prostate Cancer Genome-Wide Association Study (AAPC GWAS) Methods: Using the actual AAPC GWAS data, we divided 3334 prostate cancer cases and 3472 controls into a discovery set (1500 cases/ 1451 controls) and a target set (1903 cases/ 1952 controls) based on distinct US geographic regions. 1047986 SNPs were tested for association with prostate cancer in the discovery set using logistic regression adjusted for ten principal components. SNPs with P values lower than a P value threshold were to contribute to the score as $\Sigma \log(OR_i) \times X_i$ for each SNP i. The score was then tested for association with prostate cancer in the target population. In order to determine the sensitivity of this method to long-range hidden relatedness we simulated disease status based on the genotypes in AAPC GWAS in which only a fraction (or none) of the causal SNPs were actually in LD with the SNPs that were treated as measured in the simulations. We randomly sampled 1000 SNPs on even number chromosomes as causal SNPs, each with effect bi (~normal(0,1)) used to explain half the total individual liability for disease. Two scores were calculated. The first score was constructed using all SNPs as candidate SNPs, while the second score was using only SNPs on odd number chromosomes (and thus not in LD with any causal variants except due to residual hidden relatedness not captured by principal components). These scores were then tested for associations with simulated disease status in the target sample. P value thresholds ranging from 10-4 to 0.5 were used in score construction. Results: In the AAPC GWAS, both the statistical significance and pseudo R square increased as a greater number of SNPs were included in the score when tested in the target population. In the simulation study, the performances of both scores in the target population were strongly significant ($p<10^{-4}$) when using P value thresholds between 0.01 and 0.05 in score construction. We conclude that the method used by Purcell et al may quite sensitive to subtle sample relatedness at least when applied to geographically separate African American populations.

655F

Two-Layer Clustering Model For Linkage Disequilibrium With Applications in Population Structure, Local Ancestry Inference, And Haplotype-Phenotype Association Mapping. *Y. Guan.* Pediatrics and Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

We present a two-layer clustering model that aims to emulate the coalescent process with recombination, where a marginal coalescent tree is approximated by a hierarchy of star trees, and ancestral recombination is approximated by switching clusters within each hierarchy. The lower layer clusters represent haplotypes and the top layer clusters enforce structure on those haplotypes. Depending on the cluster-switching frequencies, such a structure may reflect difference in ancestries or more subtle difference of haplotype frequencies between case and control groups in disease association studies. In the former scenario, the population structure can be inferred using dense genotype data. Moreover, one can accurately infer local ancestries for multi-way admixed individuals. In the later scenario, one can detect haplotype-phenotype association in candidate regions. The model can be fitted for genetic data of a typical modern genome wide association study. It also has potentials in rare variants calling/imputing for next-gen sequencing data and we discuss related computational challenges and possible solutions.

656F

The effect of missing data on type I error rate and power of family-based association tests. *T. Hiekkalinna[1,2], M. Perola[1,2], J.D. Terwilliger[2,3,4,5,6].* 1) Public Health Genomics Unit, National Institute for Health and Welfare (THL), Helsinki, Finland; 2) Institute for Molecular Medicine Finland FIMM; 3) Department of Psychiatry, Columbia University, New York, NY, USA; 4) Department of Genetics and Development, Columbia University, New York, NY, USA; 5) Columbia Genome Center, Columbia University, New York, NY, USA; 6) Division of Medical Genetics, New York State Psychiatric Institute, New York, NY, USA.

Missing data is ubiquitous in family-based association (FBA) studies, as not all individuals will be alive or consent to be studied, especially parents of individuals with late onset diseases. Most FBA methods claim to work in the presence of incomplete data, but how well they behave in those circumstances is unclear. To this end, we have compared empirical type I error rates and power of several commonly used implementations of FBA tests, as a function of how much data is missing, using datasets composed of mixtures of singletons and families. We compare the performance of these tests under three hypotheses: (a) No linkage and no association; (b) Complete linkage and no association; (c) Complete linkage and association. The first two represent potential null hypotheses in (a) joint tests of linkage AND association; and (b) conditional tests of association given linkage. In power simulations, power was estimated for all methods for both joint tests and conditional tests, over a range of effect sizes for the functional locus, and a range of LD between the functional locus and a marker. The most notable result is that conditional tests of LD given linkage can have enormous type I error rates with some of the most popular methods for conditional testing, especially when the proportion of missing data is large. This can lead to an unacceptable rate of spurious conclusions about the presence of association, leading to fruitless follow up studies. Power is also shown to be optimal for methods based on full likelihood analysis of complete pedigree data, rather than methods based on the TDT. Results from a simulation-based study will be presented.

657F

Detection of phenotype-modifier genes using two-locus linkage analysis. *A. Bureau[1,2], J. Croteau[2], C. Mérette[2,3], A. Fournier[2], Y.C. Chagnon[2,3], M.-A. Roy[2,3], M. Maziade[2,3].* 1) Médecine sociale et préventive, Faculté de Médecine, Univ Laval, Québec, Canada; 2) Centre de recherche Université Laval Robert-Giffard, Québec, Canada; 3) Psychiatrie, Faculté de Médecine, Univ Laval, Québec, Canada.

In complex disorders, specific phenotypes such as disease diagnoses are often part of a broader disorder spectrum. For instance, schizophrenia (SZ), bipolar disorder (BP) and schizo-affective disorder are three diagnoses in the spectrum of major psychotic disorders. In families where multiple patients have a specific phenotype, the observation that relatives exhibit other forms of a broader phenotype suggests that some genes may increase risk for an array of phenotypes, while other causes determine which specific phenotype arises. In this context, we propose to test whether other genes determine the specific phenotype using a two-locus linkage analysis model where a gene 1 increases the risk for a broad phenotype and a gene 2 increases the risk of the specific phenotype, but only in carriers of the gene 1 susceptible genotype (i.e. gene 2 is a modifier). We deal with heterogeneity by selecting families showing evidence for linkage to the gene 1 locus. In a simulation study, we compared the proposed model to a single-locus analysis within subjects with the broad phenotype. We observed that the joint modeling of the specific and broad phenotypes had greater power to detect the modifier gene 2 than the single-locus approach (power = 0.96 vs. 0.54 under a simulation scenario including heterogeneity). This joint modeling was also more powerful than all analytic approaches considering only the specific phenotype. The strategy was then applied to a sample of 12 SZ and BP Eastern Quebec kindreds to identify genes conferring specific susceptibility to SZ or to BP by modifying the effect of a gene 1 at a locus linked to major psychosis as a broad phenotype. Among markers located within 11 regions where we previously detected linkage signals to SZ and/or BP, D8S1110 at 8p22 shows the strongest evidence of linkage to a gene predisposing specifically to BP among subjects already at risk of major psychosis because of the action of another gene at 10p13 (marker D10S245) in a subset of 6 families linked to 10p13 (conditional maximized LOD (cMOD) = 4.34, $p = 5 \times 10^{-5}$). The cMOD increases to 6.34 when including an additional 21 BP families. Some evidence of this modifying effect (cMOD = 3.45, $p = 3 \times 10^{-4}$) was also obtained when conditioning on 3q21-q23 (marker D3S2418). These results suggest that the proposed strategy is promising for detecting modifier genes conferring susceptibility to a specific phenotype in the presence of a gene predisposing to a broad phenotype.

658F

A Robust Method for Identifying the Genes that Influence an Entire Spectrum of Disease-Related Phenotypes. *A. RoyChoudhury, W.C.L. Stewart, S.E. Hodge, D.A. Greenberg.* Department of Biostatistics, Columbia University, New York, NY.

Purpose: For some complex disease studies, individuals tend to be recruited for disease-related phenotypes. However, it is often difficult to know which of these individuals should be counted as affected in the subsequent genetic analysis. This is the disease definition problem, and the power of many studies depends crucially on how this problem is addressed. Here, we present a powerful and robust method that circumvents this problem by considering all possible disease definitions. Methods: We consider the case where families are ascertained on the basis of three disease-related phenotypes, S1, S2, and S3. From these phenotypes, we consider three possible disease definitions: S1 alone; S1 or S2; and S1 or S2 or S3. This particular scenario is inspired by our earlier work where families were ascertained for having at least one of three different panic disorder syndromes. Our robust linkage detection method, RLOD, is the lod score maximized over all possible disease definitions. To assess the power of the RLOD, we analyzed simulated data where the disease-related phenotypes of individuals were influenced by both genetic and environmental factors. Specifically, the following penetrance probabilities were used: $0 = Pr(Si \mid G0, E0) < Pr(Si \mid G0, E1) < Pr(Si \mid G1, E0) < Pr(Si \mid G1, E1) = 0.31$ for i = 1, 2, 3, where G1 and E1 are genetic and environmental risk factors, respectively. Furthermore, the difference in risk is only 1% between those who have G1and E1 versus those who have G1 and E0. Hence, in our simulations, the genetic risk factor tends to dominate over the environmental risk factor. Also, to control the type I error we used simulations under the null to estimate the critical value. From the analysis of 100,000 datasets with 25 nuclear families per dataset, we find that RLOD had 90% power to detect the disease gene, compared to 85%, 25%, and 6% power for competing methods that considered S1 or S2 or S3 as affected, just S1 or S2 as affected, and S1 alone, respectively. We also simulated data from a genetic model, which was inspired from our earlier work in epilepsy. Specifically, an abnormal EEG is an endophenotype or subclinical marker related to, and required for the diagnosis of epilepsy. However, among patients with epilepsy, individuals may have any combination of myoclonic and absence seizures. In these simulations, we find a similar pattern, and note that RLOD has more than 90% power to detect the disease gene.

659F

Practical Issues for Designing Efficient Sequence-based Genetic Studies of Quantitative Traits. *R.C. Banuelos[1], D.J. Liu[1,2], S.M. Leal[1,2].* 1) Statistics, Rice University, Houston, TX; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

There is great interest in understanding the role of rare variants in complex quantitative trait (QT) etiology. Although the cost of performing next generation sequencing has greatly decreased, the expense of sequencing and analyzing large datasets is still prohibitive. Therefore, implementing efficient genetic studies are crucial for maximizing power and reducing sequencing cost. Two study design strategies can be applied: 1.) Sampling individuals with extreme traits and 2.) Combining and jointly analyzing publically available phenotyped cohorts, such as the NHLBI's Exome Sequencing Project (ESP). For most QT studies, there is usually one tail of the QT distribution that is of main clinical interest (risk tail) e.g. the high extreme of body mass index for obesity research, or high fasting glucose levels in diabetes studies. Samples from the other extreme are used as a comparison group (comparison tail). Practical issues related to designing efficient rare variant QT association studies were investigated using extensive simulations under rigorous population genetic and realistic complex trait models. It is shown that for a fixed size of whole-exome sequence samples 1.) Analyzing full QT is consistently more powerful than dichotomizing the QT and analyzing it as a binary disorder. 2.) For samples that are ascertained from the general population, using less stringent phenotypic cutoffs will require screening a much smaller number of individuals. However, the power is only slightly decreased if full QT is analyzed. 3.) When selective sampling is carried out from an existing cohort of samples, sequencing an equal number of samples from each extreme is not the optimal study design. Instead, sequencing a fewer number of individuals from the risk tail and more samples from the comparison tail can increase power. This is because of the increased difference in causal variant carrier frequency between the two tails. 4.) When a public phenotyped cohort such as ESP is available, the most powerful design is to only sequence samples from the risk tail and use the public cohort as the comparison group. In conclusion, the results and study designs presented will provide important guidance for implementing efficient sequence-based QT studies. The R code for carrying out the power calculations will be made publically available to aid investigators in customizing their study designs.

660F

Statistical power of population-based linkage analysis. *S.R. Browning[1], E.A. Thompson[2].* 1) Department of Biostatistics, University of Washington, Seattle, WA; 2) Department of Statistics, University of Washington, Seattle, WA.

Recently there has been significant interest in population-based linkage analysis, also known as identity by descent (IBD) mapping, as a method for mapping trait-associated regions using genome-wide SNP data. The underlying idea is that with dense SNP data, short segments of IBD can be detected between supposedly unrelated individuals in the sample, and non-parametric linkage analysis applied to these data could yield significant associations.

We investigate the power of this approach using a combination of theoretical results, simulations, and analysis of real data. We focus on the statistic of Purcell et al. (2007), which compares the rate of IBD in case-case pairs to the rate of IBD in other pairs (control-control and control-case pairs). For large outbred populations it is difficult to derive realistic scenarios in which IBD mapping will have an acceptable level of power. However, we show that the approach can have power in a founder population, particularly if the population has undergone recent expansion and if the disease of interest has higher prevalence in the founder population than in outbred populations. If the founder population has a high prevalence of disease, this suggests that the causal alleles have drifted to a higher allele frequency in the founder population, which increases power.

Reference: Purcell et al. 2007. Am J Hum Genet 81: 559-575.

661F

Identification of the PTPRD gene involved in genetic susceptibility to keratoconus by combining evidence of genome-wide association and genetic linkage. *Y. Bykhovskaya[1], X. Li[2], K. Taylor[2], D. Siscovick[3], A. Aldave[4], L. Szczotka-Flynn[5], S. Iyengar[5], J.I. Rotter[2], Y.S. Rabinowitz[1,2,6].* 1) Regenerative Medicine Institute, Department of Surgery, Division of Surgical Research, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 3) Departments of Medicine and Epidemiology and Cardiovascular Health Research Unit, University of Washington, Seattle, WA; 4) The Jules Stein Eye Institute at UCLA, Los Angeles, CA; 5) University Hospitals Eye Institute, Department of Ophthalmology & Visual Sciences, Case Western Reserve University, Cleveland, OH; 6) Cornea Genetic Eye Institute, Cedars-Sinai Medical Center, Los Angeles, CA.

Testing for genome-wide association (GWA) is a powerful tool for discovery of common variants associated with complex disease traits. To identify genetic locations of keratoconus susceptibility genes by testing for genetic association, we performed a comprehensive GWAS using a discovery and replication design (Li et al., manuscript submitted). A discovery panel of 222 Caucasian cases/ 3324 controls was genotyped on Illumina 370K beadchips. To select SNPs for confirmation and fine mapping, we aligned GWAS results with the previously identified linkage peaks (Li et al. 2005) by translation of genetic and physical map distances using the Marshfield genetic map and MapViewer. We identified SNPs with p-values ranging from 10-6 to 10-4 in 10 out of 11 linked regions. All SNPs selected for confirmation were genotyped in an independent replication case-control panel (304 cases/ 518 controls) and a family panel of 307 subjects in 70 families. Two SNPs, rs10959090 and rs1323486, located in the intron of the PTPRD (protein tyrosine phosphatase, receptor type D) gene at the 9p23 chromosomal region showed suggestive association in the discovery panel of the GWAS study (p=2x10-4 and 0.02, respectively) and were confirmed in the replication panel with p=0.04 and 0.03; meta p=3x10-5 and 0.003, respectively). Two additional SNPs rs10816206 and rs10491919 located in the PTPRD gene were associated in a confirmation panel with significant p=2x10-5 and 6x10-5, respectively, and in a family panel with significant p=0.03 and suggestive p=0.08, respectively. PTPRD gene codes for the receptor protein tyrosine phosphatase (RPTP) delta, a member of the three-member leukocyte antigen-related (LAR) RPTP subfamily of the protein tyrosine phosphatase family which plays a role in regulating integrity of cell-cell contacts, differentiation, and proliferation. Expression of one of the members of the LAR RPTP subfamily, LAR, has been repeatedly found to be increased in keratoconus cultures and corneas (Chiplunkar et al. 1999; Zhang et al. 2005). Additionally, phosphatase inhibition affected cell-cell contacts of corneal endothelial cells (Chen et al. 2005). Convergent findings of genetic linkage and association coupled with potential biological significance of the protein tyrosine phosphatase pathway in corneal biogenesis are supportive of a genuine effect of the variation in the PTPRD gene on keratoconus susceptibility.

662F

Genome-wide association meta-analysis of pubertal growth in males and females. *D.L. Cousminer[1], N.J. Timpson[2], D. Berry[3], J.T. Leinonen[1], W. Ang[4], N.M. Warrington[4], E. Thiering[5], J.P. Bradfield[6], E.M. Byrne[7], C. Holst[8], J. Kaprio[1,9,10], C.E. Pennell[4], O. Raitakari[11], M-R. Järvelin[12], E. Widén[1] on behalf of the EGG Consortium.* 1) Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Finland; 2) MRC Centre for Causal Analyses in Translational Epidemiology, University of Bristol, Bristol, UK; 3) Centre for Pediatric Epidemiology and Biostatistics, Institute of Child Health, London, UK; 4) School of Women's and Infants' Health, The University of Western Australia, Perth, Australia; 5) Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology, Neuherberg, Germany; 6) Center for Applied Genomics, Children's Hospital of Philadelphia Research Institute, Philadelphia, PA, USA; 7) Queensland Statistical Genetics, Queensland Institute of Medical Research, Brisbane, Australia; 8) Institute of Preventive Medicine, Copenhagen Capital Region, Copenhagen University Hospitals, Copenhagen, Denmark; 9) University of Helsinki, Hjelt Institute , Dept of Public Health, Helsinki, Finland; 10) National Institute for Health and Welfare, Dept of Mental Health and Substance Abuse Services, Helsinki, Finland; 11) Department of Clinical Physiology, University of Turku, Finland; 12) Department of Epidemiology and Public Health, Imperial College London, London, United Kingdom.

Puberty, a highly variable complex trait influenced by both genetic and environmental factors, is a childhood growth phase with implications on adult disease risk. In particular, epidemiological studies show that advanced puberty associates with increased risk for adult health outcomes like obesity, type 2 diabetes, and hormone-dependent cancers. Uncovering genes underlying these observations may shed light on predicting lifetime disease risk. A recent genome-wide association study (GWAS) uncovered many gene variants associated with age at menarche (AAM), while genes influencing male pubertal maturation remain unknown. To identify genes influencing the timing of central pubertal onset in both males and females, we ran GWAS meta-analyses on simple measurements of the pubertal height growth spurt similar in both sexes. To characterize the effect of the discovered genetic variants across pubertal growth, we utilized longitudinal height measurements available in many cohorts to investigate association between the leading signals and height or BMI at cross-sectional age bins from pre- to post-puberty. The study will also address the association between these variants and pubertal timing using Tanner breast and genital staging data. GWAS of (1)height at age 10 in girls and 12 in boys, (2)height increase from 7-adult, and (3)height increase from 14-adult revealed 9 genome-wide significant loci and 24 suggestive loci previously associated with adult stature, AAM, and/or BMI. We took forward suggestive novel signals (n= 23) and replicated a single variant (rs4788196, $p=6x10^{-11}$, n=18,737). Height analysis across puberty showed that this variant associated with tall pre-pubertal stature and declining relative height growth during puberty. rs4788196 is an eQTL in lymphoblasts for *ERK1*, encoding a MAP kinase involved in many developmental processes, including long bone growth in mice. Also significantly associated with pubertal growth was rs1172294 near *POMC*, previously associated with both childhood obesity and adult height. The BMI-increasing allele showed decreased pubertal height growth, with no effect on height prior to puberty. This study unveiled heavy genetic overlap between pubertal growth in both sexes and related traits as well as discovery of a novel variant near *ERK1*. With access to the unique resource of frequent longitudinal height measurements, we were able to describe the distinct height growth effects of each variant across puberty.

663F

New developments in the genetic fine mapping of a non-Mendelian trait through the coalescent process with recombination. *M. Dupont[1], G. Boucher[2], MH. Descary[1], F. Larribe[1].* 1) Département de Mathématiques, Université du Québec à Montréal, Canada; 2) Institut de cardiologie de Montréal, Québec, Canada.

We present new developments relating to a fine mapping methodology (MapARG) by means of the ancestral recombination graph (ARG). The coalescent theory with recombination allows us to construct genealogies that are consistent with a sample of haplotypes composed of specific markers. The likelihood of rT , the unknown location of a *Trait Influencing Mutation* (TIM), is calculated from these genealogies, through a combination of Monte Carlo approximation and importance sampling. The evaluation of this likelihood being computationally intensive, these analyses are performed using composite likelihood by means of windows of markers. As linkage disequilibrium should be present in the data, the distribution of the haplotypes should be dependent on the known phenotype. An EM algorithm has been developed to estimate the distribution of haplotypes in the population conditionally on the phenotype and the genotype. Moreover, the method can handle phenocopy and incomplete penetrance. We investigate the effect that different models of penetrance can have on the performance of MapARG. These results show that the method is somewhat efficient when the relative risks f_1/f_0 and f_2/f_0 are large enough. As the RRs increase, error rates vary along the sequence, improving in closeness to the TIM. The algorithm permits MapARG to correctly localize the TIM, even in cases where f_1/f_0 and/or f_2/f_0 are/is relatively small. We present results obtained from this methodology against the usual simple statistics used in this context. We show cases where, although the χ^2 standard test fails to indicate the correct location of the TIM, MapARG makes use of the information contained in the markers' linkage disequilibrium to give an accurate estimate of the TIM's position. Starting from a simple rare and recessive model, we showed how to enrich the method to a more general complex model with few restricting hypotheses. The method uses the coalescent with recombination in combination to the Fearnhead/Donnelly's proposal distribution to locate the position of disease gene; these models and methodologies are rich and methodologically well founded. This is a work in progress, but first results are very encouraging. The method uses as much information as currently possible, and should be more powerful than other methods, especially those based on pairwise statistics.

664F

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665F

Trans-ethnic fine-mapping of Type 2 Diabetes susceptibility loci using a "Cosmopolitan" reference panel for imputation. *M. Horikoshi[1], S. Wiltshire[1], N. Kato[2], J. Asimit[3], N. Rayner[1], N. Robertson[1], F. Takeuchi[2], A. Mahajan[1], T. Yik Ying[4], E. Zeggini[3], A. Morris[1], M. McCarthy[1], T2D-GENES.* 1) The Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom; 2) Research Institute, National Center for Global Health and Medicine, Tokyo Japan; 3) Wellcome Trust Sanger Institute, Cambridge UK; 4) Department of Statistics and Applied Probability and Department of Epidemiology and Public Health, National University of Singapore.

More than 40 type 2 diabetes (T2D) susceptibility loci have been identified in recent genome-wide association studies (GWAS). However, it has not been possible to identify the specific causal variant(s) at most of these loci. As one approach to refine the localisation of causal variants, we performed trans-ethnic meta-analysis of 13 study populations of European, South Asian and East Asian descent (14,329 cases and 22,833 controls) as part of the T2D GENES Consortium. We focused on fine-mapping of 5 established T2D loci with evidence of differential linkage disequilibrium patterns between non-African populations: *CDKAL1, CDKN2A/B, FTO, IGF2BP2* and *KCNQ1*. We combined directly genotyped and imputed data (using a "Cosmopolitan" reference panel which incorporates the variants shared across all populations from the 1000 Genomes Aug 2010 release), testing for association under an additive model. We combined these results using: (i) traditional fixed-effects meta-analysis; (ii) an in-depth "split and filter" regression analysis; and (iii) a Bayesian meta-analysis that incorporates a prior model for heterogeneity in effects between ethnic groups based on their shared ancestry. The resolution of fine-mapping was highly improved at *CDKAL1*. Fine mapping highlighted two out of the much larger group of SNPs showing equivalent evidence of association in Europeans as showing the most consistent evidence of association across ethnic groups. The two SNPS are rs9368222 ($p = 8.3x10^{-16}$) and rs7766070 ($p = 9.8x10^{-16}$) with fixed-effects OR = 1.15 [1.11-1.19] for both. There was evidence of two independent signals of association at the *KCNQ1* locus first reported in the Japanese population. The strongest association was at rs2237892 ($p = 5.3x10^{-16}$), with consistent effects across ethnic groups (fixed effects OR = 1.20 [1.15-1.27], 11.9% posterior probability of heterogeneity). The secondary association signal was at rs2237895 ($p = 3.4x10^{-11}$, OR = 1.16 [1.11-1.22]; CEU $r^2 = 0.00$ and CHB+JPT $r^2 = 0.26$ with rs2237892), with strongest effects observed in East Asians (100% posterior probability of heterogeneity). Our results highlight the potential of trans-ethnic GWAS for fine-mapping causal variants, and show promise for further refinement with additional samples from more diverse population groups, including those of African and/or Hispanic descent.

666F

A genome-wide association study for loci influencing stunting in children from Bangladesh. *J.C. Mychaleckyj[1], S.S. Rich[1], P. Concannon[1], R. Haque[2], T. Ahmed[2], X. Hou[1], Z. Li[1], W.A. Petri[3].* 1) Dept Public Health Sci, Ctr Public Health Genomics, University of Virginia, Charlottesville, VA; 2) International Centre for Diarrhoeal Disease Research, Dhaka-1000, Bangladesh; 3) Dept Medicine, Division of Infectious Diseases, University of Virginia, Charlottesville, VA.

Malnutrition is implicated in 50% of deaths due to infection and 35% of all deaths among under-five children. Stunting affects ~ 1/3 of children under age 5 and occurs in the -9 to +24 month age window. The majority of stunted children live in Asia, where the average child is born moderately stunted (height adjusted Z-score (HAZ) of -0.75). These children experience rapid growth faltering to an HAZ of -2.25 by 2 years of age. Current interventions, even if extended to all children, are predicted to reduce stunting by ~ 1/3. We are performing a genome-wide association study (GWAS) to identify genetic risk factors that may increase risk for stunting. Three cohorts of children (N=1700) are being recruited from Dhaka, Bangladesh for the GWAS. A principal components analysis of the genotype data for the initial 462 subjects recruited indicated that the study population displayed considerable similarity to Caucasian populations and clustered most closely with the Gujarati Indian (GIH) HapMap population. These results support the use of standard commercial high-density genotyping platforms in this study population (Illumina 1M and 2.5M chips) and for imputation based upon HapMap data. A preliminary analysis using height adjusted for age and sex (a surrogate for HAZ) was performed using 1M SNP genotypes and imputed to 7.8 million SNPs on the first 932 study subjects. The Bangladeshi cohorts displayed little evidence of residual population stratification in unadjusted tests of association. No common (>5% minor allele frequency) SNPs were associated with stunting at genome-wide significance. However, a number of common SNPs with P<10-6 clustered on chromosomes 2 (two loci), 3, 13 and 14. These SNPs are located in/near genes associated with lipid metabolism, signaling and immune function. Validation genotyping on a separate platform confirmed these results. Analyses using an additional 336 subjects from Bangladesh have replicated four of the five loci. Additional replication genotyping/analyses are being conducted, as is analysis of rare (MAF < 5%) variants. Knowledge of genetic risk factors that contribute to malnutrition-related phenotypes could potentially direct interventions and suggests novel therapeutic approaches.

667F

Gene-gene interaction analysis accounting for multiple level of genetic relatedness. *J. Namkung, R. Elston.* Biostatistics and epidemiology, Case Western Reserve Univ., cleveland, OH.

Population stratification is a frequent confounding factor in genetic association studies. When data comprise families of multiple ethnicities and/or an admixed population, the correlation structure becomes complex and thus accounting for it becomes more challenging. Various approaches have been reported to tackle this issue, including mixed models, transmission disequilibrium test (TDT) type statistics, and adjusting for ancestry effects using principal component analysis (PCA). However, the effect of population stratification on gene-gene interaction analysis has not been well studied. In this study, we propose an extension of the multifactor dimensionality reduction (MDR) method to analyze gene-gene interaction in the presence of various types of correlations. We use a polygenic mixed effect model to account for the familial correlation structure and PCA to obtain ancestry information for founders. We use as input to generalized multifactor dimensionality reduction (GMDR) residuals from a polygenic mixed model containing variables of ancestry information as covariates. We extensively investigate the effect of multiple levels of genetic relatedness by simulation studies. As a result, we show how greatly the performance of gene-gene interaction analysis can be improved by this procedure. We also compare the performance of our proposed approach with that of Pedigree-based GMDR (PGMDR), which is known to be immune to population structure because it conditions on parental genotypes. As an example, the proposed method is applied to data from the Framingham Heart Study, which has diverse European ethnicities.

668F

Moving beyond inferential limits of GWAS to discover genetic mechanisms in autoimmunity: how end-organ pathways contribute to pathogenesis. *L. Petukhova[1, 2], A.M. Christiano[2, 3].* 1) Deptartment of Epidemiology, Columbia Univ, New York, NY; 2) Deptartment of Dermatology, Columbia Univ, New York, NY; 3) Deptartment of Genetics & Development, Columbia Univ, New York, NY.

Recently, genome-wide association studies (GWAS) in autoimmune diseases, including our work in alopecia areata (AA), have been tremendously successful in identifying many robust associations, although the vast majority of these reside in immune response genes and reveal very little about the end-organ. We postulate that this occurs because of an inherent feature of GWAS, that the method relies upon the assumption that the disease allele will be common in the population. Since immune response genes are subject to positive selection (which increases allele frequencies), this class of genes is particularly amenable to detection by GWAS. Likewise, we postulate that AA susceptibility genes specific to the hair follicle (HF) may fail to achieve statistical significance because of low allele frequencies, thus eluding detection by GWAS. These genes should be amenable to detection by a method well-suited for rare variants, e.g. linkage. In this study, we aimed to identify HF genes in AA using convergent evidence between our GWAS and linkage studies in AA. To probe for HF genes in our GWAS data, we chose a liberal threshold for significance ($p<0.01$) and mapped the top 5000 SNPs to a set of 3347 genes. We then extracted a set of 476 genes with evidence for expression in the HF. Of these, only 5 genes contained SNPs that exceeded statistical significance in the GWAS (p<5x10-7; PPP1R14C, CREBL1, SUOX, CDK2, STX17), while the vast majority (471) contained SNPs that achieved only nominal significance. We next integrated these results with our two previous linkage analyses in AA, and found that 121 genes fell into regions with at least suggestive evidence for linkage (1<LOD<4). We found that a number of linkage peaks contain clusters of HF genes that are nested within our nominally significant GWAS findings. This study demonstrates that integration of genetic mapping approaches with expression data can allow us to gain novel insight into the genetics of autoimmunity, in particular, identifying genes of the end organ.

669F

Fine-mapping in Linkage Peaks for Osteoarthritis Biomarkers in a Large North American Pedigree. *X. Qin[1], S.H. Shah[1, 2], H.C. Chen[2], C. Haynes[1], T. Stabler[2], J.L. Huebner[2], S.G. Gregory[1,2], W.E. Kraus[2], J.M. Jordan[3], E.R. Hauser[1,2], V.B. Kraus[2].* 1) Center for Human Genetics, Duke University, Durham, NC; 2) Dept of Medicine, Duke University, Durham, NC; 3) Thurston Arthritis Research Center, UNC, Chapel Hill, NC.

Previous genome-wide linkage analysis of five osteoarthritis biomarkers in a 10-generation North American pedigree of Native American and African ancestry identified significant linkage peaks on chr. 6 for type IIA collagen N-propeptide (PIIANP max. multipoint LOD=2.25 at 50cM) and hyaluronan (HA max. multipoint LOD=1.69 at 50 cM), chr. 8 for PIIANP (LOD=4.33 at 8 cM) and for cartilage oligomeric matrix protein (COMP LOD=3.18 at 62 cM) and chr. 14 for COMP (LOD=2.57at 66 cM) (Chen et al. Arthritis Rheum. 2010). The pedigree includes 3327 members of which 333 provided both biomarker and genotype data. To further explore these results we genotyped 1536 SNPs selected to cover the common variation in these regions based on HAPMAP CEU and YRI samples. Of these,1328 (86.4%) SNPs met lab-wide quality control benchmarks. We took a multistep analysis approach starting with a simple ANOVA analysis for genotype effect on biomarker levels and identification of significant SNPs. For the significant SNPs we performed measured genotype analysis with adjustment for age and sex and for the familial relationships as implemented in SOLAR. We concentrated on SNPs with consistent statistical evidence for association that gave an ANOVA p-value <.05, SOLAR fixed effect for SNP genotype p-value <.05, and a drop in the LOD score >1 LOD unit for linkage at the SNP location comparing models with and without the fixed effect for the SNP genotype. Using this procedure we identified 4 SNPs (rs204899, rs204899 and rs411337 in TNXB and rs2794719 in HFE) for PIIANP on chromosome 6. We identified 3 SNPs (rs1438214, rs6996668, rs3860862) for PIIANP and 2 SNPs (rs10958731, rs6474169) for COMP on chromosome 8. No other regions and phenotypes produced SNPs meeting these criteria. Additional validation of this approach as well as validation of these associations in an existing GWAS data set consisting of 344 nuclear families with osteoarthritis is underway. In preliminary analyses SNP rs2794719 (HFE) showed an association (p<.04) with PIIANP in this data set. Association of a coding variant in HFE has been associated with osteoarthritis in a population-based study (Alizadeh et al. Ann Rheum Dis, 2007). These results suggest a genetic basis for OA phenotypes related to PIIANP levels.

670F

Cross-ethnic locus transferability evaluation of 26 non-HLA celiac disease risk loci in north Indians. *S. Senapati[1], G. Trynka[2], A. Sood[3], V. Midha[3], J. Romanos[2], L. Franke[2], B.K. Thelma[1], C. Wijmenga[2].* 1) Department of Genetics, University of Delhi, South Campus, New Delhi, Delhi, India; 2) Genetics Department, University Medical Center and Groningen University, 9700RB Groningen, The Netherlands; 3) Dayanand Medical College and Hospital, Ludhiana, Punjab, India.

Currently there are 26 non-HLA loci associated to celiac disease (CeD), all of which have been identified by genome-wide association studies (GWAS) in European populations. Here we investigated an ethnically different and old population from a distinct area in the north of India. The aim of the study was to gain insight into the genetic architecture of CeD in India. We performed cross-ethnic mapping using very dense genotype information from Immunochip, which enables high resolution analysis of CeD associated loci, therefore possible localization of actual disease risk variants. As we expected the linkage disequilibrium (LD) patterns between Europeans and north Indians to differ substantially, we carried out locus transferability analysis instead of direct association testing for the top GWAS SNPs. We genotyped 880 individuals from northern India (371 cases, 509 controls) and 2323 Dutch subjects (1150 cases and 1173 controls) on the Immunochip, a custom made genotyping platform comprising ~200,000 SNPs including >50,000 rare variants. The chip is densely probed for the 26 non-HLA CeD loci, by using variants from the 1000 Genomes-pilot and additional CeD re-sequencing projects. We carried out locus-wide comprehensive evaluation of association signals for all HapMap CEU variants in LD ($r^2 \geq 0.05$) with the reported European variants. Eleven loci were transferred (p-permuted= 0.004) with no significant heterogeneity in effect size estimates which signifies true locus transferability. Four of the 26 GWAS reported CeD top SNPs were also associated in north Indians: IL12A, PTPRK/THEMIS, ZMIZ1 and ICOSLG. A comparison of the LD blocks between the north Indian and the Dutch population revealed less extensive long range LD in the north Indian population. Hence, we were able to successfully narrow down the association signal for ITGA/UBE2E3 and the LPP loci to relatively smaller LD blocks. Currently we are investigating if these CeD loci are under different selection pressure in Indians and Europeans. The findings advocate the necessity of multi-ethnic approaches towards fine-mapping of disease associated loci to identify causal variants.

671F

Incorporating genotype uncertainties into the genotypic TDT for main effects and gene-environment interactions. *M.A. Taub[1], H. Schwender[2], T.H. Beaty[3], T.A. Louis[1], I. Ruczinski[1].* 1) Department of Biostatistics, Johns Hopkins University, Baltimore, MD, USA; 2) Faculty of Statistics, TU Dortmund University, Dortmund, Germany; 3) Department of Epidemiology, Johns Hopkins University, Baltimore, MD, USA.

As increasingly dense panels of polymorphic genetic markers have become available through the HapMap and the 1000 Genomes Projects, imputation has become an option for researchers to expand their genotype datasets to improve signal precision and power in tests of genetic association with disease. While methods for incorporating imputed genotype probabilities into statistical tests for case-control data are routinely used, no methods have yet been presented for efficiently incorporating uncertain genotype calls in the analysis of case-parent triad data. Here, we present the statistical framework for genotypic transmission-disequilibrium tests (gTDTs) using observed and imputed genotypes. The implementation extends our recent work in obtaining closed-form solutions for the parameter estimates in gTDTs, thus allowing for assessment of hundreds of thousands of markers within minutes. We also present an extension to assess gene-environment interactions for binary environmental variables. We illustrate the performance of our method on a large set of trios from the International Cleft Consortium.

672F

Genome-wide association analysis of skeletal maturation in healthy children from the Fels Longitudinal Study. *B. Towne[1], J. Blangero[2], J.E. Curran[2], C. Bellis[2], T.D. Dyer[2], K.D. Williams[3], E.W. Demerath[4], A.C. Choh[1], M. Lee[1], R.M. Siervogel[1], S.A. Czerwinski[1].* 1) Wright State University School of Medicine, Dayton, OH; 2) Texas Biomedical Research Institute, San Antonio, TX; 3) Temple University, Philadelphia, PA; 4) University of Minnesota, Minneapolis, MN.

Knowledge of skeletal development genetics comes mostly from studies of monogenic disorders. Few studies have examined genetic influences on normal variation in skeletal development, though the skeletal age (SA) of a normal child can vary up to 3 years from that child's chronologic age. We present here results from genome-wide association analyses of the SA of children at chronologic ages 1 to 18 years. SA data were obtained from hand-wrist radiographs taken annually from 1931 to the present of over 1,000 children from families in the Fels Longitudinal Study (Roche, 1991). Estimates of SA were made using the FELS method (Roche et al., 1988) that assesses various indicators of skeletal development appropriate for any given chronologic age. Most individuals have been genotyped with the Illumina Human 610-Quad BeadChip containing more than 550,000 SNPs. Association analyses were conducted using measured genotype analysis implemented in SOLAR (Almasy and Blangero, 1998) allowing for residual non-independence among relatives. Sample sizes ranged from n=365 at age 1 year to n=561 at age 8 years. SNP rs2120968, on 15q11 at 20,483,952 in an intron of CYFIP1, shows significant peak effects at age 3 ($p=1.1\times10^{-7}$). The minor allele (A) has a frequency of 0.24 and is associated with a 0.450 SDU decrease in SA at age 3, accounting for 7.1% of the variation in SA at that age and exhibiting significant pleiotropy across ages 1-7. SNP rs11873322, on 18q12 at 32,394,649 in an intron of FHOD3, shows significant peak effects at age 7 ($p=7\times10^{-8}$). The minor allele (A) has a frequency of 0.36 and is associated with a 0.373 SDU increase in SA at age 7, accounting for 5.8% of the variation in SA at that age and exhibiting significant pleiotropy across ages 2-13. SNP rs7629566, on 3q29 at 197,923,141 near the 5' end of PIGX shows significant peak effects at age 14 ($p=7\times10^{-8}$). The minor allele (G) has a frequency of 0.07 and is associated with a 0.598 SDU decrease in SA at age 14, accounting for 6.2% of the variation in SA at that age and exhibiting significant pleiotropy across ages 12-17. Skeletal maturation throughout childhood is a complex process. Results of this study indicate that there are significant specific genomic region effects on skeletal maturation centered on ages spanning: 1) early to mid-childhood, 2) mid-childhood to puberty, and 3) puberty to post-puberty. Supported by NIH grants R01HD012252, R01HD036342, F32HD053206, and R37MH59490.

673F

A comparison of methods to detect complex trait rare variant associations implementing the RarePower tool. *G. Wang[1,2], SM. Leal[1,2].* 1) Baylor College of Medicine, Houston, TX; 2) Rice University, Houston, TX.

There is currently great interest in detecting rare variant associations using next generation sequence data. A large number of association methods which aggregate variants across a region e.g. a gene have been developed specifically to analyze rare variant data. It is not clear which existing method is the most powerful and should be applied to test for associations using exome/genome sequence data. To compare rare variant association methods both realistic phenotype models and spectrum of variants across a region must be generated. We compared the power for 12 methods to detect associations for qualitative and quantitative traits. Power was evaluated for case-control, extreme quantitative trait sampling and population based study designs. For each method, power was determined for scenarios which included 1. analysis of a. only rare variants & b. rare (<1%) and low (1-5%) frequency variants; 2. detrimental and protective variants within a gene region; 3. misclassification a. exclusion of causal variants & b. inclusion of non-causal variants; 4. different underlying population demographic model for both Africans and Europeans and 5. gene size. It was observed that there is not a single method that is most powerful in all situations. The majority of rare variants methods had only small incremental difference in power. Rare variant association methods which were powerful in a variety of situations include the Variable Threshold (VT) method, Weighted Sum Statistic (WSS) and Kernel Based Adaptive Cluster (KBAC) method. Those methods which were developed to detect associations when both protective and detrimental variants are within an associated region (e.g. C-alpha) are usually less powerful than more general rare variant association methods. The evaluated methods vary in their computation efficiency and ability to control for confounders. Additionally, in order to optimize performance and increase power to detect associations we have we have modified several rare variant methods including the KBAC, aSum, VT and combined multivariate and collapsing (CMC) method. The RarePower tool with its user friendly graphical interface can be used to determine sample sizes which are necessary to detect rare variant associations under a large variety of complex trait and population demographic models.

674F

Developing Weighted Test Statistics for Testing Association of Rare variants Using Pairs of Affected and Unaffected Individuals. *Y. Yao[1], Y. Zhu[2], W. Guo[1], M.M. Xiong[2].* 1) Division of Intramural Research Program, National Institute of Mental Health, Bethesda, MD., USA; 2) 2.Division of Biostatics, , University of Texas School of Public Health, Houston, Texas USA.

Recently, a number of statistical methods become available for analyzing the contribution of rare variants to the development of complex traits. These methods include Combined Multivariate and Collapsing (CMC) Method, Multivariate test of collapsed sub-groups Hotelling T2 test, MANOVA, Fisher's product method, Weighted Sum Method and Kernel-based adaptive test. While the merits of these methods have been evaluated extensively for population-based association studies, none of these methods can be used to analyze the pedigree based association analysis using exome sequencing or whole genome sequencing data. It is known that many common diseases are clustered in families and one can reasonably expect that human pedigrees will be enriched with causal rare variants. However, traditional linkage analysis may not be a good tool for testing association of rare variants. There is an urgent need to develop efficient statistical methods for sequence-based pedigree analysis. We will extend collapsing principle for population-based association analysis of rare variants which collectively evaluate contribution of multiple rare variants within a genomic region to the diseases, to the pedigree analysis of rare variants. Particularly, we use pairs of affected and unaffected individuals as a study design by integrating all rare variants within a gene or a genomic region into an overall variable. We will develop family-based rare variants analysis approach by treating each affected relatives as dependent pairs and the dependency will be accounted for using correlation matrix. Specifically, similar to the weighted sum statistics for testing association of rare variants in unrelated individuals, we have developed a novel weighted statistic that compares difference in the value of the integrated overall variables between affected and unaffected individuals weighted by their IBD coefficients. Further, we use large-scale simulation to evaluate its type 1 error and power under several disease models. This method will be used to analyze a reasonable number of bipolar disorder pedigrees with exome data. Power studies and real data analysis demonstrate that the proposed statistic for sequence-based pedigree analysis may emerge as a powerful tool for genetic studies of complex diseases.

675F

Admixture mapping analysis of BP and BMI in Family Blood Pressure Program (FBPP). *X. Zhu[1], Z. Zhang[1], B. Tayo[2], A. Morrison[3], C. Hanis[3], C. Cooper[2], S. Kardia[4], D.C. Rao[5], H. Tang[6], N. Risch[7].* 1) Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Department of Preventive Medicine and Epidemiology, Loyola University Medical Center, Maywood, IL; 3) Human Genetics Center, The University of Texas School of Public Health, Houston, TX; 4) Department of Epidemiology,University of Michigan, Ann Arbor, MI; 5) Division of Biostatistics, School of Medicine, Washington University in St. Louis, St. Louis, MO; 6) Stanford School of Medicine, Stanford, CA; 7) Department of Epidemiology & Biostatistics, University of California, San Francisco, CA.

African Americans are, on average, at higher risks for hypertension and obesity, compared to European Americans. Previously we have used admixture mapping to identify candidate regions, which both contribute to disease risks and to disparity between populations. In this report, we perform admixture mapping analysis using 4012 subjects from 830 African-American families from FBPP project with GWAS data available. We first infer each individual's local ancestry using both dense SNPs and family information. We next test the association between local ancestry and BP and BMI incorporating family structure. We will report the results by admixture mapping using both the family and dense SNP data.

676F

The Rutgers Map: A third-generation combined linkage-physical map of the human genome. *T.C. Matise[1], A.Q. Nato[1], S. Buyske[2].* 1) Dept Genetics, Rutgers Univ, Piscataway, NJ; 2) Dept Statistics and Biostatistics; Dept Genetics, Rutgers Univ, Piscataway, NJ.

Accurate and comprehensive linkage maps remain critical for the success of positional cloning and genetic linkage analyses. We have created a third-generation high-resolution genetic map (The Rutgers Map) that combines physical map positions from the current genome assembly (Build 37) with recombination data from a comprehensive collection of publicly available genotype data from the CEPH pedigrees. Physical positions for the SNPs have been updated from the current version of dbSNP (Build 132). As a result of changes to the assembly and/or the SNP database, a small number of markers that were on our previous map have been dropped in the current map. Markers that have been merged in dbSNP are represented by their current rsID, and linkage analysis was used to evaluate physical map discrepancies, such as for several markers that have multiple physical positions on multiple chromosomes in dbSNP. This third-generation Rutgers map includes just under 28,000 markers, of which 60% are SNPs, 35% are STRs, and the remainder are mostly RFLPs. We have recalculated our “smoothed maps” using a quadratic fit local regression procedure. The smoothed maps provide a unique map position for every marker, which cannot be determined from the recombination data alone, and which can be readily used to interpolate a linkage map position for any marker or genetic element with a known physical position. The Rutgers Map website provides a tool for interpolating markers onto the map (http://compgen.rutgers.edu/). A major new feature of the revised map is that we have used it to interpolate a linkage-map position for 94.9% of the reference SNPs in dbSNP (N=22,181,252). The remaining reference SNPs lie outside of our map boundaries or do not uniquely map to the genome. Linkage map positions for these SNPs, as well as for SNP panels from most of the Affymetrix and Illumina GWAS panels, are available on our website. The updated Rutgers combined physical-linkage map provides a single source of linkage map positions for nearly all markers genotyped in the CEPH pedigrees.

677F

Using a Second-order Hidden Markov Model to Identify Regions of Identity-By-Descent in Exome Sequencing Data. *S. Hicks[1], S.E. Plon[2,3], M. Kimmel[1].* 1) Department of Statistics, Rice University, Houston, TX; 2) Human Genome Sequencing Center, Houston, TX; 3) Texas Children's Cancer Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas.

Identifying regions of identity-by-descent (IBD) in heritable disorders is an effective approach in identifying disease-causing mutations. Rödelsperger et al. [Bioinformatics 27: 829-836,2011] applied an inhomogeneous first-order hidden Markov model (HMM) to genotype data produced from exome sequencing to identify chromosomal regions of IBD in siblings with an autosomal recessive disorder. The goal of this study is to extend the model to a second-order HMM investigating the second-order dependence structure between the observed variant calls in siblings. This second-order HMM has to be inhomogeneous to account for position, distance and sex-specific recombination rates which are incorporated into the transitions between regions of IBD or not IBD. Predictions of IBD or not IBD at each locus are reported as well as a marginal probability of being in an IBD region. We compared the results from the first and second order HMMs using simulated and real exome sequencing data from two affected siblings. Viterbi predictions from first and second-order HMMs follow each other closely, but marginal posterior probabilities are more variable using the second-order HMM. This effect might be caused by the second order HMM revealing a finer IBD structure along the chromosomes compared to the first-order HMM. Supported by CPRIT grant RP101089 to SH, SEP and MK, and NCI T32 training grant CA096520 to SH.

678F

Developing temporal models for multiple simultaneous time series of sequenced public health genomic data. *R. Hoffmann[1], S. McLellan[2].* 1) QHS, Pediatrics, Medical College of Wisconsin, Milwaukee, WI; 2) University of Wisconsin-Milwaukee, Milwaukee, WI.

Purpose: To study the temporal changes in prevalence of bacterial taxa over a 3 year period and relate these to public health problems during the same time periods. In order to examine these relationships, it is necessary to obtain temporal models to identify common patterns of bacterial prevalence that can be adjusted for other environmental conditions such as temperature, rainfall, flow, etc. Methods: Samples were taken from two different, but related, sites in lake Michigan over a three year period. We collected 19 paired sewage influent samples from the two sites in lake Michigan that were part of the Milwaukee Metropolitan Sewerage District under different conditions over a three-year period. The service area consists of more than 10,000 km of local municipal pipes that converge into a municipal interceptor system either of the two regions. One site is a a separated residential/industrial system as well as a combined sewer system in the oldest, most urbanized area of the while the other primarily processes residential sewage. Bacterial pyrotags (individual sequences) were used to identify the taxa. Since the samples were taken during different seasons and at different points in time, determining temporal effects required methods that were both flexible and did not required regular measurements. Wavelet analysis, after filtering to reduce sampling noise, was used to determine the temporal aspects of the multivariate time series because of the irregular occurrence of the sampling points over time. Results: Analysis of 1,085,939 bacterial pyrotags (eg: individual sequences) identified a total community complexity of 1,057 different taxa. We identified 18 taxa that were in high abundance in sewage and present at minimal relative abundance in surface water. These 18 taxa accounted for 66% of the pyrotags found in sewage influent. The most common were Acinetobacter (16.1%), Aeromonas (9.8%), Trichococcus (7.7%). After fitting the wavelet models, the wavelet coefficients were clustered into 6 groups. Group 1 had 8 taxa and sub-taxa including acinitobacter tag 1. Group 2 had 7 taxa and sub-taxa including acintobacter tag 2. This group had a clear difference in time of peak and nadir from group 1. Group 3 had two taxa and sub-taxa and were partially similar to group 2. The remaining 3 taxa had distinct features that did not match the above groups nor did they match each other.

679F

Aberrant Recombination in Trisomy 21 Nondisjunction is Associated with Differential Hot-Spot Usage. *C. Middlebrooks[1], T. Oliver[1], S. Tinker[1], E.G. Allen[1], L. Bean[1], R. Chowdhury[2], F. Begum[3,4], M. Marazita[3,5], V. Cheung[6,7,8], E. Feingold[3,4], S.L. Sherman[1].* 1) Human Genetics Department, Emory University, Atlanta, GA; 2) Department of Pediatrics, University of Pennsylvania, Philadelphia, PA; 3) Department of Human Genetics, Graduate School of Public Health University of Pittsburgh, Pittsburgh, PA; 4) Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 5) Center for Craniofacial and Dental Genetics, Division of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA; 6) Departments of Pediatrics and Genetics, University of Pennsylvania, Philadelphia, PA; 7) Howard Hughes Medical Institute, University of Pennsylvania, Philadelphia, PA,; 8) Department of Genetics, University of Pennsylvania, Philadelphia, PA.

Several genomic features have been found to be associated with recombination placement. These features include CpG islands, GC content, Poly AT content, gene coverage and hot-spot location. As altered placement of recombination is associated with nondisjunction (NDJ), we sought to determine if the quantity of the aforementioned genomic features differed at sites of exchange between normally and abnormally segregating chromosomes. Potentially this would help identify features important for chiasma stabilization and proper chromosome segregation. We characterized our normally disjoined maternal chromosomes 21 from publically available family data and nondisjoined maternal chromosomes 21 from trisomy 21 offspring and their parents using 1534 genotyped SNPs along 21q. 21q was divided into three regions with the following designations and analyzed separately: centromere (13.6 -18.6 Mb), medial (18.6 - 23.6 Mb) and telomere (23.6 -48.5 Mb). Next, each region was divided into 500kb bins and the quantity (proportion or counts) of observed recombinant events and of each genomic feature was determined. Finally, linear regression was used to determine if the quantity of each genomic feature was a significant predictor of recombination within each region of 21q. This analysis included 222 meiosis I (MI), 202 meiosis II (MII) errors and 1272 euploid chromosomes 21 of maternal origin. Overall our results suggested that recombination along nondisjoined chromosomes 21 in oocytes did not fall into hot spots that have been defined by normal disjoining chromosomes. Specifically, we found that the number of hot spots at the centromeric and medial regions of chromosome 21 was directly proportional to the number of recombination events in those regions in our normal meiotic events; however, hot spots were not significant predictors of recombination placement for maternal MI or MII NDJ errors. For the first time, we have shown that aberrant recombination patterns observed among maternal nondisjoining chromosomes 21 may be the result of differential hot-spot usage. No significant differences in genomic features listed above at recombination breakpoints for disjoining and nondisjoining chromosomes were evident. Interestingly, studies have shown that variation in the PRDM9 gene is associated with differential hot-spot usage. Potentially, variation in PRDM9 leads to altered patterns of recombination on chromosome 21 that increase the risk for NDJ.

680F

Single Marker and Pathway Analysis of a Genome Wide Association Study in Obese and Lean Subjects of Northern European Ancestry. *S. Ghosh[1], J. Vivar[1], R. Davies[2], R. Dent[3], R. McPherson[2].* 1) Biomed Biotech Research Inst, North Carolina Central Univ, Durham, NC; 2) University of Ottawa Heart Institute, Ottawa, CANADA; 3) Weight Loss Management Clinic, Ottawa Hospital, Ottawa, CANADA.

Background: Genome wide association studies (GWAS) have been highly successful in identifying novel genetic loci associated with polygenic obesity but traditional single marker analysis ignores many moderately associated SNPs during multiple testing corrections. Pathway based analyses can be useful in uncovering pathways consisting of genes for which no individual SNP satisfies the traditional genome wide significance threshold (p<5e-8), but for which there exists an excess of variants with low to moderate effects on the disease. **Methods**: SNP genotyping was carried out on Affymetrix 6.0 arrays in a cohort of 985 obese and 869 lean subjects drawn from the tails of the population BMI distribution (mean BMI 43.1+8.7 and 20.3+1.84 kg/m2 for obese and lean subjects, respectively). SNPs were filtered as follows: MAF>3%, HWE<1e-6, call rate > 90%, info >0.8. Single marker association analysis was carried out via logistic regression after adjusting for gender and the first two principal components of ancestry. Analysis was performed under additive, dominant and recessive model assumptions. Pathway analysis was carried out via pathway enrichment tools such as DAVID and iGSEA4GWAS and by querying pathway databases including KEGG and PANTHER. SNPs were assigned to genes based on HapMap (NCBI build 36). A gene wide p-value was obtained from the most significant p-value of all SNPs belonging to the gene. **Results**: Single marker analysis identified several SNPs in the FTO genomic locus (16q12.2) as strongly associated to obesity (p<1e-13. No other single marker reached a genome-wide significance level of p<5e-8. Pathway analysis in DAVID identified pathways related to cytoskeletal organization, cardiomyopathy signaling, inositol phosphate metabolism, and calcium signaling, as being enriched in gene variants associated with obesity (p<1e-3). IGSEA4GWAS analysis further suggested the Wnt signaling and type II diabetes mellitus pathways (p<1e-3) as obesity-associated under additive or dominant model assumptions. **Conclusion**: The strong association of SNPs in the FTO locus to obesity is consistent with earlier results in several populations. Despite only modest associations observed for the other SNPs, pathway analysis further identified several candidate biological mechanisms that could lead to obesity from an enrichment of genetic variants of moderate to low effects. These findings can provide new hypotheses on the genetic architecture underlying polygenic obesity.

681F

Genome-wide association study identifies three genetic loci associated with Thyroid function. *R. Rawal[1], A. Teumer[2], G. Homuth[2], H. Wallaschofski[3], T. Ittermann[5], B.O. Åsvold[6], K.H. Greiser[7], D. Tiller[8], A. Kluttig[8], H. Meyer zu Schwabediss[9], A. Doering[4], C. Gieger[1], C. Meisinger[4], T. Bjøro[10].* 1) Institute for Genetic Epidemiology, Helmholtz Center, Munich/ Neuherberg, Germany; 2) Interfaculty Institute for Genetics and Functional Genomics, Ernst-Moritz-Arndt-University Greifswald, Germany; 3) Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Germany; 4) Institute of Epidemiology, Helmholtz Center, Munich/ Neuherberg, Germany; 5) Institute for Community Medicine, University Medicine Greifswald, Germany; 6) Department of Public Health, Norwegian University of Science and Technology, and Department of Endocrinology, Trondheim University Hospital, Trondheim, Norway; 7) German Cancer Research Center (DKFZ), Division of Cancer Epidemiology , Im Neuenheimer Feld, Heidelberg, Germany; 8) Institute of Medical Epidemiology, Biostatistics, and Informatics, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany; 9) Department of General Pharmacology, University Medicine Greifswald, Germany; 10) Department of Medical Biochemistry, Oslo University Hospital, and Faculty of Medicine, University of Oslo, Oslo, Norway.

Thyroid hormones play a key role in cellular growth, development and metabolism. Changes in thyroid function cause thyroid disorders, and affect many tissues and the metabolism in general. Circulating concentrations of triiodothyronine (T3) and thyroxine (T4) are tightly regulated by thyrotropin (TSH) and have a strong heritable component. A large portion of heritability is supposed to be under polygenic control, but the genes responsible are mostly unknown. In order to identify genetic loci associated with the thyroid function, we performed meta-analysis of genome-wide association study with 4907 individuals (1287 individuals from the Cooperative Health Research in the Augsburg Region study (KORA F4, Southern Germany) and 3620 participants of the Study of Health in Pomerania (SHIP)). Three genetic loci were associated with serum TSH on a genome-wide level of significance and one locus was at borderline significance level. The first locus was at 5q13.3 in the phosphodiesterase 8B (PDE8B) gene. A second locus was found at 1p36 located upstream of and within the CAPZB gene (capping protein (actin filament) muscle Z-line,beta) encoding the beta subunit of the barbed-end F-actin binding protein that modulates actin polymerization, a process crucial in the formation of pseudopodia that engulf the colloid within the follicular lumen during thyroglobulin mobilization in the thyroid. A third locus associated with TSH was upstream of the nuclear receptor subfamily 3, group C, member 2 (NR3C2) gene at 4q31. Another locus which was just at the borderline significance level, represents a “gene desert” on chromosome 16q23, located directly downstream of the predicted coding sequence LOC440389, which meanwhile has been removed from the NCBI data base as a result of the standard genome annotation processing. Experimental proof of the formerly predicted mature mRNA, however, demonstrates that LOC440389 indeed represents a real gene. Free T3 and free T4 were not found to be associated with any loci at a genome-wide significant level. The log of the T3/T4 ratio was associated at the borderline significance level with SNPs from a locus at 1p33-32 in the deiodinase, iodothyronine, type 1 (DIO1) gene (p=2.7 X 10-7). These results may increase the knowledge about genetic factors and physiological mechanisms of thyroid function.

682F

Using spatial-clustering of rare-variants in the association analysis of case/control studies: a powerful and robust approach with an application to sequencing data for nonsyndromic cleft lip with or without cleft palate. *H. Fier[1], D. Holler[1], T. AlChawa[2], K.U. Ludwig[2,3], R. Fimmers[4], M.M. Noethen[2,3], E. Mangold[2], C. Lange[1,5,6].* 1) Dept. of Genomic Mathematics, University of Bonn, Bonn, Germany; 2) Institute of Human Genetics, University of Bonn, Bonn, Germany; 3) Department of Genomics, Life and Brain Center, University of Bonn, Bonn, Germany; 4) Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany; 5) Department of Biostatistics, Harvard School of Public Health, Boston, USA; 6) German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany.

For the analysis of rare variants in sequence data, fixed and flexible threshold approaches have been proposed that collapse the rare-variant genotypes of a region into a single degree-of-freedom test statistic. While such collapsing approaches provide powerful test statistics that can incorporate information on allele frequencies and prior biological knowledge, the spatial clustering/physical distribution of rare variants in cases and controls cannot be incorporated. Based on the assumption that deleterious variants and protective variants cluster in different regions, we propose testing strategies for rare variants based on spatial-cluster methodology whose power is not affected by varying effect size directions of the variants. In simulation studies, we assess the power of the approach and compare it to exiting methodology. Our simulation studies suggest that our approach is well powered, even in situations that are ideal for standard collapsing approaches. An application to a sequencing study in patients with nonsyndromic cleft lip with or without cleft palate in which a region is followed that was implicated by a GWAS, the proposed testing strategies show highly significant findings, while standard approaches fail to detect an association.

683F

An Ensemble Pipeline to Enable Detection of Epistasis in Genomic Data. *B.J. Grady[1], T. Cattaert[2], K. Van Steen[2], M.D. Ritchie[1].* 1) CHGR, Vanderbilt Univ, Nashville, TN; 2) Systems and Modeling Unit, Montefiore Institute, University of Liege, Grande Traverse 10, 4000 Liège, Belgium.

Although our capability to gather large quantities of information on genetic variation has increased exponentially, our ability to understand the complexity of the relationship between this variation and disease has been unable to keep pace. While GWAS has had great success in uncovering novel variation associated with disease, these variants account for vanishingly small modulations in disease risk and even in aggregate they leave unexplained large amounts of the heritability estimated from family studies. One of the primary issues with our current treatment of genetic data lies in the veracity of our assumption of simplicity, with most analyses focusing only on effects of singular loci in isolation. We hypothesize that no one method is best for the analysis of all data types and that employing a hybrid approach will be beneficial. In this study we describe an ensemble procedure to exploring epistasis in genetic studies. This procedure includes filtering the data prior to analysis to reduce both computational and multiple-testing issues. For filtering, the Evaporative Cooling software and a genotypic Chi-square test are utilized. Within the genetic data passing filtering criteria, we perform separate, exhaustive pair-wise analysis utilizing the Model-Based Multifactor Dimensionality Reduction (MB-MDR) software and a Likelihood Ratio Test (LRT) containing terms for interaction between the pair of genetic loci being examined. One thousand permutations are used to determine significance of results from each method. The performance of the data analysis pipeline is evaluated by simulating 100 case-control datasets of 1000 SNPs for 1000 cases and 1000 controls over 77 different two-locus disease models with different disease penetrance and genetic effects and in which some models possess no detectable marginal effect. Ten representative models were used to further test the pipeline procedure in 2500 SNP data. We show that use of this data analysis pipeline increases the power to detect a breadth of potential genetic interactions.

684F

Bloat control methods substantially reduce computation time for detecting gene x gene interactions in ATHENA. *E.R. Holzinger, S.M. Dudek, E.S. Torstenson, M.D. Ritchie.* Ctr Human Gen Res, Vanderbilt Univ, Nashville, TN.

Recent advancements in human genetics research have spurred the increase in popularity of the genome-wide association study (GWAS) to identify the etiology of common disease. GWAS have been successful at identifying thousands of single nucleotide polymorphisms (SNPs) that contribute to hundreds of human traits; however, these variations only account for a tiny portion of the overall estimated heritability. One hypothesis is that this hidden heritability lies in non-linear interactions between multiple loci that would be missed by single-locus analyses. Testing for interactions is not a trivial task due to the computational burden of exhaustive analysis. To address this problem, our lab has incorporated two machine learning methods, Grammatical Evolution Neural Networks (GENN) and Grammatical Evolution Symbolic Regression (GESR) into ATHENA (Analysis Tool for Heritable and Environmental Network Associations). Both methods use genetic programming (GP)-based techniques to evolve a random population of solutions to find the correct susceptibility model without exhaustively testing all loci combinations or biasing on main effects. One issue with GP-based methods is that the solutions tend to bloat, or get larger but not better. This results in a substantial increase in computation time and over-fitting. Several methods have been developed to control bloat, but none have been specifically tested for human genetic data analysis. For this experiment, we compared two bloat-control techniques, prune and plant (P&P) and double tournament (DT), with no bloat control using simulated data sets with 100 SNPs (2 functional and 98 non-functional), 1000 cases, and 1000 controls. The data sets were generated using penetrance functions that included interaction and main effects for the two functional loci across four different heritabilities (0.005, 0.03, 0.01, and 0.1) and two different minor allele frequencies (0.2 and 0.4). Our results show that both P&P and DT result in a significant decrease in computation time (6-fold and 2-fold, respectively) with no negative impact on detection power.

685F

Incorporating biological knowledge for model-based clustering of genomic data: a recursively partitioned mixture model for DNA methylation data. *D.C. Koestler[1,2], B.C. Christensen[5], C.J. Marsit[3,4], K.T. Kelsey[6], E.A. Houseman[7].* 1) Department of Biostatistics, Brown University , Providence, RI; 2) Section for Biostatistics and Epidemiology, Dartmouth Medical School, Lebanon, NH; 3) Department of Pharmacology and Toxicology, Dartmouth College, Hanover, NH; 4) Norris Cotton Cancer Center, Dartmouth College, Hanover, NH; 5) Department of Community and Family Medicine, Dartmouth Medical School, Lebanon, HN; 6) Department of Epidemiology, Brown University, Providence, RI; 7) Department of Public Health, College of Health and Human Sciences, Oregon State University, Corvallis, OR.

DNA methylation is a well recognized epigenetic mechanism that has been a host to growing body of literature, typically centered on the identification and study of profiles of DNA methylation and their association with human diseases and exposures. Unsupervised clustering of DNA methylation data is often used to identify classes of samples with distinct methylation patterns and has played an integral role in our understanding of the development and progression of a vast array of different human diseases. In recent years, a number of unsupervised clustering algorithms, both parametric and non-parametric, have been proposed for clustering large-scale DNA methylation data. However, most of these approaches do not incorporate known biological relationships of measured features, and in some cases, rely on unrealistic assumptions regarding the nature of DNA methylation. We propose a modified version of a recursively partitioned mixture model (RPMM), a hierarchical model-based method for clustering high-dimensional genomic data. The modified RPMM we propose integrates information related to the proximity of CpG loci within the genome to inform correlation structures from which subsequent clustering analysis is based. Integrating biologically-informed correlation structures to enhance modeling techniques is motivated by the rapid increase in resolution of DNA methylation microarrays and the increasing understanding of the biology of this epigenetic mechanism. We have demonstrated, in simulations and several real methylation data sets, that integrating biologically informative correlation structures within RPMM can result in improved goodness of fit and clustering consistency compared to the standard RPMM which assumes class conditional independence of CpG loci.

686F

Investigating the `missing heritability' of Meconium Ileus in Cystic Fibrosis: contributions from a hypothesis-driven GWAS (GWAS-HD). *W. Li[1,2], X. Li[2], F. Lin[3], T. Chiang[2], M. Drumm[4], M. Knowles[5], G. Cutting[6], P. Durie[7], J. Rommens[3,8], L. Sun[1,9], L. Strug[2,1].* 1) Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada; 2) Child Health Evaluative Sciences, Hospital for Sick Children, Toronto, Ontario, Canada; 3) Genetics and Genome Biology, Hospital for Sick Children, Toronto, Ontario, Canada; 4) Departments of Pediatrics and Genetics, Case Western Reserve University, Cleveland, Ohio, USA; 5) Cystic Fibrosis Pulmonary Research and Treatment Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; 6) Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; 7) Physiology and Experimental Medicine, Hospital for Sick Children, Toronto, Ontario, Canada; 8) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 9) Department of Statistics, University of Toronto, Toronto, Ontario, Canada.

The Cystic Fibrosis (CF) gene modifier consortium conducted a GWAS for the early CF complication of Meconium Ileus (MI), identifying and replicating SLC26A9 and SLC6A14. Although the heritability of MI is estimated to be > 88% (Blackman et al. 2006), these two genes account for < 5% of the phenotypic variance. The GWAS-HD prioritization approach (Strug et al. 2010) has established that the collection of the 157 apical plasma membrane (APM) genes is associated with MI in two independent cohorts (Li et al. ASHG 2011), suggesting potential genetic heterogeneity in MI. We hypothesized that the APM genes account for a substantial amount of the missing heritability in MI. Here we determine which APM genes contribute to MI, and estimate the proportion of phenotypic variance these genes jointly explain. Jointly analyzing 4,029 SNPs within ±10kb of the 157 APM genes (including SLC26A9) and SLC6A14 using Lasso (Tibshirani, 1996), 48 SNPs from 36 different genes were retained in the model to predict MI. These SNPs explained ~17% of the phenotypic variance (area under the curve (AUC) = 0.73), a ~ 2.5-fold increase from GWAS findings. Permuting the phenotype and re-analyzing with Lasso resulted in a model including only two SNPs from two genes, providing an example of what would be expected by chance alone. Using the 1000 Genomes data (www.1000genomes.org) as reference, we next imputed SNPs across the APM genes to determine whether additional phenotypic variance could be explained by improved genomic coverage. Jointly analyzing 21,193 genotyped and imputed SNPs, Lasso selected 48 SNPs (37 imputed SNPs) spanning 27 unique genes (24 genes overlapped with the 36 genes identified using genotyped SNPs only), explaining ~16% of the phenotypic variance (AUC = 0.73). In summary, we identified SNPs that account for an appreciable amount of the phenotypic variance in MI, highlighting the advantage of GWAS-HD and joint-SNP analysis. These SNPs reflect a contribution by many APM genes. The inclusion of imputed SNPs did not lead to gains in percentage of variance explained or prediction accuracy. Although the APM genes are clearly associated with MI, their predictive ability is low indicating limited application towards personalized medicine. We are currently replicating the 48-SNP model in an independent CF cohort, determining causal variants, and identifying additional MI susceptibility loci.

687F

A Methodologic Proposal for Genetic Population: Surnames. *F. Loeza-Becerra[1,2].* 1) Embriología, Fac. Medicina Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, Mexico; 2) Secretaría de Salud.

In classic Genetic Popuation it's necessary to know genetic markers' inheritance, selective forces, genetic structure, geographic variations and history if we need well known the population. Surnames like `neutral alleles' are useful tool and short way to know genetic structure specially in countries where the economy is shortest. In precolumbian America, some groups had lineages and `family names' an now, surnames. We employed amerindian's surnames and its families as incruent samples and methodologic tool prior to employ HLA and others blood markers in three “tarascan” localities (60 kms between them) in Michoacan MEXICO Each one has census, pedigrees and classified surnames: Autocton ('A') if origin local or prehispanic and No-Autocton ('NA') if not; its local staying by three or more generations checking this in regional registries. HLA haplotypes were matched to families. Registered proportions A/NA, kind of surnames and frequencies intra/intercommunities, consanguinity, isonimy, difussion private ('P') if only one site or more ('C'), familial reproductive story and losses ('G' -gray- if abortus, mortinatus, etc. or `W' -white- if not). There are N=299 surnames `A' (42%) in N=1932 families, in similar composition in all three sites but many `A' had few families (viceversa in `NA'), bottlenecks and n=26 epidemics in four centuries (in two lastest, four) and disappear more 60& from `A' pioners against `NA' immigrants whom growing more by increased fertility (survivors `G'). Surnames `A' and `C' reveals ancestry, mixture and migrations. They are in Hardy-Weinberg equilibrium, simpatry and endogamy but no consanguinity; more 40% living in their original community. Maximun genetic mixture estimated by surnames was 60%; less 30% by blood genetic markers. Prehispanic component is remarkable in three localities. Surnames were incruent indicators useful to select better sites `representatives', in this case, from Tarascan (or Purépecha) ethnic group. Geography and prehispanic toponimies aren't sufficient. Surnames can to explain easy, quickly and really history and mixture and contributes to good selection for mollecular studies. Markers like HLA haplotypes was according with surnames. In this kind of peoples, surnames gives methodologic guides to integral study of populations.

688F

Combinations of phenotypes that best capture the genetic variation in CARe pilot dataset. *Y. Meng[1], L.A. Cupples[2], M. Fornage[3], B. Keating[4], E. Larkin[5], G. Papanicolaou[6], W. Post[7], A. Reiner[8], S. Rich[9], J. Rotter[10], D. Siscovick[8], J.G. Wilson[11], J.N. Hirschhorn[1].* 1) Metabolism Initiative, Broad Inst, Cambridge, MA; 2) Boston University, Boston, MA; 3) The University of Texas Health Science Center at Houston, Houston, TX; 4) University of Pennsylvania, Philadelphia, PA; 5) Vanderbilt University, Nashville, TN; 6) National Heart Lung and Blood institute, Bethesda, MD; 7) Johns Hopkins University, Baltimore, MD; 8) University of Washington, Seattle, WA; 9) University of Virginia, Charlottesville, VA; 10) University of California, Los Angeles, CA; 11) University of Mississippi, Jackson, MS.

For association studies, we typically use individual clinical endpoints or quantitative traits as the phenotypes of interest. Most studies also include a number of phenotypic measures, sometimes correlated, that can be used to characterize disease or intermediate phenotypes and that may be related to similar genetic factors. For example, body mass index (BMI), waist circumference, fat mass, percentage of fat mass (PFM) and other measures can be used to assess related aspects of obesity; LDL, HDL, lipid lowing medication, and triglycerides can be used to assess different aspects of lipid levels. Traditionally, each of these phenotypes is studied individually, but genetic variants may influence a combination of phenotypes, with perhaps different variants showing different pleiotropic profiles. In order to maximize the chance of identifying the genetic contributors to phenotype, knowing the phenotypic profile that most closely corresponds to the effects of underlying genetic factors would be beneficial. Our goal is to generate composite phenotypes that are more closely associated with validated functional SNPs than are the individual phenotypes. These new composite phenotypes could then be used in more comprehensive genotype-phenotype analyses. In effect, we are reversing the usual process of starting with a phenotype and searching through genotypes for association; rather we use methods that start with a validated genotype and search for the most closely associated composite phenotype. Using genotype data from the Candidate Gene Association Resource (CARe), we first created three sets of phenotypes from training data: the original traits, principal components (PC) of the traits, and factors of the traits. Second, for each SNP, we ran three models, where the SNP was the dependent variable, and each set of original traits, PCs, and factors were independent variables. We transformed the test datasets to PCs and factors using loading scores from the training dataset, and assigned weights using the coefficients from each training analysis to generate three composite phenotypes (multiscore, pcscore and factscore). In preliminary analyses the multiscore method showed the best power to detect associations between SNPs and multiple related traits, with a reduction in p value of 1~4 orders of magnitude compared to the results using single traits, across an array of SNP-phenotype associations, e.g. p value for rs11591147 for HDL reduced from $3.87X10^{-9}$ to $3.28X10^{-12}$.

689F

Accurate identification of low-level DNA contamination through genotyping array data as a quality control precursor to low-depth sequencing. *L. Moutsianas, RD. Pearson, KJ. Gaulton, C. Groves, W. Rayner, N. Robertson, MI. McCarthy.* Wellcome Trust Centre for Human Genetics, Univ of Oxford, Oxford, United Kingdom.

Whole genome sequencing (WGS) studies are increasingly popular as a means to interrogate genetic variation. Low-depth sequencing (4-5x coverage) offers the opportunity to identify variants in larger numbers of samples at a given cost compared to high-depth sequencing (20-30x), with little reduction in allele detection for common variants. A drawback to low-pass sequencing studies, however, is that the evidence supporting rare alleles is often limited and thus sensitive to even minor sources of error.

In some early WGS data sets, we had seen that low level cross-sample contamination during sample processing could lead to inflated heterozygosity in low-pass sequence data that was difficult to detect a priori. Genotyping arrays are relatively inexpensive and can be used as pre-screening tools, prior to sample inclusion in costly sequencing studies. Thus, we have developed a method to detect low-level human DNA contamination that can be used as a QC precursor to low-depth sequencing.

Our methodology consists of identifying variants with homozygote genotype calls from array data, grouping them by the population allele frequency of the unseen allele, and averaging the raw intensity values of the unseen alleles across each group. A regression line is then fitted to the values, where the slope is related to the amount of DNA contaminant from the specified population. We calibrated the method by creating a double dilution series of 10 known mixtures of DNA (50% - 0.1%) from two European samples obtained from the ECACC collection. Each mixture was then genotyped in duplicate on the Illumina MetaboChip array. Using our approach we were able to distinguish contaminant DNA from pure DNA samples down to the lowest tested mixture.

We provide as a resource an R package that can be used to identify DNA contamination down to 0.1% using SNP array data and population allele frequencies. We are currently extending our method to directly estimate the amount of contamination in a given sample and to determine the contaminant sample. We also provide a reference dataset from the different mixtures of 2 pure DNAs that can be used to validate, calibrate and compare contamination detection methods.

690F

Cumulative Meta-analysis for Genetic Association: When is a New Study Worthwhile? *M.A. Rotondi[1, 2], S.B. Bull[1, 2].* 1) Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada; 2) Dalla Lana School of Public Health, Toronto, Ontario, Canada.

Meta-analysis is useful to examine potential associations between complex traits and single nucleotide polymorphisms (SNPs) of interest. This approach typically detects smaller genetic associations than those that could be ascertained in any individual study. We propose a statistical technique to examine the potential impact of a newly planned association study on an existing meta-analysis. Specifically, we address the question: How large a sample size would be required to show genome-wide significance in a meta-analysis of a newly planned study together with the existing one? or alternatively: Will a planned study of size *n* be able to provide evidence of a genetic association when this study is combined with a current meta-analysis? Through the use of a simulation-based algorithm, the approach provides an empirical estimate of the power of the updated meta-analysis to detect genome-wide significance or the expected *P*-value for a specified SNP. Although such an approach is useful for both common and rare variants, as well as genome-wide association studies (GWAS) and candidate gene studies, the technique is illustrated in the context of an updated meta-analysis of case-control studies in Paget's disease. A variation of the described algorithm provides a second example; which illustrates the impact of a newly planned study on the associations of two SNPs (rs3796529 and rs2516448) with human height.

691F

Incorporating Gene Pathway Information in Genetic Association Studies. *J.L. Walker, C.J. Verzilli.* NCDE, London School of Hygiene and Tropical Medicine, London, United Kingdom.

Genome wide association studies and next generation sequencing studies have identified a number of disease susceptibility variants but, so far, these only explain a small fraction of genetic variation in disease risk. It seems likely that a substantial fraction of unexplained variation is contributed by variants which have either small effect or are very rare, for which current studies are in most cases underpowered. Existing biological knowledge could prove useful in the search for disease susceptibility variants and in disentangling genuine associations from spurious ones. This could be achieved for instance by informing the search using information on gene pathways. Several gene pathway databases now exist, which assign genes to functional categories according to their roles in shared biological processes. Here we investigate the use of prior information on gene pathway in genetic association studies. The underlying idea is to give more weight to plausible genomic regions when searching for susceptibility variants, and to boost signals that appear to be consistent with a pathway relevant to the disease studied. Gene pathways can be depicted as undirected graphs with nodes as genes, and edges connecting two nodes representing a biological functional relationship. Our approach is fully Bayesian which enables incorporation of prior information naturally. In particular, the prior of a particular gene being in model depends on whether they appear in pathway(s) relevant for the disease examined and we describe dependencies amongst genes in pathways using Markov Random Field (MRF) priors. We examine the performance of the proposed methodology using simulated data under various scenarios. We show that the probability of detecting gene-specific association is increased by having a spatially dependent MRF prior that reflect prior biological knowledge. These results were compared to single locus methods and we found that our methods increase the chances of finding associations, especially when effects are small.

692F

Robust methods for analyzing secondary phenotypes in case-control genetic association studies. *C. Xing[1], A. Allen[2].* 1) Biostatistics, Boston University, Boston, MA; 2) Biostatistics and Bioinformatics, Buke University, Durham, NC.

The case-control study is an economical approach to studying disease/exposure associations. When a disease is rare or when exposures are expensive to collect, the case-control study can represent a considerable costs savings relative to studying a cohort prospectively. For these reasons, genome-wide and whole exome association studies often utilize a case-control design. The substantial costs involved in these large-scale genetic studies also puts a premium on making the most out of these datasets. Most of these studies will measure other phenotypes, either because they are readily available, or because they are thought to be related to the underlying disease process. Studying the genetic influences of these `secondary' phenotypes may be of interest in itself or may help our understanding of the biologic pathways involved in the disease process. However, the case-control sample does not constitute a random sample from the general population. As a result, any population association between genetic variants and secondary phenotypes can be distorted in the case-control sample. Thus analysis methods that ignore the case-control design can give biased estimates of the population effect of a genetic variant on a secondary phenotype. We propose an inverse-probablility weighted estimating equation (IPWEE) approach for analyzing secondary phenotypes in case-control studies. We derive estimators that are appropriate when the disease is rare as well as estimators that utilize existing population level disease prevalence information. We evaluate our methods in an extensive simulation and compare the IPWEE approach with several existing methods. We found the IPWEE approach to perform well over a wide spectrum of scenarios. We found that IPWEE had nearly the same power as the (optimal) full-likelihood approach when the model was correctly specified. However, we found that IPWEE was substantially more robust than the full-likelihood approach, both in terms of validity and power, when the model was misspecified. The IPWEE approach is also far more computationally efficient, requiring, on average, 10 times less computing time than the full likelihood approach.

693F

An Empirical Bayes based method to identify cis-eQTLs across multiple related conditions. *C. Ye[1], B. Han[2], T. Choi[3], A. Regev[1], E. Eskin[2].* 1) Broad Institute, Cambridge, MA; 2) University of California, Los Angeles, CA; 3) Predictive Biology, Carlsbad, CA.

Large-scale eQTL studies are now being conducted where tens of thousands of phenotypes (transcript expressions) are collected across multiple related conditions (e.g. tissue, cell type, treatment or population). These studies try to identify genetic variations associated with expression levels in specific conditions. Powerful multivariate analyses (e.g. MANOVA) of the data are often prohibitive because of computational complexity, lack of statistical robustness and difficulty of interpretation. Instead, most current methods perform a series of univariate tests one transcript, one SNP and one condition at a time. This approach suffers from the “Winner's Curse” phenomenon that systematically results in limited power to detect cis-eQTLs shared between multiple conditions thus artificially inflating the proportion of condition specific cis-eQTLs. We present a novel and efficient Empirical Bayes approach that leverages the similarity between multiple transcripts and conditions to identify cis-eQTLs. We show through simulation that this method has higher power to detect cis-eQTLs shared between multiple conditions while exhibiting strong control of false discovery rate. We apply this approach to datasets collected over multiple tissue types in both mouse and human detecting more cis-eQTLs, both shared and condition specific than standard approaches at the same FDR thresholds. Computationally, we show that using a greedy algorithm to estimate a limited number of parameters, our approach is more robust and efficient than other EB based methods thus making it suitable for large-scale eQTL studies.

694F

CpGassoc - An R Function for Analysis of DNA Methylation Microarray Data. *R.T Barfield[1], V. Kilaru[2], A.K Smith[2], K.N Conneely[3].* 1) Dept of Biostatistics and Bioinformatics, Emory University School of Public Health, Atlanta, GA; 2) Dept of Psychiatry, Emory University School of Medicine, Atlanta, GA; 3) Dept of Human Genetics, Emory University School of Medicine, Atlanta, GA.

CpGassoc - An R Function for Analysis of DNA Methylation Microarray Data The analysis of DNA methylation data has recently garnered attention among researchers from a variety of backgrounds, due to the availability of high-throughput methylation microarrays. The number of CpG sites that can be analyzed is growing rapidly - for example, the latest Illumina Infinium BeadChip interrogates ~450,000 CpG sites. With the growing interest in DNA methylation and the growing volume of data analyzed, there is a need for software to perform these types of analyses. We created CpGassoc, an R package that is designed to perform association tests between CpG sites and a user-specified phenotype that can be a continuous or categorical variable. CpGassoc will fit a linear fixed or mixed effects model to model the beta values (proportion of DNA methylated) at each CpG site as a function of the phenotype and other covariates. Users can specify an unlimited number of continuous and categorical covariates, as well as a fixed or random effect to control for batch or chip effects. The output of CpGassoc is an R object containing the test statistics and p-values for each CpG site, as well as indicators of which CpG sites are genome-wide significant according to False Discovery Rate and Bonferroni approaches. The resulting R object can easily be output in a variety of text or spreadsheet formats, or can be passed to other R functions for further analysis. The CpGassoc package includes additional functions for analyzing output, including functions to assess significance empirically via a permutation test or to create quantile-quantile and CpG-specific plots based on the observed results. CpGassoc is designed to be a modular package, with the flexibility to add modules in the future. Currently we are working on modules to address issues of data cleaning and normalization, and hope to add further modules as new approaches to analysis of methylation data are developed. Because of the open source nature of R, users will have the ability to modify the functions in CpGassoc to create custom analyses or to create their own packages to complement CpGassoc. In conclusion, we have developed a useful and versatile tool for analyzing methylation microarray data that allows for a variety of different analyses, and can grow flexibly to meet the needs of a growing field.

695F

Method to Detect Differentially Methylated Loci with Case-Control Designs. *S. Wang.* Dept Biostatistics, Columbia Univ, New York, NY.

It is now understood that virtually all human cancer types are the result of the accumulation of both genetic and epigenetic changes. DNA methylation is a molecular modification of DNA that is crucial for normal development. Genes that are rich in CpG dinucleotides are usually not methylated in normal tissues, but are frequently hypermethylated in cancer. With the advent of high-throughput platforms, large-scale structure of genomic methylation patterns is available through genome-wide scans and tremendous amount of DNA methylation data have been recently generated. However, sophisticated statistical methods to handle complex DNA methylation data are very limited. Here we developed a likelihood based Uniform-Normal-mixture model to select differentially methylated loci between case and control groups using Illumina arrays. The idea is to model the data as three types of methylation loci, one unmethylated, one completely methylated, and one partially methylated. A three-component mixture model with two Uniform distributions and one truncated normal distribution was used to model the three types. The mixture probabilities and the mean of the normal distribution were used to make inference about differentially methylated loci. Through extensive simulation studies, we demonstrated the feasibility and power of the proposed method. An application to a recently published study on ovarian cancer identified several methylation loci that are missed by the existing method.

696F

New pipeline for microarray expression data analysis. *C.S. Rocha, C.V. Maurer-Morelli, I. Lopes-Cendes.* Molecular Genetics - FCM, Unicamp, Campinas, São Paulo, Brazil.

Objective: Expression profile using microarrays has become a widely used method for studying gene expression patterns. These studies produce a large amount of data which makes the analysis complex and time consuming. As the arrays' quality becomes more reliable the critical point remains the bioinformatics tools used to process and analyze the data generated in these large experiments. Therefore, we aimed to developed a pipeline that runs all the steps of data processing such as background correction, quality control, normalization, detection of differentially expressed genes and clustering analysis. In addition, our tool allows the choice of several parametric and non-parametric tests to be used for group comparisons. Furthermore, it can be used for data mining, searching for relevant information of genes as well as creating links to various public available databases. **Methods:** The pipeline is web based in a GNU/Linux operational system. A SQL database was created with the annotation data from public databases using MySQL. The tool is written in Perl using the CGI module for web application and the DBI module for data manipulation. In addition, we use several Bioconductor packages in R environment designed for statistical analysis of microarray experiments. **Discussion:** Using the tolls in the pipeline the user can perform non-parametric tests, such as RankProduct and Mann Whitney U test, as well as parametric statistical analysis such as Student's t-test. In addition, it is possible to perform corrections of p-values with the Benjamini and Hochberg or Bonferroni methods. Cluster analysis can be performed using PCA (Principal Component Analysis), Hierarchical Clustering and SOM (Self-Organizing Maps). Correlation analysis was also implemented. Starting with a list of identifiers (Probe Set IDs) the annotation tool can search a specific database for the annotation corresponding to these identifiers. To facilitate data mining we have included links to other databases such as: NetAffx, UniGene, Ensembl, SwissProt and OMIM. In addition we implemented a filter by chromosome, allowing the user to direct the search to a specific chromosome of interest. **Conclusion:** The main goal of this pipeline is to provide researchers working with microarray data a user friendly tool which includes customized statistical analysis. In addition we provide a filter by chromosome in the annotation tool which may be useful in gene identification projects.

697F

Genome-wide association study of three dimensional facial morphology identifies a variant in the PAX3 gene responsible for normal variation in face shape. *D.M. Evans[1,2], L. Paternoster[1,2], A.M. Toma[3], A.I. Zhurov[3], J.P. Kemp[1,2], B. St Pourcain[2], N.J. Timpson[1,2], G. McMahon[1,2], S.M. Ring[2], G.D. Smith[1,2], S. Richmond[3].* 1) MRC CAiTE, University of Bristol, Bristol, UK; 2) School of Social and Community Medicine, University of Bristol, Bristol, UK; 3) Department of Applied Clinical Research & Public Health, Cardiff University, Cardiff, UK.

Craniofacial morphology is highly heritable, as demonstrated by twin and family studies, however, the individual genetic variants which affect normal variation in human facial features have yet to be identified. We conducted to our knowledge the first genome-wide association study of three dimensional (3D) facial morphology in a population-based cohort in order to identify SNPs associated with normal variation in facial characteristics. 3D high resolution facial images were obtained using two Konica/Minolta laser scanners in 4747 15 year old children from the Avon Longitudinal Study of Parents and Children. 3D images were initially aligned in three reference planes and the generalised Procrustes analysis was then applied. We identified 22 landmarks on the facial shells and used their location to generate 54 3D distances. These measures were then tested for association in a discovery cohort comprising 2185 individuals who had been genotyped genome-wide on the Illumina 317K and Illumina 610K SNP chips and subsequently imputed to HapMap. Genome-wide significant association was observed between the SNP rs7559271 in the PAX3 gene and 3D distance between the nasion and the mid-endocanthion (p=2.2x10-10). This association was subsequently replicated in a further 1645 individuals from the same cohort who had been genotyped on the Illumina 550K SNP chip (p=4.4x10-7). In order to investigate the association further, we deconstructed the 3D distance into one and two dimensional distances. Association was observed in both the z (nasion prominence) and y (height of nasion relative to the mid-endocanthion) dimensions, but not the x dimension (p > 0.05). Additionally, the associations in the y and z dimensions were independent of each other, suggesting that the locus influences growth and development in the yz plane, but not the yz angle between the nasion and mid-endocanthion. There was also no association between the SNP and distance between the eyes. The PAX3 gene has previously been associated with Waardenburg syndrome, a condition which involves characteristic facial morphologies including a flattened nasal bridge and widely spaced eyes. Our study suggests that less pathogenic mutations within the PAX3 gene may produce non-pathological variation in face shape within the normal range.

698F

Novel insights into the genetics of Parkinson's disease on chromosome 17. *W. Lau, H. Elding, D. Swallow, N. Maniatis.* RESEARCH DEPARTMENT OF GENETICS, EVOLUTION AND ENVIRONMENT, UNIVERSITY COLLEGE LONDON, LONDON, United Kingdom.

Parkinson's disease (PD) is the second most common neurodegenerative disease. Several Genome Wide Association (GWA) studies have been preformed to date focusing primarily on sporadic PD. These studies have reported a relatively small number of susceptibility loci even though recent studies have indicated a large genetic contribution. Here we show significant progress by using high-resolution metric linkage disequilibrium maps to identify new genes on chromosome 17 that harbours the *MART* gene (strongest replicated GWA signal on 17q). Our analysis of the NINDS GWA data (Illumina 550, 677 cases and 538 controls) has identified the *MAPT* gene-region but with an estimated location of the casual variant downstream of *MAPT*. This localization was replicated with precision using an independent GWA on sporadic PD (Illumina 1M, 2,000 cases and 1,986 controls). Here we report several additional novel genes, including *ALOX15, RABEP1, PER1, CENTA2, THEM100, AXIN2, CD300LE, LGALS3BP*. All these signals have been replicated with great precision and most of our location-estimates point to causal variants within regulatory regions. The use of metric linkage disequilibrium maps can bring a much greater resolution and hence they can accelerate progress in identifying the genetic factors to PD and other complex disorders.

699F

Effects of genotype on TENS effectiveness in controlling knee pain in persons with mild to moderate osteoarthritis. *N. Mukhopadhyay[1], D. Schutte[2], C. Vance[4], R. Walder[4], K. Sluka[4], B. Rakel[3], M. Govil[1].* 1) Center for Craniofacial and Dental Genetics, Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA; 2) College of Nursing, Michigan State University, East Lansing, MI; 3) College of Nursing, The University of Iowa, Iowa City, IA; 4) Physical Therapy and Rehabilitation Graduate Program, College of Medicine, The University of Iowa, Iowa City, IA.

Evidence from both animal and human studies suggests that Transcutaneous Electrical Nerve Stimulation (TENS) can be an effective way to treat pain in a variety of acute and chronic conditions. The effectiveness of TENS in the treatment of osteoarthritis (OA) knee pain is less certain, and the role of genetic variants as modifiers of TENS effectiveness is relatively unexplored. The purpose of this study is to examine the extent to which genetic variability modifies the effectiveness of TENS in controlling knee pain in persons with mild to moderate OA. Seventy-five subjects (mean age 56.3 years, 61% females) diagnosed with medial compartment knee OA were recruited from the Orthopedic and Sports Medicine Department of a large Midwestern tertiary care center and were randomly assigned to three groups: 1) High Frequency TENS (HFT, 100Hz), 2) Low Frequency TENS (LFT, 4Hz), and 3) Transient Placebo TENS (TPT, current delivered for 30 sec, then ramped down to zero over 15 sec). Measures of pain were collected pre and post treatment and included subjective pain (VAS) at rest and with movement, cutaneous mechanical pain testing using von Frey filaments, heat pain threshold, and pressure pain threshold using a digital pressure algometer. Pain measures were obtained at the knee and anterior tibialis regions on both the ipsilateral and contralateral side, and the difference in pre vs. post treatment measures analyzed. On a 0-100 VAS pain scale, average reduction in intensity and distress at rest were 13.90 and 16.94 points for HFT, and 10.32 and 13.36 for LFT. TPT produced a placebo effect showing reduction of 16.84 and15.32 points. Subjects were genotyped in 11 candidate genes with a known or hypothesized role in central or peripheral pain pathways: NGFB, NTRK1, EDNRA, EDNRB, EDN1, OPRM1, TAC1, BDNF, BDKRB1, 5HTT, and COMT. Linear regression modeling, using PLINK, examined genotype effect on change in pain outcomes, comparing TPT vs. HFT and TPT vs. HFT+LFT. SNPs rs165599 (COMT) and rs6827096 (EDNRA) for heat pain threshold, and rs6715729 (TAC1) for the cutaneous mechanical test were at or below the bonferroni-corrected threshold of 0.002. The results from this study will help provide the basis for a clinically useful tool to predict TENS intervention outcomes and the targeting of subsequent interventions that are sensitive to both individual and environmental characteristics. NIH grant numbers (K99, R00) DE018085, ARRA suppl DE018085-01A2S1 and R03 NR010405-01.

700F

GWAS Analysis of Autopsy Confirmed Late-Onset Alzheimer Disease Confirms Limited Effect Heterogeneity Among Known Loci. *A.C. Naj[1], G.W. Beecham[1], E.R. Martin[1,12], P.J. Gallins[1], R. Rajbhandary[1], K.L. Hamilton[1], R. Mayeux[2,3], L.A. Farrer[4,5,6,7,8], G.D. Schellenberg[9], J.R. Gilbert[1,12], J.L. Haines[10,11], M.A. Pericak-Vance[1,12], The Alzheimer's Disease Genetics Consortium.* 1) Hussman Inst for Human Genomics, Univ Miami, Miami, FL; 2) Taub Institute on Alzheimer's Disease and the Aging Brain, Department of Neurology, Columbia University, New York, NY; 3) Gertrude H. Sergievsky Center, Columbia University, New York, NY; 4) Department of Biostatistics, Boston University, Boston, MA; 5) Department of Medicine (Genetics Program), Boston University, Boston, MA; 6) Department of Ophthalmology, Boston University, Boston, MA; 7) Department of Neurology, Boston University, Boston, MA; 8) Department of Epidemiology, Boston University, Boston, MA; 9) Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA; 10) Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN; 11) Vanderbilit Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 12) Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami, Miami, FL.

Alzheimer Disease (AD) is a highly genetic and complex neurodegenerative disease. Until recently, only *APOE* variation has been consistently associated with late-onset AD (LOAD); recent GWAS have identified additional risk loci (*CLU*, *PICALM*, *CR1*, *BIN1*, *CD2AP*, *EPHA1*, *ARID5B*, *MS4A4/6E*, *ABCA7*, and *CD33*). However, phenotypic heterogeneity may obscure the true effects of these loci. We investigated a potential source of heterogeneity, autopsy confirmationby comparing association results between autopsy-confirmed (AC) cases (n=1,472) and clinically-identified (CI) cases (n=2,693) versus all controls (n=4,752) using data from 5 ADGC datasets.). Furthermore, as misclassification may have weakened signals at truly associated variants, we examined 2,324,879 SNPs genotyped and imputed among autopsied cases and all controls for undetected novel LOAD associations. Analyses were performed with multinomial logistic regression, stratifying cases by autopsy confirmation of AD clinical diagnosis using the mlogit function of SAS (v9.2). While we confirmed associations with chromosome 19 SNPs in/near *APOE* (rs2075650- AC P=1.6×10^{-22}; CI P=3.2×10^{-29}), several other loci demonstrated differential patterns of association in stratified analyses; *CR1* SNP rs6701713 was more strongly associated among AC cases (OR=1.21, P=8.1×10^{-4}) than CI cases (OR=1.15, P=0.0026), as was *ABCA7* SNP rs3752246 (AC OR=0.82, P=0.0017; CI OR=0.88, P=0.01). Conversely, some SNPs were more strongly associated in the CI cases: *PICALM* SNP rs561655 (AC OR=1.10, P=0.041; CI OR=1.18, P=1.29×10^{-5}), and *CLU* SNP rs1532278 (AC OR=1.11, P=0.041; CI OR=1.16, P=3.5×10^{-4}). Examining only AC-control associations for novel signals, the most statistically significant associations (P<10^{-5}) included SNPs in *CR1*, such as a known missense variant, rs2296160 (OR=1.20, P=7.6×10^{-6}) and the intronic SNP rs1051029 (OR=0.72, P=5.8×10^{-7}) in the gene *ITPR1*, which is in the calcium-signaling pathway and has been shown to be down-regulated with increasing AD severity (Ravetti et al. 2010). Our stratified analyses of autopsy-confirmation status found that associations differed modestly among some but not all of the most significantly-associated SNPs in our original study, suggesting that clinical diagnosis is an excellent proxy for autopsy confirmation in genetic studies of LOAD.

701F

The NCBI dbGaP database of genotypes and phenotypes provides resources for genome-wide association studies and medical resequencing. *S. Sherry, M. Feolo, Y. Jin, M. Kimura, K. Tryka, R. Bagoutdinov, J. Paschall, L. Hao, A. Sturcke, L. Phan, N. Popova, S. Pretel, L. Ziyabari, M. Lee, Z. Wang, M. Kholodov, G. Godynskiy, N. Sharopova, S. Stefanov, M. Shumway, J. Ostell.* National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD.

The dbGaP database of Genotypes and Phenotypes (www.ncbi.nlm.nih.gov/dbgap) archives and redistributes comprehensive data sets from genome-scale biomedical research studies. Public displays of study results include data object summaries, phenotype collection documents, data browsers, analysis results and connections to other information resources. For authorized investigators, dbGaP distributes all submitted de-identified, individual-level phenotype and genetic data, and aggregate-level association results. As of June 1, 2011, 132 dbGaP studies provide individual-level data for 257,331 study participants including 125,698 phenotype measures,141 billion genotypes, and 52 terabases of sequence data in over 7000 BAM files. Any Principal Investigator (PI) registered in the NIH Federated Commons (eRA) system may request access to individual level data through the dbGaP controlled access system. In the past 12 months, 3733 access requests were submitted by 647 PIs. Completed applications are automatically routed to the PI's institutional signing official (SO) for approval, and then forwarded for review and an approval decision by an NIH Data Access Committee. 2498 were approved, with a mean time to approval of 18.8 days. Submission of the data access request constitutes agreement and acknowledgment to the terms of use for the requested data sets. These terms are detailed in the “Data Use Certification” documents and generally include agreements to adhere to indicated limitations of use; not redistribute the data; not attempt to identify or contact study participants; acknowledge intellectual property principles; and adhere to publication embargo policies. Public data in dbGaP includes study descriptions, documents, variable summaries, descriptions of controlled-access components and limited aggregate data. NCBI provides a growing collection of tools to **browse** catalogs of published association results, and **examine** multiple GWAS association results simultaneously in genome coordinates. dbGaP content is connected when possible to other public information resources, including NHGRI's Genome Wide Association Catalog, PubMed, Gene, SRA and dbSNP. The dbGaP FTP site provides all public content. There you will find study release notes, documents in XML markup and PDF formats, a report describing all of the components available through controlled access, and phenotype variable summary data.

702F

Exploiting modern computer architectures to accelerate genetic linkage analysis. *A. Medlar[1], D. Glowacka[2], H. Stanescu[1], R. Kleta[1].* 1) Department of Medicine, University College London, London, United Kingdom; 2) Department of Computer Science, University College London, London, United Kingdom.

Exact algorithms to perform genetic linkage analysis scale exponentially with the size of input. Beyond a critical limit the amount of work to do exceeds both available time and memory. Software derived from the Elston-Stewart algorithm cannot be used to analyse many genetic markers simultaneously, whereas Lander-Green-derived algorithms are limited by pedigree complexity. In circumstances beyond these limits, we are forced to either abbreviate the input in some manner (relevant to the algorithm/software employed) or else use an approximation algorithm based on stochastic sampling. Simplifying the pedigree by removing family members or the degree of consanguinity can dramatically reduce the power of the linkage study and produce ambiguous results. Approximation by stochastic simulation, greatly extends what can feasibly be analysed, but can take an immense amount of time to converge to a final result. Convergence problems are compounded by software implementations which are mostly single-threaded and, as computer processors are manufactured with increasing numbers of physical processing cores, cannot take full advantage of processing power readily available. To a limited degree we can already exploit some parallelism. Each chromosome within each pedigree is independent. In addition, we could analyse a pedigree in batches of markers using a sliding window, however this introduces error where batches meet. From another perspective, even batching limits the level of parallelism due each batch needing to be large enough to make a multi-point analysis worthwhile. A modern desktop computer (as of June 2011) can have a quad-core processor, with each core capable of running two independent threads, and a graphics cards with up to 512 stream processors. We want to better understand the workload that linkage analysis provides and how best to combine this with both the more general-purpose multi-core processor architecture versus the specialist many-core graphics processing unit (GPU) architecture. We aim to build upon existing work of descent graph samplers, by experimenting with both implementing parallel samplers for single markov-chain monte-carlo (MCMC) chains and parallel methods to combine multiple chains to understand what provides the biggest win on our target platforms.

703F

Association of COL2A1 Gene Polymorphisms with the Development of Myopia. *S. Annamaneni[1], H. Chintala[1], V. Satti[1], P. Kasu[2].* 1) Department of Genetics, Osmania University, Hyderabad, Andhra Pradesh, India; 2) Maxivision Eye Hospital, Hyderabad, Andhra Pradesh, India.

Myopia (near-sightedness) is a common, sight threatening, multifactorial ocular disorder exhibiting genetic heterogeneity, in which retinal defocus results in impaired vision. Collagens are major extracellular glycoproteins of scleral ECM of the eye and the unsual disruption, degradation or accumulation of several collagens result in altered scleral fibril architecture and loss of tissue integrity. Collagen type II (COL2A1) gene is an important positional candidate gene for myopia, the mutant forms of which are associated with connective tissue disorders and syndromes with ocular abnormalities like high myopia. In view of its functional implication, the present case-control association study (205 high myopia cases, 96 low myopia cases and 248 control cases) analysed two intronic polymorphisms of this gene (COL2A1-Hind III polymorphism in intron 33 and dinucleotide insertion/deletion polymorphism in intron 50) through PCR followed by RFLP analysis and PAGE. The genotype distribution of Hind III polymorphism (H or h allele) of COL2A1 gene in high myopia patients revealed significant increase in the frequency of hh homozygotes (48.1%) as compared to control group (38.3%) and low myopia group (20.8%). The association of hh genotype was observed with male sex in high myopia, females in low myopia, early onset high myopia, late onset low myopia, patients in vegetarian diet group, non-familial and consanguineous groups of both high and low myopia suggesting that the presence of h allele might confer risk to the development of myopia through altered scleral-collagen interactions.The genotype distribution of intron 50 polymorphism (L1 or L2 allele) among high myopia cases revealed decreased frequency of L2L2 genotype (6.34%) as compared to controls (11.3%) but showed slight elevation in comparison to low myopia (4.12%). The genotype distribution in relation to other epidemiological variables demonstrated elevated frequency of L1 allele in males with high myopia, myopia patients on vegetarian diet, high myopia cases with early age at onset and low myopia with later age at onset 10-20 yrs, familial cases of low myopia, and consanguineous group in both types of myopia. In conclusion, our results indicate that h allele of Hind III polymorphism and L1 allele of intron 50 polymorphism of COL2A1 gene might confer risk to the development of high myopia through its role in scleral remodeling.

704F

Comparing variant calling methods in target exons of a worldwide sample of 14,000 individuals. *Y.Y. Lo[1], C. Sidore[1], J. Li[2], Y. Li[3], S.L. Chissoe[4], M.R. Nelson[4], M.G. Ehm[4], G. Abecasis[1], S. Zollner[1].* 1) Department of Biostatistics, University of Michigan, Ann Arbor, MI; 2) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 3) Department of Genetics, Department of Biostatistics, University of North Carolina, Chapel Hill, NC; 4) GlaxoSmithKline, Department of Genetics, RTP, NC.

In the past years, exome sequencing studies have been shown valuable in identifying rare variants that contribute to complex phenotypic traits. The success of these studies has resulted from their ability to generate large amount of reads at high coverage and large sample size. Critical steps to understanding the contribution of rare polymorphisms to phenotypic variation include generating precise genotype calls and identifying sources of error. There are many ways of generating genotype calls, based on how information is aggregated across samples and across sites. It is important to locate possible sources of error arising from each method when sample size increases, especially at the sites with local low coverage. Here we perform a comparative analysis on a dataset consisting of the exons of 202 genes sequenced to an average depth of 27x in a sample of >14,000 individuals. In addition, SNP data from previous genome-wide association studies are available for 10,500 individuals. After careful data cleaning and read mapping, we apply several methods for calling genotypes. We use individual based and population based calling algorithms, as well as LD based methods. For calling genotypes based on LD patterns, we combine exon sequences from target genes with flanking genotype data in a 1Mb window. We evaluate the quality of the variants generated by each method and compare across methods by calculating heterozygote concordance. Also, several independent sources of data were available to measure genotype accuracy, including eight genes sequenced in 993 individuals using conventional capillary methods, 150 sample duplicates, 30 trios, and 2 trios sequenced deeply by the 1000 Genomes Project. We quantify error as a function of multiple covariates such as the underlying coverage, allele frequency and sample size. Since over 50% of the variants called are singletons, we assess in particular the quality of singletons in each method. Preliminary results show that while individual based caller generates most number of singletons, at least 25% of them are false positives. By pooling information across samples, population based and LD based algorithms have significantly better sets of singleton calls. We also find that LD based method improves most of the low-quality genotypes called by individual based or population based methods. The results of our study provide important guidelines for the design and interpretation of resequencing based association studies.

705F

Evaluation of POWERPLEX ESI 17® Amplification kit STR loci in an admixed hispano-amerindian population sample of Valparaíso, Chile. *G. Molina[1], MO. Yañez[2], J. Manríquez[1], S. Rojas[1].* 1) Unidad de Genética Forense, Servicio Médico Legal, Valparaiso, Región de Valparaíso, Chile; 2) Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile.

The PowerPlex ESI 17® (PP17, PROMEGA) amplification Kit was designed for applications in forensic genetics analysis using short tandem repeats loci (STR) polymorphism in European population (ENFSI and EDNAP recommendations). In our population, routinely, forensic genetics testing was performed using the STR loci included in AmpFℓSTR® Identifiler® PCR Amplification kit (ID, Applied Biosystems), that includes the 13 CODIS loci (FBI recommendations). Our population is an admixture between Hispanic and Amerindians. In the literature, population genetics analysis for PP17 STR loci are scarce and mainly from Europe. To obtain population genetics data and evaluate the usefulness of the loci included in PP17, we analyzed 150 unrelated individuals' samples obtained with previous informed consent, who attend to paternity testing in our unit. The sample size was calculated according to Chakraborty (1992) to obtain frequencies higher than 0.1 ((=0.95). The DNA extraction was automated performed and the STR loci was PCR amplified using PP17 and ID amplification kits, according to manufacture recommendations. Population genetics data analysis was determined using Arlequin (University of Geneva). Power of exclusion (PE) and Match Probability (MP) were calculated using PowerStats v1.2 software (PROMEGA). The SE33 loci MP was the lowest (2.20 x 10-2) and the PE (0.788) was the third highest. The other 5 new loci ((D10S1248, D22S1045, D2S441, D12S391 and D1S1653) have similar informativeness than CODIS loci. The combined MP and PE for PP17 were 3.71 x 10-19 and 0.9999998 respectively, while for ID the parameters were 5.93 x 10-17 and 0,9999993. This study showed a high percentage of heterozigocity in PP17 loci in the population studied, despite being designed for European population. In conclusion, the use of the PP17 system has better performance than ID for forensic genetics porpoises. The combined use of these two systems could resolve complex DNA testing, where ID alone is not sufficient to get a strong conclusion.

706F

Genetic Variant Of COMT (Catechol-O-methyltransferase) Associated With Risk For Dementia. *P.A. Pereira[1], M.A. Romano-Silva[1], M.A.C. Bicalho[1,2], L.A. De Marco[1], H. Correa[1], S.B. Campos[1], E.N Moraes[1,2], K.C.L. Torres[1], I.C.R.S. Bozzi[1], G.J.G.S. Pimenta[1], M.P. Mello[1], L.B. Rodrigues[1], B.R. Souza[1], D.M. Miranda[1].* 1) UFMG, Belo Horizonte, Minas Gerais, Brazil; 2) Centro Jenny Faria de Assistência à Saúde do Idoso e da Mulher, Hospital das Clínicas, Universidade Federal de Minas Gerais (UFMG), Brazil.

Objective The aim of the present study was to examine the association between polymorphisms in the COMT gene and dementia in the Brazilian population. Catechol-O-methyltransferase (COMT) catalyzes the transference of methyl group in catecholamines such as dopamine and norepinephrine in the process of metabolism of these neurotransmitters. COMT regulates the amount of dopamine in the prefrontal cortex (PFC). Substantial studies indicate a close relationship between COMT and several human psychotic disorders. The case-control method was used to study the association between dementia and genetic variants of COMT in the Brazilian population. Methods 8 tagSNPs (rs737866, rs933271, rs1544325, rs740603, rs4646312, rs740601, rs4646316 and rs165774) in the COMT gene were genotyped by PCR-Real Time in 113 outpatients with diagnosis of dementia and 118 individuals belonging to the healthy comparison group to investigate an association between the COMT gene and dementia. The participants were evaluated using the DSM-IV, MINI-PLUS and NINCDS-ADRDA. Results The statistical findings shown that seven of the remaining eight tagSNPs analyzed were found to be significantly associated with dementia at the allele level and all tagSNP at the genotype level present an association with dementia (table). Linkage Disequilibrium analysis also showed difference between cases and controls groups. Conclusion Our results support the hypothesis that the polymorphisms of COMT gene may be associated with risk/protection to development of dementia.

707F

Heritability estimation for human height using GWAS-based studies in African-Americans. *F. Chen, G.K. Chen, C.A. Haiman, D.O. Stram, the Genome-Wide Association Studies of Breast and Prostate Cancer in African-Americans.* Preventive Medicine, University of Southern California, Los Angeles, CA.

Height has an extremely polygenic pattern of inheritance. Genome-wide association studies (GWAS) have revealed hundreds of common variants that are associated with human height at genome-wide levels of significance. Each of these common variants has a very modest effect, and only a small fraction of phenotypic variation can be explained by the aggregate of these common variants. In this large study of 12,567 African-American men and women, we genotyped and analyzed 985,415 autosomal SNPs across the entire genome, from which we selected random sets of SNPs (10k, 50k, 100k, 200k, 300k) and estimated the proportion of variance in human height that is explained by the patterns of heritability captured by each set of SNPs using a standard variance components approach. We found that 5.2% (standard error (SE), 1.2%), 19% (SE, 2.3%), 30% (SE, 2.8%), 36% (SE, 3.2%) and 37% (SE, 3.4%) of variation in height can be explained by the additive heritability captured by 10k, 50k, 100k, 200k and 300k SNPs, respectively. We noted that the estimate of heritability explained increases with the number of SNPs used in the estimation process but asymptotes at around 35-40 percent at SNP numbers lower than conventional estimates of the number of variants needed to capture genetic variation in this relatively low LD population. We conclude that the additive heritability estimate is therefore most likely reflecting long-range relatedness between nominally unrelated participants rather than the variance explained by a given set of SNPs. This is the first and largest study to date that investigates the inheritance property of human height in a population of African origin using GWAS samples. Future work on this subject includes analyses focusing on further dissecting the variation of height to the genetic architecture and simulation studies aiming at unveiling the statistical picture of the inheritance pattern of height in this population.

708F

Disentangler: a visualization technique for linkage disequilibrium mapping using multi-allelic loci. *N. Kumasaka[1], Y. Okada[1], A. Takahashi[1], M. Kubo[1], Y. Nakamura[2], N. Kamatani[1].* 1) Ctr Genomic Med, RIKEN, Tokyo, JAPAN; 2) Human Genome Center, Institute of Medical Science, University of Tokyo, JAPAN.

Linkage disequilibrium (LD) mapping using multi-allelic genetic loci (such as HLA alleles) is occasionally required to identify susceptibility haplotypes for complex diseases. Here we propose a new visualization technique to map LD with multi-allelic loci. A statistical method to infer underlying haplotypes at those loci is developed and the multiple correspondence analysis (MCA, also known as homogeneity analysis) is further applied, which provides an optimal default of the inferred haplotypes on a display. Several practical examples of LD mappings with HLA alleles associated with ulcerative colitis and Crohn's disease are presented, which illustrate the potential usefulness of the method as an aid to the interpretation of disease susceptibility haplotypes at multi-allelic loci. The method is general thereby applicable for single-nucleotide polymorphisms and allele-specific copy number polymorphisms, or mixture of those in a single display.

709F

Model-based identification of pseudo-trio structures in population genomic data and application to haplotype phasing. *D. Xifara, G. McVean.* Statistics, University of Oxford, Oxford, Oxfordshire, United Kingdom.

Experimental designs that include trio data or other familial structures lead to powerful and robust approaches for the analysis of population genomic data, for example in haplotype phasing and the detection of genetic association in the presence of population structure. However, even in cohorts of unrelated individuals it is often still possible to identify individuals that share extended identity and hence recent common ancestry at one or more loci. The long range phasing method of Kong et al (2008) uses such recent ancestry to reconstruct haplotypes. However, the authors also suggested that this approach may only apply to extremely large cohorts in populations with limited genetic diversity. We have developed a model-based approach for the identification of pseudo-trios in unrelated cohorts. By combining information across multiple `pseudo-parents' we achieve accuracy in haplotype phasing comparable to current state of the art algorithms for haplotype estimation in samples of as few as 50 individuals. Furthermore, the algorithm leads to marginal phasing of samples, hence ease of parallelisation, and can be applied to cohorts of arbitrary size. We also discuss the possible use of identifying and utilising extensive relatedness in the analysis of genetic association in weakly-structured populations.

710F

The polymorphism rs642961 (IRF6) contributes differently to nonsyndromic cleft lip/palate susceptibility according to geographic regions of Brazil. *L.A. Brito[1], C.B.F. Silva[1], K.M. Rocha[1], D. Schlesinger[1], L.A. Cruz[1], L.K. Bárbara[1], M. Aguena[1], D.F. Bueno[1], N. Alonso[2], D.R. Bertola[1], D. Meyer[1], M.R. Passos-Bueno[1].* 1) Department of Genetics, University of São Paulo, São Paulo, Brazil; 2) Division of Plastic Surgery, School of Medicine, University of São Paulo, São Paulo, Brazil.

Nonsyndromic cleft lip with or without cleft palate (NSCL/P), the most common craniofacial birth defect, is a complex disease with multifactorial inheritance, with prevalence varying among populations (1:1000 in Europeans (EU), 0.3:1000 in Africans (AF) and 3.6:1000 in Amerindians (AI)). IRF6 (1q32) has been the locus of more replicated associations, with the marker rs642961 (G>A, within an IRF6 enhancer) presenting the strongest association signal in most of the studies and being appointed as a functional variant. As Brazilians constitute an admixed population mainly from EU, AF and AI populations, approaches such as structured association are recommended in order to avoid false correlations. In this study, this SNP was tested for association with NSCL/P in Brazilian population. We have also genotyped a panel of 40 biallelic insertion-deletion ancestry informative markers in 471 patients and 326 controls, in order to characterize the individual ancestry composition. Patients were from 5 different Brazilian locations (Rio de Janeiro, n=112; Maceió, n=62; Barbalha, n=52; Fortaleza, n=163 and Santarém, n=82). Ancestry contributions and association tests were performed with Structure 2.3.3 and STRAT softwares. Frequency of the at risk allele rs642961 was estimated as 0.19 (cases) and 0.13 (controls). Overall ancestry contributions were 60% EU, 22% AF and 18% AI for cases and 71% EU, 19% AF and 10% AI for controls. Individual admixture was used to stratify the association analysis and correct for effects of admixture in case-control comparison (structured association test). When the test was performed with the whole patient sample, no significant association was detected; however, analyzing each region separately, rs642961 was significantly associated only in Barbalha (P=0.005, ORhet=1.97 [95%CI 0.99-3.89], ORhom=4.85 [1.7-14.3]. The positive association found in Barbalha is consistent with the risk allele reported in other populations (A) and with the genetic contribution for NSCL/P, estimated in a previous genetic epidemiological study (heritability estimates: Barbalha=85%; Santarém=71%; Rio de Janeiro=70%, Fortaleza=64% and Maceió=45%); moreover, the higher risk conferred by the homozygous genotype suggests a recessive role for this locus. These results suggest that despite the positive association in a region of Brazil, it is not an important locus for cleft risk in our population. FAPESP, CNPq, MCT.

711F

A Novel Permutation Strategy to Correct for Population Stratification in Case-Control Studies of Rare Variation. *M.P. Epstein[1], Y. Jiang[1], K.N. Conneely[1], R. Duncan[1], A.S. Allen[2], G.A. Satten[3].* 1) Department of Human Genetics, Emory University, Atlanta, GA; 2) Department of Biostatistics and Bioinformatics, Duke University, Durham, NC; 3) Centers for Disease Control and Prevention, Atlanta, GA.

Many case-control tests of rare variation [Neale et al. PLoS Genet e1001322; Ionita-Laza et al. PLoS Genet e1001289; Li et al. AJHG 87: 728 among others] are implemented in statistical frameworks that prohibit straightforward correction for confounders such as population stratification. Confounding due to population stratification is possible in resequencing studies since rare variants likely are specific to particular ancestral groups. To correct for this confounding, we propose establishing the significance of a rare-variant test using a novel permutation procedure that preserves the population stratification within the sample. Using Fisher's noncentral hypergeometric distribution, we sample disease outcomes for subjects in a permuted dataset in a manner such that the probability a subject is selected as a case is dependent on his/her odds of disease conditional on ancestry. Our permutation framework allows for adjustment of different measures of ancestry and further can model other confounders of interest to researchers. Using simulated sequence data based on coalescent models, we demonstrate that our permutation strategy corrects for confounding due to population stratification that, if ignored, would inflate the size of a rare-variant test. As an example, we simulated case-control sequence data for African-American samples under a model where the odds of disease for a subject increased with increasing proportion of African ancestry and then applied the rare-variant test of Ionita-Laza et al. (which cannot model confounders) for analysis. When the odds ratio of disease given African ancestry was 1, 4, and 8, we estimated the type-I error rates of the naive test at nominal alpha=0.05 to be 0.051, 0.100, and 0.192, respectively. When we reanalyzed the datasets by applying our novel permutation strategy to the Ionita-Laza test (sampling subjects based on estimated odds of disease conditional on principal components), we obtained type-I error rates of 0.047, 0.053, and 0.045, respectively, which indicates our method was able to correct for the confounding. In addition to simulation results, we will also present results based on application of our approach to real sequence data from the FUSION study of type 2 diabetes. The permutation approach is applicable to any rare-variant association test used in a case-control study and is implemented in a modified version of the R package `BiasedUrn' for public use.

712F

Population structure of Hispanics in the Multi-Ethnic Study of Atherosclerosis. *A. Manichaikul[1,2], W. Palmas[3], C. Rodriguez[4], C.A. Peralta[5,6], J. Divers[7], X. Guo[8], W.-M. Chen[1,2], Q. Wong[9], K. Williams[9], K.F. Kerr[10], K.D. Taylor[8], W. Post[11,12], M.O. Goodarzi[8], M.M. Sale[1,13], A.V. Diez-Roux[14], S.S. Rich[1], J.I. Rotter[8], J.C. Mychaleckyj[1].* 1) Center for Public Health Genomics, University of Virginia, Charlottesville, VA; 2) Department of Public Health Sciences, Division of Biostatistics and Epidemiology, University of Virginia, Charlottesville, VA; 3) Department of Medicine, Columbia University, New York, NY; 4) Department of Medicine and Department of Epidemiology, Wake Forest University School of Medicine, Winston-Salem, NC; 5) Department of Medicine, Division of Nephrology, University of California San Francisco, San Francisco, CA; 6) Division of General Internal Medicine, San Francisco VA Medical Center, San Francisco, CA; 7) Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC; 8) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 9) Collaborative Health Studies Coordinating Center, University of Washington, Seattle, WA; 10) Department of Biostatistics, School of Public Health, University of Washington, Seattle, WA; 11) Division of Cardiology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 12) Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 13) Department of Medicine and Department of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville VA; 14) Department of Epidemiology, Center for Social Epidemiology and Population Health, University of Michigan, Ann Arbor, MI.

Using ~60,000 SNPs selected for minimal linkage disequilibrium, we perform population structure analysis of 1,374 unrelated Hispanic individuals from the Multi-Ethnic Study of Atherosclerosis (MESA), with self-identification corresponding to Central America (n=92), Cuba (n=50), the Dominican Republic (n=202), Mexico (n=669), Puerto Rico (n=190) and South America (n=107). By projection of principal components (PCs) of ancestry to samples from the HapMap phase III and the Human Genome Diversity Panel (HGDP), we demonstrate the first two PCs quantify the Caucasian; African; and Native American origins. The third and fourth PCs demonstrate a South-to-North axis that separates HGDP Native American samples with respect to known geographic location, and reveal corresponding differences in Native American ancestry among individuals from Mexico, Central and South America, and Puerto Rico. Using k-means clustering computed using the top PCs of ancestry, we define four subgroups of the MESA Hispanic cohort that show close agreement with self-identification, roughly labeling the clusters as primarily Dominican / Cuban; Mexican; Central / South American; and Puerto Rican. Genetic association of triglycerides with 33 SNPs in the LPL gene region, previously reported in GWAS of Caucasians but as yet unconfirmed in Hispanic populations, provides evidence of association in pooled analysis of the MESA Hispanic cohort: top SNP rs328 (Ser447Stop), P=8.1E-6. In stratified analysis, the Dominican / Cuban and Mexican subgroups had comparable estimated effects of -0.226 (SE=0.063) and -0.187 (SE=0.048) on log triglycerides (log mg/dL) per copy of the coded G allele, respectively, while estimated effects for Central / South American and Puerto Rican subgroups were closer to zero, with values -0.010 (SE=0.092) and -0.033 (SE= 0.095), respectively. Our findings highlight the genetic diversity of the MESA Hispanic cohort, and demonstrate the importance of considering this diversity in examining genetic association in the United States Hispanic population.

713F

Admixture mapping for qualitative or quantitative traits when confounding covariates and gene-environment interactions are present. *B. Zhu[1,2], D. Dunson[1], M.L. Miranda[3,4], A.E. Ashley-Koch[1].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Department of Statistical Science, Duke University, Durham, NC; 3) Nicholas School of the Environment, Duke University, Durham, NC; 4) Department of Pediatrics, Duke University Medical Center, Durham, NC.

Admixture mapping is a popular tool to identify regions of the genome associated with traits in a recently admixed population. Several methods have been introduced, primarily for dichotomous traits. We propose a two-stage approach for qualitative or quantitative traits and also consider the presence of confounding covariates and gene-environment interactions. In the first stage, the number of ancestral alleles at each selected locus is sampled across the entire genome based on a Hidden Markov Model (HMM) using a Markov chain Monte Carlo (MCMC) algorithm. The HMM is similar to the ones implemented in STRUCTURE and ANCESTRYMAP, with one key difference: we model the recombination process non-parametrically. Thus, the recombination rates are no longer homogeneous across the genome, and we can identify recombination hotspots. In the second stage, we investigate the association between the qualitative or quantitative trait with one set of ancestral allele estimates along with possible confounding covariates and gene-environment interactions, using a generalized linear model (GLM) with a shrinkage prior. The association results are combined across multiple sets of ancestral allele estimates to account for uncertainty in the ancestry estimates. This new approach is less vulnerable to false-positive discovery, since it investigates the association between the trait and all possible ancestral alleles across loci simultaneously, instead of using multiple tests each for one locus at a time as in previous approaches. Through simulation, we estimate the sensitivities and specificities of marker selection with our approach for a binary outcome with the true genetic locus imparting an odds ratio varying from 1.3 to 2.5 over a range of sample sizes from 500 to 2500. We show that, for a typical African American population (1000 subjects with an average of 80% African ancestry across the genome), our approach could identify the locus with an odds ratio 1.3 with sensitivity of 87% and specificity of 99%. Similar simulations are carried out for the continuous outcomes, although there is no direct comparison to ANCESTRYMAP for this approach. In summary, our approach provides increased flexibility for modeling in admixture analyses by allowing for continuous traits, and incorporation of covariates and higher order interactions.

714F

Multi-ethnic fine-mapping of cis expression-QTLs with fixed-effect meta-analysis. *C.P. Grace[1], J.C. Whittaker[2], J. Huxley Jones[2], A.P. Morris[1].* 1) Wellcome Trust Centre For Human Genetics, Oxford University, United Kingdom; 2) GlaxoSmithKline, Medicines Research Centre, Gunnels Wood Road, Stevenage, UK.

Genome-wide association studies (GWAS) have successfully identified novel loci contributing genetic effects to a range of complex human traits. However, localisation of the underlying causal genes and/or variants remains a challenge. One approach that may elucidate the functional basis of these findings is to integrate GWAS findings with gene expression data in relevant tissues. This is typically achieved through mapping of expression-QTLs (eQTLs) in publicly available data sets, and correlating these with signals of association from GWAS.

We have considered high-density genotyping data made available through the International HapMap Project for 270 samples across four populations: 30 CEPH trios from Utah with Northern European ancestry (CEU), 30 Yoruba trios from Nigeria (YRI), and 90 unrelated Chinese and Japanese individuals from Beijing and Tokyo (CHB/JPT). We have tested for association of up to 2.2 million SNPs with cis-expression of up to 18,000 transcripts (i.e. within 1Mb of transcription start site) in lymphoblastoid cell lines within each of these three broad ethnic groups. In the absence of heterogeneity in allelic effects on gene expression, we can increase power to detect eQTLs by combining results from the three ethnic groups through fixed-effects meta-analysis. Furthermore, differences in patterns of linkage disequilibrium between ethnic groups would be expected to improve the mapping resolution of causal variants within cis-eQTL signals.

Our trans-ethnic meta-analysis identified genome-wide significant ($p<1x10-12$) evidence of association for 4,368 cis-eQTLs. Of these, there was nominal evidence of heterogeneity (Cochran's Q-statistic $p<1x10-3$) at 513 cis-eQTLs, which is significantly higher than expected by chance (binomial test: expected: 4, 95% interval: 1 to 10). However, within many cis-eQTL signals with homogeneous effects across ethnic groups, there is a noticeable improvement in mapping resolution. For example through trans-ethnic meta-analysis, causal variants of the probe GI_21553312-S (CHURC1) (peak signal, $p = 3.85x10-304$ Cochran's Q p-value = 0.008) were localised to a 47kb interval, compared to 78kb in CEU alone.

Our results demonstrate the potential for trans-ethnic meta-analysis of cis-eQTL studies to improve mapping resolution, but also highlight the need for the development of novel methodology to take account of heterogeneity in allelic effects between distantly related populations.

715F

Independent genetic control of gene expression in whole blood and lymphoblastoid cell lines. *J.E. Powell[1], A.K. Henders[1], A.F. McRae[1], M.J. Wright[1], N.G. Martin[1], E.T. Dermitzakis[2], G.W. Montgomery[1], P.M. Visscher[1].* 1) Statistical Genetics, Queensland Institute of Medical Research, Brisbane, Qld, Australia; 2) Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva 1211, Switzerland.

The genetic control of gene expression has been well documented by a large number of studies, although, in the last few years, there has been much discussion as to the degree to which expression acts in a tissue specific manner. Given the aetiology of many complex diseases involve multiple tissues and cell types, understanding the extent of shared genetic control for gene expression between tissues is especially important. Most recent attempts to use data from multiple tissues have first mapped expression quantitative trait loci (eQTL) from individual tissues or cell types and then compared results among them, reporting the extent of eQTL overlap as an indicator of the level of common genetic control between tissues. Whilst these studies provide important information on the regulatory control of expression across tissues, their limited power means they can typically only detect eQTL with effects above a certain size, leading to studies usually only explaining a small proportion of genetic variation for gene expression. In a genetically informative design, narrow or broad sense heritability for gene expression can be estimated using the concept of identity by decent. These estimates of heritability refer to the combined effects of all causal variants that segregate in the population. Similarly, the genetic correlation between gene expression levels in different tissues can be estimated. These estimates quantify the combined effects of all causal variants on the genetic covariance. Here, we report results from a study using expression data from monozygotic twins to investigate the genetic control of gene expression in lymphoblastoid cell lines and peripheral blood mononuclear cells. We estimate the genetic correlation which is a measure of the level of common genetic control of gene expression between the two RNA sources. Our results show that, when averaged across the genome, mean levels of genetic correlation for gene expression in LCL and PBMC samples are close to zero. We support our results with evidence from gene expression in an independent sample of LCL, T-cells and Fibroblasts. In addition, we provide evidence that housekeeping genes, which maintain basic cellular functions, are more likely to have high genetic correlations between the RNA sources than non-housekeeping gene, implying a relationship between the transcript function and the degree to which a gene has tissue specific genetic regulatory control.

716F

Association analysis of LRRC20 polymorphisms with aspirin exacerbated respiratory disease. *J. Kim[1], B. Park[2], C. Park[3], H. Shin[1,2].* 1) Sogang University, Seoul, Korea; 2) SNP Genetics, Inc., Seoul, Korea; 3) Soonchunhyang University Bucheon Hospital, Bucheon, Korea.

Aspirin exacerbated respiratory disease (AERD) is characterized by so-called aspirin triad consisting of aspirin hypersensitivity, bronchial asthma and chronic rhinosinusitis with nasal polyposis. The leucine-rich repeats containing domain has been known to be conserved in many proteins involved in the innate immunity. Recently, genetic polymorphism in leucine-rich repeat containing 20 (LRRC20) has been reported to be associated with increased interferon alpha in the serum. To investigate the association between LRRC20 polymorphisms and AERD, a total of 37 common single nucleotide polymorphisms (SNPs), with minor allele frequency (MAF) over 0.05, were genotyped in 166 AERD and 429 aspirin-tolerant asthma (ATA) subjects in a Korean population. Logistic analysis revealed that LRRC20 polymorphisms and haplotypes might be risk factors for the development of AERD ($P = 0.003-0.04$). In further addition of association with forced expiratory volume in one second (FEV1) decline by aspirin provocation, minor homozygote of rs4747011 showed about 2.5-fold increase in the FEV1 decline among asthmatics compared to other genetic modes ($P = 0.01$). Despite the needs for replications in large cohorts and further functional evaluations, our preliminary findings suggest that LRRC20 polymorphisms are potentially associated with aspirin hypersensitivity in asthma.

717F

Polymorphisms in genes participating in the oxidative stress pathway are associated with airway reactivity in patients with COPD. *N.M. Rafaels[1], I. Ruczinski[2], T. Murray[1,3], R.A. Mathias[1], L. Huang[1], V. Mantese[1], C. Vergara[1], L. Gao[1], A.E. Berger[1], J. Connett[4], P.D. Paré[5,6], D. Sin[5,6], A. Sandford[5,6], D. Daley[5,6], T.H. Beaty[3], K.C. Barnes[1], N.N. Hansel[7].* 1) Division of Allergy and Clinical Immunology, Johns Hopkins, Baltimore, MD; 2) Department of Biostatistics, Bloomberg School of Public Health, Johns Hopkins, Baltimore, MD; 3) Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins, Baltimore, MD; 4) Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, MN; 5) Department of Medicine, St. Paul's Hospital, University of British Columbia, Vancouver, BC; 6) James Hogg Research Centre, Providence Heart and Lung Institute, University of British Columbia, Vancouver, BC; 7) Division of Pulmonary and Critical Care Medicine, Johns Hopkins, Baltimore, MD.

Rationale: Chronic obstructive pulmonary disease (COPD) is traditionally considered a disease of fixed airway obstruction and progressive loss of lung function contributing to significant morbidity and mortality worldwide. Airway reactivity (AR), a narrowing of stimulated air passages, in individuals with COPD from the Lung Health Study (LHS) has been associated with worse outcomes, including accelerated loss of lung function. **Methods:** We genotyped 559,660 SNPs using the Illumina Human660W-Quad v.1_A BeadChip in 4,251 European American patients with COPD from the LHS. The LHS was a randomized multicenter clinical trial designed to test the effectiveness of smoking cessation and bronchodilator administration in smokers with mild lung function impairment. AR was measured for 3,991 subjects at baseline and 3,510 subjects at year 5 of this longitudinal study and calculated as a quantitative measure, using the two-point slope then converted on the scale log10(0.681-AR). We ran a genome-wide association study (GWAS) on AR and subsequent pathway analysis using Ingenuity Systems Inc. on SNPs with a p<10-4. The most prominent pathway was the oxidative stress pathway. Here we tested the hypothesis that 340 single nucleotide polymorphisms (SNPs) in the 23 oxidative stress genes identified from the pathway analysis were associated with AR. SNP associations were tested under a linear regression model, adjusting for age, gender, height, weight, FEV1, FEV1/FVC, clinic site, and if the subject had quit smoking at year 5. The results were then combined using a correlated meta-analysis approach combining baseline and year 5 data. **Results:** Ten SNPs in the genes, *GSTM3* (rs1927328, rs1571858, and rs7483) *GSTM5* (rs929166), *ABCC4* (rs3765535, rs1729764, rs4148501, and rs1750996), and *MAP3K13* (rs12495216 and rs6803944), were associated with increased AR, after adjusting for Bonferroni correction (P = 0.00015). In addition, rs17111652 in *USP24* was significantly associated with decreased airway reactivity (P = 8.7E-8). There was a stronger association with AR at baseline for SNPs in *ABCC4* and *MAP3K13*, but stronger associations with AR at year 5 for SNPs in *GSTM3*, *GSTM5*, and *USP24*. **Conclusions:** Our findings suggest genes in the oxidative stress pathway may be a significant factor in regulating AR for patients with COPD. Analysis of imputed SNPs and additional populations is underway.

718F

Examining the relationship between smoking history and genetic susceptibility on pancreatic cancer risk. *E.J. Childs[1], L. Wang[2], S. Chen[3], A. Blackford[2], G. Parmigiani[4,5], A.P. Klein[1,2,6].* 1) Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; 2) Oncology and Pathology, Johns Hopkins Medical Institute, Baltimore, MD, USA; 3) Department of Biostatistics, University of Medicine and Dentistry of New Jersey, Piscataway, NJ, USA; 4) Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA, USA; 5) Department of Biostatistics, Harvard School of Public Health, Boston, MA, USA; 6) The Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins Medical Institute, Baltimore, MD, USA.

Pancreatic cancer is the 4th leading cause of cancer death. Its five-year survival rate of about 5% is the lowest of all major cancers. Prior segregation analysis suggests that pancreatic cancer is influenced by an autosomal dominant major gene inheritance. Well-established risk factors for pancreatic cancer include cigarette smoking and family history, with current cigarette smoking approximately doubling pancreatic cancer risk. While some studies have sought to examine the joint-effects of smoking and family history, these studies have jointly modeled both the effect of major gene inheritance and cigarette smoking. Using full pedigree data from the National Familial Pancreatic Tumor Registry, we formulated a semiparametric model incorporating autosomal dominant inheritance and estimated the independent and joint effects of major gene and cigarette smoking on the penetrance of pancreatic cancer using MCMC. Alternative models are compared using AIC and Bayes Factors. Full analysis will be presented. These data will provide the foundation for more detailed risk-assessment for high-risk pancreatic cancer families.

719F

Influence of Genetic Variants on Response to Vitamin D Administration. *Z. Dastani[1], D. Goltzman[2], T. Wang[3], L. Fu[4], D.P Kiel[5], D. Cole[6], B. Richards[1, 7].* 1) McGill Univ, Department of Human genetics and Epidemiology and Biostatistics, Montreal, QC, Canada; 2) McGill Univ, Department of Medicine, Montreal, QC, Canada; 3) Cardiology Division, Massachusetts General Hospital, Boston, USA; 4) University of Toronto, Department of Laboratory Medicine & Pathobiology ON, Canada; 5) Institute for Aging Research Hebrew SeniorLife and Harvard Medical School, Boston, USA; 6) University of Toronto, Departrments of Laboratory Medicine & Pathobiology, Medicine, and Genetics, ON, Canada; 7) Department of Twin Research and Genetic Epidemiology, King's College London.

Introduction: Vitamin D deficiency, a common worldwide disorder, is associated with several chronic diseases including diabetes, cardiovascular disease, autoimmune disorders, and cancer. 25-hydroxyvitamin D (25OHD) levels are highly heritable and a recent meta-analysis of GWAS studies from the SUNLIGHT consortium identified three loci that strongly influence circulating vitamin D levels. However, whether these variants influence dose response to vitamin D levels is unknown; and this information could be clinically useful. We therefore sought to determine if variants decreasing 25OHD levels were associated with differences in response of 25OHD levels to vitamin D administration. Objective: In this study, we aimed to test whether the genome-wide significant SUNLIGHT SNPs (rs2282679, in the GC gene; rs12785878, near the DHCR7 gene and rs10741657, near the CYP2R1 gene) which influence 25OHD levels also influence the response of 25OHD levels to vitamin D administration in 2122 individuals from Canadian Multicentre Osteoporosis study. Methods: We first adjusted naturally log transformed 25OHD levels for age, sex, body-mass index, and season of blood draw. Then change in residualized 25OHD levels calculated in people taking 400 or more IU of vitamin D per day. The range of dose of vitamin D intake was between 400-5000IU Next, we assessed the additive effect of each 25OHD decreasing allele on baseline and dose response and created an overall genotypic risk score by summing the number of risk alleles weighted by their beta-coefficients from the SUNLIGHT meta-analysis. Results: Each SNP was significantly associated with 25OHD levels in 2084 individuals (rs2282679, pvalue=3.06e-05; rs12785878, pvalue=1.24e-06, and rs10741657, pvalue=1.08e-05). The overall weighted genotype risk score was also correlated with decreasing concentrations of 25OHD () =-0.99, pvalue= 1.35e-14). A higher genotypic risk score was associated with an increase in 25OHD response to vitamin D administration () =+1.70, pvalue= 0.007) in 149 individuals with available supplementary vitamin D intake. The association persisted after adjusting for dose of vitamin D intake. Conclusion: Individuals carrying risk alleles for lower 25OHD demonstrate an enhanced response to supplementary vitamin D intake. Therefore, identifying individuals at risk may provide clinical utility for the treatment of VitD deficiency. Currently we are confirming this finding in additional samples from European populations.

720F

Genome-wide linkage scan for QTL influencing quantitative ultrasound measures of bone strength in children from the Jirel ethnic group in eastern Nepal. *K.D. Williams[1, 2], J. Blangero[3], M.C. Mahaney[3], T.D. Dyer[3], J. Subedi[4], B. Jha[5], J.L. VandeBerg[3], S. Williams-Blangero[3], B. Towne[6].* 1) Department of Anthropology , Temple University, Philadelphia, PA; 2) Department of Pediatrics , Temple University, Philadelphia, PA; 3) Department of Genetics, Texas Biomedical Research Institute, San Antonio, TX; 4) Department of Sociology and Gerentology, Miami University, Oxford, OH; 5) Tribhuvan University Institute of Medicine, Kathmandu, Nepal; 6) Department of Community Health, Boonshoft School of Medicine, Wright State University, Dayton, OH.

Most genetic studies of bone strength have been conducted in adults from Western countries using phenotypes obtained from dual-energy x-ray absorptiometry (DXA). Relatively few studies have been have been conducted in children, especially children from non-Western populations, and using phenotypic data obtained from other modalities. Here we present results from initial genome-wide linkage analyses of speed of sound (SOS) measures obtained from quantitative ultrasound (QUS) testing in healthy children from the Jirel ethnic group in Eastern Nepal who participate in the Jiri Growth Study. QUS SOS measures reflect properties of bone including cortical thickness, bone density, trabecular microstructure, and elasticity. In contrast to more commonly used calcaneal QUS measures that serve as proxies for skeletal bone strength and quality of the skeleton, this study presents results pertaining to properties of long bones. Specifically, axial QUS measures of load bearing and non-load bearing bones. In the total sample of 602 children (308 boys; 294 girls) aged 6 to 18 years, most belonging to one very large extended pedigree, the heritabilities of distal radius and midshaft tibia SOS measures were significant: SOS_{arm} h^2 = 0.33 ± 0.10, p<0.0001; SOS_{leg} h^2 = 0.36 ± 0.11, p<0.0001. Genome-wide linkage analyses were conducted in a subset of 247 children (126 boys; 121 girls) typed for ~400 STR markers spaced at an average density of 10 cM. Suggestive linkage of SOS_{arm} to markers on chromosome 10p was found (LOD = 2.03), and modestly suggestive linkage of SOS_{leg} to markers on chromosome 13q was indicated (LOD = 1.46). Because the genetic correlation between SOS_{arm} and SOS_{leg} was found in the total sample to be significantly greater than zero but significantly less than 1.0 (δ_g SOS_{arm};SOS_{leg} = 0.41 ± 0.21), bivariate linkage analysis of SOS_{arm} and SOS_{leg} was conducted. In this small initial sample of genotyped individuals, however, the joint linkage analysis of SOS_{arm} and SOS_{leg} did not yield notably different results than those observed in analyses of each trait separately. In sum, these preliminary results demonstrate significant heritabilities of QUS SOS axial bone measures in Jirel children, and the feasibility of localizing and eventually identifying in a larger study sample genes that influence these measures of bone strength.

721F

Sliding-window based testing of rare variant associations. *A. Brisbin[1], K.A. Ellsworth[2], L. Wang[2], B.L. Fridley[1].* 1) Health Sciences Research, Mayo Clinic, Rochester, MN; 2) Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN.

Single-marker based tests are underpowered for detecting association between phenotypes and rare variants, leading to the development of methods for testing association of sets of rare variants. Previously, we introduced the Difference in Minor Allele Frequency (D-MAF) method, a method for analysis of rare variants which makes no assumptions about the direction of effects, and found that it outperforms most existing methods in simulations including protective variants. Here, because including markers with no effect can reduce power, we extend our method to a sliding-window approach, and examine the effect of window size on the power of several popular methods of rare variant analysis. In addition, we use the D-MAF to test for association between variants found by next-generation sequencing of the gene FKBP5 and response to the cytotoxic drug gemcitabine in a set of lymphoblastoid cell lines.

722F

Joint modelling of repeated measures and time-to-event data in genetic association analysis of type 1 diabetes. *Z. Chen[1], A.D. Paterson[2, 3], A.J. Canty[4], L. Sun[2, 5], S.B. Bull[1, 2].* 1) Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada; 2) Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada; 3) The Hospital for Sick Children, Toronto, Ontario, Canada; 4) Department of Mathematics & Statistics, McMaster University, Hamilton, Ontario, Canada; 5) Department of Statistics, University of Toronto, Toronto, Ontario, Canada.

The longitudinal cohort design is attractive for genome-wide association studies (GWAS) of complex traits and diseases, as it enables investigation of environmental and genetic influences over time. In this design, it is typical to examine SNPs for association with multiple phenotypes of various types, e.g., repeated quantitative trait (QT) measures or time to a clinically-defined outcome. SNP associations with clinical phenotypes may be categorized as follows: (1) direct and independent of an intermediate QT, (2) indirect and induced by association with a QT, or (3) pleiotropic on both a QT and the clinical outcome. As an alternative to modelling each phenotype separately, we propose joint modelling of repeated QT measures and time to clinical outcome by an approach originally developed for HIV/AIDS clinical trials (Wulfsohn and Tsiatis, 1997), but novel to the GWAS setting. We apply it in a recent GWAS of the genetics of complications of type 1 diabetes (Paterson et al., 2010). To consider various scenarios for the relationship of a SNP with longitudinal repeated measures of HbA1c and time to diabetic retinopathy (TDR), we selected SNPs apparently associated with one or the other or both phenotypes. In each case, we compare estimates and hypothesis testing results from joint likelihood maximization to those from separate analyses, and within the joint model, we apply likelihood ratio tests (LRT) to help distinguish among direct and indirect SNP associations. For example, in separate analyses we first detected rs1358030 in the HbA1c GWAS () =0.05±0.01, p=6.7E-9), which was also associated with TDR () = 0.34±0.15, p=0.02). In joint analysis with strong global SNP association (p= 7.3E-8), rs1358030 retained association with HbA1c () =0.05±0.01, p=3.1E-8) but not with TDR () =-0.13±0.09, p=0.13). We interpret this as evidence of direct association for HbA1c and indirect for TDR. To reduce computational costs, we also applied a two-stage estimation method, in which a linear mixed model is fitted to the longitudinal HbA1c in the first stage, and in the second stage a Cox proportional hazards model is fitted to TDR with the predicted HbA1c treated as a time-dependent covariate, with similar conclusions but without a global LRT. Advantages of joint modelling include inference for joint SNP association with both phenotypes that can help to distinguish among alternative genetic architectures, and improved precision in estimation of QT association with time to event.

723F

A generalized pooled association statistic for analyzing rare variants. *A. Derkach[1], J.F. Lawless[2,3], L. Sun[1,2].* 1) Statistics, University of Toronto, Toronto, Ontario, Canada; 2) Biostatistics, Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada; 3) Statistics and Actuarial Science, University of Waterloo, Waterloo, Ontario, Canada.

There is a recent shift to analysis of rare variants due to the advances in sequencing technology and the realization that the common variants identified in many genome-wide association studies only explain a small proportion of the heritability estimated for complex human diseases and traits. Because of the limited statistical power available to detect an association between a trait and a single rare variant in studies of moderate size, the strategy of pooling variants into a composite test has been considered by several authors (e.g. Li and Leal, 2008; Madsen and Browning, 2009; Price et al., 2010). Instead of analyzing one variant at a time, the pooled approach considers multiple variants simultaneously, enriching a signal by aggregating information across variants within a gene or pathway. Most methods assume that all variants have the same direction of effect (e.g. all deleterious), but more recently, Neale et al. (2011) and Ionita-Laza et al. (2011) proposed alternative methods that directly model protective effect among the variants considered. Nevertheless, the pros and cons of each method are mostly argued in the context of ad-hoc simulation studies.

We show that previously proposed methods can be derived from the same statistical framework. More specifically, they can be constructed as a function of the same vector of statistics. One of the benefits of such a generalization is new statistics that take into account the possibility of both deleterious and protective variants. Another benefit of this generalization is it allows for theoretical calculations to facilitate the systematical evaluation of the validity and power of different methods. Lastly our generalization can handle categorical or quantitative traits, and can deal with phenotype-dependent sampling for either type of phenotype. We also compare performance of the proposed methods on the publicly available data from HapMap project. Particularly we are interested in the ability of the methods to detect population stratification with rare variants.

724F

Genetic associations of inflammatory diseases with the gene encoding septin 6. *M. Kong, Y. Kim, J. Ryu, C. Lee.* Soongsil University, Seoul, Korea.

Inflammation is a underlying protective mechanism in the body infected by foreign substances such as bacteria and viruses. Chronic inflammation, however, slowly arises when this response is not completely turned off or extinguished and can become a disease. In order to identify genes associated with a variety of inflammatory phenotypes, a genome-wide association study was conducted in a large scale cohort with 8,842 unrelated Korean individuals. Two intragenic single nucleotide polymorphisms (SNPs) of rs6603540 in Septin 6 (SEPT6, intron 1) and rs5949032 in acetylserotonin O-methyltransferase-like (ASMTL, intron 9) were associated with multiple inflammatory diseases such as degenerative arthritis, rheumatoid arthritis, and gout ($P < 0.001$). Associations of the SNPs with some inflammatory biomarkers including hematocrit were also observed ($P < 0.05$). Analysis with data partitioned by gender revealed male-specific associations of rs6603540 with rheumatoid arthritis and hyperlipidemia ($P < 0.05$). This might be attributed to a gender-specific transmission ratio distortion (TRD) considering a heterogeneity of its minor allele frequency by gender. Further analysis for their functional roles in alternative splicing showed that the rs6603540 might induce formation of cassette exon by altering splicing elements. This study suggested that SEPT6 might play a critical role in inflammatory responses by its cytokinetic function, an integral part of immune response.

725F

Combining SNP and HLA AIMs for Ancestry Analysis for an Immunogenetic Transplantation Outcome Association Study. *A. Madbouly[1], L. Gragert[1], M. Maiers[1], M. Malkki[2], E. Petersdorf[2].* 1) Bioinformatics Research, National Marrow Donor Program, Minneapolis, MN; 2) Fred Hutchinson Cancer Research Center, Seattle, WA.

The overall aim is to investigate the mechanisms through which at-risk non-HLA genetic markers e.g. immune response gene polymorphisms contribute to survival disparities in Hematopoietic Stem Cell Transplants (HSCTs). Several studies showed that survival after HSCTs depends on the patient's ethnicity, with a higher risk of post transplant mortality, relapse or graft-versus-host disease among minority patients. This led to the hypothesis that precise definition of the ancestry of transplant patients and donors will shed some light on post transplant survival disparities. In order to stratify by degree of shared ancestry a pilot study was performed to identify ancestry of transplant patients and donors genotyped for two types of ancestry informative markers (AIMs): HLA and SNPs. The cohort included 491 individuals (76 African-American (AFA), 48 Asian/Pacific Islanders (API), 243 Caucasian (CAU), 104 Hispanic (HIS) and 20 Native American (NAM)) genotyped for HLA-A, -B, -C, -DRB1 and -DQB1 and 30 autosomal SNPs from a published (Kosoy 2009) reference AIMs panel. 23 of the chosen SNPs had >40% allele frequency difference between AFA and CAU, 23 >40% difference between AFA and API, and 9 >40% difference between CAU and API. Out of the last 9 SNPs, only one had a difference >40% between CAU and South Asian (SAS) and three between East Asian (EAS) and SAS. Assignment of admixture proportions was determined using STRUCTURE v2.3.2. Preliminary results indicated high accuracy in recapturing ancestry of self-identified AFA, EAS and CAU individuals, however distinguishing SAS from CAU individuals proved challenging due to sample size disparities and the lack of sufficiently differentiating SNPs. When the pilot cohort was augmented with EAS and SAS individuals from the published reference cohort (Kosoy 2009) to balance sample proportions, SAS individuals emerged as a separate cluster distinct from EAS and CAU. We additionally used Bayesian inference to assign racial categories to the pilot cohort using HLA AIMs. The algorithm used reference haplotype frequencies and knowledge of population sizes within the Be The Match® Registry as priors to calculate the overall likelihood of a race assignment. Classifying race using HLA frequencies gave >80% recovery of self-identified race for AFA and API, with lower recovery for CAU, HIS, and NAM. We plan on using the results from the HLA ancestry classifier as a tie-breaker supplementing ancestry classification using SNP AIMs.

726F

A model that combines array and sequence data to improve genotype accuracy. *J. O'Connell[1], J. Marchini[2].* 1) The Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 2) Department of Statistics, University of Oxford, Oxford, United Kingdom.

Data sets containing large cohorts of individuals who have been genotyped on microarrays as well as sequenced at low coverage are now becoming available. Genotypes are typically called from arrays independently of sequence data (and vice versa). Whilst the genotype calls coming from modern arrays are of high quality, accuracy and call rate can still be improved by incorporating sequence data into calls. This is particularly true for low frequency variants where very few observations are available to identify clusters for the rare genotypes. Higher quality genotype calls and better call rates will reduce time spent on quality control and decrease Type I and II error in genome wide association studies. To combine sequence and array data effectively, a model must be able to identify when one data source is spurious and discard this information, relying on the alternate source. We present a Bayesian mixture model for genotype calling from array allele signal intensities, with parameter estimation and calls augmented by genotype likelihoods from sequence data. The method successfully identifies and discards spurious data and is capable of handling individuals with missing values, for example, a subset of individuals may have only been assayed on an array and not sequenced. This results in genotype calls with greater accuracy and a higher call rate than standard array-only or sequence-only methods. The implementation requires similar computational time to standard software for array-only based genotyping, providing higher quality genotype calls for minimal extra time and effort. We demonstrate these capabilities on 1000 Genomes individuals which have been genotyped on the Illumina HumanOmni2.5S chip as well as sequenced at approximately 4X coverage.

727F

Making the most of case-sibling studies. *M. Shi, D.M. Umbach, C.R. Weinberg.* Biostatistics Br, NIEHS, Res Triangle Park, NC.

In genetic association studies, properly analyzed family-based designs are robust against bias from population stratification. The case-parents design is one of the most convenient such approaches. Parents, however, are not always available, especially for diseases with onset later in life. For those diseases, the case-sibling design, where cases and one or more unaffected siblings are studied, can be very useful. This design requires the analyst to account for dependencies, for example using conditional logistic regression (CLR). We here consider an alternative analysis, which treats the case-sibling design as a nuclear family study, but with all parents missing. Under the weak assumption of Mendelian inheritance in the source population, one can carry out maximum likelihood analysis by using the expectation maximization (EM) algorithm to account for missing parental genotypes. An additional issue for this design is that many cases may not have a sibling available. The EM-based approach can also incorporate singleton cases, while a traditional logistic regression analysis would discard them. We use simulations to compare performance of CLR and nuclear family analytical approaches for studies where cases have one or two unaffected siblings. We also assess power gained by incorporating singleton cases, under several risk scenarios.

728F

The role of thromboxane A2 receptor gene polymorphisms in lung function and severity in childhood-onset asthma. *K. Takeuchi[1], Y. Mashimo[1], S. Hattori[1], N. Shimojo[2], M. Tomita[2], T. Arima[2], Y. Morita[2], K. Sato[3], S. Suzuki[3], T. Nishimuta[3], H. Watanabe[3], A. Hoshioka[4], A. Yamaide[4], M. Watanabe[5], Y. Kohno[2], A. Hata[1], Y. Suzuki[1].* 1) Department of Public Health, Chiba University Graduate School of Medicine, Chiba, Japan; 2) Department of Pediatrics, Chiba University Graduate School of Medicine, Chiba, Japan; 3) Shimoshizu National Hospital, Yotsukaido, Japan; 4) Chiba Children's Hospital, Chiba, Japan; 5) Department of Pediatrics, Toho University School of Medicine, Tokyo, Japan.

We have been studying association of matrix metalloproteinase (*MMP*) genes in the *MMP* cluster region in chromosome 11 with adult and/or childhood asthma. We were also interested in whether genetic variations have impact on pulmonary function and severity in asthma patients. We thereby investigated association of SNPs of the *MMP*s and several asthma-related genes with pulmonary function and severity in childhood-onset asthma patients. We found that one *MMP13* SNP showed a significant association with severity in childhood-onset asthma. Among asthma-related genes investigated, a SNP of thromboxane A2 receptor (*TBXA2R*) gene (c.795T/C) showed association with both pulmonary function and severity in childhood-onset asthma. Thromboxane A2 is known to be involved in platelet aggregation and constrictor of vascular and respiratory smooth muscle, therefore, the *TBXA2R* gene is a good candidate for bronchial hyperresponsiveness and airway inflammation. Because the 795T/C polymorphism is synonymous and unlikely to influence the function of the receptor, other polymorphisms being in strong linkage disequilibrium (LD) with this SNP may affect the efficiency of transcription or translation. We comprehensively surveyed SNPs of this gene including the 5' upstream and 3' downstream regions, and found that four SNPs in the intron between exon 1 and exon 1b of the gene were in LD to 795T/C. We next performed luciferase reporter assay to evaluate the effect of these SNPs on gene expression. We transfected vector constructs containing 6 different haplotypes consisting of the 4 SNPs into HEK293 cells. After 24 hours of transfection, luciferase activities were measured using Dual-Luciferase Reporter Assay System. Three haplotypes (H1, H2, H6) showed higher transcriptional activity than the rest of the haplotypes (H3, H4, H5). No single SNP was precisely associated with the activity. These results suggest that the haplotype variation of this region affects *TBXA2R* gene expression and may affect pulmonary function and severity in asthma patients.

729F

Extensions of Bootstrap Bias-Reduction to Address the Winner's Curse in Genome-wide Association Analysis of Time-to-Event Phenotypes. *J. Taleban[1], L.L Faye[1,2], A. Dimitromanolakis[1], A.D. Paterson[2,3], L. Sun[2,4], S.B. Bull[1,2].* 1) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON; 2) Dalla lana School of Public Health, University of Toronto, Toronto, ON; 3) Program in Genetics and Genome Biology, Hospital for Sick Children, Toronto, ON; 4) Department of Statistics, University of Toronto, Toronto, ON.

Adjustment of effect estimates for winner's curse inflation is critical for interpretation and replication of findings in the context of high-dimensional multiple testing, including genome-wide association studies and whole-genome sequencing. The effectiveness of a general non-parametric bootstrap bias-reduction method developed for association analysis and implemented in software (Faye et al. 2011, Sun et al. 2011) has been well demonstrated for disease status and quantitative traits in the GWAS setting. The method can adjust for both threshold and ranking selection effects arising from use of stringent genome-wide significance criteria and maximization of the association statistics over the genome, respectively. Motivated by genetic association analysis of complications in type 1 diabetes (Al-Kateb et al. 2008, Paterson et al. 2010), we extend use of the method to longitudinal study designs in which the phenotype of interest is time to observation of an event, as time to event data tend to have more power than other longitudinal measures. In this case, the phenotype is the time from baseline to clinical diagnosis of severe nephropathy in 1362 white participants of the Diabetes Control and Complications Trial (DCCT), the effect size parameter is the natural log of the hazard ratio (logHR), and the test statistic for association is the Wald statistic under additivity. In application to genetic association analysis of 1228 SNPs in 212 candidate genes with minor allele frequency (MAF) greater than 5 percent, using the freely available software BR-squared (Bias-Reduced estimates by Bootstrap Resampling), we observed 41.8-79.9 percent reduction of the logHR for 15 SNPs with nominal 1 percent significance. For the previously reported top SNP, rs17880135 in SOD1 (MAF=5.6 percent, p=8.2 x10-4, 95 percent logHR CI: 0.28-1.09), reduction in the logHR was 73.0 percent (from 0.69 to 0.19). With only 115 events observed, validity of the bootstrap in sparse data is a concern and warrants continued investigation. Using selected candidate gene SNPs, we further consider how the approach can be applied to a simple gene-based allele count for low MAF SNPs, similar to that current in sequencing applications. In principle the approach is applicable in high density genome-wide studies. The extension to survival-type models demonstrates potential for extensions to other phenotypes and to methods adapted for low frequency variants.

730F

Discovery and replication of gene-gene interactions in multiple independent Alzheimer's Disease Genetics Consortium datasets. *T.A. Thornton-Wells[1], K.D. Brown-Gentry[1], E.S. Torstenson[1], S.M. Dudek[1], L. Jiang[1], M.D. Ritchie[1], E.R. Martin[2], M.A. Pericak-Vance[2], J.L. Haines[2], Alzheimer's Disease Genetics Consortium.* 1) Center for Human Genetics Research, School of Medicine, Vanderbilt University, Nashville, TN; 2) John P. Hussman Institute for Human Genomics, Miller School of Medicine, University of Miami, Miami, FL.

Background. Alzheimer disease (AD) has a complex genetic etiology, involving heterogeneity and gene-gene interactions. Recent GWAS in AD have led to the discovery of novel genetic risk factors; however, the investigation of gene-gene interactions in GWAS has been limited. **Methods.** We conducted a gene-gene interaction analysis in one discovery dataset from University of Miami, Vanderbilt University and Mt. Sinai School of Medicine (UM/VU/MSSM) and two replication datasets: (a) the Translational Genomics Research Institute series 2 (TGEN2), and (b) the Alzheimer Disease Neuroimaging Initiative (ADNI). All cohorts were genotyped using Illumina or Affymetrix SNP microarrays, and each dataset was imputed using MaCH with HapMap Phase 2 CEU samples. We used a biological knowledge-driven approach (Biofilter) to select SNP-SNP models with a priori evidence that their genes interact or participate in common biological pathways or processes. (SNPs within 50kb of APOE were excluded.) We analyzed each SNP-SNP model using the multifactor dimensionality reduction (MDR) method and selected models with a testing balanced accuracy (testBA) ⁄ 55%. We then used a gene-centric approach for model replication. For each replication dataset, we ran MDR on all SNP-SNP models comprising genes from the selected discovery dataset models, and we selected all models with a testBA⁄ 55% in at least 2 of 3 datasets. **Results.** Six gene-gene models discovered in the UM/VU/MSSM dataset were replicated in both the TGEN2 and ADNI datasets, each with an average testBA⁄ 58% and a maximum testBA (for the best SNP-SNP model in a single dataset) of up to 72%. Seven gene-gene models replicated in only one dataset with average testBA⁄ 60% and a maximum testBA of up to 79%. ABCC9 appeared in 2 models that replicated in both datasets--one with ABCB1 (a major component of the blood-brain barrier that purportedly plays a role in A) clearance) and one with TF (which has been associated with iron overload and oxidative stress in AD). SMAD3 (which is bound by pTau in AD brain) appeared in 2 models, one of which replicated in both datasets. **Conclusions.** Using a biological knowledge-driven approach, we were able to identify SNP-SNP models of interest that replicated at the gene level across multiple datasets. Logistic regression analyses are ongoing to further characterize the models, and permutation testing will be used to obtain an unbiased estimate of significance.

731F

An evaluation of the informativeness of population genetics when making genotype calls in exome-captured next generation sequencing data. *S. Vattathil[1,2], F.A. San Lucas[1,2], Y. Fan[3], W. Yang[3], W. Wang[1], S.P. Hunger[4], M. Loh[5], M. Devidas[6], C-H. Pui[3], E. Mardis[7], M.V. Relling[3], P. Scheet[1,2].* 1) Epidemiology, University of Texas MD Anderson Cancer Center, Houston, TX; 2) The University of Texas Graduate School of Biomedical Sciences, Houston, TX; 3) St. Jude Children's Research Hospital, Memphis, TN; 4) The Children's Hospital and the University of Colorado Cancer Center, Aurora, CO; 5) Pediatrics, University of California, San Francisco, San Francisco, CA; 6) Biostatistics, University of Florida, Gainesville, FL; 7) The Genome Institute at Washington University, St. Louis, MO.

Most algorithms for making SNP and genotype calls from next-generation sequencing data do not rely solely on read counts, but incorporate additional sources of information to increase the accuracy of the calls. The use of population genetic data, such as population allele frequencies or patterns of linkage disequilibrium, helps when data quality is poor, or reads sparse. Accounting for technological artifacts, such as with sequencing error rates and base-specific quality scores, is important due to the relatively high error rates for current sequencing methods. These additional parameters may be defined in various ways. For example, allele frequencies may be calculated directly from the read data, estimated jointly with the genotypes, or measured in a larger reference population that may be somewhat genetically distinct. Technological error rates may be assumed a priori or estimated from the data. We tested several methods for defining the population and sequencing fidelity parameters within a simple calling algorithm based on allelic counts, and compared the genotype accuracies produced in each case. Our sample consisted of constitutional DNA collected from 27 individuals, with exome capture using the Nimblegen SeqCap EZ Exome v2.0 array and sequencing conducted via an Illumina HiSeq. We compared the sequence-based calls to genotypes inferred from an Affymetrix SNP 6.0 microarray experiment, and, because coverage was high (98% of sites in the target region were covered at 20x), we also assessed accuracy using the sequence data alone by “downsampling” the reads and comparing genotypes estimated from the full and reduced datasets. This allowed us to measure false-positive and false-negative rates for variant detection across multiple ranges of read depth and allele frequency.

732F

Further replication of the TRAF1/C5 genetic association with radiological damage in patients with rheumatoid arthritis. *S. Viatte[1, 4], D. Plant[1, 4], M. Lunt[1], B. Fu[1], B. Parker[1], J. Galloway[1], C. Solymossy[2], J. Worthington[1], D. Symmons[1], J. Dixey[3], A. Young[2], A. Barton[1].* 1) Arthritis Research UK Epidemiology Unit, Manchester Academic Health Science Centre, The University of Manchester, Manchester, United Kingdom; 2) Rheumatology Department, St Albans City Hospital, St Albans, United Kingdom; 3) Department of Rheumatology, New Cross Hospital, Wolverhampton, United Kingdom; 4) Equal contribution.

Background: A rheumatoid arthritis (RA) susceptibility region between the TNF receptor-associated factor-1 and complement component 5 genes (TRAF1/C5) has previously been reported to associate with radiological damage in two studies. We aimed to investigate RA genetic susceptibility markers, including variants at the TRAF1/C5 locus, as determinants of disease severity in an independent inception cohort of UK RA patients and to combine this with a previous study in a UK population. Methods: Sixty-seven RA susceptibility variants, were genotyped in 474 patients from the Early Rheumatoid Arthritis Study (ERAS) using Sequenom MassArray technology. Correlation between genetic markers and Larsen score was assessed at baseline, year 3 and year 5 follow-up. Data were combined with previously published data from the Norfolk Arthritis Register (NOAR) and analysed using longitudinal statistical models to include repeat measurements in the same individual at different time points. All analyses were adjusted for symptom duration at baseline. Results: A correlation was observed between rs2900180 at the TRAF1/C5 locus and Larsen score at year 3 (coef. 4.27 95%CI 0.49, 8.04, p=0.03) and in the longitudinal regression analysis (coef. 3.80 95%CI 0.81, 6.79, p=0.01) in ERAS. Combined longitudinal analysis of NOAR and ERAS samples increased the statistical evidence for association at the locus (coef. 2.15 95%CI 0.80, 3.49, p=0.002). Conclusions: The genetic marker rs2900180 is associated with extent of erosions, as measured by the Larsen score in the ERAS cohort. This represents the third independent study correlating genotype at the TRAF1/C5 locus with radiologic severity in RA.

733F

Association of *matrix metalloproteinase-7* and *-12* genes polymorphisms with asthma: A case-control study of *MMP-7* and *-12* in a Japanese population. *F. Yamaide[1,2], Y. Mashimo[1], N. Shimojo[2], T. Arima[2], Y. Morita[2], T. Hirota[3], S. Doi[4], K. Sato[5], S. Suzuki[5], T. Nishimuta[5], H. Watanabe[5], A. Hoshioka[6], M. Tomiita[6], A. Yamaide[6], M. Watanabe[7], Y. Okamoto[8], Y. Kohno[2], M. Tamari[3], A. Hata[1], Y. Suzuki[1].* 1) Department of Public Health, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba, Japan; 2) Department of Pediatrics, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba, Japan; 3) Laboratory for Respiratory Diseases, Center for Genomic Medicine, RIKEN, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa, Japan; 4) Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, 3-7-1 Habikino-shi, Habikino, Osaka, Japan; 5) Department of Pediatrics, National Shimoshizu Hospital, 934-5 Sikawatashi, Yotsukaido-shi, Chiba 284-0003, Japan; 6) Department of Allergy and Rheumatology, Chiba Children's Hospital, 579-1 Heta-cho, Midori-ku, Chiba, Japan; 7) The First Department of Pediatrics, Toho University Omori Medical Center, 6-11-1 Omorinishi, Ota-ku, Tokyo, Japan; 8) Department of Otorhinolaryngology and Head and Neck Surgery, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba, Japan.

[Background] Genetic variants influencing lung function or immune system may be involved in the development of asthma and/or its symptoms. Matrix metalloproteinases (MMPs), a family of secreted or membrane-bound endopeptidases, contribute to both normal and pathological tissue remodeling and also act as regulatory molecules by processing cytokines or adhesion molecules. In animal models, growing evidences suggest that MMPs play important roles in asthma phenotypes. Some *MMP* genes (e.g. *MMP9*) have recently been shown to be associated with asthma in Caucasian populations. [Objectives] We investigated whether single nucleotide polymorphisms (SNPs) in *MMP7* and *MMP12* could affect the susceptibility to and clinical phenotypes of asthma in the Japanese population. [Method] We conducted a case-control study between SNPs in *MMP7* and *MMP12* genes and asthma-related phenotypes using childhood and adult Japanese populations (653 childhood asthma patients and 423 controls, and 428 adult asthma patients and 646 controls, respectively). To investigate the effects of amino acid substitutions by SNPs on MMPs' enzymatic activity, MMP activity assays were performed using commercially available kits based on fluorescence resonance energy transfer (FRET) peptide. We also evaluated the effect of 3'UTR SNP in *MMP7* on its mRNA stability and the effect of SNP in *MMP12* on its antimicrobial activity. [Result] We found that, in the Japanese population, SNPs of *MMP7* (rs10502001, G/A, Arg77His; rs14983, C/T, 3'UTR) (P = 0.006; odds ratio (OR), 1.46; 95 % confidential interval (CI), 1.126 - 1.903) and *MMP12* (rs652438, A/G, Asn357Ser) (P = 0.015; OR, 1.60; 95 % CI, 1.002 - 2.556) showed significant association with adult and childhood asthma, respectively. We also found that the SNP (rs652438) in *MMP12* was associated with severity in adult asthma (P = 0.010). Using supernatant from cultured HEK293 cells stably transfected with the pcDNA3.1(+)-MMP7 or MMP12 as MMP proteins, we evaluated activation kinetics, rate of proteolytic cleavage of FRET peptide, Michaelis constant, and substrate specificity of the enzyme. In this system, we couldn't detect the functional effects of amino acid substitutions by SNPs on the enzymatic activity. [Conclusion] Our association study suggested that genetic variants of *MMP7* and *MMP12* conferred risk for development of asthma in the Japanese population. We are now investigating the effect of the SNPs on mRNA stability and antimicrobial activity.

734F

RVTests: Rare-variant test package. *X. Zhan[1,2], Y. Hu[1,2], G. Abecasis[1,2].* 1) Department of Biostatistics, University of Michigan, Ann Arbor, MI; 2) Center of Statistical Genetics, University of Michigan, Ann Arbor, MI.

Next-Generation Sequencing(NGS) technology brings scientific community unprecedented amount of data. Existing tools are not maturely developed to conveniently handle sequencing data and are inefficient in helping researcher study the association between rare variant and phenotypes. With these considerations in mind, we developed RvTests, an open sourced C++ statistical package, that focuses on speed, accuracy and flexibility in manipulate large sequencing data set and provides interfaces for statisticians to develop their own statistical methods. We have integrated well-known statistical procedures, including exact test, Li's CMC method, Madsen-Browning's weighted method and et al, in the logistic regression framework. We also ease the procedure of exporting sequencing data for mature GWAS analysis tools, including PLINK, Merlin, and VCF. We will describe typical work flows that can be simplified by our package and will illustrate several of the features currently under active development.

735F

Combining Association Tests for Rare Variant Analysis. *H. Zhou, K. Hao, C. Molony.* Merck Research Laboratories, Boston, MA.

The fast advancement of the next-generation sequencing technology has enabled us to identify rare variants with large genetic effects, which otherwise could hardly be captured by previous genome-wide association studies. Due to the low frequency of rare variants, statistical methods used for common variant analysis might not have optimal performance in the next-generation sequencing studies. Several alternative strategies, such as collapsing approach (TC), weighted-sum method (TW), and variable-threshold approach (TV), have been proposed to jointly analyze a group of variants within a gene and test for associations. However, these methods may not be robust when non-causal variants are included or when both protective and risk variants are pooled in the analysis. Extending our previous method developed for multiple common variant analysis, we present a testing approach that combines single rare variant association test p values. This combination is based on the summation of single test p values, giving greater weight to those with smaller p values. The performance of the combining approach was evaluated using realistic sequence-level data generated by FREGENE. Two scenarios were modeled: one panmictic population and one population incorporating bottlenecks, periods of growth and subdivision. For each population, one neutral model and one complex selection model were simulated. We compared the combining method to alternative tests (TC, TW, and TV) in identifying susceptibility genes under these scenarios. It is shown that the combining method is preferred and robust against the existence of non-causal variants or causal variants with opposite effects. This study demonstrates that the proposed method can be an optimal test for rare variant analysis.

736F

Critical comparison of deep resequencing analysis methods in a population sample of over 3000 subjects. *L. Li[1], L. Warren[1], J. Shen[1], V.E. Mooser[2], D.M. Waterworth[2], J.C. Whittaker[3], M.R. Nelson[1].* 1) Statsitical Genetics, GlaxoSmithKline, Res Triangle Park, NC, USA; 2) GlaxoSmithKline, Upper Merion, PA, USA; 3) GlaxoSmithKline, Stevenage, UK.

Rare variants are widely believed to contribute to phenotypic variation independently from or in conjunction with common variants. However, the methods used for analysis of common variants lack the power to detect associations due to rare variants even in very large samples. The rapid development and adoption of next-generation sequencing technologies has spurred corresponding innovation in statistical methodology. More than 20 analysis methods have been proposed, most aimed at assessing the collective effect of multiple rare variants within genes or other genomic regions of interest. Within such a large pool of analysis options, there is no method that is universally most powerful, so how do we choose which methods to apply? How do we interpret conflicting association results from different methods? Although answers to these questions can be addressed in part through simulation, rigid assumptions strongly influence the results and limit our ability to draw general conclusions. Here we present insights gained from analysis of 202 drug target genes resequenced in over 3000 European and Indian Asian population samples from the CoLaus and LOLIPOP studies, extensively phenotyped for cardiovascular and metabolic-related continuous and binary traits. We analyzed 42 traits, including several previously known or suspected associations, with a broad range of newly developed methods and predictions of functionality from PolyPhen and SIFT. We found many examples of widely concordant and discordant inferences among the methods and have investigated their underlying differences. We will compare analysis results from the different methods, share our reflections on their relative strengths and weaknesses and discuss the challenges and opportunities in working with such deep resequence data.

737F

Keloid susceptibility loci 1q41, 3q22.3, and 15q21.3 replicated in a Nigerian population. *S. Hooker Jr[1], P.B. Olaitan[2], O.M. Oluwatosin[3], S.A. Fadiora[2], S. Ademola[3], V. Odesina[4], S.M. Leal[1], E.J. Reichenberger[5].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Surgery, Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Osun State, Nigeria; 3) Department of Surgery, University of Ibadan, Ibadan, Oyo State, Nigeria; 4) HIV Adherence Program, University of Connecticut Health Center, Farmington, CT; 5) Department of Reconstructive Sciences, University of Connecticut Health Center, Farmington, CT.

Keloids are overgrown scars that stem from abnormal wound healing, usually as a result of injury. Keloids have a higher incidence in darker-skinned individuals with the greatest prevalence being found in individuals of African ancestry. Although keloids were first described centuries ago their pathogenesis is poorly understood. To better understand the genetic etiology of keloids, recently a genome-wide association (GWA) study was performed using a Japanese population (Nakashima et al., Nat Genet 2010). This study identified four variants on chromosomes 1q41, 3q22.3 and 15q21.3 which were associated at GWA significance level p-values. Two of the significant SNPs in the 3q22.3 region are not in linkage disequilibrium and therefore represent two separate regions. Only one of the SNPs lies within a known gene, NEDD4 on chromosome 15. We genotyped 96 tagSNPs within these regions in 286 cases and 267 controls, all of whom are Yorubans that were ascertained in Nigeria. Analysis was performed within a logistic regression framework using an additive allelic effect model controlling for both age and sex. Within these regions multiple SNPS were found to be associated with Keloids, the most significant findings for each region were with markers rs753961 (chr1: 220.3 Mb) OR = 0.58 ($p = 3.45 \times 10^{-3}$); rs13064974 (chr3:140.1Mb) OR = 1.32 ($p = 2.6 \times 10^{-2}$) ; rs940187 (chr3:140.3Mb) OR = 1.33 ($p = 2.7 \times 10^{-2}$) ; and rs8031043 (chr15: 53.9 Mb) OR = 0.68 ($p = 2.82 \times 10^{-3}$). For the two polymorphic SNPs which were genotyped in both studies, rs873549 (1q41) and rs940187 (3q22.3), the minor alleles increased the risk of keloid development in both studies. This is the first study to characterize the genetic susceptibility of keloids in a sub-Saharan African population.

738F

Genotype at the NOS3 G894T SNP is not associated with acute mountain sickness upon ascent to 4380 m or during brief exposure to an equivalent normobaric hypoxia. *M.J. MacInnis[1, 2], E.A. Carter[1], M.S. Koehle[1, 2], J.L. Rupert[1].* 1) School of Kinesiology, University of British Columbia, Vancouver, British Columbia, Canada; 2) Division of Sports Medicine, University of British Columbia, Vancouver, British Columbia, Canada.

Introduction: Acute mountain sickness (AMS) develops upon exposure to altitude and normobaric hypoxia (simulated altitude), and evidence suggests that susceptibility to AMS is partially genetic. The endothelial nitric oxide synthase gene, *NOS3*, was previously associated with AMS; however, the association has not been replicated, and its physiological basis is unclear.

Methods: Subjects were recruited for two separate experiments. Nepalese subjects (n=77) were recruited and assessed at the 2010 Janai Purnima festival, Gosainkunda, Nepal (4380m), and Caucasian subjects (n=19) were recruited and assessed during a 6-hour exposure in a normobaric hypoxia chamber (12% O_2; ~4400m). The Lake Louise Score (LLS) was used to diagnose AMS (AMS+/AMS-) and to quantify AMS severity, and PCR-RFLP was used to determine genotype at the *NOS3* G894T SNP. Heart rate (HR) and oxygen saturation (SpO_2) were measured in both conditions, and exhaled NO was measured in the normobaric hypoxia chamber using a NObreath analyzer (Bedfont Scientific, UK).

Results Variants of the NOS3 G894T SNP were not associated with susceptibility to AMS during exposure to altitude (p=0.84) or normobaric hypoxia (p=0.053). There was also no difference in genotype frequencies when the two populations were combined (p=0.34). Genotype was not associated with LLS, SpO_2, or HR during exposure to altitude (p>0.05) or normobaric hypoxia (p>0.05). Exhaled NO during normobaric hypoxic exposure was unrelated to the G894T genotype (p>0.05).

Discussion: The *NOS3* G894T SNP was previously associated with AMS in a Nepalese cohort, but we did not replicate this association in a separate Nepalese cohort at altitude or in a Caucasian cohort exposed to simulated altitude. The relatively small sample sizes resulted in too little power to detect the expected small-moderate effect; however, the overall distribution of genotypes was consistent with a protective role for the G allele, and the Caucasian cohort was near statistical significance. The discordance between the two Nepalese cohorts might be due to stratification resulting in unexpected heterogeneity, and we are currently using AIMs to investigate this possibility. The physiological basis of the previous association remains unclear.

739F

The Functional MICA-129 Polymorphism is Associated with Psoriatic Disease Independently of HLA-B and C. *R.A. Pollock[1], V. Chandran[1], J. Barrett[2], L. Eder[1], F. Pellett[1], C. Yao[1], M. Lino[1], S. Shanmugarajah[1], V.T. Farewell[2], D.D. Gladman[1].* 1) Psoriatic Arthritis Program, Toronto Western Research Institute, Toronto, Ontario, Canada; 2) MRC Biostatistics Unit, University of Cambridge Institute of Public Health, Cambridge, UK.

Objective: The major histocompatibility complex class I chain-related gene A (MICA) is located 47kb centromeric to HLA-B. MICA alleles can be classified into high and low-affinity binders of the natural killer/T-cell receptor NKG2D, based on a functional polymorphism at amino acid 129 (A/G>Met/Val). Our aim was to determine whether the high affinity MICA-129 Met allele is increased in psoriatic arthritis (PsA) patients compared to patients with psoriasis without arthritis and controls. Methods: 248 unrelated Caucasian PsA patients, 250 psoriasis subjects without arthritis, and 249 healthy controls were allelic typed for MICA using PCR-SSP and for HLA-B and C by PCR-SSO reverse line blot. All PsA patients satisfied CASPAR criteria and psoriasis subjects were examined by a rheumatologist to exclude PsA. MICA-129 Met/Val genotypes were assigned from allelic typing using DNA sequences available from the IMGT/HLA database (release 3.1.0). Univariate logistic regressions and chi squared tests were performed to determine the effect of MICA-129 genotype on group membership. Multivariate logistic regressions were also performed using the Val/Val genotype as the reference category, to adjust for the presence of risk alleles HLA-B*13, B*27, B*38, B*57, C*01, C*02, C*06, and C*12. Results: Univariate analyses showed that the presence of a Met allele significantly increased the risk of developing psoriatic disease (OR=1.6, p=1.5x10-3), psoriasis without arthritis (OR=1.7, p=5.3x10-3), and PsA (OR=1.6, p=7.7x10-3). Multivariate analyses showed that after adjustment for significant HLA-B and C alleles, homozygosity for the Met allele (genotype Met/Met) significantly increased risk of psoriatic disease (OR= 3.8, p= 1.0x10-4), psoriasis without arthritis (OR = 2.8, p= 6.1x10-3), and PsA (OR = 2.4, p= 2.7x10-2). Heterozygosity (Met/Val) did not affect risk. There were no significant differences in MICA genotypes between patients with PsA and psoriasis without arthritis. Conclusions: Individuals with a high-affinity Met residue at MICA-129, particularly those who are homozygous for the Met allele (Met/Met), have an increased risk of developing psoriatic disease, psoriasis without arthritis, and PsA independently of the presence of HLA-B and C risk alleles. This study is currently being validated in independent cohorts of patients and new results will be presented.

740F

Polygenic and SNP-specific Genotype x Age Interaction Effects on Telomere Length. *J.W. Kent[1], S. Kumar[1], M. Carless[1], T.D. Dyer[1], V.P. Diego[1], J. Charlesworth[2], M.P. Johnson[1], A.G. Comuzzie[1], M.C. Mahaney[1], L. Almasy[1], J.E. Curran[1], E.K. Moses[1], H.H.H. Goring[1], J. Blangero[1], S. Williams-Blangero[1].* 1) Dept Gen, Tx Biomed Res Inst, San Antonio, TX; 2) Menzies Tasmania Res Inst, Hobart, TAS, Australia.

Genotype x environment interaction may contribute to differential risk of age-related physiological decline and progression of diseases of senescence. Telomeres are terminal chromosomal structures consisting of a repeated DNA sequence and its associated proteins; age-related reduction in telomere length is associated with genomic instability and cell senescence/death. We have previously reported GWA and linkage analysis of telomere repeat/single-copy sequence ratio (TSR), an efficient measure of telomere length, in peripheral blood mononuclear cells in 1,232 Mexican Americans in ~40 extended families. Here we compare these results to those for TSR measures in 1,089 Nepali Jirel in a single highly extended pedigree. TSR is heritable in both cohorts (Mexican Americans: h2 = 0.25 +/- 0.05, p = 5.2x10-12; Jirel: h2 = 0.22 +/- 0.05, p = 1.0x10-7. Family data permit polygenic genotype x age (GxA) analyses, with age taken as a proxy for lifetime exposure to environmental and physiological stressors. There is significant evidence of polygenic GxA in both cohorts (Mexican Americans, p = 0.00025; Jirel, p = 0.024), suggesting that at least some genes responsible for variation in TSR are differentially affected by environmental stress. We further compare GWA and linkage evidence from both ethnically distinct cohorts, as well as evidence for SNP-specific GxA for TSR.

741F

Gene polymorphisms associated with optic nerve vertical cup-to-disc ratio are risk factors for primary open angle glaucoma. *F. Mabuchi[1], Y. Sakurada[1], K. Kashiwagi[1], Z. Yamagata[2], H. Iijima[1], S. Tsukahara[1].* 1) Dept Ophthalmology, Univ Yamanashi, Chuo, Yamanashi, Japan; 2) Dept Health Sciences, Univ Yamanashi, Chuo, Yamanashi, Japan.

Purpose: To assess whether the gene polymorphisms associated with optic disc vertical cup-to-disc ratio (VCDR) are risk factors for primary open angle glaucoma (POAG). Methods: Four hundred and twenty-five Japanese patients with POAG, including normal tension glaucoma (NTG, n = 213) and high tension glaucoma (HTG, n = 212), and 191 control subjects without glaucoma were analyzed for 7 gene polymorphisms associated with VCDR; rs1063192 (*CDKN2B*), rs10483727 (*SIX1*), rs17146964 (*SCYL1*), rs1547014 (*CHEK2*), rs1900004 (*ATOH7*), rs1926320 (*DCLK1*), and rs12015126 (*RERE*). The allele frequencies were compared between the patients with NTG or HTG and the control subjects. A logistic regression model was used to study the effects of the risk alleles when comparing the POAG patients with control subjects. Demographic and clinical features, including maximum intraocular pressure (IOP), in patients with POAG were compared between the genotypes. Results: The T allele frequency of rs1063192 (*CDKN2B*) and the A allele frequency of rs1900004 (*ATOH7*) were significantly higher (p = 0.0023 and p = 0.028 respectively) in patients with NTG than in the control subjects (86.2% vs. 77.7% and 39.4% vs. 31.9% respectively). Adjusted for age, gender, refractive error, and IOP, an almost 1.7 and 1.6 times increased risk of POAG was found with the T allele of rs1063192 (P = 0.032, odds ratio 1.73, 95% confidence interval 1.05 to 2.87) and the A allele of rs1900004 (P = 0.012, odds ratio 1.64, 95% confidence interval 1.12 to 2.41) respectively. The ages at diagnosis of the NTG patients with the TT genotype of rs10483727 (*SIX1*) and the CT or TT genotypes of rs1926320 (*DCLK1*) were significantly younger (P = 0.017 and P = 0.040 respectively) than those of the NTG patients without these genotypes. The ages at diagnosis of the HTG patients without CC genotype of rs12015126 (*RERE*) were significantly younger (P = 0.037) than those of the HTG patients with the CC genotype. There were no significant associations between the maximum IOP and the genotypes in patients with POAG. Conclusions: *CDKN2B* and *ATOH7* gene polymorphisms are considered to be non-IOP related genetic risk factors for POAG. *SIX1*, *DCLK1*, and *RERE* gene polymorphisms influence the phenotypic features in patients with NTG or HTG, and may contribute to POAG risk.

742F

A rare penetrant mutation in CFH confers high risk of age-related macular degeneration. *S. Raychaudhuri[1,2,3], O. Iartchouk[3], K. Chin[4], P.L. Tan[5], A. Tai[6], S. Ripke[2,7], S. Gowrisankar[3], S. Vemuri[3], K. Montgomery[3], Y. Yu[4], R. Reynolds[4], D.J. Zack[8], B. Campochiaro[8], P. Campochiaro[8], N. Katsanis[5], M.J. Daly[2,7], J.M. Seddon[4,9].* 1) Department of Medicine, Brigham and Women's Hospital, Boston, MA; 2) Program in Medical and Population Genetics, Broad Institute, Cambridge, MA; 3) Partners HealthCare Center for Personalized Genetic Medicine, Boston, MA; 4) Ophthalmic Epidemiology and Genetics Service, New England Eye Center, Tufts Medical Center, Tufts University School of Medicine, Boston, MA; 5) Center for Human Disease Modeling and Departments of Cell Biology and Pediatrics, Duke University, Durham, NC; 6) Study Center on the Immunogentics of Infectious Disease, Department of Pathology, Tufts University School of Medicine, Boston, MA; 7) Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, MA; 8) McKusick-Nathans Institute of Genetic Medicine, Department of Ophthalmology, Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD; 9) Department of Ophthalmology, Tufts University School of Medicine, Boston, MA.

Two common variants within CFH, the Y402H variant and the intronic rs1410996 SNP explain 17% of the total variance of age-related macular degeneration (AMD) liability. However, definitive proof for the involvement of CFH, as opposed to a neighboring transcript, as well as information about the potential mode of action of susceptibility alleles are lacking. Under the premise that rare functional variants might establish the causality of CFH and provide mechanistic insights, we used genotype and high throughput sequencing data to query the CFH region in a large cohort of AMD patients. We discovered a rare high-risk CFH haplotype. Sequencing introns, exons, and flanking regions of the CFH gene (100kb) in 84 individuals, including heterozygotes for the rare high-risk haplotype, revealed the presence of an R1210C mutation on that haplotype. This allele has been implicated previously in atypical hemolytic uremic syndrome, and results in loss of C-terminal ligand binding. We genotyped the R1210C mutation in 2,423 AMD cases and 1,122 controls with a TaqMan assay and found this rare allele to be associated with disease ($p=7.0x10^{-6}$), to exhibit high penetrance (present in 40 cases versus 1 control), and to track significantly with a six year earlier onset of disease ($p=2.3x10^{-6}$). This result suggests that loss of function alleles at CFH likely drive AMD risk. To our knowledge, this finding represents one of the first instances where a common variant for a complex disease has led to the discovery of a rare mutation with a large effect.

743F

Interaction between genetic and epigenetic variation affects regulatory mechanisms at the asthma-associated locus 17q12-q21. *S. Moussette[1,2], S. Berlivet[2], M. Ouimet[3], D.J. Verlaan[4,5,3], V. Koka[4], A. Al Tuwaijri[2,5], D. Sinnett[3,6], T. Pastinen[4,5], A.K. Naumova[1,2,5].* 1) The Research Institute of MUHC, Montreal, Quebec, Canada; 2) Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada; 3) Sainte-Justine University Health Centre, Montreal, Quebec, Canada; 4) McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, Canada; 5) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 6) Department of Pediatrics, University of Montreal, Montreal, Quebec, Canada.

Phenotypic variation results from variation in gene expression, which may be modulated by genetic and/or epigenetic factors. To understand the molecular basis of human disease, interaction between genetic and epigenetic factors needs to be taken into account. The asthma-associated region 17q12-q21 harbors three genes, *ZPBP2*, *GSDMB* and *ORMDL3*, that show allele-specific differences in expression levels in lymphoblastoid cell lines and CD4+ T-cells. Genetic association between *cis*-regulatory polymorphisms and expression levels of *GSDMB* and *ORMDL3* reaches genome-wide significance, whereas the genetic *cis*-effect associated with *ZPBP2* expression has lower significance level[1]. Here we report the molecular dissection of allele-specific transcriptional regulation of the genes within the chromosomal region 17q12-q21 using *in vitro* transient transfection, formaldehyde-assisted isolation of regulatory elements, chromatin immunoprecipitation and sodium bisulfite DNA methylation assays. We found that a single nucleotide polymorphism produces an allelic effect on the activity of *ZPBP2* promoter region *in vitro*, and also leads to nucleosome repositioning on the asthma-associated allele. Analysis of epigenetic states shows that variable methylation of exon 1 of *ZPBP2* masks the strong genetic effect on *ZPBP2* promoter activity. In contrast, the *ORMDL3* promoter is fully unmethylated, which allows detection of genetic effects on its transcription. We conclude that the *cis*-regulatory effects on 17q12-q21 gene expression result from interaction between several polymorphisms and epigenetic factors. Moreover, our data suggest that GWAS studies are more likely to detect association for genes whose regulatory elements are not methylated.

1.Verlaan, D.J. *et al*. Allele-specific chromatin remodeling in the *ZPBP2*/*GSDMB*/*ORMDL3* locus associated with the risk of asthma and autoimmune disease. *Am J Hum Genet* **85**, 377-93 (2009).

744F

Epigenetic changes subsequent to fetal exposure to dioxin. *A. Paoloni-Giacobino[1,2], E. Somm[3], C. Stouder[1].* 1) Dept Gen Med & Development, Geneva Univ Med Sch, Geneva 4, Switzerland; 2) Swiss Center for Applied Human Toxicology, University of Geneva Medical School, 1211 Geneva 4, Switzerland; 3) Department of Pediatrics, Geneva University Hospital, 1211 Geneva 14, Switzerland.

The endocrine disruptor dioxin, a by-product of numerous industrial processes that, being resistant to degradation, accumulates in the environment, is a potent reproductive and developmental toxicant. Paternal exposure to relatively low doses of dioxin was reported to decrease the sex ratio and maternal exposure during pregnancy to decrease ano-genital distance, prostate weight and sperm count in the male offspring. Dioxin might act by interfering with hormone secretion and/or action. Its toxicity might also be mediated by epigenetic changes in DNA methylation. This study addressed possible effects of dioxin on the methylation pattern of imprinted genes. Pregnant female mice were administered daily between days 9 and 19 of gestation 2 different doses of dioxin (2 or 10 ng/kg) and possible methylation changes in the differentially methylated domains of 2 maternally- (Snrpn and Peg3) and 1 paternally- (Igf2) imprinted genes were investigated in of 8-week-old male F1 offspring. The tissues analyzed were the sperm, the liver and the skeletal muscle. No change was observed in the level of methylation of Snrpn, whereas that of Peg3 was decreased in the liver at both dioxin doses and that of Igf2 was decreased in the sperm and in the liver but only at the higher dose of dioxin and increased in the muscle at both dioxin doses. Interestingly, the hypermethylation of Igf2 in the muscle was paralleled by a decreased mRNA expression of this promyogenic gene. The possibly direct toxic effects of dioxin on somatic cells might in particular affect muscle growth in the offspring, whereas an effect of dioxin on the imprinted gene Igf2 in the sperm, suggests a mechanism for transgenerational effects of this toxicant and possibly of other compounds with endocrine disrupting properties.

745F

Measures of global DNA methylation in post-mortem Alzheimer's disease brain. *Y.G. Patel[1], J. Mill[2], J.F. Powell[1].* 1) MRC Centre for Neurodegeneration Research, Institute of Psychiatry, Kings College London, UK; 2) Psychiatric Epigenetics Group, MRC SGDP Research Centre, Institute of Psychiatry, Kings College London, UK.

A role for epigenetics in the complex aetiology of the neurodegenerative disease Alzheimer's disease (AD) has been proposed. The most studied epigenetic mark is DNA methylation (the addition of methyl groups to cytosine located in CpG dinucleotides) and is known to change over time with aging and may also reflect more subtle changes in gene expression. An increasing number of measures of global DNA methylation have become available in recent years from ELISA (Enzyme-Linked ImmunoSorbent Assay) based assays to those adapted to quantitative analysis by pyrosequencing at CpG dinucleotides. Furthermore methylation status of repetitive elements (i.e. Alu and LINE1) is a major contributor to global DNA methylation patterns and has already been investigated in a variety of human diseases including cancers.

We have studied both global DNA methylation (by ELISA) and the more specific CpG dinucleotide based methylation of repetitive elements (at LINE1 and Alu elements) in post-mortem brains of AD cases and controls (20 AD cases and 20 controls). Preliminary results from comparing affected (Superior Temporal Gyrus) and unaffected (Visual Cortex) areas of AD brain and age-matched cognitively normal controls show a trend for a decline in global methylation and a significant decline at LINE1 CpGs in both affected and unaffected tissue. These changes in global methylation may reflect changes in tissue composition as a consequence of the neurodegenerative process and/or an affect of the oxidative and the inflammatory processes active in the degenerating Alzheimer's brain.

746F

The effects of alcohol and smoking on genome wide methylation patterns in women. *R. Philibert[1], J. Plume[2], S. Beach[3].* 1) Psychiatry, University of Iowa, Iowa City, IA; 2) Neuroscience and Genetics Programs, University of Iowa, Iowa City, IA; 3) The University of George, Athens, GA.

Smoking is associated with a wide variety of adverse health outcomes including depression, diabetes and heart disease. Unfortunately, the molecular mechanisms through which these effects are conveyed are not clearly understood. To examine the potential role of epigenetic factors in this process, we examined the relationship of smoking to both genome wide methylation patterns and gene expression in lymphoblast DNA from 165 female subjects from the Iowa Adoption Studies with respect to current smoking status using the Illumina 450 Methylation Bead Chip. We found that current smoking status was associated with significant genome wide changes in methylation at the single CpG residue and multi-residue resolution. Pathway analysis of differentially methylated genes demonstrated that the differentially methylated transcripts mapped to gene pathways known to involved in smoking related disease. Expression analysis of selected differentially methylated transcripts was informative and suggests that expression analyses will be necessary for the rigorous interpretation of methylation results. We conclude that smoking is associated with genome wide changes in DNA methylation and that the understanding of this area of epigenetics will shed new insight into smoking related disease.

747F

Disease-associated epigenetic changes in monozygotic twins discordant for schizophrenia and bipolar disorder. *R. Pidsley[1], E.L. Dempster[1], L.C. Schalkwyk[1], S. Owens[2], A. Georgiades[2], F. Kane[2], S. Kalidin[2], M. Picchioni[2], E. Kravariti[2], T. Toulopoulou[2], R. Murray[2], J. Mill[1].* 1) SGDP Centre, Institute of Psychiatry, London, United Kingdom; 2) Psychosis Studies, Institute of Psychiatry, London, United Kingdom.

Studies of major psychosis (schizophrenia and bipolar disorder) have traditionally focused on genetic and environmental risk factors, although recent research indicates a role for epigenetic processes in mediating susceptibility. Because monozygotic (MZ) twins share a common DNA sequence, the study of discordant twins represents an ideal design for investigating the contribution of epigenetic factors to disease etiology. We performed a genome-wide screen of DNA methylation on peripheral blood DNA obtained from a sample of MZ twin-pairs discordant for major psychosis (n=22 twin-pairs, 44 individuals). Numerous loci demonstrated disease-associated DNA methylation differences between twins discordant for schizophrenia and for bipolar disorder, and jointly for twins discordant for major psychosis. Pathway analysis of our top loci highlighted a significant enrichment of epigenetic disruption to biological networks and pathways relevant to disease and neurodevelopment. The top psychosis-associated DMR is located in the promoter of ST6GALNAC1, overlapping a previously reported rare genomic duplication observed in schizophrenia. This region was significantly hypomethylated in affected twins (p=4.03E-04). We subsequently assessed this region in post-mortem brain tissue from schizophrenia patients and controls finding marked hypomethylation across an extended region in ~13% of schizophrenia patients tested. Overall, our data provide further evidence to support a role for DNA methylation differences in the etiology of both schizophrenia and bipolar disorder.

748F

Germline *KILLIN* (*KLLN*) mutations or epimutations in *PTEN* mutation-negative Cowden syndrome and in patients with apparently sporadic renal and breast cancers. *E. Pontzer[1], K. Bennett[1], P. Funchain[1], J. Mester[1], H. Neumann[2], C. Eng[1].* 1) Genomic Medicine Institute, Cleveland Clinic, Cleveland, OH; 2) Section of Preventive Medicine, University of Freiburg Medical Center, Freiburg, Germany.

KLLN and *PTEN*, on 10q23, share a bidirectional promoter and are transcribed in opposite directions. Like PTEN, KLLN plays a role in the cell cycle and is transactivated by p53. Autosomal dominant Cowden syndrome (CS) is characterized by hamartomas and high risk of breast, thyroid and other cancers. While *PTEN* is believed to be the major CS gene, our recent study of 3042 probands with classic CS or CS-like (CSL) phenotypes revealed 25% of CS and 5% of CSL probands with germline pathogenic *PTEN* mutations. Linkage studies which pointed to 10q23 indicated no genetic heterogeneity. Our pilot study revealed *KLLN* germline hypermethylation (epimutation) in 37% of 123 *PTEN* mutation negative CS/CSL patients (Bennett JAMA2010) and in 56% of 41 sporadic clear cell renal cell cancer (ccRCC) patients (Bennett GCC2011). We hypothesized that both germline *KLLN* epimutation and mutation play roles in cancer predisposition. Mutation analysis of 136 *PTEN* mutation negative CS/CSL individuals, revealed a single germline *KLLN* mutation p.V17I. Through bisulfite sequencing and COBRA analysis of 154 *PTEN* mutation negative CS/CSL patients, 50 (32%; 95% confidence interval [CI] 24.63%-39.37%) were found to have germline *KLLN* epimutation, not seen in 50 controls. We demonstrated that *KLLN* promoter epimutation decreases *KLLN*, but not *PTEN*, expression 250-fold which can be reversed by demethylating agents. We also found that CS/CSL patients with *KLLN* epimutation have >2-fold increased prevalence of breast and renal cancers but without differences in thyroid or endometrial cancer prevalence. Because of the increased frequencies of breast and renal cancers in CS/CSL patients with *KLLN* epimutation, we analyzed *KLLN* for mutations and epimutations in individuals with apparently sporadic breast cancer (BC) or ccRCC. Of 52 patients with ccRCC, 2 (3.8%) had germline *KLLN* p.G44R mutations; of 67 ccRCC patients, 26 (39%; 95%CI27.32%-50.68%) were found to have *KLLN* epimutation. Of 20 patients with sporadic BC, one had a *KLLN* p.G44R mutation. No *KLLN* mutations were found in 57 controls. Our observations suggest that germline *KLLN* epimutation may be a new mechanism for CS/CSL with a high risk of breast and renal cancers. Further, germline *KLLN* epimutation may represent a low penetrance predisposition mechanism for ccRCC. These etiomechanisms not only will help in diagnosis, risk assessment and predictive testing, but also suggest future exploration of demethylating agents for prevention.

749F

Differential CpG site methylation classifies psoriatic and normal skin. *EDO. Roberson[1], Y. Liu[1], C. Ryan[2], C. Joyce[1], S. Duan[1], L. Cao[1], A. Martin[3], W. Liao[4], A. Menter[2], A. Bowcock[1,3].* 1) Dept of Genetics, Washington University, St. Louis, MO, U.S.A; 2) Dept of Dermatology, Baylor University Medical Center, Dallas, TX, U.S.A; 3) Dept of Medicine, Division of Dermatology, Washington University School of Medicine, St. Louis, MO, U.S.A; 4) Dept of Dermatology, University of California, San Francisco, CA, U.S.A.

Psoriasis is a chronic, inflammatory, immune-mediated disorder primarily affecting the skin as well as other organs, including joints. Psoriasis is relatively common in Caucasian populations with a prevalence of 1% or more, less common in Asian populations (0.1%) and very rare in African populations. Differential gene expression studies have identified over 1,300 transcripts altered in psoriatic involved skin compared to normal skin. However, a global epigenetic profile of psoriatic skin has not been described. We have performed the first genome-wide study of altered CpG site methylation in psoriatic skin. We determined the methylation levels at 27,578 CpG sites in skin samples from individuals with psoriasis (12 involved, 8 uninvolved) and 10 unaffected individuals using Illumina methylation beadarrays. CpG methylation of involved skin significantly differed from normal skin at 1,108 sites. Twelve of these CpG sites mapped to the epidermal differentiation complex, upstream or within genes that are highly up-regulated in psoriasis. Hierarchical clustering of 50 of the top differentially methylated (DM) sites separated psoriatic from normal skin samples, and also performed well at classifying psoriatic involved from psoriatic uninvolved skin. For some of the analyzed samples expression arrays had previously been performed, and correlation between methylation and expression was calculated. Sites with inverse correlations between methylation and nearby gene expression include those of KYNU, OAS2, S100A12, and SERPINB3, whose strong transcriptional up-regulation are important discriminators of psoriasis. We observed a small number of CpG sites with differential methylation in psoriatic uninvolved skin versus normal. We confirmed CpG methylation differences by pyrosequencing CpG sites in the region of three DM CpG sites. Multiple clinical trials have demonstrated the efficacy of TNF blockade for the treatment of psoriasis. When we examined the effect of this treatment on global CpG methylation we observed that after a month, methylation levels had started to change in the direction seen in uninvolved skin. Hence, psoriasis diagnosis and treatment response might be predicted by methylation changes in skin of patients.

750F

DNA methyltransferases expression and methylation status in adult offspring of hyperhomocysteinemic dams supplemented with methionine. *V.C. Silva[1], E.J. Haseyama[2], M.T.C. Muniz[3], V. D'Almeida[2].* 1) Pediatrics, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil; 2) Psychobiology, Universidade Federal de São Paulo, São Paulo,São Paulo, Brazil; 3) Universidade de Pernambuco, Recife, Pernambuco, Brazil.

Aim: Epigenetic regulation is crucial in the development of organisms, since it is required to achieve either stable genes expression or repression at various stages of development. DNA methylation is the most studied epigenetic mechanism and it is accomplished through specific enzymes called DNA methyltransferases, which transfer a methyl group to the cytosine of CpG dinucleotides. Human epidemiologic and animal model data indicate that nutrition and other environmental stimuli influence prenatal epigenetic development pathways and thereby induce permanent changes in metabolism and chronic disease susceptibility. As methionine-homocysteine cycle alterations are described in some disease and could have a role on fetal programming through changes on S-adenosylmethionine (SAM) levels, the aim of this study was to investigate the effects of methionine supplementation, during gestation and lactation, on expression of methyltransferases and global methylation pattern. Methods and Results: One month before pregnancy, 13 Swiss female mice were distributed into 2 groups: control (CT=6) group and methionine supplemented (MS=7) group (1% of methionine in water ad libitum). After 20 days, plasma homocysteine levels from MS group were approximately 50% higher than CT group (CT= 4.45 μmol/L, MS= 8.71 μmol/L; p=0.0001). Three months old male offspring (CT=15, MS=11) were euthanized by decapitation, the liver was harvested and DNA and mRNA isolated by Wizard® Genomic DNA Purification Kit and Trizol® method, respectively. Gene expression of *Dnmt1*, *Dnmt3a* and *Dnmt3b* was quantified by real time PCR using *Gapdh* as the housekeeping gene and data were analyzed by 2-ΔΔCT method. Hepatic DNA global methylation was measured by Imprint® Methylated DNA Quantification Kit. T-test was used to compare the results considering significant $p<0.05$. Our results demonstrated that the treatment with methionine throughout the pregnancy and lactation had no significant effect on the hepatic male expression of *Dnmt1* (p=0.844), *Dnmt3a* (p=0.794) and *Dnmt3b* (p=0.963), and neither on hepatic DNA global methylation (p=0.183). Conclusion: Despite dams hyperhomocysteinemia, we can suggest that the organism develops compensatory mechanisms during high methionine intake which results on similar methyltransferases gene expression and also global methylation in exposed adult offspring compared to controls. Sources of research support: FAPESP, CNPq and AFIP.

751F

Neonatal DNA Methylation Patterns Associate with Gestational Age. *A.K. Smith[1,2], J.W. Schroeder[2], K.N. Conneely[3], J.C. Cubells[1,2,3], V. Kilaru[1], D.J. Newport[3,4], B.T. Knight[3,4], Z.N. Stowe[3,4,5], P.A. Brennan[6], J. Krushkal[7], F.A. Tylavsky[7], R.N. Taylor[5], R.M. Adkins[8].* 1) Psychiatry & Behavioral Sciences, Emory University, Atlanta, GA; 2) Genetics and Molecular Biology Program, Emory University, Atlanta, GA; 3) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA; 4) Emory Women's Mental Health Program, Emory University School of Medicine, Atlanta, GA; 5) Department of Gynecology and Obstetrics, Emory University School of Medicine, Atlanta, GA; 6) Department of Psychology, Emory University, Atlanta, GA; 7) Department of Preventive Medicine, University of Tennessee Health Science Center, Memphis, TN; 8) Department of Pediatrics, University of Tennessee Health Science Center, Memphis, TN.

There is an increased risk for adverse neonatal outcome with declining gestational age (GA), and epigenetic alterations, including changes in DNA methylation, may contribute to the relationship between GA and adverse health outcomes in these offspring. To test this, we evaluated the association between GA (range: 32.3-43.4 weeks) and methylation patterns in neonatal DNA extracted from umbilical cord blood. DNA methylation at >27,000 CpG sites was examined in two prospectively-characterized cohorts: 1) a clinical cohort consisting of 259 neonates from women with a history of neuropsychiatric disorders, delivered between 2000 and 2010 and 2) a population-based cohort consisting of 197 neonates of uncomplicated mothers, delivered between 2007 and 2010. GA was determined by obstetrician report and maternal last menstrual period. The associations between proportion of DNA methylated and GA were evaluated by fitting a separate linear mixed effects model for each CpG site, adjusting for relevant covariates including neonatal sex, race, birthweight percentile and chip effects. CpG sites in 39 genes were associated with GA (false discovery rate <.05) in the clinical cohort. The same CpG sites in 18 genes replicated in the population-based cohort, with each association in the same direction. Notably, these CpG sites were located in genes previously implicated in labor and delivery (e.g., *ESR1*, *AVP* and *OXT*) or that may influence the risk for adverse health outcomes later in life (e.g. *DUOX2*, *CAPS2* and *CASP8*). All associations were independent method of delivery or induction of labor. The current results suggest that neonatal DNA methylation varies with gestational age even in term deliveries. The consequences of these changes warrant further investigation of their potential contribution to clinically significant postnatal adverse outcomes.

752F

Causal relationships between genotype, methylation and expression in whole blood of healthy controls. *K.R. van Eijk[1,2], S. de Jong[1,2], T. Langeveld[1], E. Strengman[1], E. Janson[1], J.H. Veldink[3], L.H. van den Berg[3], R.S. Kahn[2], C.G.F. de Kovel[1], M.P.M. Boks[2,4], S. Horvath[5], R.A. Ophoff[1,2,5,6].* 1) Department of Medical Genetics, University Medical Center Utrecht, The Netherlands; 2) Department of Psychiatry, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, The Netherlands; 3) Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, The Netherlands; 4) Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands; 5) Department of Human Genetics and Biostatistics, University of California, Los Angeles, USA; 6) Center for Neurobehavioral Genetics, Neuropsychiatric Institute, University of California, Los Angeles, USA.

Epigenetic mechanisms are important for regulating the cellular machinery including gene expression. DNA methylation is one form of epigenetic control that is most commonly studied. However, little is known about the complex relationship between DNA methylation and gene expression. This study is an effort to decipher the causal relationship between DNA methylation and gene expression. For this study we used (Illumina) array-based genome-wide DNA methylation and gene expression data obtained in whole blood of 148 healthy controls. To examine the association of DNA methylation and gene expression a linear regression was performed with DNA methylation as a predictor of gene expression levels. Age and gender were used as covariates. Significance thresholds were adjusted for cis (maximum distance of 500 kb between the probes) and trans (genome-wide). Available genome-wide SNP data was used to investigate the genetic control of DNA methylation and gene expression at these loci. Finally the edges (links) between the top 25 DNA methylation probes and corresponding gene expression probes were oriented by using underlying cis-acting SNPs as causal anchors. Specifically, the network edge orienting (NEO) software was used to calculate Local Edge Orienting (LEO) scores based on local structural equation models. We observed both positive and negative associations between gene expression probes and DNA methylation probes. For cis 3,617 significant associations were found, of which 1,934 negative (53.5%) and 1,683 positive (46.5%). Of the methylation and expression probes associated with each other 239 methylation probes and 219 expression probes are regulated by SNPs. After calculating LEO scores for 159 probe/SNP combinations, 50 combinations (31%) have a LEO score above 0.8, indicating good model fit, for the model SNP>Methylation>Expression and 12 combinations (7.5%) have a LEO score>0.8 for the model SNP>Expression->Methylation. Patterns in causality were found to coincide with LD blocks of genetic markers. Overall the results show that DNA methylation levels and gene expression levels are both positively as well as negatively correlated. In addition, about 10 percent of the DNA methylation and gene expression probes are regulated by genetic variants. For some relationships a causal direction could be assigned. These results provide insight into the complex and diverse relationship between DNA methylation and gene expression.

753F

Epigenetic regulation at the NR3C1 gene promoter and PTSD. *V. Vukojevic[1], A. Papassotiropoulos[1], D. de Quervain[2].* 1) Molecular Neurosciences, Biozentrum/University of Basel, Basel, Switzerland; 2) Cognitive Neurosciences, Faculty of Psychology/University of Basel, Basel, Switzerland.

Background: Human memory is a genetically complex trait. Candidate gene and genome-wide studies (GWAS) led to the identification of gene loci associated with human memory capacity. Despite the success of this approach a significant amount of phenotypic variation remains to be explained. The study of epigenetic mechanisms, such as DNA methylation, is expected to improve our understanding of the molecular underpinnings of this complex trait. Methods: Via direct bisulfite sequencing and SMART qPCR we investigated the promoter methylation of NR3C1 (nuclear receptor subfamily 3, group C, member 1), which is known to be important for cognitive processes related to post-traumatic stress disease (PTSD). Genetic variability of the NR3C1 locus was tested with the Affymetrix 6.0 SNP array. Results: Human emotional memory performance and brain activation during emotional memory tasks are associated with NR3C1 promoter methylation profiles. DNA methylation of the NR3C1 promotor 5' CpG NGFI-A factor-binding site is negatively correlated with such PTSD-related phenotypes as severity of intrusions and total PDS score in survivors of the Rwandan genocide. Conclusion and outlook: We conclude that DNA methylation of the NR3C1 gene promoter is associated to PTSD-related phenotypes. Currently we are expanding our search to the epigenome-wide level to identify additional and -possibly- interacting epigenetic loci.

754F

Pharmacoepigenetic impact of Chronic systemic steroid use on genome-wide DNA methylation in the International COPD Genetics Network. *E.S. Wan[1], W. Qiu[1], B.J. Klanderman[1], D.A. Lomas[2], S.I. Rennard[3], A. Agusti[4], A. Baccarelli[5], W.H. Anderson[6], E.K. Silverman[1], D.L. DeMeo[1].* 1) Channing Laboratory, Brigham and Women's Hospital, Boston, MA; 2) Cambridge Institute for Medical Research, University of Cambridge, Cambridge,UK; 3) University of Nebraska, Omaha, NE; 4) Hospital Clinic Universitat De Barcelona, Barcelona, Spain; 5) Harvard School of Public Health, Boston, MA; 6) GlaxoSmithKline, Research Triangle Park, NC.

Rationale: DNA methylation can reflect both endogenous factors, such as aging, & exogenous factors, such as smoking, diet, and medication use. Systemic steroids are a powerful class of medications administered therapeutically for a variety of indications in medical practice, often for protracted courses. Despite widespread use, the mechanisms of action of systemic steroids are not fully understood. We hypothesize that chronic systemic steroid use may be associated with distinct genome-wide DNA methylation patterns. Methods: We examined probands (n=382) enrolled in the International COPD Genetic Network (ICGN): subjects were 45-65 y.o., had ⁄ 5 pack-years of smoking, and a post-bronchodilator FEV_1 <60% predicted with an FEV_1/FVC <90% predicted. Genome-wide DNA methylation data was obtained using the Illumina HumanMethylation27 BeadChip on DNA isolated from peripheral blood leukocytes. We conducted an analysis comparing probands who reported chronic systemic steroid use (n=60) with probands who did not (n=322) using beta regression as implemented in R. All analyses were adjusted for age, sex, batch, and FEV_1 % predicted. Results: Using a Bonferroni adjusted p-value cutoff of 1.88×10^{-6} to denote genome wide significance, 2,322 CpG sites were differentially methylated in probands who reported chronic systemic steroid use compared to probands who did not report steroid use. CpG sites in the low density lipoprotein receptor related protein 3 (*LRP*), lymphotoxin alpha (*LTA*), & aldehyde dehydrogenase 3 family member B1 (*ALDH3B1*) were among the most highly associated differentially methylated loci. We conducted a gene set enrichment analysis in DAVID which demonstrated significant enrichment in extracellular & plasma membrane components, defense, inflammatory, and wound healing response, cytokine activity, cellular adhesion & communication, signal transduction, coagulation, platelet activation, cell migration & chemotaxis related processes. Conclusions: Chronic systemic steroid use in subjects with moderate to severe COPD is associated with variable DNA methylation patterns throughout the genome. Gene set enrichment analysis identified both expected and novel pathways impacted by differential methylation and may lend insight into the mechanism of action of & variable responses to this commonly administered medication. Funding: T32 HL007427 (S.T.Weiss), R01 HL089438 (D.L.D.)& the Doris Duke Clinical Scientist Development Award (D.L.D.).

755F

Genome-wide DNA methylation changes in fragile X syndrome. *T. Wang[1,2], R. Alisch[1], P. Chopra[1], J. Mowrey[1], B. Lynch[1], V. Patel[1], C. Collins[1], K. Conneely[1,3], J. Visootsak[1], B. Coffee[1], S. Warren[1].* 1) Department of Human Genetics, Emory University, Atlanta, GA; 2) Genetics and Molecular Biology Graduate Program, Emory University, Atlanta,GA; 3) Department of Biostatistics, Emory University, Atlanta,GA.

Fragile X syndrome (FXS) is the most common form of inherited intellectual delay and is caused by an expansion of a CGG repeat located in the 5' untranslated region (UTR) of FMR1, leading to hypermethylation and gene silencing of this locus. While the dynamic methylation patterns of this full mutation allele are well characterized, DNA methylation changes elsewhere in the genome has not been examined. Here, we quantitatively profiled whole blood-extracted DNA from six full mutation FXS patients and 170 controls for their methylation levels at 485,764 CpG dinucleotides throughout the genome. Following a differential methylation analysis, we find 79,330 loci with a significant differential methylation at an FDR of 0.05 . Of these differentially methylation loci (DML), only the FMR1 locus is hypermethylated. The remaining DML show statistically significant but subtle hypomethylation, suggesting that the hypermethylation of FMR1 full mutation may act as a `methylation sink,' reducing methylation of methyl-sensitive alleles throughout the genome. In addition, considerably more homogeneous cells-induced pluiripotent stem (iPS) cells derived from two Fragile X fibroblasts and two normal fibroblasts exhibit hypermethylation at four genomic regions distinct from the FMR1 locus. Together, these data indicate that, depending upon the cell type and/or differentiation status, the full mutation allele of FMR1 may influence DNA methylation elsewhere in the genome.

756F

Nicotine dependence-associated DNA methylation changes measured by a methylation array-based approach. *H. Zhang[1,3], A.I. Herman[1,3], H.R. Kranzler[4], R.F. Anton[5], H. Zhao[2], W. Zheng[2], J. Gelernter[1,3].* 1) Department of Psychiatry, Yale University School of Medicine, New Haven, CT; 2) Department of Biostatistics, Yale University School of Public Health, New Haven, CT; 3) VA Connecticut Healthcare System, West Haven, CT; 4) Department of Psychiatry, University of Pennsylvania School of Medicine and Philadelphia VAMC, Philadelphia, PA; 5) Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina, Charleston, SC.

Tobacco use, including cigarette smoking, may increase the risk for cardiovascular and pulmonary disease and cancer in part through epigenetic modification of genes. We investigated peripheral blood DNA methylation levels of 384 CpGs in promoter regions of 82 candidate genes (mainly involved in dopaminergic, serotonergic, opioidergic, GABAergic and glutamatergic neurotransmission) in 285 African Americans [AAs; 122 cases with nicotine dependence (ND) and 163 controls] and 249 European Americans (EAs, 115 ND cases and 134 controls) using Illumina GoldenGate methylation array assays. Effects of ND on DNA methylation were analyzed using multivariate linear regression with sex, age, ancestry proportion and alcohol or drug dependence as covariates. The q-value method was applied to control the false discovery rate in multiple testing. Six CpGs in AAs and nine CpGs in EAs showed a nominally significant difference in DNA methylation levels between ND cases and controls (6 CpGs in AAs: $0.018 \le P \le 0.034$, $q>0.05$; 7 CpGs in EAs: $0.015 \le P \le 0.043$, $q>0.05$). Additionally, there were sex effects on DNA methylation. In male AAs, six CpGs (GRIN1^2, GRIN1^4, MBD1^1, SLC6A1^3, HTR1A^8, and OPRD1^6) were hypermethylated in cases ($1.7\times10^{-8} \le P \le 0.009$, $6.5\times10^{-6} \le q \le 0.594$) compared to controls. In particular, CpG GRIN1^2, which is located in the promoter region of GRIN1 and the potential binding site (GGCGCG) of transcription factor ZF5, was more highly methylated in AA male cases (mean±SEM: 0.174±0.008) than in AA male controls (mean±SEM: 0.011±0.019) ($P=1.7\times10^{-8}$, $q=6.5\times10^{-6}$). In female AAs, two CpGs (GABRG2^5 and GRIN2B^5) were more highly methylated in cases than in controls ($P=0.004$, $q=0.743$ for both sites). However, in male EAs, 11 CpGs (HTR1B^9, SLC6A4^5, CHRNB4^7, DRD1^8, GRIN2C^5, GRIN2B^3, POMC^5, OPRK1^2, GABRB1^2, MECP2^1 and MAOA^4) were less highly methylated in cases than in controls ($0.0007 \le P \le 0.008$, $0.077 \le q \le 0.158$). In contrast, in female EAs, seven CpGs (HTR1A^2, OPRD1^3, OPRL1^4, POMC^5, GABRB1^3, DNMT3A^2 and GAD2^5) were more highly methylated in cases ($0.003 \le P \le 0.010$, $q>0.050$) than in controls. In summary, DNA methylation levels were found to be influenced by ND, race and sex. These preliminary findings need to be verified using independent sample sets and methods which can measure methylation levels of denser CpG sites in the promoter region of genes.

757F

Epimutations and transcriptional dysregulation of SHANK3 in brains associated with autism spectrum disorder. *L. Zhu[1], X. Li[1, 6], A. Towers[2], X. Wang[1], J. Goldstein[1], H. Yang[5], Y. Jiang[1, 2, 3, 4].* 1) Pediatrics, Duke University, Durham, NC., U.S; 2) Program in Genomics and Genetics, Duke University, Durham, NC., U.S; 3) Program in Cell and Molecular Biology, Duke University, Durham, NC., U.S; 4) Duke Institute for Brain Science, Duke University, Durham, NC., U.S; 5) Department of Biostatistics and Bioinformatics, Duke University, Durham, NC., U.S; 6) Molecular Genetics department, Shanghai Jiao Tong University, Shanghai, China.

Although a genetic component is strongly implicated in the etiology of autism spectrum disorders (ASD), the molecular basis is poorly understood in the majority of cases; single gene mutations and chromosomal microdeletions or duplications are found in about 10% of idiopathic ASD cases. Microdeletion and de novo frame shift mutations of the synaptic protein gene SHANK3 have been strongly implicated as a cause of ASD. We hypothesize that epigenetic dysregulation of the SHANK3 gene in brain contributes to ASD susceptibility. We performed DNA methylation profiling of 5 SHANK3 CpG islands (CGI-1 to CGI-5) in cerebral cortex and cerebellum tissues from 52 ASD patients and 32 controls. Compared to controls, ASD cerebellum samples had a significantly higher percentage of methylation in CGI-2 (control: 4.1±6.6%; ASD: 10.1±18.3 %, p=5.03e-13), CGI-3 (control: 45.6±10.1%; ASD: 52.4±12.6%, p= 5.45e-09), and CGI-4 (control: 2.1±5.8 %; ASD: 9.4 ± 14.9 %, p=1.11e-43) using the t-test. In the cerebral cortex, ASD samples had a significantly higher percentage of methylation in CGI-2 (control: 4.5±7.6%; ASD: 24.3±19.6%, p= 5.82e-64) using t-test; no difference was found for the other CGIs. Heat map analysis revealed significantly increased methylation of CGI-2 and CGI-4 in 12 to 15% of ASD brain tissues. In Silico analysis, RNA expression, and luciferase reporter assay revealed that SHANK3 has 5 intragenic promoters and extensive alternative splicing of coding exons. The CGI-2, CGI-3, and CGI-4 correspond to promoter 3, 5, and 6 respectively. The pattern of alternative splicing and expression of different isoforms of SHANK3 were altered in ASD brains with increased methylation. The chromatin immunoprecipitation (ChIP) assay revealed that the active chromatin marker H3K4me3 is significantly decreased but the repressed chromatin marker H3K27me3 is significantly increased in the CGI-4 region in ASD samples with increased methylation. Aberrant methylation at global level and other candidate ASD candidate genes were not found in these ASD brain tissues. This study is the first to show that epimutations of SHANK3 are strongly associated with susceptibility to ASD. We propose that isoform-specific transcriptional dysregulation of synaptic protein genes in brain, due to epimutation, may contribute significantly to the molecular basis of ASD.

758F

MicroRNA-Regulated Gene Networks in Human. *S. Banfi[1,2], V.A. Gennarino[1], G. D'Angelo[1], G. Dharmalingam[1], S. Fernandez[3], G. Russolillo[4], R. Sanges[5], M. Mutarelli[1], A. Ballabio[1,6], P. Verde[3], M. Sardiello[1,6].* 1) TIGEM, Fondazione Telethon, Naples, Naples, Italy; 2) Medical Genetics, Department of General Pathology, Second University of Naples, Naples, Italy; 3) Institute of Genetics and Biophysics “A. Buzzati Traverso”, CNR, Naples, Italy; 4) CNAM - Laboratoire Cédric & Chaire de Statistique Appliquée, Paris, France; 5) Bioinformatics, Animal Physiology and Evolution, Stazione Zoologica Anton Dohrn, Villa Comunale, Naples, Italy; 6) Department of Molecular and Human Genetics, Baylor College of Medicine, Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, Texas USA.

Transcriptional regulatory networks control proliferation, differentiation and metabolism of eukaryotic cells through two main classes of regulators: transcription factors (TFs) and microRNAs (miRNAs). However, while the contribution of TFs is well established, our current understanding of the processes regulated by miRNAs remains limited. Here, we carried out a comprehensive analysis of human miRNA regulatory networks based on Co-expression Meta-analysis of miRNA Targets (CoMeTa). CoMeTa integrates expression data from hundreds of cellular systems and multiple tissues, to capture the relationships between the regulatory dynamics of genes. The CoMeTa analysis of 675 human miRNAs provided a dramatic improvement in our ability to pinpoint their bona fide target genes, as compared to first-generation methodologies, which were based solely on sequence and evolutionary analyses. CoMeTa outputs were used here to correlate miRNAs to specific biological pathways. This analysis revealed that many critical biological processes are controlled by miRNAs through regulation of specific networks of co-expressed target genes. CoMeTa data were also used to build a global map of miRNA communities based on gene co-targeting, which revealed synergistic regulation of cohorts of genes involved in similar processes. The use of CoMeTa, coupled to experimental in vitro assays, allowed us to identify three novel putative regulators of TGF) signalling, which represent potential therapeutic targets for treatment of invasive tumours. Based on our findings, we propose CoMeTa as a general paradigm for second-generation procedures to infer targets, biological roles and network communities of miRNAs.

759F

Comprehensively Functional Studies of miR-199a in Testicular Germ Cell Tumors. *S. Gu[1], Y.K. Suen[1], H.H. Cheung[2], W.Y. Chan[1,2].* 1) School of Biomedical Sciences, The Chinese University of Hong Kong, HKSAR, Hong Kong; 2) Lab of Clinical and Developmental Genomics, NICHD, NIH, Bethesda, MD.

microRNAs (miRNAs) regulate gene expressions by pairing to the mRNAs of protein-coding genes to direct their posttranscriptional repression. Previous studies showed that hypermethylation silences the expression of miR-199a-2 in testicular germ cell tumors (TGCTs). It has been shown that miR-199a-2 is a tumor suppressor, normally modulating a spectrum of genes involved in TGCTs, and PODXL (podocalyxin-like protein) is one of such targets. We aim to find other downstream target(s) of miR-199a-2 besides PODXL and examine their roles in tumorigenesis. In addition, the relationship among these targets of miR-199a-2 and the overall regulation of tumorigenesis by miR-199a-2 will be studied. In order to find potential targets of miR-199a-2 in TGCTs, two stably transfected testicular tumor cell strains (NT2), one with an expression vector carrying control marker GFP and one with a vector carrying miR-199a-2 precursor downstream of the marker GFP were used as in vitro models. Total cellular proteins of the cells were analyzed by two-dimensional gel electrophoresis. Differentially expressed proteins were isolated and subjected to mass spectrometry analyses. Difference in gene expression was investigated using expression chips. In addition, target prediction programs were employed to identify target candidates of miR-199a-2. Combining the results of these methods, several potential targets of miR-199a-2 were obtained for further verification by Western Blot and qPCR. One of such targets is involved in chromatin modification. We are in the process of verifying direct interaction between miR-199a-2 and the potential targets by luciferase receptor assay. The effects of silencing of the potential targets on the behavior of TGCT cells will also be examined.

760F

Comprehensive evaluation of genome-wide SNP, mRNA expression and microRNA expression relationships in human cell lines. *RS. Huang[1], HK. Im[2], D. Ziliak[1], B. LaCroix[1], ME. Dolan[1], NJ. Cox[1], E. Gamazon[1].* 1) Dept Med, Univ Chicago, Chicago, IL; 2) Dept Health Studies, University of Chicago, Chicago, IL.

Given the major role of gene and microRNA (miRNA) expression in biological, physiological and pathological processes, we set out to comprehensively and systematically evaluate the relationship between miRNA expression, mRNA expression and genetic variation. Genome-wide miRNA and mRNA expression were characterized in Phase I/II unrelated 54 CEU (Utah residents with northern and western European ancestry) and 55 YRI (African from Ibadan, Nigeria) HapMap lymphoblastoid cell lines (LCLs) using the Exiqon miRCURYTM LNA arrays and the Affymetrix GeneChip® Human Exon 1.0 ST array, respectively. Genome-wide analysis identified 100 and 114 miRNAs whose expression levels are negatively correlated with 558 and 633 mRNA expression phenotypes in CEU and YRI samples, respectively ($p<10^{-4}$). A large number of these potential miRNA-mRNA binding relationships are also predicted by miRbase. We then investigated the role of genetic variation in mediating these relationships. First, we identified SNPs that are located in the 3'UTR regions of genes and may affect miRNA bindings. Importantly, 25 and 25 SNPs located in the 3'UTR of target genes were identified as cis-acting eQTLs ($p<0.01$) to 16 and 18 of their neighboring genes in CEU and YRI, respectively. Replication studies for the cis-acting eQTL and miRNA-mRNA relationships were conducted in an independent set of Phase III HapMap CEU and YRI samples. Functional study was then performed on selected miRNA/mRNA pairs to confirm the differential miRNA binding affinity due to the presence of these SNPs. Secondly, we mapped miRNA expression levels to 7,458 and 12,413 SNPs as miRNA-eQTLs ($p<10^{-8}$) in CEU and YRI, respectively through a genome-wide association study. Of these miRNA-eQTLs, 47 and 12 were also found to be mRNA-eQTLs for 318 and 150 genes in CEU and YRI ($p<10^{-4}$), suggesting that either genetic variation is mediating the observed relationship between miRNA and mRNA or a SNP may regulate miRNA expression thereby regulating mRNA expression. This study extends our understanding of the genetic regulation of the transcriptome and suggests that genetic variation may influence interactions between miRNAs and mRNAs greater than has previously been appreciated.

761F

A parallel study of mRNA and microRNA profiling of peripheral blood in young adult women. *C. Huang[1], S. Simone[2], S. Gadd[3], N. Jafari[4].* 1) Preventive Med, Northwestern Univ, Chicago, IL; 2) Dept. of Pediatrics, Northwestern Univ, Chicago, IL; 3) Dept. of Pathology, Northwestern Univ, Chicago, IL; 4) Center for Genetic Medicine, Northwestern Univ, Chicago, IL.

Backgound: Aging is a complex process that involves the interplay of genetic, epigenetic, and environmental factors. Identifying aging-related biomarkers holds great potential for improving our understanding of complex physiological changes, thereby providing a means to investigate the mechanism by which aging influences various diseases. Method and Results: We performed a parallel study of microRNA and gene expression profiling of peripheral blood in a group of healthy young adult women, among which 13 were aged 22-25 and 9 were aged 36-39 years old. We identified a significantly distinct pattern of microRNA, but not gene expression profiling, between these two young adult women groups. We also performed correlation analysis of expression levels between all pairs of age-associated microRNAs and genes and identified a weak global correlation between these two types of expression levels. A significant involvement of estrogen regulation was observed by pathway analysis of the most differentially expressed microRNAs that included miR-155, -18a, -142, -340, -363, -195, and -24. Conclusion: Our results suggest that the change in global microRNA expression in the peripheral blood is associated with normal aging in young adult women. This change may precede global gene expression changes. The association of differentially microRNAs with estrogen regulation may inform future studies to investigate the regulatory mechanism of these microRNAs on estrogen-associated diseases.

762F

Epigenetic Regulation of Ocular Growth: Genome-wide Profiling of Scleral micro-RNAs, Potential Role and Implications for Myopia. *R. Metlapally[1], P. Gonzalez[2], F.A. Hawthorne[3], K. Tran-Viet[3], C.F. Wildsoet[1], T.L. Young[2,3].* 1) School of Optometry, UC Berkeley, CA, USA; 2) Duke Eye Center, NC, USA; 3) Duke Center for Human Genetics, NC, USA.

Ocular growth during myopia development is driven by a retino-scleral signaling cascade with the sclera (outer coat) ultimately guiding ocular elongation via active extracellular matrix remodeling. Micro-RNAs are small non-coding RNAs that can regulate gene expression by base pairing with the 3' UTR of target sequence/s, and serving as nodes of signaling networks. To date, there are no studies of micro-RNAs in the sclera. We hypothesized that the sclera, like most tissues, expresses micro-RNAs, some of which play an active role in modulating genes during ocular growth regulation. We profiled micro-RNAs in the human sclera from rapidly growing human fetal eyes and stable adult donor eyes using high-throughput microarray and quantitative PCR analyses. Scleral samples from normal human fetal (24 wk gestation) and age-matched adult donor eyes (n=3, each group) were obtained, and RNA extracted using the miRVANA kit. Genome-wide micro-RNA profiling made use of the Agilent Human miRNA Microarray platform. miRNA target predictions were obtained using Microcosm, TargetScan and PicTar algorithms. Follow-up experiments using TaqMan® MicroRNA Assays targeting micro-RNAs showing either highest significance, detection, or fold differences, and collagen specificity, were applied to tissue from posterior and peripheral regions (n=7, each group). Microarray data were analyzed using miRInform, and quantitative PCR data with 2^-deltaCt method. Approximately 300 micro-RNAs were found to be expressed by human sclera (298 & 353 detected in at least one adult and fetal sclera sample respectively), with several micro-RNAs showing differential regulation ($p<0.01$, min $p=1.5 \times 10^{-6}$). In follow-up experiments, mir-214, let-7c, let-7e, mir-103, mir-107, and mir-98 showed increased expression in fetal tissue (fold changes 1.5 to 4, $p<0.01$). No significant differences were observed in micro-RNA expression between posterior and peripheral regions within either age group. This is the first study of micro-RNA expression in human sclera. The sclera expresses several micro-RNAs, some of which show age-related differential regulation, higher in rapidly growing fetal eyes, consistent with a role in ocular growth regulation. A link between these scleral micro-RNAs and myopia, which also involves accelerated eye growth is plausible but yet to be established. Genome-wide mRNA profiling is underway to understand the pathways involved and the correlation with micro-RNA signatures.

763F

Identification of the chromatin regions coated by non-coding *Xist* RNA. *H. Kugoh[1], K. Murakami[2], E. Ohshiro[1], T. Ohhira[1], M. Oshimura[1].* 1) Biomedical Science and Chromo., Regenerative Medicine, yonago city, Tottori, Japan; 2) Laboratory for Pluripotent Cell Studies, RIKEN Center for Developmental Biology (CDB), Kobe, Japan.

The non-coding RNA (ncRNA) genes, which did not encode proteins, produce functional RNA molecules that play an important role of gene regulation, including X chromosome inactivation (XCI) and RNA interference (RNAi). Of these, the non-coding XIST RNA has been particularly well characterized. XIST RNA is known to act as a regulatory molecule in XCI, which accomplishes dosage compensation in mammals. *XIST* RNA coats and spreads along the X chromosome; recruits the silencing complexes, including histone deacetylase, histone methyltransferase, and the polycomb group protein complex; and eventually establishes X chromosome inactivation by induction of heterochromatin formation. Although the majority of X-linked genes in mammalian cells indicate loss of expression by silencing of the entire chromosome on XCI, some genes were expressed in both active and inactive X chromosome. However, it was incompletely defined how *XIST* RNA actually affects on silencing genes and genes escaped from inactivated X chromosome, respectively. We developed a modified RNA tagging and recovery of associated proteins (TRAP) method to study the association between *Xist* RNA and its target genes. In mouse cells, *Xist* RNA was detected on the *Ube1x* gene, but not on Jarid1c and *Utx* genes, which escape from XCI. Using this technique we were able to show that the *Xist* RNA molecule is not present on active genes that escape from XCI, but is present on genes inactivated by XCI, suggesting that this method is a powerful tool for functional analysis of ncRNA.

764F

Evidence for genetic, epigenetic and exogenous regulation of Inflammasome and related genes. *A.A. Awomoyi[1], A. Papp[2], G. Smith[2], W. Sadee[2], M. Wewers[3].* 1) 6209, Lone Oak Dr, Bethesda, MD; 2) Pharmacology Dept, Ohio State University Medical Center, Columbus, OH; 3) Davis Heart and Lung Res. Inst and Internal Medicine, Ohio State University Medical Center, Columbus, OH.

Multiprotein complexes, termed inflammasome and related genes, which are assembled in a stimulus dependent manner, are central to the innate host response. Dysregulated inflammasome functioning is associated with numerous diseases and disorders. Naturally occurring genetic variations within any of the inflammasome genes have the potential to modulate the activation and assemblage of this complex. One important variation that, to our knowledge, has not yet been systematically investigated in the context of inflammasome function is that regulatory polymorphisms in specific inflammasome genes may result in altered mRNA expression. This is detectable as an allelic mRNA expression imbalance in subjects heterozygous for a marker SNP in the transcribed exonic region of the genes. To search for regulatory mechanisms influencing inducible gene expression, we selected 6 of these genes; NLRP1, NLRP3, PYCARD, MEFV, CASP1 and CASP12. We searched public databases and identified TagSNPs in exonic regions with high frequency of heterozygosity within these genes. We used a single base extension technique (SNaPshot) to measure allele mRNA expression ratios in human blood monocytes and MCSF differentiated macrophages in the presence or absence of LPS from heterozygous individuals. Allele mRNA expression was compared to the corresponding genomic allele ratios measured in the same subjects. A relative deficit in one allele compared to the alternate allele suggests a cis acting regulatory element within the gene locus of interest. Using this approach, we predict that specific inflammasome genes will be shown to be regulated at least in part by cis-acting genetic elements. We show that similar genotypes were expressed differently. Out of 20 human healthy volunteers 4 heterozygotes exhibit monoallelic expression of Pyrin at the exon 2 SNPID rs224225. This we found to be due in part to promoter DNA hypermethylation. This genomic region is known to be excised from full length transcript during or following gene transcription. Those monoalleleic expressions that are not found to be due to promoter region DNA hypermethylation, extracellular stimulus such as LPS is able to restore the heterozygous state. We demonstrate for the first time the multiple interacting affects of genetic, epigenetic and exogenous factors on a biological process such as Inflammasome Complex activity. This finding has profound implications for interpretation of genetic linkage and association studies.

765F

sFRP1 and sFRP2 epigenetic association with osteoblastic differentiation of mesenchymal stem cells. *M. Mahmoodinia Maymand[1], M. Norouzinia[2,3], S. Rezaei[1], M. Farshdousti Hagh[3].* 1) Stem cell Dept, Sarem Cell Research Center, Tehran, Ekbatan, Iran; 2) Department of Medical Genetics, Tarbiat Modaress University, Tehran, Iran; 3) Department of Hematology, Tarbiat Modaress University, Tehran, Iran.

Mesenchymal stem cells (MSCs) have an extensive potential to proliferate and differentiate into osteoblasts. Wnt/) -catenin signaling pathway play key role in osteogenesis. Wnt inhibitors on the other hand can result in osteoporosis. The role of gene expression regulation of Wnt signaling in osteoblast differentiation has been shown in several studies. However, the exact mechanism of this regulation remains to be cleared in this study we sought to investigate the role of epigenetic regulation at the DNA level in promoter regions of sFRP1 and sFRP2 during osteoblastic differentiation of mesenchymal stem cells. Expression pattern of these genes has also been studied in relation to methylation changes. MSC from bone marrow was isolated, expanded and characterized in vitro by flowcytometry, differentiation potential and culture characteristics. Osteoblastic differentiation factors such as Dexamethasone and ascorbic acid were used to induce differentiation. Osteoblastic markers such as ALP and osteocalcin as well as Alizarin red immunostaining showed the success of differentiation. sFRP1 and sFRP2 promoter methylation status was determined using Methylation Specific PCR in multipotent state and during different stage of osteoblastic differentiation. We show that sFRP1 and sFRP2 promoters are totally unmethylated in MSC. Methylation pattern during osteoblastic differentiation shown no de novo methylation. RT-PCR confirms the result of MSP on sFRP1 and sFRP2. It has been shown that Canonical Wnts and BMPs cooperatively induce osteoblastic differentiation through a GSK3) -dependent and) -catenin-independent mechanism. Mesenchymal stem cells seem to be mainly unmethylated in the indifferentiated state. However we have shown before that some genes like as ROR2 are epigenetically methylated at DNA level during the differentiation. In tis research show that sFRP1 and sFRP2 are expressed and its expression maintained through osteoblastic differentiation through mechanisms other than methylation of DNA. Histone modifications and transcription factor based regulation of gene expression seem to be good candidates for further research.

766F

Regulation of chromatin structure on human subtelomeres during cellular senescence. *P.E. Thijssen[1,2], P.E. Slagboom[2], B.T. Heijmans[2], S.M. van der Maarel[1].* 1) Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands; 2) Department of Molecular Epidemiology, Leiden University Medical Center, Leiden, Netherlands.

Telomeres erode due to successive rounds of DNA replication in cycling cells and stochastic damage of oxygen radicals. *In vivo* telomere lengths roughly correlate with donor age, but show high inter- and intra-individual variability. *In vitro*, progressive telomere loss is one of the triggers of the tumor suppressive mechanism of cellular senescence. More recently it has been reported that the chromatin structure of telomeres and the adjacent subtelomeres changes with telomere length reduction. Fifth generation $Terc^{-/-}$ mice show dramatic telomere loss, a reduction in heterochromatin markers H3K9me3, H4K20me3 and CpG methylation, and an increase in acetylation of lysine residues. The effect of telomere dynamics on subtelomeric chromatin make-up in human cells is less well understood. Subtelomeric chromatin changes can have phenotypic consequences as implicated by the loss of compaction leading to improper expression of a subtelomeric retrogene in the progressive muscular dystrophy FSHD. To gain more insight in the interplay between telomere shortening, cellular senescence and subtelomere chromatin structure, we set out to map chromatin changes on two subtelomeres in senescing WI-38 human primary fibroblasts. Using ChIP followed by Q-PCR, we systematically interrogated the chromatin make-up at 7qter and 11qter up to 250 kb proximal from the telomere. On both subtelomeres and telomeres we observe a ± 2 fold decrease in the heterochromatin marker H3K9me3, concurrent with a 2-3 fold increase in the transcriptional regulator H3K27me3. H4K16ac, implicated in yeast telomere biology and chromatin higher order compaction, shows a steep decrease with cellular senescence. In the studied 250 kb window we did not observe a clear effect of distance to the telomere on the chromatin changes. In addition, we did not observe uncontrolled spreading of the shelterin component TRF2 onto the subtelomere. In summary, the subtelomeres of chromosome 7q and 11q show relative opening by a loss of H3K9me3, possibly compensated by a gain in transcriptional repressor H3K27me3 and a decrease in H4K16ac. We are currently interrogating the expression of transcripts from the region studied, including TERRA transcripts emanating from 7q and 11q.

767F

PARP-1 and SATB-1 bind to the TT>A SLE associated regulatory downstream of TNFAIP3. *F. Wen[1], M. Kinter[2], J.M. Guthridge[1], P.M. Gaffney[1].* 1) Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) Free Radical Biology and Aging, Oklahoma Medical Research Foundation, Oklahoma City, OK.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by dysregulated interferon responses and loss of tolerance of self-antigens resulting in systemic inflammation and organ failure. The tumor necrosis factor alpha inducible protein 3 (TNFAIP3) gene encodes the ubiquitin-modifying enzyme A20, which negatively regulates NF-2B activity. We recently described a functional TT>A polymorphic dinucleotide, 42kb downstream of the TNFAIP3 promoter, as a prime candidate causal polymophism responsible for association with SLE in subjects of European and Korean ancestry that demonstrates reduced affinity for a nuclear protein complex that includes NF-2B. In order to further characterize this nuclear protein complex we affinity purified proteins from LPS stimulated THP-1 cells using oligonucleotide probes specific for the wild type (TT) or polymorphic (A) sequence followed by mass spectrometry. Two primary proteins poly (ADP-ribose) polymerase 1 (PARP-1) and special AT-rich sequence binding protein 1 (SATB-1) were isolated. The identities of these proteins were confirmed by Western blotting in independent experiments. PARP-1 functions in DNA replication and chromatin remodeling, and also facilitates diverse inflammatory responses by promoting pro-inflammatory gene expression. SATB1 functions in tethering chromatin to the nuclear membranes at matrix attachment regions thus facilitating long-range regulation of gene transcription. These results suggest that the TT>A polymorphism may predispose to SLE through the recruitment of chromatin modifying proteins that modulate long-range transcriptional regulation of A20.

768F

Can repetitive element methylation be used as a surrogate measure of gene promoter-region methylation? *E.M. Price[1,2], M.S. Penaherrera[2], D.E. McFadden[2,3], M.S. Kobor[2], W.P. Robinaon[2].* 1) Obstetrics and Gynaecology, University of British Columbia, Vancouver, British Columbia, Canada; 2) Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada; 3) Pathology, University of British Columbia, Vancouver, British Columbia, Canada.

Two repetitive elements (REs) - LINE1 (L1) and Alu make up close to 50% of the human genome and are hypothesized to modulate gene expression and genomic evolution in beneficial, neutral and detrimental ways. Hundreds of thousands of REs are present throughout the human genome and generally maintain a stable level of methylation through life, which is thought to prevent their transposition. Recently, studies of human disease and environmental exposure have capitalized on these characteristics and used methylation of L1 and/or Alu elements as surrogate measures for global DNA methylation. However, there has been little evidence to support this claim and no investigation for whether it holds true across different tissues. We assessed L1 and Alu DNA methylation by bisulfite pyrosequencing as indirect measures of global DNA methylation and compared them to an average methylation of promoter regions across the genome as assayed by the Illumina Infinium HumanMethylation27 BeadChip array (measuring 27,578 CpGs in 14,475 genes). These measures were evaluated in villi from 41 control placentae (20 1st trimester; 11 2nd trimester; and 10 3rd trimester) in addition to organ tissues from 11 control 2nd trimester fetuses (11 brain, 11 kidney, 10 muscle samples) and 10 adult female control blood samples. Statistical analyses of associations were carried out using Spearman rank-order correlation and Mann-Whitney tests. L1s in 1st, 2nd and 3rd trimester placenta were significantly less methylated than in all other tissue types ($p<0.001$). There was a trend for increased average gene promoter methylation with gestational age, while L1 methylation remained stable. Illumina average DNA methylation was correlated with L1 methylation ($R_s=0.51$, $p<0.001$) but not Alu in highly methylated tissues (fetal organ and female blood samples) but not moderately methylated tissues (placental samples). Specifically, there was a strong correlation between L1 and Illumina average methylation in 2nd trimester placenta ($R_s=0.77$, $p<0.01$) and brain tissue ($R_s=0.76$, $p<0.01$). Thus, L1 methylation may be a better reflection of methylation at gene promoters across the genome. However, we caution that even though L1 methylation is correlated with Illumina DNA methylation in some of our study groups, each of L1, Alu and Illumina DNA methylation is a different and distinct measure of genomic methylation. Thus, all three may be influenced by environmental factors in different or complementary ways.

769F

Skewing of X-chromosome inactivation in tissues: is HUMARA assay in blood a reliable proxy? *M. Hrebicek, J. Minks, J. Sikora, D. Musalkova, R. Dobrovolny, E. Richterova.* Inst Inherited Metabolic Disor, Charles Univ, Prague 2, Czech Republic.

X-inactivation, the mechanism by which females compensate double dosage of X-linked genes, leads to transcriptional silencing of one X chromosome in each somatic cell. In some females, one of the X chromosomes is preferentially inactivated. Such a "skewing" of X-inactivation, routinely measured in peripheral blood leukocytes by HUMARA assay, may be associated with adverse phenotypes and therefore serves as a useful diagnostic parameter. To expand the limited literature, we have tested the fidelity with which HUMARA assay in blood captures skewing in different tissues as well as allelic expression ratios of X-linked genes. Skewing of X-inactivation in whole blood and 20 other tissues in five elderly females revealed that mean standard deviation of X-chromosome inactivation ratios among the assayed tissues was 8.2 percent. Of note, as much as 16 percent variation was observed between pairs of specimens collected from the same tissue. Segregation of skewing of XCI ratios with the germ layer from which the tissues were derived was not observed as mean standard deviation (S.D.) of skewing of XCI ratio among all tissues (8.0) was comparable to the mean S.D. calculated within tissues originating from ectoderm (8.3), mesoderm (7.7) and entoderm (5.1). We further probed allelic expression ratios of 10 X-linked genes in 63 females in blood. In 94.6 percent of samples, the results differed from the HUMARA-based results by <20 percent. While the departure of an individual gene or tissue from skewing measured by HUMARA assay in blood can be substantial and cannot be excluded unless probed in situ, this assay usually provides a fair estimate of the inactivation of other genes in other tissues. We conclude from our data that HUMARA assay in blood, often the only feasible approach for testing X-inactivation skewing, is a useful surrogate for skewing of X-inactivation in tissues. Research was supported by Internal grant Agency of Ministry of Health, Czech Republic grant NR 8361-3 and received also institutional support from VZ MŠM CR 0021620806.

770F

The Role of Cohesin in Imprinting and Escape from X-inactivation. *J. Kalish[1], S. Lin[2], M. Bartolomei[2].* 1) Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA.

Cohesin genes are associated with human disease. Specifically, mutations in cohesin subunits and related proteins (SMC1A, SMC3, and NIPBL) are seen in patients with Cornelia de Lange Syndrome (CdLS). The pathogenesis of CdLS does not appear to be related to sister chromatid cohesion and chromosome segregation, but is likely due to the role of cohesin in gene regulation. Cohesin co-localizes with CTCF and other factors and may help mediate transcriptional regulation at several imprinted loci. CTCF has been shown to bind at the boundaries of several genes that escape X-inactivation, acting as a chromatin insulator. Liu et al demonstrated increased cohesin binding on the X chromosome at hypomethlyated regions compared to autosomes in normal patients but in CdLS patients less cohesin is present at hypomethylated regions on the X chromosome. This suggests a role for cohesin in gene regulation specifically on the X chromosome. Renault et al reported linkage of SA2, a cohesin component located on the X chromosome, with familial skewing of X-inactivation, suggesting a role for cohesin in X-inactivation choice. We have shown that RNAi targeting of CTCF and cohesin leads to elevated mRNA expression of imprinted genes while maintaining imprinted expression, suggesting a non-allelic role for CTCF and cohesin in imprinting. We are currently using siRNA knockdown of CTCF and cohesin to test the hypothesis that these genes have a role in gene escape from X-inactivation.

771F

Elucidating the role of noncoding RNAs in initiation of mouse imprinted X chromosome inactivation. *E. Maclary, M. Hinten, M. Freedman, M. Pedraza, E. Buttigieg, C. Harris, S. Purushothaman, S. Kalantry.* Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI.

In XX female mammals, genes along one of the two X-chromosomes are transcriptionally silenced in order to equalize the dosage of X-linked genes to that expressed in XY males in a process known as X-chromosome inactivation. Classically, initiation of X-inactivation in the mouse model system is thought to be controlled in cis by two noncoding RNAs: Xist and Tsix. Xist is expressed only from the inactive-X, and is proposed to trigger epigenetic silencing by recruiting protein complexes that alter chromatin structure. The antisense partner of Xist, Tsix, is exclusively expressed from the active-X, and is thought to forestall inactivation of the active-X by inhibiting Xist transription. We have recently shown, however, that the initiation of imprinted mouse X-chromosome inactivation, a process during which the paternally-derived X-chromosome is preferentially inactivated, is not globally disrupted in the absence of Xist. Analysis of X-linked gene expression in early mouse embryos lacking Xist on the paternally-derived X-chromosome show that these embryos still initiate silencing of paternal X-linked genes. This Xist-independent silencing calls into question the cascade of epigenetic events that characterize the two Xs, including the role of Tsix. We therefore set out to test if Tsix expression is also dispensable during the initiation phase of imprinted mouse XCI. Preliminary results, employing RNA fluorescence in situ hybridization and allele-specific RT-PCR, suggest that Tsix is not required to prevent inactivation of the maternally-derived X-chromosome during the initiation phase of imprinted X chromosome inactivation. Our data therefore argue that alternate mechanisms, including novel X-linked noncoding transcripts, regulate the initiation of imprinted mouse X chromosome inactivation. To discover novel noncoding X-linked transcripts that are candidates to trigger or prevent imprinted X-inactivation, we plan to use RNA-seq technology.

772F

Assessing patterns of placental development using X-chromosome inactivation (XCI) and gene methylation profiling. *M.S. Peñaherrera, L. Avila, R. Jiang, R.K.C. Yuen, C.J. Brown, W.P. Robinson.* Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada.

A considerable degree of within-placenta variability in DNA methylation level has been reported for a number of loci. X-chromosome inactivation (XCI) skewing has also shown considerable site-to-site variability within a placenta. In an attempt to understand the three dimensional growth and development of the placenta, we utilized chorionic villi samples collected from a set of 4 control placentas for which 3-7 sites divided into 4 different placental depths were collected. First, we assessed XCI skewing using the assay for the Human Androgen Receptor gene (HUMARA). We found a significant degree of variability for XCI skewing between sites within each placenta; however, there was a high degree of correlation of skewing values for samples obtained from the same site but spanning the different placental depths (fetal to maternal side of the placenta). This demonstrates that placental development is highly clonal by depth and that cells within a villous tree are likely derived from a small number of precursors. To determine if methylation patterns associated with other genes/regions would also show a greater degree of between-site than within-site differences, we studied methylation at genes/regions previously found to show variable methylation within a placenta including: *H19/IGF2* ICR1; the promoters of *KISS1*, *CASP8*, *PTPN6*, and *APC*; and indicators of global genome methylation such as LINE-1 elements. Methylation at these sites was quantified using pyrosequencing. ANOVA analysis of the methylation results showed that both LINE-1 and *APC* have greater between-than within-site variation, while *PTPN6* and *H19/IGF2* ICR1 show no effect of site or depth on methylation. The promoter methylation of some genes such as *KISS1* and *CASP8* shows significant between-site differences in some, but not all, placentas. Methylation levels that vary to a greater degree between sites but are highly correlated at different placental depths of the same site may reflect that, to some degree, methylation in those genes/regions is stably maintained during the growth phase of the placental villous trees. On the other hand, for genes/regions that do not follow this pattern, such as *H19/IGF2* ICR1, it is possible that methylation is modified post-differentiation or is perhaps more subject to variation through environmental influences.

773F

A conserved role of (-adducin (ADD-1) in memory, synaptic plasticity, and in AMPA-type glutamate receptor dynamics. *V. Vukojevic D.[1], L. Gschwind[2], C. Vogler[1], P. Demougin[1], D.J.-F. de Quervain[2], A. Papassotiropoulos[1], A. Stetak[1].* 1) Molecular Neurosciences, Biozentrum/University of Basel, Basel, Switzerland; 2) Cognitive Neurosciences, Faculty of Psychology/University of Basel, Basel, Switzerland.

Identifying the molecular mechanisms that underlie learning and memory are one of the major challenges in neuroscience. Taken the advantages of the nematode C. elegans, here we investigated the role of (-adducin (add-1) in associative learning and memory. We found that loss of add-1 selectively impairs short- and long-term memory. Furthermore, (-adducin is required in vivo for long-term consolidation of synaptic plasticity, and sustained increase of AMPA-type glutamate receptor (GLR-1) content in the synapses but also plays an important role in long lasting changes of GLR-1 turnover dynamics. The role of ADD-1 is splice-form and tissue specific and it controls the storage of memories likely through capping of the fast growing barbed end of actin. In addition to C. elegans, genetic variability of the human ADD1 gene was significantly associated with episodic memory performance. Taken together, our findings support a common molecular role for (-adducin in memory from nematodes to humans and may provide a powerful clinical approach for the treatment of memory related diseases.

774F

Expression of Claudin-3 on Perineurial fibroblasts of Sporadic Neurofibroma. *M.H. Sulaiman[1], A. Dodson[2], C.A Kudi[1], J.O Hambolu[1], S.A Ojo[1].* 1) Vet Anatomy, Ahmadu Bello University, Zaria, Nigeria; 2) School of Cancer studies, Division of Surgery and Oncology, University of Liverpool.

INTRODUCTION; Neurofibromatosis type 1 (NF1) is one of the common autosomal disorders in human with an estimated birth incidence of 1/3500 people worldwide. Schwann cells are reported to constitute the major cell type (40-80%) in neurofibromas because they exhibited biallelic mutation of the NF1 gene. Its protein product, neurofibromin negatively regulate Ras. Therefore, in Schwann cells loss of function of neurofibromin may promote cell proliferation. In peripheral nerves, Schwann cell-axon relationship is thought to be isolated from the adjacent tissues by the perineurium, which creates a diffusion barrier formed by the tight junction. Tight junctions (TJ) are specialized cell-cell point of adhesion at the apical region of epithelial and endothelial cells. These junctions create a barrier that regulate paracellular transport of solutes (barrier function) between cells and also restrict lateral diffusion (fence function) of molecules so as to maintain cell polarity. Tight junction proteins have been suggested to constitute an essential part of this barrier in perineurial and endothelial cells. AIM OF THE STUDY; This study was undertaken in to demonstrate the functional integrity of the perineurial fibroblasts tight junction in sporadic neurofibromas using rabbit anti claudin-3 polyclonal antibody. METHODS; Prior informed patient consent was obtained, and the study has and ethical approval (06/1505/137) that was granted by the Liverpool Research Ethics Committee. Standard immunohistochemistry method was used. Briefly, routine histological procedures(haematoxylin and eosin staining) was first carried out followed by staining using cell markers and tight junctions antibody staining using commercially prepared antibodies (Claudin-3) on experimental and control tissues. RESULTS; The sporadic benign neurofibromas exhibited an immunolabelling pattern with anti-claudin-3 that was intensely positive for membranous staining of perineurial fibroblasts as was recorded for the normal peripheral nerve. Additionally, nuclei staining were observed in Schwann cells of the sporadic neurofibromas. CONCLUSION; We suggests that the perineurial fibroblast tight junctions in sporadic neurofibroma is not disrupted and may be intact due to full compliments (lack of haplo) of the NF-1 gene in sporadic neurofibroma.

775F

Expression of PPARbeta, C/EBPalpha and C/EBPbeta in Psoriasis. *S. Emre[1], N. Unver[1], F. Sarikaya[1], G. Erkin[2], A. Karaduman[2].* 1) Medical Biology, Faculty of Medicine, Ankara, Ankara, Turkey; 2) Department of Dermatology, Faculty of Medicine, Hacettepe University, Ankara-TURKEY.

Psoriasis is a disorder with genetic and immunologic background. Abnormal epidermal proliferation and differentiation characterize the inflammatory skin disease psoriasis. The important roles of PPAR) and C/EBPs transcription factors in regulating overlapping pathways in keratinocyte strongly suggest that both families of transcription factors closely interact to regulate the differentiation program of the skin. This work examined the expression of PPAR) in lesional and nonlesional psoriatic skin compared to normal skin by immunohistochemical analyses . It also aimed to investigate whether the expression of PPAR) and C/EBPs were altered in lesional and nonlesional psoriatic skin compared to normal skin by using quantitative real time RT-PCR methods. Our results analyzed that PPAR) are strongly expressed in interfollicular epidermis. In particular, PPAR) is localized to nuclei throughout the entire subrabasal part of lesional psoriatic skin. The results also showed that the expression of hyperproliferation marker Keratin 6A and late keratinocyte differentiation marker Involucrin are upregulated in psoriatic skin. Next, it demonstrated the expression of PPAR) is significantly upregulated in hyperproliferative lesional skin from psoriasis patients. It also showed that the expression of C/EBP(and C/EBP) are decreased in lesional psoriatic skin. This study reveals the importance of the regulatory interplay among PPAR) , C/EBPs in the control of proliferation and differentiation in keratinocyte cell during psoriasis pathogenesis. .

776F

Tissue Inhibitor of Metalloproteinases-4 gene expression is enhanced in human osteoarthritic synovial membranes and is upregulated by TGF-) in Chondrocytes. *M. Zafarullah[1, 2], W. Huang[1], M. El Mabrouk[1], J. Sylvester[1], F. Dehnade[2].* 1) Medicine, University of Montreal, Montreal, Quebec, Canada; 2) CHUM Notre-Dame Hospital, Montreal, Quebec, Canada.

INTRODUCTION: Tissue inhibitors of metalloproteinases (TIMPs) inhibit matrix metalloproteinases (MMPs) implicated in arthritic cartilage destruction. TIMPs also have anti-angiogenic, growth-promoting and proapoptotic activities in diverse systems. TIMP-4 gene therapy diminishes experimental arthritis and periodontitis in rats. TIMP-4 gene polymorphism is associated with osteoarthritis in Korean population. We studied the previously unknown status of TIMP-4 gene expression in human joint tissues and its regulation by arthritis-associated cytokines. METHODS: RNA from human knee synovial membranes and synovial fibroblasts or chondrocytes was extracted by homogenization in guanidinium isothiocyanate solution. TIMP-4 mRNA expression was measured by semi-quantitative RT-PCR analysis in comparison with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. TIMP-4 protein levels from cytokine-treated chondrocytes were measured by western blotting. Chondrocytes were also treated with extracellular signal-regulated kinase (ERK) pathway inhibitor, U0126 or Sp1 inhibitor, mithramycin alone or with transforming growth factor beta (TGF-) 1). Total cellular proteins (20 μg/lane) were separated by SDS-PAGE and Western blots reacted with rabbit Anti-carboxy terminus human TIMP-4 polyclonal antibody (Chemicon AB816) that detects a ~29-kDa band, which co-migrates with the purified human TIMP-4 protein. RESULTS: TIMP-4 RNA expression originating from synovial fibroblasts was significantly (2.4 fold; $p<0.001$) elevated in 8 osteoarthritic (OA) versus 7 non-arthritic synovial membranes. Non-arthritic and OA femoral head and knee chondrocytes displayed substantial but variably constitutive expression of the TIMP-4 mRNA. In articular chondrocytes, TGF-) 1, oncostatin M (OSM) and interleukin 17 (IL-17) upregulated TIMP-4 RNA or protein expression while interleukin-1 beta (IL-1)) and tumor necrosis factor alpha (TNF-( ) did not, suggesting differential regulation by arthritis-associated cytokines. TGF-) 1 induction of TIMP-4 expression was inhibited by U0126 and mithramycin. CONCLUSIONS: Increased TIMP-4 gene expression in OA synovial membranes and cartilage may be due to induction by TGF-) 1, OSM and IL-17 suggesting its pathophysiological role in tissue remodeling in human joints. TGF-) 1 induction of TIMP-4 expression is mediated partly by ERK signalling pathway and Sp1 transcription factor.

777F

Molecular characterization of phenotypes associated with PEX7 deficiency: Rhizomelic Chondrodysplasia Punctata type 1 (RCDP1) and adult Refsum disease (ARD). *S. Jiralerspong[1], G. Nimmo[1], A. Moser[2], S. Steinberg[2], N. Braverman[1].* 1) Dept. of Human Genetics, McGill University-Montreal Childrens Hospital Research Institute, Montreal, Quebec, Canada; 2) Dept. of Neurogenetics and Neurology, Kennedy Krieger Institute, Johns Hopkins Med. Ctr., Baltimore, Maryland, USA.

RCDP1 is a peroxisome biogenesis disorder caused by defects in PEX7, the peroxisome receptor for PTS2 targeted matrix proteins: thiolase, phytanoyl-CoA hydroxylase (PhyH) and alkylglycerone-phosphate synthase (AGPS). We previously reported severe, intermediate and mild phenotype groups based on residual tissue plasmalogens and reflecting amounts of AGPS imported into the peroxisome. In the mild group, plasmalogens are near normal and the phenotype resembles ARD, due to deficient PhyH import. Here we evaluated intraperoxisomal amounts of AGPS, PhyH, thiolase and PEX7 proteins by immunoblotting in 24 PEX7 deficient fibroblast lines, representing all 3 phenotype groups. We show that 30-60% of AGPS is correctly targeted inside the peroxisome in the mild patient group, was absent in the severe group, but also appeared absent in the intermediate group. We propose that AGPS import in the intermediate group accounts for residual plasmalogen levels observed in patients, but may be below the sensitivity of western analysis. For PhyH, 10-20% is imported into the peroxisome in both mild and intermediate groups, but negligible in the severe group. Thiolase import was undetectable in nearly all severe patients, but showed a high level of variability (0-75%) in our set of intermediate and mild patients, indicating additional, unknown factors that influence thiolase import. We found reduced amounts of PEX7 protein generated by the majority of alleles in each group. The highest recovery of AGPS and PhyH import occurred in an ARD patient, shown to express residual amounts of wild type PEX7 transcript from a `leaky' splice site mutation. Treatment with trimethylamine chemical chaperones to encourage conformational correction of PEX7 missense proteins failed to increase PTS2 protein import. Filipin staining showed accumulation of perinuclear cholesterol in patient fibroblasts with absent AGPS import, an increase not observed in fibroblasts from our PEX7 hypomorphic mice with 30% residual AGPS import, even after loading with LDL. We conclude that certain PEX7 missense alleles, as well as residual amounts of wild type PEX7, can rescue the RCDP phenotype by restoring sufficient (⁄ 30%) AGPS import. These same PEX7 alleles, although associated with some PhyH import, cannot prevent the development of ARD over time.

778F

***RET* mutational spectrum in Hirschsprung's disease: evaluation of 601 Chinese patients.** *S.S. Cherny[1], M.M. Garcia-Barceló[2], T.Y.Y. Leon[2], M.T. So[2], P.C. Sham[1], P.K.H. Tam[2].* 1) Psychiatry, Univ Hong Kong, 21 Sassoon Road, Hong Kong; 2) Surgery, Univ Hong Kong, 21 Sassoon Road, Hong Kong.

Hirschsprung's disease (HSCR) is a developmental disorder characterized by the absence of ganglion cells in the lower digestive tract. Aganglionosis is attributed to a disorder of the enteric nervous system (ENS) whereby ganglion cells fail to innervate the lower gastrointestinal tract during embryonic development. There is significant population variation in the incidence of the disease, and it is most often found among Asians (2.8 per 10,000 live births). HSCR most commonly presents sporadically (80% of the cases), with a recurrence risk of 4%, and more males than females affected (4:1). Both rare (<1% in the population) and common germ line variants of the RET gene, acting either alone or in combination, are the main cause of the disease. Yet, while *RET* common variants are strongly associated with the commonest manifestation of the disease (male, short segment, and sporadic forms), rare coding sequence (CDS) variants are more frequently found in the lesser common and more severe forms of the disease (females, long or total colonic aganglionosis, and familial). Here we present a rare variant (RV) screening of the CDS and intron/exon boundaries of the *RET* gene in 607 Chinese sporadic HSCR patients, the largest number of patients ever reported. We have found a total of 61 different heterozygous RVs (50 novel) distributed in 100 patients (16.64%). These include 14 silent, 29 missense, 5 nonsense amino-acid changes, 4 frame-shifts, and one in-frame amino-acid deletion in the exonic region and two splice-site deletions, 4 nucleotide substitutions and a 22 bp deletion in intronic or untranslated regions. The exonic variants were mainly clustered in the sequence encoding the extracellular domain of the RET protein. All RVs were predicted to alter the protein function. The highest frequency of rare variants was found among those patients with the most severe form of the disease (24% in long or total vs 15% in short-segment). Phasing of the RVs with the *RET* risk-haplotype suggested that *RET* RVs do not underlie the undisputable association of RET common variants with HSCR. None of the variants was found in 250 Chinese controls.

779F

Looping Interactions and Noncoding Transcription Support Complexity of Distant *Bmp2* Enhancers in Bone. *E.M. Broeckelmann[1], S. Pregizer[1], G.E. Crawford[2], D.P. Mortlock[1].* 1) Vanderbilt University, Nashville, TN; 2) Duke University Medical Center, Durham, NC.

Bmp2 is a member of the TGF-) superfamily of secreted signaling molecules that not only plays a critical role in pattern formation and morphogenesis during embryonic development, but also promotes osteoblast differentiation and bone formation throughout life, thus making it a vital factor in the maintenance of bone health. Association studies have implicated *Bmp2* polymorphisms in the pathogenesis of osteoporosis and osteoarthritis, and BMP2 is indispensable for the initiation of fracture repair, which is illustrated by the fact that *Bmp2* conditional knockout mice have significantly reduced bone mass and exhibit spontaneous fractures that are unable to heal. Previously, our transgenic analyses in mice have shown that *Bmp2* gene expression is controlled by numerous distant cis-regulatory elements in a tissue- and time- specific manner and further identified a 656bp evolutionarily conserved element (ECR1) 156kb downstream of the promoter, which specifically functions as *Bmp2* enhancer in osteoblasts. However, while ECR1 drives robust transgene expression in most endochondral bones, it exhibits remarkable anatomical specificity in that it is inactive in cranial intramembranous bones, suggesting the existence of (an) additional osteoblast enhancer(s). In order to further examine the specific role of ECR1 *in vivo*, we deleted ECR1 from a *Bmp2*-containing BAC transgene. Transient transgenic analysis in mouse embryos shows that ECR1 enhancer activity is essential for transgene expression in all bones, although in isolation it is only sufficient to drive expression in a limited subset of osteoblasts. Additional evidence suggesting an active role for multiple enhancers in the regulation of osteoblast-specific *Bmp2* expression was obtained in Chromosome Conformation Capture (3C) assays in MN7 cells (a murine osteoblast cell line), which reveal looping interactions of the *Bmp2* promoter with not only the ECR1 locus, but also with a new candidate locus ~120kb upstream of *Bmp2*. This corresponds to the location of a long noncoding RNA that could also be shown to be expressed in MN7 cells. Lastly, DNase hypersensitivity data from human osteoblasts have been mapped onto the murine *Bmp2* locus and serve as an independent tool to identify additional candidate enhancer loci, some of which overlap with previously identified regions of interest.

780F

A novel functional, regulatory PPAR. polymorphism. *M. Claussnitzer, V. Glunk, H. Hauner, H. Laumen.* Else Kröner-Fresenius-Centre for Nutritional Medicine, Technical University Muenchen, Freising, Germany.

Introduction: Obesity and type 2 diabetes result from a complex interaction of environmental factors acting on a susceptible genetic background. The PPAR. 2 variant Pro12Ala has been reproducibly associated with improved insulin sensitivity and BMI variability, although the Ala12 allele results in reduced PPAR. 2 transcriptional activity. This might be explained by a counteracting regulatory SNP in strong linkage disequilibrium (LD) to the PPAR. tagSNP. **Methods:** 13 HapMap SNPs in LD (r^2 = 0.8) with the PPAR. Pro12Ala tagSNP were bioinformatically analyzed by Genomatix software. Using luciferase assay and electrophoretic mobility shift assay (EMSA) the transcriptional regulatory function of the SNP was assessed. PPAR. mRNA levels were analyzed in homozygous EBV-LCL cells by RT-PCR. **Results:** Based on a bioinformatic approach the SNP rs4684847(C>T) was selected for functional characterization. This polymorphism is located 6 kb upstream of the PPAR. 2 promoter in perfect LD to the Pro12Ala variant. The minor T allele drove a significantly higher relative luciferase expression compared to the major allele in 3T3-L1 adipocytes and C2C12 myocytes. EMSA experiments revealed an allele-specific DNA-protein binding. Specificity of this complex was confirmed by competition experiments. Allele- and isoform-specific regulation of endogenous mRNA expression in homozygous EBV-LCL cells was exclusively evident for PPAR. 2, but not for PPAR. 1. **Conclusion:** The contradictory finding that the Ala12 allele results in reduced transactivation capacity though it is associated with increased insulin sensitivity and altered BMI might be partially explained by this cis-regulatory polymorphism.

781F

Time Course for Production of the Putative Inducer of MYOC Transcription (IMT) Protein. *C. Marrs[1], K. Scott[1], F. Rozsa[1], M.I. Othman[1], J.E. Richards[1,2], H. Pawar[1].* 1) Ophthalmology & Visual Sciences, Kellogg Eye Center, University of Michigan, Ann Arbor, MI; 2) Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI.

Purpose: Induction of myocilin (MYOC) transcript and protein is known to occur in human trabecular meshwork (TM) cells in response to long term dexamethasone (DEX) treatment, and excess MYOC protein is seen in TM in about 50 percent of primary open-angle glaucoma (POAG) cases (Lutjen-Drecoll et al. IOVS 1998;39:517). This raises important questions about the role of increased MYOC in POAG, one of the leading causes of blindness worldwide, and the circumstances under which MYOC can be induced. It has previously been shown by Shepard and colleagues (IOVS 2001;42:3173) that the use of cycloheximide to block protein translation results in failure to induce increased transcription of the MYOC gene by DEX treatment in TM cells. This implies the existence of an intermediate protein, which we refer to as Inducer of MYOC Transcription (IMT), that is necessary for induction of increased MYOC transcript. The goal of this project was to identify the time period during which the putative IMT protein is synthesized. Methods: Primary culture human TM cells were grown to 75 percent confluence and induced with DEX as previously reported (Rozsa et al. Mol Vis 2006;12:125). Cells from DEX treated and untreated wells were harvested at 0, 0.1, 2, 6, 10, 12, 18, 24, and 48 h time points. Total RNA was extracted using the Trizol® Reagent, quantified using Nanodrop spectrophotometer, reverse transcribed, and then the MYOC transcript was quantified by TaqMan® assay. Results: Using MYOC expression normalized to a beta-actin control, we observed that DEX treatment of primary culture TM cells led to a slight, brief drop in MYOC transcription at 0.1 h, followed by a return to baseline level up until about 10 h. MYOC gene transcription first showed signs of induction by a 1.6-fold at 12 h, followed by continued increases of ~3.4-fold by 18 h, ~ 4.8-fold by 24 h, and ~8.6-fold at 48 h. Conclusion: Even though elevation of intraocular pressure in response to DEX takes weeks to manifest, we find that induction of transcription begins between 10 h and 12 h of exposure to DEX. This implies that the putative IMT protein required for DEX induction of MYOC must be expressed within that first 12 h period.

782F

Identification of a sciatic-nerve specific enhancer regulating expression of the PMP22 gene. *P. Patel[1], S. Choi[1], D. Hawkins[2], B. Ren[3].* 1) Inst Gen Med, Univ Southern California, Los Angeles, CA; 2) Dept. of Medicine/Medical Genetics, University of Washington, Seattle, WA; 3) Ludwig Institute for Cancer Research, Univ of California, San Diego, CA.

Charcot-Marie-Tooth is the most common inherited peripheral neuropathy in humans with an estimated prevalence of 1 in 2,500. Defects in at least 39 genes lead to CMT. CMT1A is characterized by slowly progressive distal muscle atrophy and weakness, absent deep tendon reflexes, and deformity of the feet. A 1.5Mb duplication including the peripheral myelin protein gene (PMP22) underlies CMT type 1A (CMT1A) in >70% of CMT patients making it the most frequent sub-type. Animal models over-expressing PMP22 recapitulate the disease. Thus, reducing expression of PMP22 could be a therapeutic strategy for CMT1A. We have therefore, sought to dissect the regulation of the gene as a first step towards discovering small molecules that may be useful in modulating expression of the gene in CMT1A patients. Chromatin immunoprecipitaion (ChIP) is a powerful method to identify binding sites on genomic DNA for proteins such as transcription factors, or to assess modifications of genome structure by histone-binding in a region of the genome, which is now known to influence gene transcription epigenetically. DNA regions bound by histone 3 methylated at lysine 4 (H3K4me1) and acetylated at lysine 27 (H3K27ac) are regarded as putative enhancer elements. We therefore, conducted ChIP analysis of an ~200kb region encompassing the PMP22 gene using chromatin purified from rat sciatic nerve to identify putative enhancers. The immunoprecipitated chromatin was hybridized to a custom NimbleGen 385K chip array on which 87 rat genes, 10 mouse genes, and 9 human genes related to myelination and their flanking sequences were tiled. The input DNA was labeled with Cy-3 and the test DNA was labeled with Cy-5 and after hybridization, the respective signals obtained by scanning and analyzed using NimbleScan. Fourteen regions were identified as candidate enhancer elements and along with two negative control regions from other regions of the genome. These regions were cloned into a luciferase reporter vector, pGL3-basic and pGL3-TK (herpes virus thymidine kinase promoter) and evaluated for expression in a rat Schwann cell line, S16 to determine if they can enhance luciferase expression. These studies have identified an enhancer element within the first intron of the gene that is currently being characterized. These results will be presented.

783F

Abnormal quantitative Real-Time PCR (qPCR) Quantification in *TSC1/TSC2* genes: Results in a Cohort of Patients with Tuberous Sclerosis. *N. Lannoy[1], JF. Durant[2], I. Abinet[1], T. Corbisier[1], A. Jansen[3], K. Pelc[4], MC. Nassogne[5], K. Keymolen[6], JL. Gala[2], B. Delle Chiaie[7], S. Gaillez[8], Ch. Verellen[1], Y. Sznajer[1], N. Lannoy is supported by a grant from "Michelle stichting, scientific foundation.* 1) Centre de Génétique Humaine, Cliniques Universitaires Saint-Luc U.C.L, Brussels, Belgium; 2) Centre de Technologies Moléculaires Appliquées , Cliniques Universitaires Saint-Luc U.C.L, Brussels, Belgium; 3) Pediatric Neurology, UZ Brussel, V.U.B, Brussels, Belgium; 4) Pediatric Neurology, Hôpital Universitaire des Enfants Reine Fabiola, U.L.B, Brussels, Belgium; 5) Pediatric Neurology, Cliniques Universitaires Saint-Luc U.C.L, Brussels, Belgium; 6) Centrum for Medische Genetica, UZ Brussel, V.U.B, Brussels, Belgium; 7) Centrum for Medische Genetica, UZ Gent, Gent, Belgium; 8) Centre de Génétique Humaine, CHU Sart-Tilman, Liège.

Introduction Tuberous sclerosis complex (TSC) represents an inherited autosomal dominant condition characterized by progressive occurrence of skin lesions and multisystemic hamartoma. Mutation or rearrangement in *TSC1* or TSC2 gene encoding hamartin and tuberin has been identified in patients who fulfilled the clinical criteria (Roach 2004). Despite systematic sequencing of all coding exons, splice site junctions and search for large genomic rearrangements, a causative mechanism is not found in up to 20 percent; suggesting genetic heterogeneity or that maybe other regulatory regions modification inside these two genes might be responsible for the disease.In order to test this last hypothesis, qPCR quantification of TSC mRNA was performed in 17 unrelated patients with TSC phenotype. Methods Patients referred for molecular diagnosis of TSC were enrolled based on the validated clinical criteria. In patients where first line of investigation did not allow to identify mutation (sequencing of all 21/41 exons, respectively) nor deletion/duplication (MLPA) in *TSC1 or TSC2* gene, RNA sample was collected for determining gene expression variations on a LightCycler® 480 System. Relative mRNA quantification was applied to determine the amount of target mRNA in samples. Relative comparison between each sample was achievable using the 2-+;+Ct method and)-actin housekeeping gene for normalization of gene expression. Additionally, TSC genes promoters and UTR regions were analysed on DNA patients by sequencing. Results On the initial cohort of 91 index patients diagnosed with TS, 17 were eligible for RNA expression studies. Abnormal level (decreased, increased or complete absence) of expression in either one or in the two genes was identified in 15 affected patients. 6 patients were identified with expression anomaly in both genes; 6 in *TSC1* and 3 in *TSC2* gene. In two families, expression in both genes was comparable to normal population. Sequencing of promoter and 5'/3'UTR regions of TSC genes did not reveal any sequence distortion. Conclusion Expression anomalies in TSC genes were evaluated in 17 unrelated families with a clinical diagnosis of tuberous sclerosis after mutation in coding sequences and del/dupl were rule out. This complementary molecular investigation allows postulating for a causative role of so far unidentified regulatory regions important for tuberin and hamartin expression. *Ref: Roach S. J Ped Neurol 2004;19:644.*

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784F

Mutations in *LRP5* cause primary osteoporosis without features of OI by reducing Wnt signaling activity. *J. Korvala[1], H. Jüppner[2], O. Mäkitie[3], E. Sochett[4], D. Schnabel[5], S. Mora[6], D. Deraska[7], W.G. Cole[8], H. Hartikka[1], L. Ala-Kokko[1,9], M. Männikkö[1].* 1) Oulu Center for Cell-Matrix Research, Biocenter and Department of Medical Biochemistry and Molecular Biology, University of Oulu, Oulu, Finland; 2) Departments of Medicine and Pediatrics, Massachusetts General Hospital and Harvard Medical School, USA; 3) Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland; 4) Hospital for Sick Children, University of Toronto, Canada; 5) Department for Pediatric Endocrinology and Diabetes, Otto-Heubner-Centrum für Kinder- und Jugendmedizin, Charite, University Medicine Berlin, Germany; 6) Laboratory of Pediatric Endocrinology, BoNetwork, Division of Metabolic and Cardiovascular Sciences, San Raffaele Scientific Institute, Milan, Italy; 7) Department of Medicine, Winchester Hospital, Winchester, MA, USA; 8) Division of Orthopaedic Surgery, Hospital for Sick Children, University of Toronto, Toronto, ON, Canada; 9) Connective Tissue Gene Tests, Allentown, PA, USA.

Primary osteoporosis is a rare childhood-onset skeletal condition whose pathogenesis is largely unknown. We have previously shown that primary osteoporosis can be caused by heterozygous missense mutations in the *LRP5* gene. The role of *LRP5* was further investigated by analyzing the gene in 18 children and adolescents who had evidence of osteoporosis (manifested as reduced BMD, recurrent peripheral fractures and/or vertebral compression fractures), but who lacked the clinical features of osteogenesis imperfecta (OI) or other known syndromes linked to low BMD. Fifty-one controls were also analyzed. Methods included direct sequencing and MLPA. *In vitro* studies were performed to examine the effect of two novel and three previously found primary osteoporosis mutations on the activity of the canonical Wnt signaling and on expression of Tph1 and 5-Htr1b. Two novel *LRP5* mutations (c.3446T>A; p.L1149Q and c.3553G>A; p.G1185R) were identified in two patients and their affected family members. *In vitro* analyses showed that one of these novel mutations and two previously reported mutations (p.C913fs, p.R1036Q) significantly reduced the activity of the canonical Wnt signaling pathway. Such reductions may lead to decreased bone formation, and could explain the bone phenotype. Gut-derived Lrp5 has been shown to regulate serotonin synthesis by controlling the production of serotonin rate-limiting enzyme, Tph1. *LRP5* mutations did not affect Tph1 expression, and only one mutant (p.L1149Q) reduced expression of serotonin receptor 5-Htr1b ($p < 0.002$). Our results provide additional information on the role of *LRP5* mutations and their effects on the development of juvenile-onset primary osteoporosis, and hence the pathogenesis of the disorder. The mutations causing primary osteoporosis reduce the signaling activity of the canonical Wnt signaling pathway and may therefore result in decreased bone formation. The specific mechanism affecting signaling activity remains to be resolved in future studies.

785F

Tissue-specific loss of gene expression mediated by a common upstream SNP. *K. Lower, C. Derry, J. Hughes, D. Garrick, D. Vernimmen, H. Ayyub, R. Gibbons, D. Higgs.* MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK.

It is estimated that approximately 30% of genes are differentially expressed from each allele, and that the majority of this allele-specific expression occurs in a tissue-specific manner. Studies have shown that this observed allele-specific expression can primarily be explained by genetic variation, such as SNPs, within cis-acting regulatory elements. Whilst a functional effect can be predicted when a SNP is found within a known transcription factor recognition site, it is not yet clear to what extent sequence variation within the local area of transcription factor binding sites can affect the regulation of gene expression. Elucidating the functional effect of sequence polymorphisms, hundreds of which have now been linked to common human diseases through genome-wide association studies, is integral to reconciling these studies with the pathology of disease. We have previously shown that in humans the gene NME4, contained on the short arm of chromosome 16, has acquired a non-conserved, functional erythroid-specific transcription factor (GATA-1) binding site. The presence of this site results in recruitment of erythroid-specific transcription factors, and results in the production of not only an alternative erythroid-specific transcript, but also mediates an interaction with the evolutionarily conserved upstream (globin regulatory elements (REs), which lie approximately 300kb upstream of NME4. The physical interaction between these two GATA-1 binding sites ((globin REs and NME4) results in an ~8-fold upregulation of both the erythroid-specific transcript (eNME4) and full length NME4. Here we describe in detail the functional effect of a common SNP within NME4, which results in both the complete loss of expression of eNME4 and the loss of upregulation of NME4. One variant of this polymorphism completely abrogates GATA-1 binding in vivo, despite lying 65 base pairs upstream of the predicted canonical GATA-1 binding site. Importantly, the presence of this SNP has no effect on NME4 expression in non-erythroid tissues. This provides an example of the potentially dramatic, tissue-specific effects, which can arise from common variation, and may account for a significant proportion of normal variation and disease risk within the human population. In addition, this study highlights the importance of carrying out functional characterisation of common non-coding polymorphisms, associated with disease risk, in clinically relevant tissues.

786F

TNF-alpha-308A allele is associated with the risk of Hepatitis B infection. *M. Mansoori[1], A. Tahmasebifar[2], R. Abdolmohammadi[1], M. Shahbazi[3].* 1) Golestan University of Medical sciences, Advanced Biomedical Faculty & Cellular and Molecular Medicine Research Center, Taleghani Hospital, Gorgan, Iran, MSc; 2) Golestan University of Medical sciences, Advanced Biomedical Faculty & Cellular and Molecular Medicine Research Center, Taleghani Hospital, Gorgan, Iran, MD-PhD; 3) Golestan University of Medical sciences, Advanced Biomedical Faculty & Cellular and Molecular Medicine Research Center, Taleghani Hospital, Gorgan, Iran, MSc-PhD.

TNF-alpha-308A allele is associated with the risk of Hepatitis B infection Background and aim: Tumor necrosis factor-alpha (TNF-a) plays a vital role in host immune response to HBV. The cytokine production in individuals has a capacity that contains a major genetic component. The researches showed the evidences that suggest TNF-a gene polymorphisms associated with resistant to or susceptibility of chronic HBV infection. The purpose of this study was to examine whether TNF-a promoter polymorphisms are associated with the resistant to HBV infection or not. Methods: A total number of 744 Iranian people were classified in two different groups; HBV infected cases (n=261), who were HBS-Ag-positive, and healthy controls (n=483). TNF-a promoter polymorphism at position -308G>A was determined and the distribution of genotypes in HBV infected cases and healthy controls were compared. Results: The frequency of TNF-a-308GG genotype in healthy controls was 47.2 percent, significantly higher than 28 percent in HBV infected patients ($p<0.05$). While the frequency of TNF-a-308A (A/A and A/G)genotype in healthy controls was 52.8 percent, significantly lower than 72 percent in HBV infected patients ($p<0.05$). The frequency of TNF-a-308A allele in Iranian people was remarkably higher in comparison to the world population. Conclusion: TNF-a-308 G/G polymorphism was associated with HBV resistant, whereas TNF-a-308A (A/A or A/G) polymorphism was associated with chronic HBV infection. It is probably that, the -308 G/G polymorphism of TNF-a gene promoter region plays an important role in the resistant to HBV infection in Iranian population.

787F

Identification of genetic variations in human Glycine N-acyltransferase using 454® pyrosequencing. *F.H. O'Neill, C. Herfurth, A.A. van Dijk.* Biochemistry, North-West University, Potchefstroom, South Africa.

Humans are continuously exposed to harmful compounds. These compounds may be of xenobiotic, e.g. pharmaceutical drugs, or endogenous origin. Detoxification is the process by which these harmful compounds are neutralised and excreted from the body. Glycine N-acyltransferase, (EC 2.3.1.13; GLYAT) is a key enzyme in the second phase of detoxification and detoxifies compounds containing a carboxylic acid group by conjugating them with glycine. The conjugation renders the compound more water soluble and allows for its excretion in urine. It is believed that, similar to what has been observed for the Cytochrome P450 group of enzymes, variations within the GLYAT gene may lead to altered enzyme activity that can affect the efficacy of detoxification. The aim of this study was to identify genetic variations within the GLYAT gene of a cohort of individuals whose GLYAT activity has been biochemically screened.

A number of potential participants were identified via screening of their phase I and II biochemical detoxification profiles. Eighteen of the identified individuals, all of whom were of Caucasian origin, agreed to participate in the study. DNA, isolated from blood samples obtained from the participants, was used as a template to amplify the GLYAT gene via PCR. The 23.2kb GLYAT gene was amplified in four parts and the amplicons sent for GS FLX Titanium (Roche) pyrosequencing at Inqaba Biotec (Pretoria, South Africa). The sequencing results were analysed with the Lasergene software package (DNAStar). A minimum depth of coverage of 20 times was a prerequisite for an area to be analysed for variants.

Analysis of the sequence data revealed 94 variations within the GLYAT gene, 29 of which were novel variations. The novel variations consisted of 10 nucleotide changes, seven insertions and 12 deletions. Four of the novel variations occurred within the 3' untranslated region of exon six while the rest were all located within the introns. Identification of these variants is an important step towards establishing a correlation between variations within the gene and altered enzyme activity.

788F

TNF-alpha-238A allele is associated with the risk of Hepatitis B infection. *A. Tahmasebifar[1], M. Mansoori[2], R. Abdolmohammadi[3], M. Shahbazi[4].* 1) Golestan university of Medical sciences, Advanced Biomedical Faculty and Cellular and Molecular Medicine Research Center, Taleghani Hospital, Gorgan, Iran, MD-PhD; 2) Golestan university of Medical sciences, Advanced Biomedical Faculty and Cellular and Molecular Medicine Research Center, Taleghani Hospital, Gorgan, Iran, MSc; 3) Golestan university of Medical sciences, Advanced Biomedical Faculty and Cellular and Molecular Medicine Research Center, Taleghani Hospital, Gorgan, Iran, MSC; 4) Golestan university of Medical sciences, Advanced Biomedical Faculty and Cellular and Molecular Medicine Research Center, Taleghani Hospital, Gorgan, Iran, Ms-PhD.

TNF-alpha-238A allele is associated with the risk of Hepatitis B infection Background and aim: Chronic hepatitis B virus (HBV) infection is a global health problem with an estimated 350 million people chronically infected worldwide. The virus itself, environment factors, ethnic differences and genetic susceptibility have also been reported to have some influences on the progression of this liver disease. Interestingly, the clearance of HBV infection is associated specifically with HLA-DR13 in several ethnic groups. Several cytokines have been identified to participate in the process of viral clearance via host immune response to HBV. In particular, Tumor necrosis factor-((TNF-( ) is an important cytokine in the pathogenesis of HBV infection. The gene of TNF-(is located 850 kb telomeric of the class II HLA-DR locus of the short arm of chromosome 6. The amount of cytokine production seems to be affected by the polymorphisms in the regulatory region. There have been a number of studies on the association between chronic HBV, HBV clearance, HBV resistance and HBV susceptibility and TNF- promoter polymorphisms (-1031T/C, -863C/A, -857C/T, -308G/A, and-238G/A).We hypothesize that genetic variation in the TNF-(promoter polymorphisms may affect the resistance and clearance of HBV infection. Methods: A total number of 744 Iranian people were classified in two different groups; HBV infected cases (n=372), who were Hepatitis B Surface-Antigen-positive (HBS-Ag-positive), and healthy controls (n=372). TNF-a promoter polymorphism at position -238G>A was determined and the distribution of genotypes in HBV infected cases and healthy controls were compared. Results: The frequency of TNF-(-238GG genotype in healthy controls was 57.6 percent, significantly higher than 37.7 percent in HBV infected patients ($p<0.05$). While the frequency of TNF-(-238A (A/A and A/G) genotype in healthy controls was 42.4 percent, significantly lower than 62.3 percent in HBV infected patients ($p<0.05$). The frequency of TNF-(-238A allele was remarkably elevated in Iranian population as compared to the other groups throughout the world reported in literature. Conclusion: TNF-(-238 G/G polymorphism was associated with HBV resistant, whereas TNF-(-238A (A/A or A/G) polymorphism was associated with chronic HBV infection. Therefore, the -238 G/G polymorphism of TNF-(gene promoter region may play an important role in the resistant to HBV infection.

789F

Extensive variation in U4atac snRNA, a minor spliceosome component that is mutated in the severe human developmental disease MOPD I. *D.E. Symer[1,2,3,4], K. Akagi[1,2], H. He[1,2], R.C. Dietrich[5], A. Robertson[6], W. Li[1,2], J. Li[1,2], J.A. Westman[3], C.B. Burge[6], A. de la Chapelle[1,2], R.A. Padgett[5].* 1) Human Cancer Genetics Program, Ohio State Univ Comp Cancer Center, Columbus, OH; 2) Department of Molecular Virology, Immunology and Medical Genetics, Ohio State University, Columbus, OH; 3) Department of Internal Medicine, Ohio State University, Columbus, OH; 4) Department of BIomedical Informatics, Ohio State University, Columbus, OH; 5) Department of Molecular Genetics, Lerner Research Institute, Cleveland Clinic, Cleveland, OH; 6) Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.

We recently showed (ref. 1) that mutations in human U4atac small nuclear RNA (snRNA), a component of the U12-dependent spliceosome, are responsible for the devastating developmental disorder, microcephalic osteodysplastic primordial dwarfism (MOPD I). Functional splicing studies showed that this disease is caused by biallelic mutations in the mature RNA region of U4atac snRNA, reducing minor splicing function to between 3 and 8% of normal activity. MOPD I patient-derived fibroblasts show defective U12-dependent splicing but normal U2-dependent splicing, while introduction of a wild type *RNU4ATAC* snRNA gene into such cells restores U12-splicing. We sequenced the *RNU4ATAC* snRNA gene from a large number of healthy, diverse individuals, revealing a surprisingly high frequency of single nucleotide polymorphisms (SNPs) within and adjacent to it. We also found extensive variation in and near three other U12-dependent spliceosomal snRNA genes, *RNU11*, *RNU12* and *RNU6ATAC*, in diverse human genomes. Each variant identified in healthy adults appears to occur in heterozygosity and at low allele frequencies. Analysis of transcriptome datasets and functional testing both demonstrated that each of these minor snRNA genes is encoded by a single functional copy in the human genome. Numerous pseudogene copies of these snRNAs display significantly less variation within human populations than do the snRNA genes themselves. Of the many naturally occurring SNPs identified in U4atac snRNA, several are predicted to disrupt basepairing to different extents. An assay of in vivo function confirmed that many of these variants can disrupt U12-dependent splicing, in some cases at similar levels seen for the MOPD I disease-associated mutations. These results suggest that functionally deleterious variants can occur at unexpectedly high frequency in essential snRNA components of the minor spliceosome, and provide new insights into the ongoing evolution of RNA splicing. They also suggest that additional disease-associated mutations in snRNAs remain to be identified. 1. He et al. (2011) Science 332, 238-240.

790F

Quantitative analysis of the human beta cell transcriptome. *A.C. Nica, J.C. Irminger, S.E. Antonarakis, P.A. Halban, E.T. Dermitzakis.* Genetic Medicine & Development, University of Geneva, Geneva, Switzerland.

Gene expression shapes phenotypic differences among cell-types, individuals and populations. Immediately affected by DNA sequence variation, transcript abundance provides a direct link between genotype and organismal phenotypes, helping thus interpret some of the currently known disease-predisposing loci. As most phenotypes manifest themselves only in certain tissues, it has become apparent that mRNA profiling is most informative in the context of a trait-relevant cell-type. Towards this end, we provide here the first detailed description of the human beta cell transcriptome using deep coverage RNA sequencing (RNA-seq). We employ a unique method described previously (Parnaud G et al, Diabetologia 2008) to obtain a highly purified (approx 90%) population of beta cells from a mixed preparation of islets derived from human cadaveric pancreata. We sequence each sample in one Illumina HiSeq 2000 lane, providing thus an unbiased transcriptome characterisation at unprecedented resolution, down to even just one transcript copy per cell. We quantify and catalogue all beta cell expressed genes, both coding and non-coding (including microRNAs) at the exon, transcript and gene level. In addition, we identify novel alternatively spliced variants. While the limited sample size does not allow proper association mapping of expression quantitative trait loci (eQTLs), we will determine allele-specific expression (ASE) signals in each heterozygote site. These, in combination with haplotype homozygosity information, enable us to detect rare eQTLs and assess the overall properties of proximal (cis) effects. We shall further make use of the observed inter-individual variation in beta cell expression levels by integrating and interpreting diabetes-associated loci. Altogether, the fine structure and properties of the human beta cell transcriptome constitute a powerful resource aiding our understanding of diabetes genetics.

791F

Detection and validation of MCF7 fusion transcripts, chimeric transcripts and novel proteins using paired end RNA Seq with the 5500 Genetic Analysis System. *R. Nutter, C. Barbaraciou, O. Sakarya, A. Siddiqui.* Life Technologies Applied Biosystems, Foster City, CA.

Our understanding of the structure and function of RNAs is rapidly expanding due to the implementation of the high throughput sequencing application termed RNA Seq. We have used RNA Seq to study in great detail the transcriptome of MCF7, a well studied breast cancer cell line. We have constructed cDNA libraries from polyA RNA isolated from the MCF7 cell line and used high throughput, paired end sequencing using the 5500xl Genetic Analysis System to study the transcriptome in depth. The data generated was analyzed using Lifescope™ v2.0 software followed by the publicly available software programs Cufflinks and SIFT to find novel RNA forms not present in normal human cells. Our approach has identified a number of novel RNA forms and predicted proteins from a large paired end dataset. We found a total of 25 fusion transcripts in our dataset, some are known but many have not previously been reported. All novel fusion transcripts have been validated using custom TaqMan® assays. When we compared our results to RNAs from a number of other cancer lines, we found examples of recurrent fusions in a number of lines. Deep analysis of the fusion transcripts suggests specific regions of transcripts are more favored than others as sites where fusions occur. Cufflinks and SIFT were used in addition to Lifescope™ software to identify alternative splicing events and chimeric transcripts and predict novel proteins from this large dataset. This is the first demonstration of the potential power of using very large datasets with these software tools to more fully describe the transcriptome of a cancer cell line and reliably predict novel transcripts as well as proteins derived from these transcripts. For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

792F

The creation of BAC transgenic mice to explore the transcriptional and behavioral effects of a putative functional polymorphism in the human dopamine) -hydroxylase gene. *D. Perdomo[1, 2], T. Berg[5], K. Mercer[4], P. Szot[3], C.L. Liles[2], D. Wenshenker[2], J. Cubells[2,4].* 1) Graduate Program in Genetics and Molecular Biology, Emory University, Atlanta, GA 30322; 2) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA 30322; 3) 3. Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98108; 4) Department of Psychiatry and Behavioral Sciences, Atlanta, GA 30322; 5) 5. Ce-M-M-Research Center for Molecular Medicine of the Austrian Academy of Sciences, Lazarettgasse 14, AKH BT 25.

BACKGROUND: Dopamine beta-hydroxylase (D) H) is the catecholamine biosynthetic enzyme that converts dopamine to norepinephrine, and is expressed specifically in noradrenergic cells. Serum D) H activity is a stable heritable phenotype that is highly variable across individuals, and a large fraction of this variability appears to be controlled by rs1611115, a single nucleotide polymorphism 1021 bp upstream of the translational start site of the DBH gene 1 The “T” allele of rs1611115 associates with low serum D) H activity, but transient transcription experiments with reporter constructs suggested the “T” allele associated with greater transcriptional activation2. Here, we report initial results of our efforts to create a mouse model capable of supporting analysis of the functional effects of rs1611115 on DBH gene regulation. METHODS: We are generating human BAC transgenic mice bearing either the “C” or “T” allele at -1021C-T, in the context of greater than 100 kb of human sequence. Those lines are then crossed with mice completely lacking endogenous mouse D) H (Dbh -/-). RESULTS: We have successfully generated two lines of mice with the low activity “T” allele of human DBH and crossed them onto a Dbh -/- background. In situ hybridization results reveal that human DBH is specifically expressed in the locus coeruleus, the major brain noradrenergic nucleus, indicating that all regulatory elements necessary for cell type-specific expression in the brain are present in the BAC. Furthermore, the transgene rescued the embryonic lethality, delayed growth, and ptosis phenotypes of Dbh-/- mice. We are currently generating lines of mice with BACs bearing the “C” allele of DBH, and will compare DBH transcription, catecholamine neurochemistry, and behavior with the “T” allele BAC transgenic mice.

793F

IMPLICATION OF THE ESE TRANSCRIPTION FACTORS (EPITHELIUM-SPECIFIC ETS-LIKE FACTORS) IN CFTR GENE EXPRESSION. *A.T. de Becdelièvre[1,2,3], A. Hinzpeter[1], N. Martin[1], B. Costes[1], P. Fanen[1,2,3].* 1) U955, INSERM, Créteil, France; 2) Université Paris-Est, Faculté de Médecine, UMR-S 955, Créteil, France; 3) Service de Génétique, Hopital Henri Mondor, APHP, Créteil, France.

Cystic fibrosis (CF) (MIM#219700), the most common lethal autosomal recessive disease in Caucasians, is caused by the dysfunction of an epithelial chloride channel encoded by the *CFTR* gene (OMIM*602421). CF affects respiratory, digestive and reproductive systems with a variable severity, correlated to the amount of functional CFTR in each organ system. Of the 1800 mutations identified so far, less than 1% are located in *CFTR* regulatory regions and only a few transcription factors has been shown to regulate *CFTR* gene expression. ESE transcription factors are tissue-specific proteins which are up-regulated in bronchial epithelial cells in response to inflammation (1), a characteristic feature in the lungs of CF patients.**Aim**: We aimed to evaluate the influence of members of the ESE transcriptions factors on CFTR expression. **Methods**: Different sizes of the *CFTR* promoter subcloned upstream of the luciferase reporter gene were co-transfected in IB3-1 epithelial cells (CF cells) with either ESE-1, ESE-2 or ESE-3. Endogenous CFTR expression was measured by qRT-PCR after over-expression of the ESE proteins and interaction between ESE-1 and the *CFTR* promoter was assessed by chromatine immunoprecipitation (ChIP) in Calu-3 cells. **Results**: Whereas ESE-2 and ESE-3 over-expression did not affect promoter activity, ESE-1 had a 2 to 6 fold enhancer activity depending on the tested construct. Bioinformatic tools and direct site mutagenesis enabled to identify the major binding site of ESE-1 at position -37 bp of CFTR promoter, which was validated by ChIP. Finally, over-expression of ESE-1 in Calu-3 cells doubled endogenous *CFTR* expression while over-expression of ESE-2 or ESE-3 had no significant effect. **Discussion - Conclusions**: Members of the ESE protein family appear of special interest in cystic fibrosis, as a recent GWAS associated the locus of *ESE-3* (*EHF*) with lung disease severity in CF patients (2). We showed that ESE-1, a transcription factor induced by pulmonary inflammation, increases *CFTR* gene transcription. Sequence variations within its binding site or altering ESE-1 functional level could affect *CFTR* expression in patients, possibly accounting for phenotype variability. Deciphering ESE and *CFTR* interactions mechanisms could participate to a better understanding of the CF pulmonary disease. (1)Wu et al, Cell Research, 2008 (2)Wright et al, Nature Genet, 2011. Supported by INSERM and the French Association “Vaincre La Mucoviscidose”.

794F

Analysis of HSP70B' as a potential direct target gene of the FOXC1 transcription factor. *Y.A. Ito[1], F. Berry[1,3], M.A. Walter[1,2].* 1) Department of Medical Genetics, University of Alberta, Edmonton, AB, Canada; 2) Department of Ophthalmology, University of Alberta, Edmonton, AB. Canada; 3) Department of Surgery, University of Alberta, Edmonton, AB. Canada.

FOXC1 mutations cause human Axenfeld-Rieger Syndrome (ARS), an autosomal-dominant disease that affects the anterior segment of the eye. ARS patients have an elevated risk to develop earlier-onset glaucoma, a progressively blinding condition that is usually associated with increased intraocular pressure (IOP). Structures of the anterior segment of the eye, such as the trabecular meshwork (TM), are important in maintaining the aqueous flow pathway and thus, regulating IOP. The TM outflow pathway tissue is subjected to constant stress. Healthy TM cells have protective mechanisms to cope and adapt to various stresses, including oxidative stress. However, chronic exposure to stress may overwhelm cellular defense mechanisms, resulting in progressive diseases such as glaucoma. The identification and characterization of direct target genes of FOXC1, specifically those genes that are stress-responsive, are essential in understanding how disruptions in FOXC1 can result in ARS and glaucoma. Previous work in our laboratory using microarray technology has led to the identification of potential genes that are regulated by FOXC1. Heat shock protein 70 (HSP70B') is one gene identified in the microarray as a potential FOXC1 target. Although the function of HSP70B' is largely unknown, HSP70 proteins are typically involved in protecting cells during stress response. HSP70B' was an interesting gene to further study because there is increasing evidence that a variety of stresses, including oxidative stress, may contribute to the development of glaucoma. First, HSP70B' was further confirmed as a FOXC1 target gene by examining HSP70B' RNA levels in human trabecular meshwork (HTM) cells. HSP70B' RNA levels are decreased by 1.6 fold when FOXC1 is knocked down using siRNA technology ($P<0.05$). Under H_2O_2-induced oxidative stress conditions, there is at least four times as much HSP70B' RNA compared to non-oxidative conditions, suggesting that HSP70B' is stress-responsive. HTM cells are less resistant to oxidative stress when FOXC1 is knocked down, suggesting that FOXC1 is involved in the cellular stress pathway. Interestingly, FOXC1 knockdown resulted in a 1.6 fold increase in HSP70B' protein levels ($P<0.05$) in oxidatively stressed HTM cells. In fact, the highest level of apoptosis was observed in cells expressing the highest level of HSP70B'. Thus, these experiments suggest that FOXC1 regulation of HSP70B' protein is important in mediating the cellular stress response pathway.

795F

Host factors associated with LINE-1 and its retrotransposition. *J.L. Goodier, L. Cheung, P.K. Mandel, H.H. Kazazian.* McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

LINE-1 retrotransposons constitute one-fifth of human DNA and have helped shape our genome. A full-length L1 encodes a 40 kD RNA-binding protein (ORF1p), and a 150 kD protein (ORF2p) having endonuclease and reverse transcriptase activities. Despite the immense significance of L1s for genome evolution, much about their biology remains unknown, including cellular factors involved in the complex process of retrotransposition. By means of immunoprecipitation of tagged L1 ORF1p or ORF2p expressed from full-length retrotransposition-competent L1 constructs followed by MS sequencing, we identified proteins associated with the L1 ribonucleoprotein particle. These included helicases, RNA transport proteins, chaperone proteins, and splicing factors. We have assessed the co-localization of these cellular proteins with L1-enoded proteins and RNA. Employing a well-established GFP-reporter retrotransposition assay, as well as a novel retrotransposition assay based on secreted luciferase, we also assayed the effects of overexpression or siRNA-suppression of these proteins on cell culture retrotransposition. These studies suggest candidate cofactors that interact with the L1 to modulate its activity, and increase our understanding of the means by which the cell coexists with these genomic “parasites”.

796F

Retrotransposition of marked SVA elements by human L1s in cultured cells. *D. Hancks, J. Goodier, P. Mandal, L. Cheung, H. Kazazian Jr.* Institute for Genetic Medicine, The Johns Hopkins School of Medicine, Baltimore, MD.

Human retrotransposons generate structural variation and genomic diversity through ongoing retrotransposition and non-allelic homologous recombination. Cell culture retrotransposition assays have provided great insight into the genomic impact of retrotransposons, in particular, LINE-1(L1) and Alu elements; however, no such assay exists for the youngest active human retrotransposon, SINE-VNTR-Alu (SVA). Here we report the development of an SVA cell culture retrotransposition assay. We marked several SVAs with either neomycin or EGFP retrotransposition indicator cassettes. Engineered SVAs retrotranspose using L1 proteins supplemented in trans in multiple cell lines, including U2OS osteosarcoma cells where SVA retrotransposition is equal to that of an engineered L1. Engineered SVAs retrotranspose at 1-54 times the frequency of a marked pseudogene in HeLa HA cells. Furthermore, our data suggest a variable requirement for L1 ORF1p for SVA retrotransposition. Recovered engineered SVA insertions display all the hallmarks of LINE-1 retrotransposition and some contain 5' and 3' transductions, which are common for genomic SVAs. Of particular interest is the fact that 4 out of 5 insertions recovered from one SVA are full-length, with the 5' end of these insertions beginning within 5nts of the CMV promoter transcriptional start site. This assay demonstrates that SVA elements are indeed mobilized in trans by L1. Previously intractable questions regarding SVA biology can now be addressed.

797F

THE TRANSMEMBRANE PROTEIN 126A GENE (OPA7), A MITOCHONDRIAL LOCATED mRNA, ENCODES A MITOCHONDRIAL INNER MEMBRANE PROTEIN. *S. HANEIN[1,2], M. GARCIA[3], V. SERRE[1,2], L. FARESTAIE[1,2], I. PERRAULT[1,2], S. GERBER[1,2], J. COTTINEAU[1,2], N. DELPHIN[2], A. SCHMITT[3,4], J.-M. MASSE[3,4], A. ROTIG[1,2], F. DEVAUX[3], A. MUNNICH[1,2], J. KAPLAN[1,2], J.-M. ROZET[1,2].* 1) Genetics and epigenetics of neurometabolic diseases and birth defetcs, INSERM U781-Paris Descartes University & Institute of genetic diseases IMAGINE, Necker - Enfants Malades University Hospital, Paris, France; 2) Genetics Department, Necker - Enfants-Malades University Hospital, Paris, France; 3) Structure, dynamique et évolution des réseaux génétiques, UMR 7238 CNRS-Université Pierre et Marie Curie, Paris, France; 4) Inserm, U1016, CNRS, UMR8104, Institut Cochin - Paris Descartes University, Paris, France.

Purpose: Hereditary optic neuropathies are a heterogenous group of inherited disorders affecting the retinal ganglion cells and their axons which form the optic nerve. To date, four modes of inheritance were described: autosomal dominant, autosomal recessive, mitochondrial and X-linked. Leber hereditary optic neuropathy (MIM 535000) and autosomal-dominant optic atrophies (adOA, MIM165500) are by far the two main forms. Nonsyndromic autosomal-recessive optic atrophies (arOA) are rare conditions which existence has been long debated. Recently, we have identified the first gene, to our knowledge, responsible for this condition, TMEM126A (OPA7, MIM612988) in four families originating from the Maghreb. TMEM126A is highly conserved in higher eukaryotes, strongly expressed in retinal cellular compartments enriched in mitochondria suggesting that TMEM126A may be a mitochondria-localized mRNA (MLR) protein and might be essential in the early nucleation process of large mitochondrial complexes. TMEM126A is supposed to encode a mitochondrial transmembrane protein but its function remains to be elucidated. In an effort to further understand the physiopathologic mechanisms of arOA and to investigate the mechanism of action of TMEM126A protein we address the sub-cellular localization of both TMEM126A mRNA and endogenous protein. Methods: A polyclonal antibody targeting the TMEM126A protein has been generated and its specificity was validated. Fluorescent in situ hybridation, cellular fractionation, mitochondrial membranes association study, mitochondrial sub-compartmentalisation analysis by both proteolysis assays and transmission electron microscopy were carried out. Results: We present molecular and biochemical evidences showing that TMEM126A is translated in the vicinity of mitochondria and encodes a mitochondrial protein strongly associated to the mitochondrial inner membranes. Transmission electron microscopy approach further localizes TMEM126A protein to the inner mitochondrial membrane likely close to the cristae. Conclusion: OPA7 gene encodes a mitochondrial inner membrane protein close to cristae. This result is a first step toward to a better understanding of the mechanism of action of this crucial protein for retinal ganglion cells and provides further arguments to support the importance of mitochondrial dysfunction in the pathogenesis of inherited optic atrophies.

798F

A rare deletion of *IKBKG/NEMO* promoter B in Incontinentia Pigmenti patient reveals the role of *IKBKG* promoterA during embryonic development. *F. Fusco[1], A. Pescatore[1], M.I. Conte[1], M. Paciolla[1,2], M.B. Lioi[2], M.G. Miano[1], M.V. Ursini[1].* 1) IGB-ABT-CNR, Naples, Italy; 2) University of Basilicata, Potenza, 85100, Italy.

Nuclear factor-kB (NF-kB)is a key transcription factor that regulates innate and adaptive immunity as well as ectodermal development. Mutations in the coding region of the *IkBKinaseG/NF-kB Essential MOdifier* (*IKBKG/NEMO*)gene cause X-linked Ectodermal Dysplasia with Immunodeficiency (EDA-ID,MIM300291) in male and Incontinentia Pigmenti (IP,MIM308300) in female. This gene, in Xq28,overlaps *G6PD* gene by a conserved bidirectional CpG promoter (promoterB) ubiquitously expressed. In addition, *IKBKG* gene has its own promoter, promoterA, at long distance to its coding region. We identified a promoterB deletion in one IP patient and we showed the IP skin pathogenesis in that case is due to the absence of promoterB and to physiological downregulation of promoterA in keratynocites differentiation. The observation that promoterA is more active in the stem cells prompted us to investigate on its role during embryonic development. We predicted by bioinformatic analysis the conserved human-mouse transcription binding sites typically expressed in embryonic stem cells, and we observed that most of them were involved in hematopoietic development. We focalized our attention on Prep1 homeodomain transcription factor belonging to the TALE superclass that includes Meis1-3, Prep2 and Pbx1-4. *TALE* genes in mouse show to be essential for hematopoietic stem cells,and *Prep1* is responsible for protecting the embryo very early in development. We performed functional studies by co-trasfecting the promoterA with Prep1 and Pbx proteins in HeLa cells and, using luciferase reporter assay, we observed activation of the *IKBKG* promoterA expression only in presence of both TALE factors. ChIP assay on the factor Prep1 protein extracts of fetal liver of mice at the embryonic E14.5 stage, indicated that *IKBKG* is a direct *in vivo* target of Prep1 during the hematopoiesis. We suggest that a coordinate regulation between promoterA and promoterB in early embryogenesis is required for proper *IKBKG* gene expression during the immunosystem development. On the other hand, such a role of IKBKG in immunosystem is strongly supported by the severe immunodeficiency observed in EDA-ID patients carrying mutations of *IKBKG*. This study demonstrates how a rare genomic rearrangement responsible for IP can give the cue to uncover novel mechanisms controlling the *IKBKG* gene expression.The study is supported by Telethon GGP08125 and AssociationIncontinentia Pigmenti France (http://www.incontinentiapigmenti.fr).

799F

A Challenge to Genotype to Phenotype Relationships: What are the Implications of the Discovery that Post-Genomic Events can Influence Phenotypic Expression? *C. Alvarado[1,3], B. Gottlieb[1,2,3], L.K. Beitel[1,2,3,4], M. Trifiro[1,2,3,4].* 1) Lady Davis Institute for Medical Research, Montreal, Quebec, Canada; 2) Segal Cancer Centre, Jewish General Hospital, Montreal, Quebec, Canada; 3) Dept of Human Genetics, McGill University, Montreal, Quebec, Canada; 4) Dept of Medicine, McGill University, Montreal, Quebec, Canada.

A central dogma of modern genetics has been that phenotypic expression can be predicted by genomic sequencing. This principle has been the basis for much of our understanding of disease ontogeny. Up until now the concepts of variable expressivity and penetrance have been used to explain problems in genotype to phenotype relationships. However, possible mechanisms involved in these concepts have remained unidentified. The assumption has been that any such variation in the relationship is within the `normal' parameters of the relationship. To test this assumption, we decided to examine the genetics of the androgen receptor (AR) gene, a locus-specific gene, in which mutations are responsible for the rare condition known as androgen insensitivity syndrome (AIS). We examined a total of 140 patients with a clear AIS phenotype, and found that in over 40% a mutation in their AR coding exons could not be identified. Further, similar results have been reported in two other AIS databases. In the absence of any other gene being identified as causing AIS to date, the question arises as to whether post-genomic events may play a critical role in determining AIS phenotype. Over the past few years a number of such events and factors have indeed been identified that can significantly affect the relationship between genotype and phenotype. These include DNA and RNA editing, alternative splicing, DNA methylation and interactions involving coactivators and corepressors. In particular, the discovery of widespread RNA editing in over 10,000 genes in normal individuals has suggested that the belief that a specific genotype will result in a specific phenotype needs to be carefully reexamined. Clearly, our reliance on genomic information to predict disease phenotype, such as in genome wide association studies, needs to be reconsidered, which is perhaps why until now, such studies have failed to produce the breakthroughs originally predicted. In light of all the possible post-genomic sources of phenotypic variation and the apparent disconnect between genotype and phenotype in many diseased individuals, we have proposed a new model of the genotype to phenotype relationship that we believe more accurately reflects the actual complexity of this relationship. The term `personalized medicine' has become a key concept of many in discussing the future approaches in treatment of disease, however, it seems unlikely that such a goal can be achieved relying solely on genomic analysis.

800F

Retrotransposition of a unique piece of LCR7 played a key role in the generation of Williams-Beuren syndrome-associated LCR during the primate evolution. *A. Shimizu[1], S. Minoshima[2], N. Shimizu[3].* 1) Department of Molecular Biology, Keio University School of Medicine, Tokyo, Japan; 2) Department of Photomedical Genomics, Basic Medical Photonics Laboratory, Medical Photonics Research Center, Hamamatsu University School of Medicine, Hamamatsu, Japan; 3) Advanced Research Center for Genome Super Power, Keio University, Tsukuba, Japan.

Background: Segmental duplications (SDs) are thought to accelerate the diversity of genome organization during evolution. The SDs in general form repeats of DNA fragments with various sizes, facilitating the birth-and-death event of creating new genes. However, the SDs occurred at a remote location often mediate large hemizygous deletions at meiosis. This is often caused by the unequal nonallelic homologous recombination (NAHR), giving rise to several genetic disorders. Williams-Beuren syndrome (WBS; OMIM 194050) is characterized by distinct facial changes, growth deficiency, mental retardation, and congenital heart defect, in association with infantile hypercalcemia. The WBS is caused by the large hemizygous deletion (1.6 Mb or 1.8 Mb) at 7q11.23 including several contiguous genes. These deletions were mediated by the chromosome 7 specific low copy repeats (LCR7s). Many studies have proposed the mechanism of LCR-mediated deletion or duplication in the primates, however, how these LCRs have evolved from ancestral loci and how they spread over the mammalian genome is still unclear. Results: Here, we present examined an evolutional history of the WBS chromosomal region in the mammalian genome, and defined twenty-six homologous sequence units (LCR7-a~LCR7-z) in the WBS region of human genome. This The unique piece of LCR7 (LCR7-k) flourished during the primate evolution (Chimpanzee, Orangutan, Baboon, Macaque and Gibbon), and it was repeated twenty-one times in the human genome. Interestingly, one particular gene or its paralogs were found in every piece of LCR7-k. Moreover, at least seven of 21 are considered active gene although their functions are not yet clear. The Ka/Ks ratio of those active genes for human vs chimpanzee was as high as 3.29. Conclusions: Our observations suggest that retrotransposition of a unique piece of LCR7-k, played a key role in the generation of WBS-associated LCR in the human lineage. As an evolutionary consequence, only human was given the risk for causing the congenital disorder WBS.

801F

Genome-wide signatures of `rearrangement hotspots' within segmental duplications in humans. *M. Uddin, M. Sturge, D.D. O'Rielly, L. Peddle, P. Rahman.* Faculty of Medicine, Discipline of Medicine and Genetics, Memorial University, St. John's, Newfoundland, Canada.

The catalogue of segmental duplications comprises approximately 5% of the human genome where specific regions are prone to genomic rearrangements. Evidence suggests involvement of multiple events in the origin of rearrangements such as non-allelic homologous recombination (NAHR), non-homologous end joining (NHEJ), fork stalling and template switching (FoSTeS), and microhomology-mediated break-induced replication (MMBIR). The primary objective of this study was to create a genome-wide high resolution map (i.e., >100bp) of `rearrangement hotspots' to facilitate the identification of regions capable of mediating pathogenic deletions or duplications in humans. A hierarchical method was employed to fragment segmental duplications into multiple smaller segmental duplication (SD) units. Combining an end space free pairwise alignment algorithm with a `seed and extend' approach, an exhaustive search of 409 million alignments was performed to detect complex structural rearrangements within the reference guided assembly of NA18507 human genome with 18x coverage. This included previously identified 4.8 MB novel sequence from de novo assembly within this genome. The results of this study revealed that intra-chromosomal recombination is enhanced in genic compared with agenic duplicated regions. We have identified 1,963 rearrangement hotspots within the duplicated regions overlapping 166 genes. Furthermore, duplicated gene nucleotide variants (DNVs) were enriched within the hotspots region. These regions show increased NAHR event frequency as the origin of copy number variations (CNVs) and pathogenic duplications/deletions. Among the detected hotspots, 20% were clustered within the proximal and distal segmental duplications breakpoints flanked by the pathogenic deletions/duplications that have been mapped for 24 NAHR mediated genomic disorders. FISH Validation of selected complex regions shows >90% concordance with in silico localization of the highly homologous derivatives. The generation of genome-wide signatures of `rearrangement hotspots' likely serve as templates for NAHR and may provide a powerful approach towards the understanding of the underlying mutational mechanisms for development of constitutional and acquired diseases.

802F

CAGI: The Critical Assessment of Genome Interpretation, a community experiment to evaluate phenotype prediction. *S.E. Brenner[1], S. Repo[1], J. Moult[2].* 1) Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720; 2) IBBR, University of Maryland, Rockville, MD 20850.

The Critical Assessment of Genome Interpretation (CAGI, `kā-jē) is a community experiment to objectively assess computational methods for predicting the phenotypic impacts of genomic variation. In this assessment, participants are provided genetic variants and make predictions of resulting phenotype. These predictions are evaluated against experimental characterizations by independent assessors. The CAGI experiment culminates with a community workshop and publications to disseminate results, assess our collective ability to make accurate and meaningful phenotypic predictions, and better understand progress in the field. A long-term goal for CAGI is to improve the accuracy of phenotype and disease predictions in clinical settings.

At the first CAGI workshop in December 2010, Pauline Ng, Iddo Friedberg, Sean Tavtigian, Gad Getz, and Sean Mooney made detailed assessments of 108 prediction entries from 8 countries. The CAGI 2010 datasets included rare variants identified from resequencing in cancer cases and controls; nonsynonymous point mutations within a human metabolic enzyme; clinical phenotypes associated with complete human genomes and exomes; cancer cell-line pharmacogenomics; effects of double-mutants in reactivation of p53; and mechanisms underlying GWAS disease associations. The meeting revealed the relative strengths of different prediction approaches, showing some that worked consistently well, while other classes worked only on special types of problems. Even with the simplest dataset, involving nonsynonymous mutations in a human metabolic enzyme, yielded great variability of the results: the best groups had a Spearman rank correlation of ~0.5 with the correct results, while predictions submitted by some groups were only as good as random or worse. Several predictions for the cancer case / control dataset significantly segregated the individuals into their respective cohorts, and some methods performed better than the method initially applied by the dataset author. Overall, CAGI 2010 highlighted the need for customized approaches for specific problems. We are currently completing further analysis of the prediction quality. The CAGI 2011 prediction season runs during summer 2011, with assessment in the fall, and a meeting to be held in the Bay Area in December 2011. We continue to welcome new experimental datasets for CAGI and all predictors. Current information is at the CAGI website at http://genomeinterpretation.org.

803F

Refining susceptibility loci for chronic obstructive pulmonary disease with lung eQTLs. *M. Lamontagne[1], C. Couture[1], M. Laviolette[1], Y. Bossé[1,2], The Merck-Laval-UBC-Groningen Lung eQTL consortium.* 1) Centre de recherche Institut universitaire de cardiologie et de pneumologie de Québec, Quebec, Quebec, Canada; 2) Department of Molecular Medicine, Laval University, Quebec, Canada.

Chronic obstructive pulmonary disease (COPD) is the fourth cause of chronic morbidity and mortality in the United States and Canada. COPD is characterized by airflow obstruction that is not fully reversible. Recent genome-wide association studies (GWAS) have identified robust susceptibility loci associated with COPD and lung function. However, the genetic mechanisms mediating the risk within these loci remain to be found. The goal of this study is to identify causal variants and genes within susceptibility loci associated with COPD and lung function measurements. Genome-wide gene expression profiles of 500 non-tumor lung specimens were obtained from patients undergoing lung surgery. Blood-DNA from the same patients were genotyped for 1,2 million SNPs. Following genotyping and gene expression quality control filters, 409 samples were analyzed. The first step of the analyses was to identify SNPs affecting gene expression in the lung or lung expression quantitative trait loci (eQTLs). The second step was to overlay lung eQTLs to susceptibility loci of COPD and lung function derived from previous GWAS. The first step of the analyses is now completed. A total of 49,667 cis and 1,713 trans eQTLs were identified. Analyses are ongoing to link these eQTL results with published GWAS. Preliminary results suggest that lung eQTLs are located in susceptibility loci of lung function and COPD. Notably, an eQTL on chromosome 5q33.1 governing the expression levels of FBXO38 was identified. This locus was previously associated with abnormal lung function (i.e. FEV1/FVC ratio) and our results suggest that the risk is mediated through the regulation of FOXO38 in the lungs. In summary, the results of this study suggest that lung eQTLs reside in genomic regions previously associated with COPD and lung function, which is an important step to understand the molecular basis of COPD. This study also supports the use of eQTLs in a relevant tissue to prioritize causal genes within loci identified as harboring susceptibility gene variants in GWAS.

804F

Stimulation effects on eQTLs in peripheral blood leukocytes in peripheral blood leukocytes. *K. Löytynoja[1], E. Dukes[1], M. Zucchelli[2], J. Vendelin[1,3], A. Parmar[1], E. Einarsdottir[1], J. Kere[1,2,3,4], D. Greco[1], P. Saavalainen[1].* 1) Dept Medical Genetics and Research Program for Molecular Medicine, University of Helsinki, Helsinki, Finland; 2) Dept Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden; 3) Clinical Research Centre, Karolinska University Hospital, Huddinge, Sweden; 4) Folkhälsan Institute of Genetics, Helsinki, Finland.

BACKGROUND: Expression quantitative trait loci(eQTLs) are loci of genetic variation that control gene expression by proximal (cis) or distal (trans) effects, and may contribute to phenotypic differences. Previous studies have identified multiple such loci and revealed differences between cell types and tissues. Our aim was to identify eQTLs potentially relevant in immune related traits using primary human leukocytes under immunological stimuli. MATERIALS AND METHODS: Spearman rank correlations were calculated for Agilent whole human genome (44K) expression data of in vitro LPS-stimulated (6h) peripheral blood mononuclear cells (PBMCs) and anti-CD3/CD28 stimulated (6 h)CD4+ T cells, along with unstimulated controls, and Illumina 610 quad SNP data of 39 healthy blood donors (17 males, 22 females). Exact p-values were computed for eQTLs with p 1.0E-8 and sex specific eQTLs. RESULTS AND CONCLUSIONS: Preliminary results show highest number of eQTLs for unstimulated cells, and lower number of eQTLs after stimulation. In line with previous studies we found females to show higher number of eQTLs in comparison to males, and ciseQTLs in general to have lower p-values than the more numerous trans eQTLs. Males had higher number of eQTLs in T cells than in PBMCs, whereas females had no difference in eQTL counts between the cell types or conditions. Most of the eQTLs found in more than one subset were shared between females and the combined data set. We have successfully replicated several previously reported indicating enough power of the sample size for common alleles. However, we expect 10 % of the variation in expression to result from SNPs in probe binding sites. Our data provides information of global interactions within genome in immune stimulated and unstimulated PBMC and T cells, and sheds light on sex specific shared between females and the combined have successfully replicated several previously reported eQTLs, indicating enough power of the sample size for common alleles. However, we expect 10 % of the variation in expression to result from SNPs in probe binding sites. Our data provides information of global interactions within genome in immune stimulated and PBMC and T cells, and sheds light on sex specific eQTLs in humans.

805F

Comprehensive NextGen Clinical Sequencing of the Human Mitochondrial Genome (mtDNA) with Low Level Heteroplasmy Analysis: 1+ Years of Diagnostic Experience Highlights the Clinical Importance of Homoplasmic as well as Heteroplasmic Variants. *C. Buzin[1], R. Boles[2], W. Scaringe[1], C. Boysen[1].* 1) MEDomics, LLC, Azusa, CA; 2) Los Angeles Childrens Hospital.

BACKGROUND: Disorders of the mitochondrial DNA (mtDNA) differ clinically from most genetic diseases due to maternal inheritance and the presence of hundreds to thousands of genome copies within a cell (heteroplasmy). Most “classical” mtDNA disorders are often thought to be caused by heteroplasmic mutations, and heteroplasmy is often screened for when evaluating patients for mtDNA disorders. Previous evidence suggesting that mtDNA has a mutation rate roughly 18x higher than nuclear DNA, would predict that low heteroplasmy levels could be common in normal individuals, and thus significantly confound clinical interpretation of mtDNA heteroplasmic variants found in patients with suspected mitochondrial disease. In addition, homoplasmic mtDNA mutations are also established as the cause of some clinical conditions, although differentiation from the multiple homoplasmic polymorphisms found in nearly all humans can be challenging. RESULTS: Heteroplasmic and homoplasmic variants were detected by comprehensive clinical NextGen sequencing of the mitochondrial genome derived from blood and saliva; each sample was sequenced thousands of times. Surprisingly, analysis of the first 50 consecutive clinical samples reveals that about half are without any heteroplasmic variants at levels of 1% or more. In addition, most of the heteroplasmic variants seen are unlikely to be of functional significance. Thus some previous patients who were candidates for painful and expensive muscle sampling (biopsy) for biochemical analysis, were reevaluated for mitochondrial genome diseases by the referring physician, and subsequently tested by blood sampling for nuclear mitochondrial diseases or for other diseases in the differential diagnosis. In addition, some muscle biopsies were eliminated because genomic sequencing demonstrated homoplasmic mutations that likely contribute to, or drive the clinical phenotype, analogous to the manner in which homoplasmic mutations can cause diseases like Leber's Hereditary Optic Neuropathy or hereditary deafness. CONCLUSIONS: While some cases may illustrate how current ignorance is more profound than knowledge, comprehensive NextGen sequencing (or massively parallel sequencing) and deep heteroplasmy analysis of the mitochondrial genome derived from blood is often clinically useful and sometime eliminates the need for muscle biopsies.

806F

Whole-genome definitive haplotypes of SNPs and CNVs determined by genotyping duplicated haploid genomes. *T. Tahira[1], Y. Kukita[2], K. Yahara[3], M. Sonoda[1], K. Yamamoto[1], N. Wake[4], K. Hayashi[1].* 1) Div. Genome Analysis, Med Inst Bioreg, Kyushu Univ. Fukuoka, Japan; 2) Research Inst., Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan; 3) Div. Life Science System, Fujitsu Kyushu Systems Inc. Fukuoka; 4) Dpt. Gynecology and Obstetrics, Graduate School of Medical Sciences, Kyushu Univ.

The majority of the variation of human genome, in terms of the extent genomic regions are involved, is accounted for by copy number variations (CNVs), and some of them are implicated in the disease susceptibility. Finding such disease-responsible CNVs requires detailed knowledge of haplotype structure of CNVs and SNPs for the ethnic groups to which the study subjects belong. However, delineating such haplotype structures is difficult if diploid cells without pedigree information are studied. We have been determining definitive haplotypes of Japanese by analyzing complete hydatidiform moles (CHMs), using high-density DNA arrays. CHMs are tissues carrying duplicated haploid genomes derived from single sperms. This makes CHMs more advantageous as materials over conventional diploid cells in detecting CNVs, because greater signal to noise ratios in hybridization signals are expected, and data interpretation is not bothered by possible heterozygosities of overlapping CNV segments. We genotyped 84 CHM genomes using Affymetrix SNP 6.0 and Illumina 1M-duo, and obtained a definitive haplotype map that included 1.7 million SNPs. For 1.1 million common SNPs (MAF ／ 0.05), we calculated linkage disequilibrium and created the map of LD-bins in which strongly correlated SNPs (pairwise r^2／ 0.8) were grouped and represented by tagSNPs. Genotype data (signal intensities) were analyzed for CNVs using the Circular Binary Segmentation algorithm. Segments were then filtered based on their mean log-ratios. Number of segments defined as CNV were 8002 (Affymetrix) and 3715 (Illumina). These segments were merged based on mutual overlap and 2258 CNV region (CNVR) were identified. We compared 1030 CNVRs detected in at least 2 samples in our study with 436 CNPs (MAF>0.02) identified by HapMap 3 project. One fourth of our CNVRs overlapped with HapMap CNPs. Most of our CNVR (83%) overlapped with structural variation registered in the Database of Genomic Variants (v10). Whole-genome definitive haplotype of SNPs and CNVs determined in this study (D-HaploDB: http://orca.gen.kyushu-u.ac.jp/) provide a useful resource to relate genome variations to complex diseases.

807F

Detailed Metabolic Studies Identify Endophenotypes in Rare Obesity and Overgrowth Disorders. *W. Gibson[1, 2], J. Sou[1, 2], S. Gyawali[3], A. Fam[1, 2], R. Billings[1, 2], S.L. Babich[1, 2], L. Musa[1, 2], J. Friedman[1], S. Lear[3], S. Jones[4,5], D.D. Weaver[6], K. Boycott[7], P. Eydoux[1, 8] FORGE Consortium Canada.* 1) Dept Medical Genetics, BC Children's Hospital, Vancouver, BC, Canada; 2) Diabetes Research Program, Child and Family Research Institute, Vancouver, BC; 3) School of Kinesiology, Simon Fraser University, Vancouver, BC; 4) Michael Smith Genome Sciences Centre, Vancouver, BC; 5) BC Cancer Agency; 6) Dept Medical & Molec Genetics, Indiana University, Indianapolis; 7) Dept Medical Genetics, Children's Hospital of Eastern Ontario, Ottawa; 8) Cytogenetics Laboratory, BC Children's Hospital.

Recent advances in genome-wide analysis have identified previously-unknown metabolic pathways that control body weight. Study of patients and families with monogenic obesity and overgrowth disorders typically finds rare DNA variants that confer a high risk for obesity, whereas genome-wide association studies identify variants that confer a high population-attributable risk for increased adiposity (i.e. % body fat). We have been studying patients with rare overgrowth, obesity and lipodystrophy syndromes with a combination of genome-wide methods (Affy 6.0 SNP arrays and Whole Exome Sequencing in selected families). Because these disorders perturb the normal relationship between lean mass, fat mass and metabolic risk, we believe that Body Mass Index should be supplemented with direct quantitation of food intake, lean tissue mass, metabolic rate and activity level, as well as measurement of total and depot-specific fat mass. We have recruited several individuals with “paradoxical endophenotypes” - persons in whom metabolic risk appears abnormally high despite low adiposity. Our data on healthy adults (N=19, Age range 24-60) has found a mean resting energy expenditure (REE) of 30.6 kilocalories per kilogram of lean body mass per day (kCal/kgLBM; 95% CI 26-35). We have found several people whose REE is elevated after adjusting for lean body mass (range: 37-90 kCal/kgLBM/d). In lean individuals with low muscle mass and normal food intake, this may indicate a long-term risk for wasting and sarcopenia, as has been reported in Amyotrophic Lateral Sclerosis. In lipodystrophic individuals with high muscle mass and normal/elevated food intake, such a finding may indicate the body's attempt to dispose of excess intrahepatic and intramuscular fat by oxidation. Thus, data on body composition and metabolic rate may add value to the clinical delineation of patients with rare disorders of body weight. These data could assist in medical management, by showing a response to pharmacological and/or dietary interventions. For example, high-calorie supplements might be prescribed to build muscle in patients with low lean mass and elevated metabolic rate. Alternatively, a lowering of resting energy expenditure might be an early marker of metabolic benefit from dietary manipulation in patients with lipodystrophies. Supported by CIHR's Institute of Nutrition, Metabolism & Diabetes (OG PCN-102990), by the BC Clinical Genomics Network and by the FORGE consortium.

808F

Functional genomic study on circulating B cells underlying defects in bone quality in postmenopausal Caucasian women. *V.S. Hanumanthu, L. Li, X.H. Peng, Y. Wang, R.R. Recker, P. Xiao.* Osteoporosis Research Center, Creighton University, Omaha, NE.

The commonly used measurement BMD (bone mineral density) is not the only real-life factor and may not even be the most important factor that determines skeletal fractures. Bone quality (BQ) describes the more important determinant of fractures. BQ can be defined in a hierarchical cellular, tissue, and structural levels. Excessive osteoclastogenesis may be one important mechanism that regulates bone quality. B cells play an important role in osteoclastogenesis via secretion of osteoclast-related factors, such as RANKL (receptor activator of NF-2B ligand), OPG (osteoprotegrin) and TGF-) 1 (transforming growth factor). Our previous functional genomic study also found importance of circulating B cells in the etiology of postmenopausal low BMD. However, little is known about circulating B cells in the etiology of defects in BQ. In the present study, we aim to screen functional genes in circulating B cells associated with defects in BQ in postmenopausal Caucasian women. We recruited 50 postmenopausal Caucasian women at age 45-70 with osteopenia, 25 with fragility fractures during the previous 5 years and 25 non-fracture controls with age, sex and BMD matched. We isolated circulating B cells and extracted their total RNA. Expression profiling for RNA sample from each subject was performed by Affymetrix Human Gene 1.0 ST array to identify genes that are differentially expressed. Significance of gene expression was measured using paired t-test. We found 223 genes that were differentially expressed among the two groups. Among them, chemokine ligand 17 (CCL17) ($p = 0.017$) and TYRO3 protein tyrosine kinase (TYRO3) ($p = 0.048$) were up-regulated in the fracturing group and found to have functional significance in osteoclastogenesis upon reviewing existing literatures. Studies found that increased secretion of CCL17 in human peripheral blood monocytes stimulated osteoclastogenesis and activated TYRO3 in mouse mature osteoclasts increased bone resorption. Further data analysis and qRT-PCR will be conducted to verify the findings. This is the first study to elucidate the role of circulating B cells in regulating human BQ.

809F

Exome sequencing of extended late-onset Alzheimer's disease (LOAD) families identifies rare variants associated with AD risk. *M.A. Kohli[1], A.C. Naj[1], E.R. Martin[1], G.W. Beecham[1], K.L. Hamilton[1], P.L. Whitehead[1], J.R. Gilbert[1], J.L. Haines[2], S. Zuchner[1], M.A. Pericak-Vance[1].* 1) John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL, USA; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN, USA.

Over the past five years, identifying risk genes for Alzheimer's disease (AD) has focused on testing the `common disease-common variant' (CDCV) hypothesis, in part because the tools to do so (e.g., large-scale SNP genotyping chips) have become widely available. While common variants like the , 4 allele of *APOE* clearly play a role in AD, there is a growing realization that the CDCV hypothesis is unlikely to explain all the genetic effect underlying AD. One alternative hypothesis invokes multiple rare variants (RV) in one or more genes, each with stronger individual effects than common variants in genes identified under CDCV. We identified a subset of 5 pedigrees from 61 extended multi-generational, late-onset AD (LOAD) families to attempt identification of rare variants. The five pedigrees average six AD-affected individuals, and have been screened to exclude known familial AD mutations in *APP*, *PS1*, and *PS2* genes. No *APOE* , 4/, 4 individuals were included. All affecteds in the five pedigrees underwent genome-wide genotyping (Illumina OMNI 2.5 arrays). Whole-exome sequencing was performed on 2-4 affected individuals per pedigree comprising 2-6 cousin or avuncular pairs. Based on genotyping results we calculated extended identity-by-descent (IBD) haplotypes per pedigree and determined that 0.7% to 18.1% of the genome fell within regions of >80% IBD sharing among affected relatives making these regions the most likely to harbor variants that lead to the LOAD burden. Novel missense variants in *FBXL22* and *G6PC2* were observed within the IBD sharing regions of each pedigree and were specific to each family. Neither gene has been associated with LOAD. Both variants were confirmed by Sanger sequencing and showed nearly complete segregation. Moreover, missense variants were identified that fell in genes recently associated with LOAD ($P<10^{-5}$) in a large genome-wide association study (GWAS, Naj et al. 2011). These changes include a rare missense variant in the *PTPRD* gene and a common missense change in *CD33*. We have identified unique rare variants that segregate with LOAD in these large families. Our results validate that combing genome-wide genotyping to determine IBD sharing with whole exome sequencing data in extended families can identify candidate rare variants in both known and novel LOAD genes. This approach holds promise for any complex disease where such families can be identified.

810F

PHARMACOGENOMICS AND DRUG METABOLIMS. *A.Patricia. lozano Medellin.* pharmaceutical chemistry, National university, bogota, Colombia.

Pharmaceutical chemistry is a discipline that has enabled to provide the necessary medicines and vaccines for human welfare through the development of the human genome project has opened a window for using pharmacogenomics and pharmacogenetics get closer to the development of new drugs pharmacological targets which are detected with great precision with thanks to the techniques of medicinal chemistry.Several genetic causes and at different levels in the mechanism of action of a drug may be responsible for inter-individual variations in response to drug treatment. These include, first, differential pharmacokinetics due to genetic variations in the processes of absorption, distribution, metabolism, pro-drug activation, inactivation of the active generation of biologically active metabolites), and excretion of a particular drug. The rapid metabolism of certain drugs is caused by duplication, or multiduplicatión amplification of genes coding for CYP2D6. To date we have identified 2, 3, 4, 5 and 13 copies of these genes in tandem. These allelic variants confer a phenotype “ultra-rapid metabolizer” on their hosts. The rapid scientific progress has identified the key enzymes responsible metabolism occurred in phases I and II. One of the biggest obstacles is presented in pharmacological treatment currently is for the extensive interindividual variation is observed in drug metabolism. These variations may lead to different situations, such as treatment failure of drug therapy, onset of effects adverse or even toxic effects. The incidence of fatal adverse effects triggered by drugs cause over 100,000 deaths per year in the United States, placing between 4 and 6 cause of death in recent times.

811F

Identification of the genetic and molecular basis of adult neuronal ceroid lipofuscinosis (NCL4) using novel genomic methods. *L. Noskova[1,2], V. Stranecky[1,2], H. Hartmannova[1,2], A. Pristoupilova[1,2], H. Hulkova[1,2], M. Elleder[1,2], H. Jahnova[1], K. Hodanova[1,2], S. Kmoch[1,2].* 1) Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague and General Teaching Hospital, Prague, Czech Republic, Ke Karlovu 2, Prague 2; 2) Center for Applied Genomics, Prague.

Neuronal ceroid lipofuscinoses (NCLs) are heterogenous group of most common inherited neurodegenerative disorders with incidence of between 1 and 5 per 100 000 in different countries. They are characterized by neurodegeneration and accumulation of autofluorescent lipopigments in both neural and peripheral tissues. Most of NCLs presenta as infantile or juvenile forms, to date approximately 280 NCL causing mutations have been found in eight genes.Adult NCLs either recessive (Kufs` disease) or dominant (Parry disease) are considered rare and their genetic and molecular basis is still unknown. We identified Czech family with autosomal dominant ANCL presenting with myoclonic epilepsy, generalized tonic clonic seizures, progressive cognitive deterioration and depressive symptomatology starting in a third decade of life, normal activity of palmitoylthiosterase 1 in leucocytes and characteristic neurolysosomal storage of autofluorescent material with ultrastructural appearance of granular osmiophilic deposits (GRODs) in brain. To map the disease loci, we used Affymetrix GeneChip® Mapping 10K 2.0 Arrays, genotyped available family members and performed linkage analysis. We also performed gene expression analysis in leucocytes isolated from four patients using Illumina HumanRef-8 v2 Expression BeadChips and exome sequencing on SOLID™ 4 System in one proband. By combination of these approaches and several methods of bioinformatic analysis of exome data, we obtained list of unique changes in candidate regions. Sequencing verification and segregation of found changes with the fenotype is in progress. This study was supported by the grant from the Ministry of Education of the Czech Republic (MSM0021620806) and by the grant of Charles University GAUK 299911.

812F

Identifying Common and Infrequent Genetic Risk Variants in Type 1 Diabetes in a Family Based Study. *S. Onengut-Gumuscu[1], W.M. Chen[1], M. Szpak[1,2], J.C. Mychaleckyj[1], P. Concannon[1], S.S. Rich[1], the T1DGC.* 1) Center for Public Health Genomics, University of Virginia, Charlottesville, VA, USA; 2) Faculty of Biology, University of Warsaw, Warsaw, Poland.

Type 1 Diabetes (T1D) is a complex disease that results from the immune mediated destruction of the insulin secreting beta-cells of the pancreas. We have recently reported the results of a genome-wide association (GWA) study in T1D and a meta-analysis of these data in combination with two prior GWA reports. The total sample set included 7,514 cases and 9,045 controls. We identified 18 novel genomic regions that were associated with T1D in our meta-analysis. In conjunction with those from previous linkage and association studies, there are now more than 40 confirmed genomic regions harboring risk loci for T1D. Aiming to fine map all reported T1D risk loci in a systematic approach, we have densely genotyped all T1D regions in 2,835 affected sib pair (ASP) families and 494 T1D trio families (total of 12,983 samples) previously recruited by the Type 1 Diabetes Genetics consortium (T1DGC) using a custom genotyping panel (ImmunoChip). The ImmunoChip content focused not only on known T1D risk loci but also on risk loci from other immune-mediated diseases, and included 186 risk loci. Common and infrequent SNPs included on the ImmunoChip were chosen from the 1000 Genomes Project pilot CEU population variants as well as those contributed by investigators through disease specific resequencing projects. After completing Illumina design metrics, a total of 196,524 SNPs were included on the ImmunoChip. On average, 643 SNPs were genotyped in each non-HLA T1D risk locus (over 6,000 SNPs covered the HLA region). Approximately 12 % of the SNPs genotyped had minor allele frequencies (MAF) between 0.01 and 0.05 (less frequent) and 14 % of the SNPs were rare (MAF < 0.01). A total of 35 of the previously reported T1D risk loci exhibited strong evidence of association in the ASP and trio T1D families where 22 of the risk loci had P-value < 1×10^{-4} and 13 of the risk loci had P-values between $1x\ 10^{-4}$ and 1×10^{-3}. The ImmunoChip platform has allowed us to refine associations in T1D risk loci simultaneously and lead to identification of independent common and infrequent T1D risk variants contributing to disease development. The biological influence and detailed functional role of each risk variant will need to be further elucidated in future functional studies.

813F

Copy Number Variants of GSTM1 gene in Rheumatoid Arthritis. *E. Petit-Teixeira[1], M.S. Ben Kilani[1], L. Michou[1], T. Bardin[2], F. Cornelis[1,2,3,4].* 1) GenHotel-EA3886 Laboratory, University of Evry-Val d Essonne, Evry , France; 2) Federation of Rheumatology, Lariboisiere Hospital, APHP, Paris, France; 3) Clinical Genetics Unit, Lariboisiere Hospital, APHP, Paris, France; 4) Adult Genetics Unit, CHSF, Corbeil, France.

Rheumatoid Arthritis (RA) is a chronic inflammatory disease that affects primarily joints manifesting as pain, stiffness and synovitis leading in turn to articulation destruction. This autoimmune disease has a complex etiology not entirely elucidated. The major genetic factor, HLA-DRB1 gene, is characterized by several shared epitope (SE) alleles associated with RA. Part of environmental component in RA is explained by tobacco exposure. Detoxifying process of carcinogen components from tobacco is a key step under control of several genes. Among them, glutathion S-transferases (GST) family genes encode peroxydases acting on secondary cytotoxic metabolites for cell protection. One member of this family, GSTM1 gene, is characterized by Copy Number Variations (CNV). Our aim was first to analyze CNVs of GSTM1 gene in relation with tobacco exposure among RA patients. Second, genetic, clinical and biological data (presence of erosion and of autoantibodies) were considered to analyze the relationship between CNVs and RA. Finally, we studied transmission of CNVs in trio families. CNVs identification was performed using fluorescent specific probe and dedicated analysis (Applied Biosystems). Results were confirmed by specific amplifications. Relation between CNVs, tobacco and others factors in 240 RA patients was determined by Odds Ratio with 95% Confidence Interval and with logistic regression. Transmission disequilibrium and genotype relative risk were tested in trio families with one RA patient and its two healthy parents. Homozygous GSTM1 deletion was observed in 52% of RA patients. This deletion is not associated with tobacco exposure (TE+) in RA patients (43% TE+ versus 53% TE-, OR = 0,69 [0.41-1.15]), but with presence of the HLA-DRB1 SE in the subgroup of RA patients TE+ (56% HLA-DRB1 SE positive versus 14% HLA-DRB1 SE negative, OR = 2.87 [1.15-7.17]). A trend of transmission disequilibrium in trio families was observed for GSTM1 deletion. Furthermore, CNVs characterization revealed several cases of GSTM1 copy loss through transmission. Several studies described associations of GSTM1 deletion with RA considering tobacco exposure and severity of the disease. Our study is the first to characterize CNVs GSTM1 in RA trio families. The familial material could highlight mechanisms of deletion for this gene and the association with RA. CNVs characterization should be the next step to elucidate new genomic factors in complex diseases such as RA.

814F

VAR-MD: Advanced analysis of whole exome sequencing for detection of hemizygosity. *M. Sincan[1,2], D.R. Simeonov[1], T.C. Markello[1], D.A. Adams[1], C. Toro[1], C. Tifft[1], W.A. Gahl[1], C.F. Boerkoel[1].* 1) Medical Genetics Branch, NIH/NHGRI, Bethesda, MD; 2) NIH Undiagnosed Diseases Program, NIH, Bethesda, MD.

Whole Exome Sequencing (WES) is increasingly used in diagnoses of rare Mendelian diseases, but methods are required to comprehensively analyze and prioritize huge numbers of variants. We have developed a tool that can evaluate WES variants and produce a ranked list of potential causative mutations in the family. This tool 1) employs a simple, tab-delimited input file containing the annotated variants shared within the family; 2) utilizes family structure and affected status, 3) makes use of public data sets for further annotation and analysis; 4) makes use of the short read alignments of the exomes in bam format for additional analyses; and 5) for the first time, detects hemizygosity. This tool was highly successful in prioritizing and ultimately identifying pathological mutations causing spinocerebellar ataxia type 28, GM1-gangliosidosis, and spastic paraplegia type 35 (SPG35) in different patients enrolled in the NIH Undiagnosed Diseases Program. Of particular interest was the case of a boy with SPG35. Previous WES analytical tools read the mother's genotype as homozygous for the normal FA2H allele while the father was found to carry a point mutation. However, the maternal allele actually carried a deletion of exons 3-7. Since monoallelic deletions cause continuous loss of heterozygosity across the deletion, we interrogated the BAM alignment files of the family for unusual runs of homozygosity. We confirmed that this was indeed the case for this family, and incorporated this process into our WES filtering analysis. This advanced tool for WES analysis (VAR-MD), which has broad applicability and includes the innovation of detecting copy number variants, is available to the public at http://research.nhgri.nih.gov/.

815F

Large-scale transcriptome analysis using RNA-seq reveals new insights into psoriasis biology. *L. Tsoi[1], R. Nair[2], B. Li[1], J. Ding[1], J. Gudjonsson[2], P. Stuart[2], T. Tejasvi[2], H. Kang[1], G. Abecasis[1], J. Elder[2,3].* 1) Department of Biostatistics, University of Michigan, Ann Arbor, MI; 2) Department of Dermatology, University of Michigan, Ann Arbor, MI; 3) Ann Arbor Veterans Affairs Hospital, University of Michigan, Ann Arbor, Mi.

Psoriasis is a genetically determined chronic inflammatory disease of the skin and joints that affects ~0.2-2% of the world's population. To further our understanding of the biology of psoriasis, we utilized high-throughput RNA sequencing technology (RNA-seq) to assay the transcriptomes of lesional and normal skin. We sequenced 92 lesional and 82 normal punch biopsy samples of human skin, each with an average of ~38 million single-end 80-bp reads. Comparison of 42 samples examined with both RNA-seq and our recent microarray-based studies showed systematic differences in estimated expression levels. Based on RNA-seq data, we identified 3,577 differentially expressed genes between the two tissues, and further investigation showed that the genes identified only by RNA-seq have markedly lower expression than those also identified by microarray, indicating that RNA-seq has higher resolution for low abundance transcripts. Pathway analyses revealed differentially expressed genes were enriched for genes involved in cytokine-cytokine receptor interaction and immune system processes. Furthermore, cytokine genes that are functionally important for psoriasis, such as IFNG, IL17, IL22 and IL24, which were not detected as differentially expressed by microarray analyses, were all identified as differentially expressed using RNA-seq. Using a specially tuned variant calling algorithm, we also performed variant calling using the RNA-seq data. Among the nonsynonymous, synonymous, and nonsense polymorphisms identified, 49%, 63%, and 33% of them overlap with the records in dbSNP respectively. The transition to transversion ratios (Ts/Tv) for the novel variants found are comparable to the known variants and, comparing individual genotypes with previous GWAS data, we obtained an average of 98.4% concordance rate, indicating the high accuracy of variant discovery and genotype calling from RNAseq. The increased resolution and power provided by RNA-seq will allow a more comprehensive view of global gene regulation and interaction in psoriasis, and the ability to identify novel disease-associated expressed variants should provide new insights into its pathogenesis.

816F

Identification of the gene causing IFTDC. *M. Weterman[1], P. Barth[2], G. Nuernberg[3], O. Milanesi[4], P. Nuernberg[3], B. Poll-The[2], F. Baas[1].* 1) Neurogenetics/LVGA, K2-213, AMC/UvA, Amsterdam, Netherlands; 2) Pediatric Neurology, Emma Children's Hospital, AMC, Amsterdam, Netherlands; 3) Cologne Center for Genomics, Koln, Germany; 4) Pediatrics, University of Padova, Padova, Italy.

Infantile Fiber Type Disproportion and Cardiomyopathy (IFTDC) is a rare pediatric muscular disorder, characterized by general muscle weakness with onset shortly after birth and dilated cardiomyopathy. It was initially reported in six apparently unrelated Dutch families. All patients died between 4 and 6 months of age. Muscle biopsies of four of the patients showed small type I and normal type II fibers. An extensive search for defects related to disturbances in energy metabolism, respiratory chain defects and disorders of mitochondrial fatty acid metabolism, in frozen muscle biopsies and cultured fibroblasts, was negative. We therefore tried to identify the cause of this disease using a genome wide approach. Genomic DNA was used for linkage analysis on an Affymetrix 250K SNP microarray. Parametric analysis, initially with a reduced marker panel of 20K SNPs markers, assuming consanguinity with a second cousin marriage, and autosomal recessive inheritance with full penetrance and low frequency led to the identification of a homozygous region of 2.2 Mb on chromosome 12 that was shared between all patients. Detailed analysis of the region using all markers gave a LOD score of 10.82. Sequence capture of the region of interest followed by next generation 454 sequencing only yielded homozygous changes within the coding regions of genes that were already reported as polymorphisms. Although over 95% of the coding regions were covered, 31 of 398 exons had a coverage that was either too low or totally absent. Sanger sequencing of these gaps yielded three homozygous changes, one was a known polymorphism, one was located in the untranslated region of the corresponding mRNA, and the third mutation was a splice site mutation in a myosin gene, making this gene a strong candidate for the disease. Identification of compound heterozygosity for frame shift mutations in the same gene in an Italian patient with the same clinical phenotype, supports the pathogenic role of this gene in IFTDC.

817F

Deep sequencing of the human TCR. and TCR) repertoires provides evidence that TCR) rearranges after () , .+ T-cell commitment. *C.S. Carlson[1], A. Sherwood[2], C. Desmarais[2], R.J. Livingston[2], J. Andriesen[2], M. Haussler[3], H. Robins[1].* 1) Pub Hlth Sci, Fred Hutchinson Cancer Res Ctr, Seattle, WA; 2) Adaptive TCR Corporation, Seattle, WA; 3) Faculty of Life Sciences, University of Manchester, Manchester,United Kingdom.

The two main lineages of T lymphocytes develop from multi-potent precursors in the human thymus. The most common type in blood are () T cells, which bind to antigenic peptides displayed on the surface of cells by human leukocyte antigen (HLA) molecules. Far less well understood are .+ T cells, which do not bind HLA:peptide complexes and are more prevalent in the gut mucosa. For both lineages, their ability to recognize a diverse array of antigens is mediated by a rearranged receptor on their surface, the T cell receptor (TCR), composed and of an (and) chain for () T cells or a . and + chain for .+ T cells. The canonical model for commitment from the precursor to one these two lineages assumes that . , +, and) chains rearrange prior to commitment to () or .+ fate. A crucial step towards better understanding the role of .+ T cells is to work out the developmental process. To test the standard model and to understand the .+ TCR repertoire, we use high-throughput sequencing to catalog millions of TCR. and TCR) chains from peripheral blood () and .+ T cells, from three unrelated individuals. Almost all sampled () and .+ T cells have rearranged TCR. sequences. While sampled () T cells have a diverse repertoire of rearranged TCR) chains, less than 10% of .+ T cells in peripheral blood have a rearranged TCR) chain. Our data indicate that TCR. rearranges in all T lymphocytes, consistent with TCR. rearranging prior to T cell lineage commitment, while rearrangement of the TCR) locus is restricted, and occurs after T cell precursors commit to the () T cell lineage. This result explains the conundrum in T cell leukemia and lymphoma that TCR. is almost always rearranged and TCR) is only rearranged in a subset of cancers. As high-throughput sequencing of TCRs is translated into the clinic for monitoring minimal residual for leukemia/lymphoma, our data suggests the sequencing target needs to be TCR . .

818F

Measurement of T cell repertoire diversity in the peripheral blood by novel multiplex PCR and next-generation sequencing methods. *J. Han, C. Gunter, C. Sanders, Q. Yang, C. Wang.* HudsonAlpha Institute for Biotechnology, Huntsville, AL.

An individual's collection of B and T cells determine their ability to respond to infection and malignancies; each cell contains one of millions of possible combinations of V, D, and J gene segments, comprising an immune repertoire. Studies from our lab and others suggest that immune repertoire diversity might be an effective indicator for determining overall health status and disease prognosis. We have previously developed an ARM-PCR/HTS (amplicon rescued multiplex PCR/high throughput sequencing) method allowing us to semi-quantitatively and comprehensively analyze the TCR repertoire[1]. Using this technology, we sequenced TCR) CDR3 fragments amplified from cDNA molecules isolated from three T cell subsets: regulatory (Tr, CD4+CD25+), helper (Th, CD4+CD25-), and cytotoxic (Tc, CD8+) cells from peripheral blood samples. Based on these, we propose a diversity index called D50 as a measure of the diversity of a sequenced immune repertoire. In simple terms, D50 is the minimum percentage of distinct CDR3s accounting for at least half of the total CDR3s.

We compared D50 values between lung, colon, and breast cancer samples to age-matched healthy individuals, who had no known illnesses at the time of blood donation, and found that the D50 values in all three T cell subsets are lower by a statistically significantly amount ($p<0.05$) in comparison to their healthy control counterparts. In addition, D50 values of Tr cells were better able to distinguish normal from cancer samples than those of either Tc or Th cells. Given that loss of immune repertoire diversity is associated with various diseases, we suggest that the D50 test could be used as a screening test to evaluate health status. In addition, the expression profile of disease-specific CDR3s may be used as biomarkers for early diagnosis, prognosis, or disease management.

1. Wang, C., et al. High throughput sequencing reveals a complex pattern of dynamic interrelationships among human T cell subsets. Proc Natl Acad Sci U S A 107, 1518-1523.

*Han and Gunter are co-first authors.

819F

Sex-specific Genetic Architecture of Systemic Lupus Erythematosus. *T. Hughes[1], A. Adler[1], J.T. Merrill[2,3], J.A. Kelly[1], K.M. Kaufman[1,2,4], A. Williams[5], C.D. Langefeld[5], G.S. Gilkeson[6], J. Martin[7], G.S. Alarcon[8], E.E. Brown[8], R.P. Kimberly[8], J.C. Edberg[8], R. Ramsey-Goldman[9], M. Petri[10], J.D. Reveille[11], L. Vila[12], P.M. Gaffney[1], K.L. Moser[1], T.J. Vyse[13], M.E. Alarcon-Riquelme[1,14], J.A. James[1,2], R.H Scofield[1,2,4], J.B. Harley[15], B.C. Richardson[16], A.H. Sawalha[1,2,4].* 1) Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; 2) Department of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA; 3) Clinical Pharmacology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; 4) US Department of Veterans Affairs Medical Center, Oklahoma City, OK, USA; 5) 5Department of Biostatistical Sciences, Wake Forest University Health Sciences, Medical Center Blvd, Winston-Salem, NC, USA; 6) 6Department of Medicine, Division of Rheumatology, Medical University of South Carolina, Charleston, SC, USA; 7) 7Instituto de Parasitología y Biomedicina López-Neyra, Consejo Superior de Investigaciones Científicas, Granada, Spain; 8) Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA; 9) Department of Medicine, Northwestern University, Chicago, IL, USA; 10) Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA; 11) Department of Medicine, University of Texas-Houston Health Science Center, Houston, TX, USA; 12) Department of Medicine, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico, USA; 13) Divisions of Genetics and Molecular Medicine and Immunology, Infection and Inflammatory Disease, King's College London, Guy's Hospital, London, UK; 14) Center for Genomics and Oncological Research Pfizer-University of Granada-Junta de Andalucia, Granada, Spain; 15) Rheumatology Division and Autoimmune Genomics Center, Cincinnati Children's Hospital Medical Center; and US Department of Veterans Affairs Medical Center, Cincinnati, OH, USA; 16) Division of Rheumatology, University of Michigan; and US Department of Veterans Affairs Medical Center, Ann Arbor, Michigan.

Background: Systemic lupus erythematosus is a sexually dimorphic autoimmune disease which is more common in women, but affected men often experience a more severe disease. The genetic basis of sexual dimorphism in lupus is not clearly defined. Here, we examine sex-specific genetic effects among lupus-susceptibility loci. Methods: A total of 18 lupus-associated autosomal genetic loci were genotyped in a large set of lupus patients and controls of European descent, consisting of 5,932 female and 1,495 male samples. Sex-specific genetic association analyses were performed. Sex-gene interaction was further validated using both a parametric (logistic regression) and non-parametric (multifactor dimensionality reduction) methods. We examined aggregate differences in sex-specific genetic risk by calculating a cumulative genetic risk score for lupus in each individual and comparing average genetic risk between men and women. Results: We observe a significantly higher cumulative genetic risk for lupus in men than in women. We report significant sex-gene interaction in the HLA region and IRF5 genes whereby male lupus patients possess a significantly higher frequency of risk alleles than their female counterparts. We also report that the genetic effect observed in KIAA1542 is specific to female lupus patients, and does not seem to play a role in lupus in men. Conclusions: Our data indicate that men require a higher cumulative genetic load to develop lupus compared to women. This observation suggests that the higher prevalence of lupus in women, and the more severe lupus phenotype in men could be related to autosomal genes.

820F

Identification of novel genes in human primary immunodeficiency diseases using exome sequencing. *S. Khan[1], B. Wakeland[1], C. Liang[1], N. van Oers[1], M. De la morena[2], E. Wakeland[1].* 1) University of Texas Southwestern Medical Center, Dallas, Texas; 2) Children's Medical Center, Dallas, Texas.

Severe immunodeficiencies are rare in the human population (less than 1 in 70,000) and their genetic etiology is very difficult to decipher. More than 150 genes have been linked to various forms of primary immunodeficiencies and most of these are the consequence of a monogenic defect and hence follow a simple mendelian inheritance. In this study we utilized whole exome sequencing strategy to identify the causative genetic lesions in patients with severe immunodeficiencies of unknown etiology. This strategy has proven to be a powerful, efficient strategy for identifying genes underlying rare Mendelian disorder. We subjected five families with one or more children who have severe PID with an unknown genetic basis to whole exome sequencing using SureSelect Human All Exon Kit from Agilent technologies and sequenced in a single lane of an Illumina GAIIx. On average 4.5 Gbp of sequence data was generated per sample of which 2.54 Gbp of the sequence mapped specifically on the tiled exons. On average individual genomes differed from the reference sequence by 64,449 total SNVs. To distinguish potentially deleterious mutations from other variants, only nonsynonymous, variants and short coding region insertions or deletions were considered. We will present the analysis for one of the probands with severe combined immunodeficiency with no detectable peripheral T cells at ASHG 2011. The causative variants for the PID exhibited by the proband was sought using several filtering strategies that led to a list of 29 candidate variations that were novel, deleterious mutations that were homozygous or compound heterozygous only in affected child within a PID family. Based on the functional impact of SNV on the gene and a consideration of the expression of these genes within immune cell lineages or relevant tissues, a final list of 6 potential genetic mutations that could cause this specific SCID has been generated. These mutations are now being validated using standard Sanger sequencing. The future studies will characterize the impact of these variants on their expression in T cells and other tissues by RNA-SEQ analysis of PBMC from the parents and or siblings that are heterozygous for the candidate variant. We hope that these data will identify novel genes and their functional role within the human immune system.

821F

A genetical genomics approach to understanding the regulatory mechanisms of host-pathogen interactions in humans. *M.N. Lee[1,2,3], C. Ye[1], A.C. Villani[1], P. De Jager[1,3], A. Regev[1], N. Hacohen[1,2,3].* 1) Broad Institute, Cambridge, MA; 2) Massachusetts General Hospital, Boston, MA; 3) Harvard Medical School, Boston, MA.

While naturally occurring genetic variants are known to modulate gene expression (e.g. eQTL), limited studies have evaluated the influence of these variants on pathogen responses using relevant human primary immune cells. Results from such gene-environment studies will be crucial to further understand disease etiology of a variety of disorders including infectious diseases, autoimmune diseases, and cancer. We will present a systematic study evaluating variation of gene expression responses across a healthy cohort of 30 individuals using monocyte-derived dendritic cells (MoDCs) activated through bacterial and viral sensors that mediate innate immune responses to pathogens. The cohort consists of equal numbers of Caucasians, Asians and African-Americans; half female and half male for each ethnicity. We will describe a combined information-theoretic and functional genomics strategy to identify key components of the pathogen response with natural genetic variants that may be associated with disease susceptibility.

822F

Identification of Novel Imprinted Genes Expressed in Mouse Frontal Cortex Using RNA-Sequencing. *K. Wigg[1], E. Dempster[2], C. Barbacioru[3], L. Gomez[1], Y. Feng[1], P. Monnier[1], R. Logan[1], J. Eubanks[1], C. Barr[1,4].* 1) Genetics and Development, Toronto Western Hosp, Toronto, ON, Canada; 2) Institute of Psychiatry, Kings College London, London UK; 3) Applied Biosystems (Life Technologies), Foster City, California, USA; 4) Program in Neurosciences and Mental Health, Hospital for Sick Children, Toronto, Ontario, Canada.

Current data support approximately 90 validated imprinted genes in mice, but recent estimates indicate that this number may be well over 1,000. Many of the genes are imprinted in a tissue/cell type specific pattern and little is known of these genes. Previous evidence indicates that imprinted genes influence the development of specific brain regions and we sought to identify imprinted genes contributing to the development of the frontal cortex. We developed a strategy using crosses and reverse crosses of mouse strains to determine the parent-of-origin of expressed genes. We used next generation sequencing of RNA (RNA-seq) from the frontal cortex of the adult F1 mice to identity transcripts differing in expression between the crosses. RNA from the two parental lines was also sequenced to determine strain specific polymorphisms and determine the parent-of-origin of the transcripts. We identified transcripts that were differentially expressed between the crosses showing parent-of-origin effect in the frontal cortex including known imprinted genes (e.g. Meg3, Snurf/Snrpn, Ube3a, Rasgrf1) and genes with no prior evidence for parent-of-origin effects. Using a FDR of 0.01, and requiring that the same parent-of-origin bias was evident in 3 biological replicates, we identified 109 transcripts with parent-of-origin effects. The finding of novel imprinted genes indicates that the number of imprinted genes with tissue specific effects is underestimated and requires further studies to identify the full complement of imprinted genes in brain. The understanding of parent-of-origin effects in specific brain regions will make a significant contribution to our understanding of the role of imprinting in brain function and behavior.

823F

Genetic analysis of the Th17-related genes in cerebral malaria. *S. Marquet[1], B. Poudiougou[2], U.E Ndoumbe[1], A. Traore[2], A.A Oumar[3], S. Cabantous[1], O. Doumbo[2], A.J Dessein[1].* 1) INSERM U906, Université de la Méditerranée, Marseille, France; 2) Department of Epidemiology of parasitic disease, University of Bamako, Bamako, Mali; 3) Centre des oeuvres universitaires, University of Bamako, Bamako, Mali.

The pathogenic mechanisms of cerebral malaria (CM) are unclear but are thought to involve cytokine-mediated inflammation enhanced by parasite sequestration in microcirculation. In previous work, we have shown that Interferon-. and Interleukin-12 protect against CM whereas IL-4 may be a risk factor for severe malaria. By conditional logistic regression analysis, three genotype combinations of two IL4 polymorphisms influencing IL-4 levels are associated with different risk of severe malaria. Actually, our research is focus on the recently discovered Th17 cytokine response which is shown to play a crucial role in regulating tissue inflammatory reactions. To investigated the contribution of Th17-related genes in susceptibility to CM, 96 Tag SNPs have been selected in IL1B, IL6, TGFB1, IL23A, IL17A, IL17F, IL22, IL6ST, IL17RA, and IL27RA genes and tested by Illumina Golden Gate genotyping Assay. The univariate analysis in the first cohort of 152 nuclear families was performed using the Family-based association test package (FBAT). The analysis revealed significant or suggestive associations with disease for 18 polymorphisms. To follow-up these results, study of 87 additional families, who were independent of the initial study sample and were from the same population, are ongoing. When the association will be confirmed, additional polymorphisms in linkage disequilibrium with associated SNPs could be tested. Finally, functional analysis will be performed to determine the molecular role of these markers in disease development.

824F

Significance of polymorphisms within the *SLC11A1* (*NRAMP1*) gene that is associated with *Mycobacterium avium* complex infection. *B.R. Sapkota[1,2], M. Hijikata[1], I. Matsushita[1], G. Tanaka[3], A. Mabuchi[2], K. Tokunaga[2], N. Keicho[1].* 1) Department of Respiratory Diseases, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan; 2) Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; 3) Department of Respiratory Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

Background: *Mycobacterium avium* complex (MAC) disease is caused by the infection with a group of microorganism in nontuberculous mycobacteria (NTM) *viz. Mycobacterium avium* and *Mycobacterium intracellulare*. Pulmonary MAC infection is a rare disease manifested with common respiratory symptoms and typical radiographic findings. Although genetic variants in the *SLC11A1* (*NRAMP1*) gene have been associated with mycobacterial diseases, these finding have not been extensively validated in pulmonary *Mycobacterium avium* complex (MAC) infection. This study investigates linkage disequilibrium (LD) pattern within the *SLC11A1* gene and its association with pulmonary MAC infection. **Methods and Findings:** Altogether, 19 polymorphic loci in *SLC11A1* were genotyped to define LD blocks and haplotype structures in European descendents and Japanese population, and Tag SNPs were chosen. A total of 6 polymorphic sites were genotyped in 111 pulmonary MAC cases and 211 controls from Japan. Furthermore, allelic expression imbalance of mRNA of *SLC11A1* gene was evaluated in peripheral blood cells from Japanese heterozygous individuals. Frequencies of major haplotypes were different between these two populations. One unique haplotype accounting for 4% was observed only in the Japanese population with a variant (allele 7) of 5' GT repeat. We observed an association between T allele of rs2279014 and protection from MAC disease when comparing allele frequencies with an odds ratio of 0.582 (95% CI 0.379 - 0.894, p = 0.013). In subgroup analysis, this SNP was associated with late onset (> 50 age group) of disease (p = 0.029) and also associated with patients without co-morbidity (p = 0.028). Of the haplotypes carrying three loci (rs17235409, rs17235416 and rs2279014), haplotype `GIC' was significantly associated with the MAC disease (Permutation p value = 0.008). However, no significant difference in allele-specific expression levels of mRNA was observed. **Conclusion:** Haplotype frequency differences were observed between European descendents and Japanese population, and Tag SNPs were selected in the Japanese population. A significant association of rs2279014 with pulmonary MAC infection was observed. To our knowledge, rs2279014 SNP has not been reported in association with any other diseases. The statistical significance was modest and will require further study for conclusive validation.

825F

Analysis of de novo genomic events implicates a zinc-finger protein in controlling chilhood carcinogenesis. *J. Hussin[1,2], V. Bruat[2], Y. Idaghdour[1,2], F. Casals[1,2], V. Saillour[2], J.F. Spinella[2], M. Larivière[2], S. Busche[3], B. Ge[4], T. Pastinen[3,4], D. Sinnett[1,2], P. Awadalla[1,2].* 1) Faculty of Medecine, University of Montreal, Quebec, Canada; 2) Ste-Justine Hospital Research Centre, Montreal, Quebec, Canada; 3) Department of Human Genetics, McGill University, Montreal, QC, Canada; 4) Genome Quebec Innovation Centre, Montreal, QC, Canada.

Cancer is the leading cause of death by disease among children in western countries, with nearly 15,000 new pediatric cases diagnosed each year in North America. While the overall incidence rate of cancer in children continues to rise steadily, its etiology remains elusive. In particular, little is known about the contribution of *de novo* events, occurring during meiosis, to the genetic instability that drives pediatric cancer. To characterize the importance of these genetic events in controlling carcinogenesis in children, we used a family-based analytical framework, coupled with cohort validation studies, to identify new variants and recombination breakpoints using genomic data. Through the in-depth analysis of a unique case, a family with two siblings having hyperdiploid childhood acute lymphoblastic leukemia (ALL), we called *de novo* point mutations and indels from high-throughput sequencing data, performed fine-scale dissection of meiotic recombination events and examined tumor-specific mitotic recombination patterns and chromosomal breakpoints. We successfully identified candidate *de novo* mutations, having predicted deleterious phenotypic impact, and we located recombination events in genes known to be involved in cancer, which could be indicative of potential driver mutations in childhood ALL. Furthermore, the abnormal placement of maternal meiotic recombination events allowed us to discover the presence, in the mother, of a rare allele of a chromatin-modifying gene, encoding a C-terminal tandem array of C2H2 zinc fingers (ZnF). This meiosis-specific gene has been identified as a key player in regulating recombination in mammals. We further observed an excess of rare alleles at that locus in a cohort of parents with children having childhood ALL. Since variation in the ZnF array domain in humans has been shown to influence meiotic instability and to be implicated in some pathological genome rearrangements, it may therefore play an important role in childhood tumorigenesis.

826F

Title: Linkage disequilibrium analysis of polymorphisms of FRAS1 Related Extracellular Matrix 1 (*FREM1*) in susceptibility to HIV-1 infection in a Sub Saharan African female sex worker cohort. *J.F. Tuff[1], N. Kaplonski[1], V. Ly[1], B. Liang[1], S. Tyler[1], J. Kimani[3], M. Luo[1,2], F.A. Plummer[1,2].* 1) National Microbiology Laboratory, Winnipeg, MB, Canada; 2) University of Manitoba, Medical Microbiology, Winnipeg, MB, Canada; 3) University of Nairobi, Medical Microbiology, Nairobi, Kenya.

Objective: A genome wide association study of the Pumwani Sex Worker (ML) cohort identified a single nucleotide polymorphism (SNP; rs1552896) in *FREM1* to be significantly correlated with the HIV-exposed Seronegative (HESN) phenotype. In this study, our goal was to identify polymorphisms within *FREM1* that are in linkage disequilibrium (LD) with rs1552896 and characterize causative genetic variants contributing to HESN phenotype. Design: Twenty-two overlapping PCR amplicons were designed to span *FREM1* (176,330 bp) and amplified in 69 ML members. The amplicons were sequenced with Genome Sequencer FLX™ (Roche) and assembled with Sequencher 4.8 (Gene Codes). Statistical measures of LD (Logarithm of the Odds (LOD) score, D', and r2) between polymorphisms were determined with Haploview v 4.2. Results: We identified 918 *FREM1* polymorphisms in this cohort, 447 of which are newly discovered. Twelve of these polymorphisms were in LD with rs1552896, within a 32Kbp LD block. These polymorphisms consisted of 8 intronic SNPs (rs112356286, rs74549063, rs2818932, rs12337418, rs62537679, rs1889050, rs62537685, and rs11788565), 3 novel microsatellite variations, and a non-synonymous exonic SNP (rs2779500). *FREM1* SNP rs2779500 results in a leucine residue replacing a valine residue in the second CSPG domain of FREM1 splice isoform 1 and 2. Conclusions: It is possible for a polymorphism to be associated with a disease phenotype not because it is biologically causal, but because it is in LD with a causal polymorphism. We therefore sought to identify all polymorphisms in LD with *FREM1* SNP rs1552896, a SNP previously shown to be highly associated with the HESN phenotype. In doing so, we have identified 12 additional polymorphisms whose possible contribution to the HESN phenotype in the ML cohort warrants future investigation.

827F

Genome-wide Linkage Scan in an Indian Family with Split-hand/foot Malformation narrowed down the SHFM3 region to 10q25.1-q25.3. *A. Ali[1], R. Raman[2].* 1) Centre for Genetic Disorders, Banaras Hindu University, Varanasi, India; 2) Department of Zoology, Banaras Hindu University, Varanasi, India.

Split-hand/foot Malformation is a complex disorder with considerable variable expressivity and genetic heterogeneity. However, its inheritance pattern mostly indicates autosomally dominant monogenic pattern. Recently, examples of X-linked and autosomally recessive patterns have been seen where involvement of more than one interactive genes has been speculated. Genome-wide linkage scan of a 5-generation SHFM family, using 10K SNP arrays (Affymetrix, USA), shows a single peak with LOD score 3.01 at the region 10q25.1-q25.3. A few earlier reports have also identified 10q24-q25 as one of the critical regions for SHFM3. Recombination in one of the affected children between SNPs rs1361356 and rs952965 enables us to narrow down the critical locus to a 0.46 cM region, which falls upstream of the gene SORCS1 which has not yet been shown as a candidate gene for SHFM. The study was supported by a grant from the Department of Biotechnology, New Delhi to RR.

828F

Genome-wide linkage analysis for longevity in European nonagenarian siblings: Genetics of Healthy Ageing Study (GEHA). *M. Beekman[1,2], P.E. Slagboom[1,2], H. Blanche[3], M. Perola[4], A. Hervonen[5], V. Bezrukov[6], E. Sikora[7], L. Christiansen[8], S. Schreiber[9], A.J.M. De Craen[1,2], T.B.L. Kirkwood[10], I.M. Rea[11], M. Poulain[12], J.M. Robine[13], S. Valensin[14], M.A. Stazi[15], G. Passarino[16], L. Deiana[17], S. Gonos[18], O. Törnwall[4], Q. Helmer[1], F. Martella[1], R.G.J. Westendorp[1], M. Lathrop[3], J.W. Vaupel[19], K. Christensen[8], J. Gampe[19], A. Nebel[9], J.J. Houwing-Duistermaat[1,2], C. Franceschi[14] On behalf of the GEHA consortium.* 1) Leiden University Medical Centre, LUMC, Leiden, The Netherlands; 2) Netherlands Consortium for Healthy Ageing, The Netherlands; 3) Foundation Jean Dausset, CEPH, France; 4) The National Institute for Health and Welfare, THL, Helsinki, Finland; 5) Tampere School of Public Health, Tampere, Finland; 6) Institute of Gerontology, Kiev, Ukraine; 7) Nencki Istitute for Experimental Biology, NENCKI, Warszawa, Poland; 8) Epidemiology, Institute of Public Health, University of Southern Denmark, Odense, Denmark; 9) Institute of Clinical Molecular Biology, Christian-Albrechts-University Kiel (CAU), Germany; 10) Newcastle University, UNEW, United Kingdom; 11) Queens University of Belfast, QUB, United Kingdom; 12) Catholic University of Louvain, UCL, Belgium; 13) INSERM, Health & Demography, CRLC, France; 14) University of Bologna, Interdepartmental Centre "Luigi Galvani" CIG, UNIBO, Italy; 15) Istituto Superiore di Sanità, ISS, Rome, Italy; 16) University of Calabria, UNICAL, Italy; 17) University of Sassari, UNISS, Italy; 18) National Hellenic Researcher Foundation, NHRF, Athens, Greece; 19) Max Planck Institute for Demographic Research, MPIDR, Rostock, Germany.

Nonagenarians and their first degree family members have a life-long survival advantage that can be attributed to a lower risk of coronary artery disease and type-2 diabetes despite the fact that they carry as many GWAS-identified disease risk alleles as the general population. We hypothesized that long-lived families carry alleles that lower disease susceptibility. To identify such longevity alleles, we performed a genome-wide linkage scan among 2118 nonagenarian Caucasian sibling pairs that have been recruited in fifteen study centers of eleven European countries in the Genetics of Healthy Ageing (GEHA) Study. In the joint nonparametric linkage analysis we observed four regions that show linkage with longevity; chromosome 14p11.2 (LOD=3.47), chromosome 17q21.32 (LOD=2.95), chromosome 19p13.2 (LOD=3.76) and chromosome 19q13.32 (LOD=3.57). Since the 19q13.32 region spans the *APOE* gene, known to contribute to longevity and mortality, we analyzed whether the APOE, 2, 3, 4 alleles contributed to the linkage. An analysis in which association and linkage were additively modeled, using LAMP, showed that together the APOE, 2 ($P \leq 0.0001$) and the APOE, 4 ($P=0.020$) alleles explain the linkage at 19q13.32. To fine map these linkage results at the 14p11.2, 17q21.32 and 19p13.2 loci we used GWAS data in the nonagenarian siblings that contributed to the linkage results to test for associations explaining the linkage. For the association analyses 1058 unrelated nonagenarian cases were compared with 8776 younger controls of similar geographical origin as the cases. At 14p11.2 we tested 1023 SNPs for association, at 17q21.32 2969 SNPs, at 19p13.2 1850 SNPs and at 19q13.32 3805 SNPs. Using a fixed effect meta analysis approach, rs2075650 at 19q13.32 appeared the only SNP within these four linkage regions significantly associated with longevity. This SNP is in linkage disequilibrium with the APOE, 2, 3, 4 polymorphisms and is known to reflect the effect of the *APOE* gene on longevity. We conclude that besides the effect of the *APOE* gene on longevity, at least three other loci play a role located at 14p11.2, 17q21.32 and 19p13.2. Since the linkage results are not explained by common variants, we suggest that rare variants at these regions contribute to human familial longevity.

829F

Genetic mapping of loci regulating transcripts related to stress responses. *A. Jasinska, N. Freimer.* Dept Psychiatry, Univ California, Los Angeles, Los Angeles, CA.

The genetic component of individual vulnerability to stress and stress-related disorders remains unclear. Genetic differences among individuals underlie individual responses to environmental stressors at both molecular (gene expression) and higher organismal (behavioral and cognitive) levels. Identification of stress gene expression traits and their regulatory expression quantitative trait loci (eQTL), may contribute to understanding individual differences in stress response. Here we report genetic mapping of eQTL related to the regulation of these responses. We investigated gene expression levels before and after a severe stressor which was evenly applied to all members of an extended pedigree of about 500 vervet monkeys, the Vervet Research Colony (VRC). In 2008 the entire pedigree was relocated from UCLA to Wake Forest University. We analyzed linkage for pre-move and post-move expression levels, as well as for change in expression levels between these time points. We identified two loci, ANXA11 and TFE demonstrating cis and trans regulation respectively.

830F

Copy-Number Variation In Finnish Multiple Sclerosis Families. *V. Leppa[1,2,3], O. Pietilainen[1,2], L. Peltonen[1,2,4], A. Palotie[1,4], J. Saarela[1].* 1) Institute for Molecular Medicine FIMM, University of Helsinki, Helsinki, Finland; 2) National Institute for Health and Welfare, Helsinki, Finland; 3) Helsinki Biomedical Graduate School, University of Helsinki, Helsinki, Finland; 4) The Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

Multiple sclerosis (MS) is a debilitating complex disease of the central nervous system. It is characterized by multifocal demyelination, inflammation and neuronal degeneration. Both environmental factors and genetic background affect the development of the disease. The etiology of MS remains largely unknown. Prevalence of MS is 1/1000 in Finland, but roughly 2-fold in the Southern Ostrobothnia (SO) region and shows familial clustering. This is likely to be caused by long lasting isolation of this region. Genome-wide association studies (GWAS) have revealed over 50 common SNPs with modest odds ratios that are associated with MS. These SNPs are thought to be in linkage disequilibrium with the potential causative variants. Copy-number variants (CNVs) are larger and thought to be more likely causative themselves. CNVs have not yet been intensively studied in MS. Our aim is to study CNVs in the Southern Ostrobothnian MS families and to determine if the CNVs are inherited and co-segregate with the disease. We have genotyped 157 samples (41 cases and 116 family members) from 10 multiplex families and 36 regional control samples using the Illumina HumanHap670 chip. CNVs were called using PennCNV and QuantiSNP softwares. 89% of regional controls and 91% of family samples passed QC, and six family members were retyped and passed subsequent QC (raising family success rate to 95%). CNV calls from PennCNV and QuantiSNP were compared and only CNVs that were detected using both methods were included (81% of initial PennCNV calls on average). The family-based QC has not yet been completed. In addition to families, we have data available from 600 Finnish MS cases and 3000 Finnish cohort samples to verify our initial findings and to monitor for CNV population frequencies. All putatively associated CNVs will be confirmed using traditional methods and tested for association in the whole Finnish MS material. We will study the expression of genes within and surrounding the CNV in individuals carrying different amounts of copies to assess the functional relevance. In case of *de novo* variations, we will use public databases (e.g. Genome Variation DB) to identify the potential segmental duplications or similar repetitive sequences potentially predisposing to CNVs. In addition to CNV analysis we will use the GWAS SNP data to assess the genetic load of known MS associated variants within the multiplex families.

831F

Exome sequencing identifies novel candidates for familial combined hyperlipidemia. *A.C.Y. Mak, P.L.F Tang, R. Deo, C.R. Pullinger, M.J. Malloy, J.P. Kane, P.Y. Kwok.* Cardiovascular Research Institute, University of California, San Francisco, CA.

Familial combined hyperlipidemia (FCH), the most prevalent hyperlipidemia, is a complex metabolic disorder characterized by variable occurrence of high low-density lipoprotein (LDL) and high triglycerides - a condition that is commonly associated with coronary artery disease (CAD). Over the years, numerous studies have contributed to the elucidation of the genetic basis of FCH, which is still largely unknown. The need to search for additional genetic variants remains due to the highly variable and complex genetic profile specific to each affected family. Exome sequencing provides a fast and comprehensive study of genetic variants that are associated with this disorder. Using the Illumina HiSeq2000 platform and the TruSeq exome enrichment protocol, we performed exome sequencing on 3 affected individuals from a large 60-member family with history of FCH and low levels of high-density lipoprotein cholesterol. The data obtained had a high target coverage of 94%, including 422 genes or loci previously found to be associated with lipid metabolic pathways, blood cholesterol (HDL and LDL) or triglycerides concentrations, or risk of CAD. On average, 31,000 variants were found within target regions in each individual. After removing synonymous and non-frameshift variants, 862 variants in regions conserved in 44-species were identified to be common in all three affected individuals. This includes 32 nonsynonymous variants in genes related to lipid metabolism. Among the 862 variants identified, 4% are novel non-synonymous or premature stop variants that possibly represent rare variants responsible for the FCH phenotype. Verification and exome sequencing of additional individuals from this family are underway. This partly includes the sequencing of a normal daughter of the current affected mother. This work will further help identify specific variants that provide protective functions against lipid disorders.

832F

A pipeline of targeted resequencing data analysis applied to a family with hearing loss. *J. Cai, J. Samanich, D. Moskowitz, R. Calder, J. Greally, B. Morrow.* Genetics, Albert Einstein College of Medicine, New York, NY.

Re-sequencing of exonic regions of human genome has become a popular strategy to discover variants associated with human disease. To efficiently provide interpretation of data, we developed an automatic pipeline for SNP/InDel identification and annotation that is added to WASP (http://wasp.einstein.yu.edu/index.php/Main_Page). The SNP/InDel identification module starts from alignment using BWA algorithm, de-duplication with Picard tools to the GATK framwork (including local re-alignment, base quality recalibration, per-base alignment quality adjustment and Unified Genotyper). For the SNP/InDel annotation module, basic functional annotation of SNPs/InDels is performed by ANNOVAR software, which implements regional classification (e.g. exon vs intron) and lists related genes. If the variants are within exons, functional consequences are appended, including nonsynonymous/synonymous SNP, stopgain/stoploss and frameshift/nonframeshift insertion/deletion. For a nonsynonymous SNPs, the effect of amino acid change is predicted to be benign or damaging using BLOSUM62, SIFT and Polyphen2. For a known SNP/InDel from the 1000 Genomes project or dbSNP, the alternative allele frequency or heterozygosity is added respectively. We applied this pipeline to a targeted resequencing of hearing loss genes (roughly 1 Mb) from one family with two deaf children and normally hearing parents. Based on Call Quality (>=30), Read Depth (>=5 per sample) and filtering SNPs around InDels, we identified 9957 SNPs and 1099 Indels. We evaluated the presence of recessive mutations and we found compound heterozygous variants in the gene TRIOBP, a known deafness gene (DFNB28). One was a nonsynonymous SNP (c.C5224T:p.L1742F), Leucine to Phenylalanine change (damaging) at chr22:36466887 (hg18) and the other was a frameshift deletion (c.1613_1615del) at chr22:36450122-chr22:364501224. Neither were in dbSNP131. The nonsynonymous SNP was not in the 1000 genome project sequences either and the other mutation was reported allele frequency 0.037 (InDel release, 2010.07).

833F

Novel Features of Neural Retina Transcriptome Revealed by High-throughput RNA-Sequencing. *E. Gamsiz, S. Nagpal, E. Morrow.* Molecular Biology Cell Biology Biochemistry, Brown University, Providence, RI.

Neural retina is a favorable model to study neurogenetic disorders as there are greater than 100 genes in which mutations cause Mendelian forms of visual impairment. The transcriptome is the full set of transcripts expressed in a specific cell or tissue at a certain stage. RNA-Sequencing (RNA-Seq) on Next Generation Sequencing (NGS) platform is a promising technology to profile the transcriptome providing thorough and sensitive information on expression levels and alternative splicing events. In this study, we have applied high-throughput RNA sequencing to the whole set of mRNAs isolated from 21 day old murine neural retina. Isolated two cDNA libraries were sequenced by paired end chemistry via Illumina Genome Analyzer IIx. In average, 50 million of 60 bp paired end reads were obtained from each library. Using a pipeline of Bowtie, TopHat, Cufflinks and custom-made scripts, we identified 15251 genes and 20558 transcripts expressed in both libraries. Of the 15251 genes, 3665 were found to have alternative splicing events. Gene abundances were quantified in Fragments Per Kilobase of exon model per Million mapped fragments (FPKM) and exhibited a range between 0.125 and 2481.5. Rhodopsin was identified as the most abundant gene of all expressed in our samples. We also demonstrated interesting features of retinal disease genes. 159 genes in total associated with retinal diseases were taken from Retinal Information Network (RetNet) (http://www.sph.uth.tmc.edu/retnet/sum-dis.htm). Retinal disease genes were found to be among the most abundant expressed genes in the transcriptome (Chi-Square 72.07, DF=1, p<0.0001). We also studied genes from several gene ontology (GO) groups such as axonogenesis, neuron development, neurite morphology, neurotransmitter secretion, synaptic vesicles, synapse part and voltage-gated channel activity in neural retina transcriptome and demonstrated some other remarkable features. For example; we demonstrated that neural retina expresses a specific and restricted set of synaptic vesicle genes and large percentage of genes related with voltage-gated ion channels were highly expressed in both replicates. Our study presents the most comprehensive view of the transcriptome of the murine neural retina to date using novel, massively-parallel sequencing technologies. These data may also serve as a resource for the community of researchers investigating gene expression in the neural retina in mouse.

834F

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835F

Extensive transcriptional diversity identified through massively parallel DNA and RNA sequencing of eighteen Korean individuals. *Y. Ju[1,2], J. Kim[1], S. Kim[1], D. Hong[1], H. Park[3], S. Lee[1], C. Lee[3], J. Seo[1,2].* 1) Genomic Medicine Institute, Medical Research Center, Seoul National University College of Medicine, Seoul, Korea; 2) Macrogen Inc., Seoul 153-023, Korea; 3) Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115, USA.

Massively parallel sequencing technologies have identified broad spectrum of diversity of human genome. Here, we sequenced and correlated 18 genomes and 17 transcriptomes of unrelated Korean individuals. To prevent misalignment of RNA short-reads bearing splicing junctions, which increases error rate in RNA sequencing, we aligned the short-reads onto a set of cDNA sequences, generated using ~160,000 mRNA sequences obtained from Refseq, UCSC and Ensembl databases. We identified 4,414 regions as final unknown transcripts, which do not overlap any known genes or pseudogenes. Of these, 111 were detected from all the 15 samples. These transcripts cover 2.74 Mb of the human genome, and are located 4.7 kb from the nearest gene and the median length of these 4,414 unknown transcripts was 360 bp. Of the 4,414 novel RNA-derived genic regions, 19.5% (862) showed homology (≥ 20 amino acids long with ≥ 80% identity) to known protein sequences. We then examined gender differences in expression level of X chromosomal genes to explore X-inactivation profiles. We found 23 genes, including XIST, PNPLA4, HDHD1A, NGFRAP1 and GPR34, that show higher levels of gene expression in females compared to males. Interestingly, 5 genes that appear to be escaping X-inactivation had not been previously shown to escape X-inactivation in human fibroblasts. To investigate nucleotide change during transcriptional processes, we looked for evidence of RNA variants that did not correspond exactly with their derived genomic sequence. Applying conservative filter criteria to genome and transcriptome sequences of the same individuals, we identified 1,809 sites of such transcriptional base modifications (TBMs). On average, the lymphoblast cell lines of each individual showed ~ 500 sites of TBM variants. Of these 1,809 TBMs, 74.1 % (N=1,341) were nucleotide transition. 81.7% (N=1,096) of the transitions were A to G (N=985) and C to T (N=111) modifications on the coding-strand, which could be explained by previously known molecular mechanisms of A to I and C to U RNA editing. Next, to investigate allele-specific expression, or allelic expression imbalances, we compared the allele-specific read-counts from genome and transcriptome sequence data of individuals heterozygous in a tested SNP. From our dataset, we found 580 nsSNP sites that show preferential expression of one allele compared to the other.

836F

Massively parallel sequencing and High-throughput of sorted human chromosomes 21 and Y. *Y. Kuroki[1], A. Toyoda[2], S. Kondo[1], Y. Nishida[3], O. Ohara[1], A. Fujiyama[2, 4].* 1) Laboratory for Immunogenomic, RIKEN Research Center for Allergy and Immunology, Yokohama, Japan; 2) Comparative genomics laboratory, National Institute of Genetics, Mishima, Japan; 3) United Centers for advanced Research and Translational Medicine, Tohoku University School of Medicine, Sendai, Japan; 4) Principles of Informatics Research Division, National Institute of Informatics, Chiyoda-ku, Japan.

Previous studies revealed striking differences of the genomic constitution and evolutionary aspect between autosomal chromosome 21 and male-specific chromosome Y, in addition to the differences identified the between human and chimpanzee genomes (Watanabe et al., 2004, Kuroki et al., 2006). In these works, we demonstrated that the nucleotide differences between human and chimpanzee were 1.5 times higher in chromosome Y than that of chromosome 21, and that the numbers and contents of the retrotransposons, the frequency of the genome rearrangements such as insertion/deletion and inversion, and the degree of the conservation in the syntenic regions were different between Chrs 21 and Y. To further clarify the fundamental characteristics in the genome structure and function between autosome and sex chromosome (Y chromosome) during the course in the evolution of human genome, we started extensive comparative analysis for the chromosomes 21 and Y among primates and other mammals. At first, we constructed pipeline for the sequencing of purified whole chromosomes and bioinformatics analyses. Roughly three million each of flow-sorted Chrs 21 and Y were subjected for sequencing by using Solexa/GAII analyzer. The average read depth for each chromosome exceeded more than 50X coverage, and these data enabled us to identify the SNVs, indels, and the sequences which did not included in the current version of the human reference genome assemblies, Build 37 or hg19. The strategy and the analytical pipeline for the chromosome-based genome analysis, and the novel findings will be presented.

837F

Candidate gene analysis in Behcet's disease using massively parallel sequencing. *S. Lee[1], S. Kim[5], H-G. Yu[5], J-I. Kim[1,2,3], J-S. Seo[1,2,3,4].* 1) Biomedical Sci, Seoul National Univ, Seoul, Korea; 2) ILCHUN Genomic Medicine Institute, Medical Research Center, Seoul National Univ, Seoul, Korea; 3) Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine, Seoul, Korea; 4) Macrogen Inc., Seoul, Korea; 5) Department of Ophthalmology, Seoul National University Hospital, Seoul, Korea.

Behcet's disease is thought to be triggered by environmental factors in patients with presidposing genetic background. HLA-B51 has shown strong association with Behcet's disease with relative risk of 5 to 20. As the contribution of HLA-B51 to the pathogenesis of Behcet's disease is less than 20%, the other genetic factors remain to be proven. Many candidate genes including TNF-alpha, ICAM-1, IL-1, CTLA-4, SUMO4, etc. were studied to identify associations with Behcet's disease. Recently, genome-wide association studies (GWASs) using more than 300K SNP chips identified association at IL10 and IL23R-IL12RB2 locus. However, recently performed GWASs based on SNP genotyping in many complex diseases have identified only a small fraction of the heritable variation. One possible explanation is that many rare variants, which are difficult to identify via GWASs, contribute substantially to the genetic variation of these diseases. Therefore, it has been suggested that sequencing candidate genes may be an efficient methods to investigate the contribution of rare variants to the phenotype. We performed targeted exon sequencing of 145 candidate genes using exon capture array followed by massively parallel sequencing in 32 Behcet patients and 32 controls. We called more than 100 SNPs with significant differences in frequency. These SNPs are located in FCGR3A, NOD1, IL25, IL12RB2, etc. We also identified several rare variants, including nonsynonymous rare variants which are predicted to be harmful by SIFT analysis. In conclusion, target enrichment and massively parallel sequencing technologies seem to provide valuable information on genetic predisposition in Behcet's disease.

838F

Non-coding and coding mutations associated with Kabuki syndrome identified by comprehensive, massively parallel sequencing of the 50 kilobase locus around *MLL2*. *S. Ng[1], K. Buckingham[2], M. McMillin[2,3], M. Bamshad[1,2,3], J. Shendure[1].* 1) Genome Sciences, University of Washington, Seattle, WA; 2) Pediatrics, University of Washington, Seattle, WA; 3) Seattle Children's Hospital, Seattle, WA.

We report on the identification of potential coding and non-coding mutations associated with Kabuki syndrome, both at and beyond the mixed lymphoid leukemia (*MLL2*) locus. By sequencing the entire locus (coding, intronic and flanking regions) using targeted capture and massively parallel sequencing, we can efficiently screen for both coding and non-coding variants, and gain a comprehensive picture of all variation within the region. This is informative for finding causal mutations, and also for identifying samples that are truly mutation negative, which in turn are useful to search for additional causative loci.

Previously, we had used exome sequencing to implicate *MLL2* in Kabuki syndrome[1], and also screened the coding exons of this gene (26 kb) using Sanger sequencing[2]. Likely causal mutations were found in 82 patients, but not in 22 others (21%). It is uncertain if the latter are actually *MLL2*-mutation-negative and hence effective candidates for a second locus search; they may instead harbor non-coding, structural or missed coding mutations at the *MLL2* locus. Using probes against the whole *MLL2* locus, including regulatory regions (50 kb total), we subjected sample libraries (100 ng gDNA starting) to 48-plex target enrichment and massively parallel sequencing. Initial results show high sensitivity: all causal mutations from sufficiently sequenced positive controls were recovered (78%, >90% bp passing filter). Also, 8 mutations were identified in 13 other well covered mutation-unknown samples - 1 nonsense, 1 missense, 2 coding indels), 2 variants in splice-sites and 2 in introns (GERP > 3). The remaining 5 samples had no potential pathogenic mutations despite >90% coverage, and represent samples that are most likely to be *MLL2*-mutation-negative. Based on this success, we are using this approach in lieu of Sanger sequencing to ascertain mutation status in a further 57 previously unscreened samples. The *MLL2*-mutation-negative samples are being used to identify additional loci for Kabuki syndrome through exome sequencing to find shared genes with mutations, and we are also sequencing a single parent-child trio to pinpoint *de novo* mutations. 323 potential mutations have been found in the proband of the trio (none in *MLL2*), and filtering by parental data will further reduce this list considerably.

Refs: 1) Ng et al., 2010, *Nat Gen*, doi:10.1038/ng.499; 2) Hannibal et al., 2011, *AJMG*, in press.

839F

Agilent Technologies SureSelect™ Human All Exon v3: High Performance Target Enrichment System for Human Exome Sequencing on Illumina and SOLiD platforms. *C. Pabón-Peña[1], E. Lin[1], B. Novak[1], M. Hamady[1], F. Useche[1], M. Visitacion[1], A. Giuffre[2], H. Ravi[2], S. Joshi[2], J. Ong[2], B. Arezi[3], S. Happe[2], D. Roberts[1], E. Leproust[1].* 1) Agilent Technologies, Genomics Research and Development, Santa Clara, CA; 2) Agilent Technologies, Genomics Research and Development, Cedar Creek, Texas; 3) Agilent Technologies, Genomics Research and Development, La Jolla, CA.

The introduction and widespread adoption of next generation sequencing technologies have reduced high-throughput sequencing costs by several orders of magnitude and enabled numerous whole-genome analyses. Nevertheless, the expense and operational capacity necessary for large scale whole genome studies is still prohibitive for many laboratories. Since large portions of the genome consist of repeat elements and regions of unknown phenotypic value, targeted exome capture combined with massively parallel sequencing has become one of the most viable options to gain novel insights into the genetic causes of inherited disorders. By focusing on the protein-coding regions of the human genome, scientists are now able to more efficiently identify both common and rare polymorphisms that are more likely to result in cellular dysfunction and exhibit significant penetrance in disease association studies, such as those implicated in the etiology of Mendelian disorders and complex diseases like cancer or neurological pathologies. We hereby describe our latest SureSelect Human All Exon design developed in collaboration with the Broad Institute. Performance improvements of multiplexed samples are demonstrated with respect to capture efficiency, uniformity, reproducibility of enrichment, and ability to detect SNPs, insertion/deletions, and CNVs on Illumina (Genome Analyzer IIx and HiSeq2000), and Life Technologies SOLiD platforms. The high specificity, accuracy, and excellent cross-platform sequence coverage demonstrates the utility of the SureSelect All Exon v3 for a wide variety of research and clinical applications that demand the most comprehensive view of functionally relevant regions of the human genome.

840F

High-throughput sequencing of a neuromuscular disorder panel for application on diagnostic testing: a 2x2 comparison between target enrichment methodologies and sequencing platforms. *S.L. Pereira[1], B. Baskin[2], E. Cheran[1], Z. Hu[1], K. Ho[1], L. Lau[1], S.W. Scherer[1], P.N. Ray[2].* 1) The Centre for Applied Genomics and Division of Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Division of Molecular Genetics, Dept Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, Ontario, Canada.

Current diagnostic method for targeted gene re-sequencing is PCR amplification and Sanger sequencing. This procedure is labor intensive, expensive and time consuming, thus not economically feasible as a diagnostic test where many genes need to be analyzed. We investigated the sensitivity and specificity of two targeted enrichment methods (RainDance Technologies (RD) and Agilent SureSelect (SS)) to be used as comprehensive clinical diagnostic test for neuromuscular diseases using Next Generation Sequencing (NGS) platforms. We selected a panel consisting of 803 exons from 44 genes for target enrichment and high throughput sequencing of four individual affected by neuromuscular diseases. The target areas cover 198 Kb using RD, and 155 Kb using SS. Samples were sequenced on an Illumina GAII (2x76-bp), and SOLiD 4 (50-bp). The target coverage for the GAII and SOLiD was 100-120X and 50-75X respectively, and solely reflects the comparison of paired versus single read data. The two capture methods performed similarly, with ⁄ 95% and 76-98% of the target-bases with 10X and 30X coverage, respectively. GC-rich (>80%) exons were under-represented in the sequenced data regardless of the NGS platform or capture method used. Between 187 and 234 SNPs were detected for the 4 samples, most of which concordant between sequencing platforms and capture methods. Among discordant SNPs, <7% of the calls are specific to a NGS platform or capture method. The neuromuscular disease test panel proposed here provides an excellent strategy to test the power of targeted enrichment for massively parallel sequencing and to translate this technology into clinical diagnostics. Although the two target methods and NGS platforms perform equally well for this purpose, other parameters needs to be taken into consideration: RD requires a higher initial capital investment and incurs higher costs for sample preparation compared to SS, but RD samples can be ready for sequencing 2-3 days earlier than SS samples. The costs of running the NGS instruments may be comparable for runs of similar read length, but the time required to generate the data may vary between 4 to 16 days depending on read lengths and if performing a single or paired end run. The amount of data and results created requires a bioinformatician to deal with the analysis, and trained technical and clinical personnel for data interpretation based on read depth, read quality, alignment scores, and other parameters used in the analysis.

841F

Semiconductor Sequencing for Life. *J. Rothberg, J. Leamon.* Ion Torrent, South San Francisco, ca.

Ion Torrent has invented the first device—a new semiconductor chip—capable of directly translating chemical signals into digital information. The first application of this technology is sequencing DNA. The device leverages decades of semiconductor technology advances, and in just a few years has brought the entire design, fabrication and supply chain infrastructure of that industry—a trillion dollar investment—to bear on the challenge of sequencing. The result is Ion semiconductor sequencing, the first commercial sequencing technology that does not use light, and as a result delivers unprecedented speed, scalability and low cost. Ion Torrent sequencing uses only natural (label-free) reagents and takes place in disposable semiconductor microchips that contain sensors that have been fabricated as individual electronic detectors, allowing one sequence read per sensor. The system performance has been demonstrated by sequencing four bacterial genomes, ranging in genomes size and GC content from Vibrio fisheri (4.3Mb genome, 38% GC) to Escherichia coli K12 - (4.6Mb genome, 51%GC) and DH10b (4.7Mb genome, 51%GC) to Rhodopseudomonas palustris (5.5Mb genome, 65%GC). Besides comprising the first genomes sequenced with post-light technology, the genomes are remarkable in the lack of data bias, which is as good, or better, than existing commercial platforms. Ion's technological applicability to routine human sequencing has also been demonstrated by utilizing Ion chips to sequence a human genome. We will show how the technology has scaled in just a few months from 1.2 million sensors in the first-generation Ion 314 chips to 6.1 and 11 million sensors in the second-generation Ion 316 and 318 chips respectively—all while maintaining the same 1- to 2-hour runtime. Additionally, Ion has successfully accomplished design, manufacture and sequencing using chips possessing the smaller 1-micron diameter well, enabling further increases in well density and sequencing throughput in subsequent chip designs. Because the heart of the system is a novel, disposable sensor, built and assembled using standard semiconductor fabrication methodologies, able to sequence without the need for intermediate enzymes or the constraints of having to image using light, the cost of genome sequencing will continue to fall with each successive generation of denser chips according to Moore's law.

842F

Assessing the Effects of Whole Genome Amplification on Exome Sequencing. *J. Smith, I. Stanaway, E. Turner, C. Igartua, K. Patterson, S. McGee, T. Shaffer, C. Poel, B. Paeper, Q. Yi, K. Sawatzki, B. Munson, I. Robertson, M. Mynsberge, S. Austin, C. Shepard, S. Paquette, M. Tackett, J. Furlong, P. Robertson, D. Siegel, E. Johanson, E. Johnson, E. Phillips, M. Rieder, D. Nickerson.* Genome Sci, Univeristy Washington, Seattle, WA.

Traditional approaches for exome sequencing require significant amounts of input DNA from 5 - 10 microgram to successfully produce high quality exome sequencing libraries. However, many stored samples have limited DNA quantities and these input amounts limit their usefulness for second-generation sequencing methods. Whole Genome Amplification (WGA) is a cost-effective method to increase the amount of input DNA, but the effects on exome library construction, which already uses an enrichment strategy, could lead to coverage and uniformity bias. We have conducted a systematic review of WGA compared to unamplified controls. We amplified 100 ng of genomic DNA and compared it with standard exome library preparation protocols using 5 ug of input DNA. Libraries were prepared using standardized Illumina adaptors and captured using the Roche/Nimblegen EZ-Cap v2.0. We compared uniformity and read depth across exome target regions on multiple samples and assessed the ability to accurate call Single Nucleotide Variants (SNV), Indels, and Copy Number Variants (CNV). Interestingly, SNV and Indel variant calling was highly concordant between methods with rates > 99.99%, although read depths were lower and more variability of coverage was seen. The ability to accurately call CNVs from the WGA data was impaired by the large variance in the read depth and specificity was significantly reduced. Our results show that WGA has little effect on exome sequencing and SNV detection with caveat is that it will impact the ability to call structural variants, thus careful consideration should be made before utilizing this procedure.

843F

X-chromosome exome sequencing of a nonsyndromic intellectual disability family with multiple affected male sibs. *S. Kantarci[1,2], W.L. Donahue[3], P. Bayrak-Toydemir[3], L. Al-Gazali[4], N. Bissar-Tadmouri[5].* 1) Department of Pathology, Beth Israel Deaconess Medical Center, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Department of Pathology, University of Utah, ARUP Laboratories, Salt Lake City, UT; 4) Department of Paediatrics, United Arab Emirates University, Al-Ain, United Arab Emirates; 5) Department of Basic Medical Sciences, University Of Sharjah, College of Medicine, Sharjah, United Arab Emirates.

Intellectual disability (ID) is a lifelong condition with significant limitations both in intellectual functioning and in adaptive behavior, affecting ~1-3% of the general population. About 10-15% of ID is attributed to X-linked traits, with higher male to female ratio among milder cases. Non-syndromic intellectual disability (NSID) is characterized by intellectual impairment as the only feature without major physical abnormalities, dysmorphism, or neurological abnormalities. About 80% of known genes responsible for NSID reside on X-chromosome (>90 genes). In this study, we enrolled a nonconsanguineous Arab family with four affected male sibs and five unaffected female sibs. The affecteds have mild to moderate ID as the sole clinical feature. The affected sibs, with prior normal standard karyotype and Fragile-X molecular testing, were 15, 10, 8, and 6 years old at the time of recruitment. We utilized X-linked exome sequencing approach to identify a NSID-causing gene in this family. Using Agilent's SureSelect human X-chromosome kit, we captured the X chromosome genes (~7,674 exons) of one of the affected sibs for DNA sequencing. The captured region was sequenced as paired end with 50x coverage using Illumina HiSeq 2000. A total of 3,790 SNP and indel variants were annotated on this DNA sample using the GenomeQuest whole genome analysis software. After filtering, 66 novel variants (not reported in dbSNP database) were found to fall inside protein coding regions of 13 X-linked genes. We are in the process of further analyzing data. Deciphering the genetic basis of NSID is necessary for further understanding of human cognitive development. Revealing new pathways might allow improvements in diagnostic testing, genetic counseling, and future therapeutic interventions in families with NSID. Our study demonstrates the utility of next generation technology to help identify genes for genetics disorders of unknown etiology.

844F

Whole-genome expression profiling of skeletal muscle reveals potential link between insulin resistance and diabetes in over weight west Africans. *H. Xu[1], A. Adeyemo[1], A. Elkahloun[5], J. Adeleye[2], W. Balogun[2], H. Huang[1], J. Zhou[1], G. Chen[1], D. Shriner[1], C. Adebamowo[3,4], S. Chandrasekharappa[5], C.N. Rotimi[1].* 1) Center for Research on Genomics and Global Health, National Institutes of Health (NIH), 12 South Drive, MSC 5635, Bethesda, MD 20892-5635; 2) Department of Medicine, College of Medicine/ University College Hospital, Ibadan, Nigeria; 3) Institute of Human Virology, Abuja, Nigeria; 4) Department of Epidemiology and Preventive Medicine, University of Maryland, Baltimore, Maryland; 5) Cancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland.

Obesity is one of the major risk factor for type 2 Diabetes (T2D). However we know very little about the changes in the transcriptome of the major tissue targets of insulin action (skeletal muscle and liver) in the early stages of obesity. Here, we used the Affymetrix HU-133 Plus 2.0 Arrays to examine genome-wide expression in skeletal muscle obtained by biopsy from West Africans. Twenty-one male non-diabetic subjects (mean age=56 years, mean body mass index (BMI)=23.8 kg/m^2, all Yoruba ethnicity) were enrolled from Ibadan, Nigeria. After controlling for covariates, 128 transcripts were correlated with log transformed BMI (partial correlation coefficient > 0.6, p<0.005). Seventy one transcripts were differentially regulated between overweight (BM⁄ 25 kg/m^2) and normal weight subjects (BMI<25 kg/m^2) at an absolute fold change>1.4 and a p<0.05. Principal component and cluster analyses showed clear separation of these two groups of subjects based on their gene expression profiles. Notably, 13 out of the 71 transcripts have been reported to influence the risk of developing diabetes (KCNQ5, HLA-DQA1, MEIS2, SPG7, PTGDS, TSPAN15, FAM78B, FAM102B, PMP22, SERPINA5, LPP, OSBPL3, HLA-DPB1).In addition, we examined the differences in gene expression profile between individuals with high HOMA_IR (a homeostatic model assessment of insulin resistance) and those with low HOMA IR index. We found that SERPINA5 was significantly differentially expressed between high compared to low HOMA_IR subjects. Interestingly, Elbein SC et al (2011) reported recently that, gene expression of SERPINA5 was associated with insulin resistance in skeletal muscle of European Americans. Furthermore, Gene Set Enrichment Analysis identified the G protein signaling pathway as the most significantly upregulated pathway in overweight subjects (p<0.001, FDR 0.03). To our knowledge, this study is the first to use expression profiling in skeletal muscle to identify genes that may underlie the well documented association between early weight gain, insulin resistance and diabetes in West Africans. We showed that, compared to normal weight, overweight subjects have notable changes in muscle gene expression in pathways that are dysregulated in T2D. By integrating these data with genetic association, it may be possible to identify novel biomarkers that may facilitate more robust understanding of early intervention targets in obesity, insulin resistance and T2D.

845F

Whole Transcript Expression Microarray Profiling and Sequencing of Normal Colon and Adenocarcinoma Total RNA. *S. Fulmer-Smentek[1], B. Mullinax[2], E. Lin[1], J. Venneri[2], V. Kulkarni[1], C. Pabón-Peña[1], B. Novak[1], A. Bergstrom Lucas[1].* 1) Genomics R&D, Agilent Technologies, Santa Clara, CA; 2) Genomics R&D, Agilent Technologies, La Jolla, CA.

The ability to measure the alternative splicing of exons from RNA that leads to the translation of different proteins is useful for understanding biological mechanisms and classifying tissue and tumor types for research. Several methods are available to interrogate alternative RNA splicing, including exon microarrays, targeted RNA sequencing and whole transcriptome RNA sequencing. While all three methods provide the ability to identify alternative splicing between two samples, each brings distinct advantages. The SurePrint G3 exon microarray workflow includes catalog and custom microarrays, a whole transcript amplification method and tailored analysis software. Exon-level probes on the microarrays were designed from the highest quality content of public databases including RefSeq and Ensembl. Exon array data are analyzed for gene level and exon level expression using GeneSpring GX 11.5 software, enabling whole transcript profile comparisons within two days. Targeted RNA Seq methods allow for in depth analysis of a subset of transcripts of interest via RNA sequencing, while whole transcriptome RNA sequencing allows for hypothesis neutral discovery of alternative splicing, but at a relatively high cost and low throughput. We investigate here the correlation of exon microarrays with the various RNA sequencing methodologies, and demonstrate the high reproducibility and wide dynamic range of expression across a variety of RNA inputs for the exon microarrays. The whole transcriptome exon microarray workflow was further used to detect alternative splicing of exons between cancerous and normal cells resulting in gene and exon expression profiles consistent with the current literature. The results presented demonstrate the compatibility and utility of the various alternative splicing analysis methods.

846F

Deep sequencing of small RNAs from human skin reveals major alterations in the psoriasis miRNAome. *C.E. Joyce[1], X. Zhou[2], J. Xia[2], C. Ryan[3], B. Thrash[3], A. Menter[3], W. Zhang[2,1], A.M. Bowcock[1,4].* 1) Department of Genetics, Washington University School of Medicine, St. Louis, MO, 63110, U.S.A; 2) Department of Computer Science and Engineering, Washington University, St. Louis, MO, 63130, U.S.A; 3) Department of Dermatology, Baylor University Medical Center, Houston, TX, 77030, U.S.A; 4) Division of Dermatology, Department of Medicine, Washington University School of Medicine, St. Louis, MO, 63110, U.S.A.

Psoriasis is a chronic, inflammatory skin disease for which there is currently no cure. Transcriptome analyses have revealed ~1300 mRNA expression changes in psoriatic skin, but much less is known about the role of noncoding RNAs. Aberrant miRNA expression has been implicated in a number of human diseases, including cutaneous diseases such as psoriasis, atopic dermatitis, and melanoma. We have completed a comprehensive analysis of the miRNAome in normal, non-lesional psoriatic, and lesional psoriatic skin. We generated 6.7x10^8 qualified small RNA reads, representing the largest small RNA sequencing dataset derived from any human tissue to date. The global landscape of miRNAs in normal and psoriatic skin included 1) 717 known miRNAs and 176 cognate miRNA*s, 2) 284 novel miRNA loci, 3) miRNA variants that differ at the 3' or 5' termini, termed isomiRs, 4) a number of previously undescribed miRNA*s from known miRNA loci, and 5) a low frequency edited miRNAs. We validated the endogenous expression and processing of three novel miRNAs in skin and other human cell lines or tissues. Of particular note was the discovery and validation of a novel antisense miRNA derived from the miR-203 locus, which has a role in epidermal differentiation. The expression level of this novel antisense miRNA was 2.7-fold higher in psoriatic skin than normal skin. An additional 80 known and 17 novel miRNAs were two- to 42-fold differentially expressed in psoriatic skin. Differentially expressed miRNAs reflected defects in keratinocyte differentiation, immune cell quiescence, and angiogenesis, all processes which are known to influence psoriasis pathogenesis. In situ hybridization of differentially expressed miRNAs revealed stratified epidermal expression of an uncharacterized keratinocyte-derived miRNA, miR-135b, as well as the epidermal infiltration of the hematopoietic-specific miRNA, miR-142-3p, in psoriatic lesions. This study lays a critical framework for functional characterization of miRNAs in skin and therapeutic application of miRNAs for the treatment of cutaneous diseases.

847F

Identification of trait- and disease-relevant genetic polymorphisms in miRNA target sites. *T. Kwan[1,2], S. Busche[1,2], B. Ge[2], S.H. Chen[2], L. Karemera[2], M. Georges[3], T. Pastinen[1,2].* 1) Human Genetics, McGill University, Montreal, PQ, Canada; 2) McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, Canada; 3) Faculty of Veterinary Medicine, University of Liege, Belgium.

MicroRNAs (miRNAs) are ~22 nucleotide long noncoding RNAs that control the expression and function of eukaryotic genomes by acting as adaptors to specifically recognize and regulate targeted mRNAs. Candidate studies suggest that genetic polymorphisms in miRNA target sites (poly-miRTS) are associated with various disorders like Tourette's syndrome, hypertension, or the risk to developing breast cancer. We aim to create a genome-wide catalogue of poly-miRTS to provide the fundamental basis for the characterization of miRNA-related genetic alterations with an impact on human traits and disease, and to unravel the mechanisms of action of some causal poly-miRTS. To identify poly-miRTS on a genome-wide scale, we will monitor RNA levels and differences in allelic expression (AE) with and without active miRNA gene regulation. To shutdown miRNA-mediated gene regulation we interfere with the miRNA processing machinery by silencing Drosha, DGCR8, Exportin-5, and/or Dicer. Importantly, interference with any of these genes is not expected to prevent siRNA processing. Our present results show that silencing any single gene within the miRNA machinery does not substantially inhibit the level of mature miRNAs. However, successful silencing of Drosha, Exportin-5 and Dicer leads to an ~80 % reduction of mature miRNAs. RNA-sequencing and SNP genotyping experiments are currently ongoing. To circumvent off-target effects in assessing the global impact of silencing miRNA expression, we will monitor genome-wide differences in allelic rather than total gene expression. We anticipate that this will effectively eliminate non-specific effects and allows focusing on interaction of miRNA binding and genetic variants at binding sites.

848F

High-throughput identification of direct microRNA targets. *H.C. Martin, S. Wani, A. Steptoe, S.M. Grimmond, N. Cloonan.* Queensland Centre for Medical Genomics, Institute for Molecular Bioscience, University of Queensland, St Lucia, QLD, Australia.

Several studies have reported associations between SNPs in microRNAs or their predicted binding sites and human disease, and aberrant expression of microRNAs has been linked to multiple cancer types. In order to understand how dysregulation of a particular microRNA contributes to disease etiology, it is essential to know which mRNA transcripts it targets. Most of the existing high-throughput methods for elucidating these are confounded by the secondary effects of microRNA over-expression or repression, and the numerous computational algorithms for predicting targets have very high false positive rates. We optimized a protocol to capture direct microRNA-mRNA interactions, and applied this to ten human microRNAs known to play a role in cancer. Essentially, cells were transfected with synthetic biotinylated microRNA duplexes, the protein-microRNA complexes were purified out using streptavidin beads, and their associated mRNAs were analysed by microarray. We also extended the method to precisely locate the microRNA-binding site by combining an RNAse protection assay with massive-scale sequencing. Our results confirm that a single microRNA can directly bind to hundreds of transcripts. There was some evidence that many interactions were being mediated by the central region of the microRNA rather than the seed region, which is generally thought to be essential. The inferred targets were enriched in specific biological pathways, of which many were related to tumorigenesis, and our results further suggest several new avenues for investigation into the functional roles of these microRNAs. We have also found support for the hypothesis that isomiRs work in concert with their canonical partners to regulate the same molecular networks. This work is likely to improve our understanding of microRNA targeting and thus facilitate the characterization of normal microRNA function and annotation of GWAS hits, ultimately leading to a better understanding of the role of these molecules in disease.

849F

Whole exome resequencing reveals an unexpected amount of variability with possible functional consequences in human microRNAs. *J. Santoyo-Lopez[1,2,5], J. Carbonell[2], E. Alloza[2], S. Borrego[3,4], M. Ruiz-Ferrer[1,4], P. Arce[1], I. Medina[2], A. Vela[1], S. Perez[1], R. Fernandez[1], S.S. Bhattacharya[1,6], G. Antiñolo[1,3,4], J. Dopazo[1,2,5,7].* 1) Andalusian Center for Human Genomic Sequencing, Seville, Spain; 2) Department of Bioinformatics and Genomics, Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain; 3) Unidad de Gestión Clínica de Genética, Reproducción y Medicina Fetal. Instituto de Biomedicina de Sevilla (IBIS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain; 4) Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Seville, Spain; 5) Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Valencia, Spain; 6) Andalusian Molecular Biology and Regenerative Medicine Centre (CABIMER), Seville, Spain; 7) Functional Genomics Node (INB), Valencia, Spain.

Targeted exome resequencing projects produce enormous amounts of data on protein coding sequences as well as other interesting functional elements of the genome, which includes microRNAs. These non coding RNAs are key components of the gene regulatory network in a wide range of species and operate by base complementarity. Due to this mode of action it is commonly believed that such regulatory elements were highly conserved. The analysis of the information available on 23 exomes from healthy southern Spain population, sequenced in the context of the Medical Genome Project, has uncovered an unexpected amount of variability in microRNAs. A total of 558 variants were found in 291 different miRNAs, 131 of which are known to be involved in almost 200 diseases. Among these, 487 (87%) variants were described for the first time in this study. This figure almost doubles the number of known variants in microRNAs and constitutes a remarkably high ratio of discovery. Different parts of the mature structure of the microRNA were affected by variants, which suggest a potential functional effect in the variability found. The average number of variants per individual found within miRNAs positions was of 118. Despite miRNAs were thought to be a highly conserved genomic element, our study has uncovered an unexpectedly high level of variability.

850F

Endothelium-derived Adropin is a Mediator Associated with oxLDL-Induced Endothelial Cells Dysfunction via Elevating microRNA-29a/b Levels. *M. Shen[1], K. Chen[1,3], M. Lu[2], S. Juo[1,3], T. Lee[1,3].* 1) Department of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 3) Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.

Adropin, encoded by the Energy Homeostasis Associated (Enho) gene mainly expressed in the liver and brain, was originally thought to participate in maintaining metabolic homeostasis. It was also shown to exert direct effects and protective role on the vascular endothelium, including angiogenesis, anti-apoptosis, likely via VEGFR2 up-regulation and downstream eNOS activation through the VEGFR2-PI3K-Akt pathways. Due to its detectable expression in vascular endothelial cells such as HUVECs and implications in vascular function, we therefore investigated the patho-physiological significance of endothelium-derived, endogenous adropin in vascular endothelium. This endothelium-derived, endogenous adropin appears to function in regulating VEGFR2 signaling associated with vascular homeostasis. Whereas in the pathological case, pro-atherogenic oxidized LDL (oxLDL) was able to down-regulate adropin as well as VEGFR2 expression in HUVECs at mRNA and protein levels from qRT-PCR and Western blot analyses. Due to the presence of several putative microRNA binding sites in the adropin 3'UTR, we speculated whether microRNA-mediated silencing can account for the mechanism underlying oxLDL-triggered adropin down-regulation. Among the candidate microRNAs targeting to adropin 3'UTR, the levels of miR-29a and -b were elevated in response to oxLDL exposure. Additionally, co-transfection of either miR-29a or -b mimics abrogate the luciferase activity of luciferase reporter construct carrying adropin 3'-UTR. However this suppressive effect was not observed in the construct carrying mutated miR-29a/b binding site, which further supports the regulatory role of miR-29a/b. Furthermore, both miR-29a and -b mimics also down-regulate VEGFR2 expression in HUVECs. All these observations indicate that elevated miR-29a and -b upon oxLDL treatment contribute to the adropin down-regulation, which may in turns impair VEGF/VEGFR2 signaling and lead to endothelial cells dysfunction. Interestingly, the pull down assay indicated the directly physical interaction of adropin and VEGFR2 that probably affects the downstream VEGFR2 expression and activates VEGFR2 signaling. In this study we not only unravel the regulatory role of adropin in vascular endothelium but also provide a rational molecular basis for the pathogenesis of atherosclerosis.

851F

TaqMan®-based miRNA profiles classify expression in breast cancer and leukemia. *L. Wong, N. Mulakken, D. Ridzon, P. Brzoska, C. Chen.* Genomic Assays R&D, Life Technologies, 850 Lincoln Centre Drive, Foster City, CA, 94404, USA.

MicroRNAs (miRNAs) are small endogenous RNA molecules that play an important role in the regulation of developmental and physiological processes in animals and plants. Studies indicate that miRNAs are involved in the multilevel regulation of gene expression targeting a battery of mRNA genes. Researchers have discovered that miRNAs are efficacious biomarkers for the classification of tumors and other cell types, as well as prediction of outcome for many diseases because of their evolutionary conservation, unique expression signatures, relative stability, and abundance. To identify potential candidates involved in tumor progression in humans, we examined the expression of 750 miRNAs using Applied Biosystems TaqMan® MicroRNA Assays in five human breast cancer cell lines, and two human leukemia cell lines. Data from leukemia cell lines were compared to two cell lines of normal peripheral blood mononuclear cells (PBMC), and data from breast cancer cell lines were compared to normal breast tissue. In addition, the two groups of cancer samples were examined relative to the expression levels of 38 different normal tissues. We confirmed several miRNAs that were previously identified to be associated with cancer. For example, let-7 family showed significant differential expression between breast cancer cell lines and normal breast tissue. We also determined a group of miRNAs that were consistently differentially expressed relative to the normal samples in all cancer cell lines. In addition, we identified miRNAs that are uniquely expressed in breast cancer but not in Leukemia cell lines. Further, miRNA families that are represented frequently in the list of significant miRNAs in both types of cancers were determined. These findings provide insight into possible common and distinct pathways between breast cancer and leukemia and the role of miRNAs in human cancer.

852F

Examining fine-scale dynamics of mitochondrial transmissions in humans. *B. Dickins[1], H. Goto[1], E. Afgan[2,5], I.M. Paul[3], J. Taylor[2,5], A. Nekrutenko[4,5], K. Makova[1].* 1) Department of Biology, The Pennsylvania State University, University Park, PA; 2) Department of Biology, Emory University, Atlanta, GA; 3) Department of Pediatrics, Penn State College of Medicine, Hershey, PA; 4) The Huck Institute for Life Sciences and Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA; 5) The Galaxy Project, http://usegalaxy.org.

Mitochondrial inheritance is unusual because it is uniparental and because, despite this, multiple alleles in varying proportions subsist in the populations of genomes present within a cell, tissue or individual: a situation defined as heteroplasmy. *De novo* mutations (DNMs) and heteroplasmy frequency shifts (HFSs) both contribute to the complex dynamics exhibited in this inheritance channel for which a thorough quantitative understanding is lacking. Given the uses to which mitochondrial DNA (mtDNA) sequence data is put, improving our knowledge would constitute a valuable contribution to fields as diverse as evolutionary biology, forensics and cancer diagnostics.

To this aim, we have sequenced mtDNA at high coverage from blood and buccal tissue of 19 individuals from six families with a total of 13 maternal transmission events. While massively parallel sequencing is, in principle, well-suited to the analysis of heteroplasmy (and polymorphism in general), we have strived to develop an analysis pipeline that is resistant to noise generated during sample amplification, library preparation, sequencing and mapping. Using simulations and re-sequencing of clonal DNA, we devised a broadly applicable set of criteria for detecting heteroplasmic sites above the 2% level and our analysis is easily repeatable through our Galaxy website where data and tools can be accessed, copied and modified.

Our analysis of data collected so far has revealed nine heteroplasmic sites and 15 mutation events (DNMs and HFSs) allowing us to describe the fine-scale dynamics of mitochondrial heteroplasmy. Because we have made use of DNA from more than one tissue, we are also able to infer patterns of change within individuals as well as within families. In general heteroplasmies appear to be relatively uncommon events while significant HFSs are more common.

853F

Characterization of the porcine imprinted and non-imprinted genomic domains orthologous to the human Prader-Willi syndrome chromosome region. *R.D. Nicholls[1,2], D.W. Lewis[2], K.L. Weichler[1], B.J. Henson[1], S.M. Gollin[2], R.S. Prather[3].* 1) Department of Pediatrics, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA; 2) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 3) Division of Animal Sciences, University of Missouri, Columbia, MO.

Prader-Willi syndrome (PWS) is a multisystem disorder caused by loss of function of a cluster of ~12 paternally-expressed, imprinted genes in human chromosome 15q11.2. Cardinal features of PWS include neonatal failure to thrive, abnormal body composition, short stature with growth hormone deficiency, and childhood-onset hyperphagia and obesity, among other endocrine and behavioral abnormalities. Although mouse models of PWS recapitulate some of the clinical components of the disorder, none develop early onset hyperphagia or the severe obesity of the human disease. Therefore, alternative animal models are needed to study the biomedical basis and therapeutic approaches for the eating disorder and obesity. Miniature pigs may provide an ideal model for PWS, since they have a more similar body size, physiology, anatomy, and genome to human than does the mouse, and hence may be more susceptible to development of obesity. Furthermore, technologies exist in the pig to produce genetic models of disease. While the pig genome sequence is close to being finished, nonetheless, the PWS-orthologous region is poorly represented. Using sequence databases to screen for phylogenetically conserved sequences from the PWS domain, we generated *in silico* a BAC contig spanning large portions of the pig PWS-homologous imprinted and non-imprinted domains. Ten of the imprinted genes have been identified from partially sequenced BACs and ESTs. We identified unsequenced BAC clones that span the ~150-kb *cis*-acting imprinting center (IC) or extend from partially sequenced regions towards the IC. Five of these BACs are being sequenced by the Wellcome Trust Sanger Institute. These new sequences will allow us to identify regulatory elements controlling genomic imprinting in this domain and that control neuronal gene expression of the *Snurf-Snrpn*-snoRNA bicistronic mRNA and downstream cluster of small nucleolar RNAs. Also, BAC clones for imprinted and non-imprinted regions are being FISH-mapped, with the IC-*Snurf-Snrpn* locus mapping to *Sus scrofa* chromosome 1q18. This work will identify the genetic structure, including imprinted genes, transcriptional and imprinting *cis*-regulatory elements, and the chromosome evolutionary breakpoints in the PWS-orthologous domain in pig, a region of significant biological, agricultural, and medical interest. *Supported by the Foundation for Prader-Willi Research and RAC (Children's Hospital of Pittsburgh)..*

854F

Improved Base Calling for 5500 Series SOLiD Sequencers. *C. Yang, M. Jiang, E. Wang.* Life Technologies, Foster City, CA.

Recent advances in SOLiD systems have revolutionized genomics, making it possible to quickly produce huge amount of sequences at substantially lower costs than traditional Sanger sequencing. Improved probe and ligation chemistry have resulted in enhanced coverage with fewer sequencing gaps in both GC- and AT-rich regions. Meanwhile, there exist several challenging obstacles that have to be overcome in order to push sequencing accuracy even higher. For example, it has been a well known issue that later ligation cycles have much higher error rate than earlier ligation cycles. One of the factors contributing to such phenomena is through-cycle residual build-up, which results in the change of the bead intensities captured by the instrument camera. In this work, we developed an efficient algorithm to dynamically model and correct the residual effect to improve color call. Specifically, the bead intensity at a given cycle is modeled as the sum of three components: 1) the underlying theoretical intensity vector at the current cycle, 2) the residual effect from the immediate previous cycle as the product of the residual coefficients and the intensity vector of the previous cycle, 3) a vector term representing the background difference between the two cycles. Among the three terms, both the residual coefficients and the background difference terms are channel-dependent. Once the model is solved mathematically through least square fitting technique, the residual and the background difference are subtracted from the current cycle to recover the underlying intensity, from which more accurate color calls can be achieved. In practice, this complete workflow consists of three steps: 1) a chosen color caller feeds the initial color call values into the model; 2) the underlying intensity values are recovered by the model; 3) the recovered intensity values are feed into the color caller to refine the color calls. A subset of the beads are used for model fitting and the solved model parameters are applied to all the beads in the same panel for performance reason. The above algorithm has been implemented in 5500 series SOLiD sequencers and tested on multiple data sets. Compared with results obtained without residual correction, the new base caller showed about 10 percent of total and 50 percent of perfect matched throughput increase and it has been observed that such improvement resulted from lowering errors in later ligation cycles from cycle modeling correction.

855F

Genome-wide mutation detection using the Mouse Diversity Genotyping Array: Evidence for a mutation signature associated with a premature aging phenotype. *S.T. Eitutis, A.E. Wishart, K.A. Hill.* Department of Biology, The University of Western Ontario, London, Ontario, Canada.

The somatic mutation theory of aging implicates mutation accumulation with aging-associated diseases. However, genome-wide analysis of mutation load in individual tissues of humans is limited by tissue availability. Mouse mimics of human disease permit direct hypothesis testing, and a new advance in single nucleotide polymorphism (SNP) array-based genotyping provides the first high-resolution and low-cost approach to genome-wide mutation detection. Using the Mouse Diversity Genotyping Array (MDGA) providing a 623,124 SNP probe mutation target, we report the first application of this array for somatic mutation detection in a mouse mimic of human aging-associated neurodegenerative disease. The *harlequin* (*hq*) mouse contains a hypomorphic mutation in the *Apoptosis-inducing factor* (*Aif*) gene resulting in a premature aging phenotype with mitochondrial dysfunction and neurodegeneration. The MDGA was used to determine the frequency and distribution of mutations between splenic and cerebellar DNA from two 8-month and two 15-month-old *hq* brothers and two 8-month-old AIF-proficient brothers. Putative mutations were detected as differences in SNP calls between two tissues of the same mouse. The *de novo* mutations in a tissue were identified as genotype calls different from the genetic background of the mice. Mutation frequency was higher in *hq* compared to AIF-proficient mice ($p<0.001$). Inter-animal variation in mutation frequency was lower in both tissues of the *hq* mice ($p<0.001$). Putative mutations in *hq* mice were over-represented on chromosomes 12, 13, and 14 in both tissues compared to AIF-proficient mice ($p<0.001$). These data suggest that the mutational mechanisms operating in the *hq* mouse appear to be similar in spleen and cerebellum and are suggestive of an endogenous mutagen with a specific signature or DNA sequence context yet to be identified. The postmitotic cerebellum in contrast to the highly replicative spleen may contribute to the degenerative phenotype of the mutation burdened *hq* cerebellum. To date, no disease phenotype has been observed for the *hq* spleen. Detailed searches for a preferred sequence context have ruled out common culprits: selectively neutral sequence, G+C content, single nucleotide composition, sequence repeats and CpG dinucleotides. Resequencing of chromosomes 12, 13 and 14 may reveal the mutation signature to help elucidate the role of accumulated mutations in this mouse model and perhaps with human aging and neurodegeneration.

856F

Evidence for an altered profile of copy number changes in the cerebellum of the *harlequin* mouse mimic of human aging-associated neurodegeneration. *A.E. Wishart, S.T. Eitutis, K.A. Hill.* Department of Biology, The University of Western Ontario , London, Ontario, Canada.

An interesting paradox exists in the pursuit of understanding the genetic basis of complex phenotypes such as aging and neurodegenerative disease. In humans, technological advances and demand have fuelled low-cost, high-resolution methodologies including high-resolution SNP arrays and whole genome sequencing, but hypothesis testing is severely limited by tissue availability. In the mouse, array-based approaches were very low-resolution, and genome sequencing remains high-cost due to comparably lower demand, but hypothesis testing has the freedom of tissue availability and control over mouse genotype. The Mouse Diversity Genotyping Array (MDGA) is a new high-resolution 623,124 SNP array with over 900,000 additional probes for copy number detection capable of genome-wide assessment of associations between copy number variation and complex disease. We used the array for a novel approach to mutation detection and specifically to identify copy number changes (CNCs) across the genome with comparison between two somatic tissues of the same mouse. The burden and nature of CNCs were examined in two male *harlequin* (*hq*) mice deficient in *Apoptosis-inducing factor* (*Aif*) resulting in mitochondrial dysfunction, neurodegeneration and premature aging in comparison with with two AIF-proficient brothers. Genomic segmentation analysis using Partek® Genomics Suite identified different profiles in *hq* and AIF-proficient mice. Total CNC burden is similar between *hq* and AIF-proficient mice for both cerebellum and spleen. The *hq* mice have significantly more gains in copy number in the cerebellum ($p<0.001$). The *hq* mice have more similar profiles of CNCs in contrast to greater variation in the AIF-proficient mice. The occurrence of CNCs across chromosomes was similar to that expected for a uniform distribution. Our novel MDGA approach to CNC analysis has demonstrated success in identifying a potential mutation type associated with a tissue-specific disease phenotype.

857F

Recent Natural Selection in Primate Non-coding Sequences. *B. Giardine, K. Chen, R. Harris, F. Chiarmonte, R.C. Hardison.* The Pennsylvania State University, Center for Comparative Genomics and Bioinformatics, University Park, PA, USA.

Non-coding regions under selective pressure indicate functional importance and can contribute to phenotypic diversity among species. To date few genome-wide studies have looked for signatures of selection outside of protein-coding regions. We employ a variation of the McDonald-Kreitman approach, using widely distributed ancestral repeats as the putative neutral mutation class, to detect recent selection in humans using two different time scales of hominoid evolution. The increasing availability of SNP data from more individuals and a variety of human populations supports more robust analyses of selection and diversity than were previously possible. Preliminary results show that the distribution of adaptive-selection signals varies among and along chromosomes; furthermore, regions showing strong deviations from neutrality tend to overlap with segmental and gene duplications that are enriched with other footprints of adaption. This genome-wide scan enables us not only to find signals of natural selection close to genes, but also those distal to coding regions. The resulting regions showing selection signatures can aid further studies to expand our understanding of the functional impacts of non-coding sequences under natural selection.

858F

MTHFR and SCN1A genes polymorphism in Iranian patients with migraine. *M. Ansari[2], M.S. Fallah[1], AA. Owji[3], S. Saeidi[3], M. Moghadasi[4], A. Ebrahimi[1], N. Hatamnejadian[2], B. Sedaqati Khayat[2], F. Rezaei[1].* 1) Kawsar Human Genetics Research Center (KHGRC), Tehran, Iran, Islamic Republic of,; 2) Department of Clinical Biochemistry, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of,; 3) Department of Biochemistry, Shiraz University of Medical Sciences, School of Medicine, Tehran, Iran, Islamic Republic of; 4) Department of Neurology, Iran University of Medical Sciences, Tehran, Iran, Islamic Republic of.

Introduction Migraine is a common debilitating neurologic disorder and familial clustering raises the probability that genetic factor might play a role in migraine susceptibility. Many genes including CACNA1A, ATP1A2, SCN1A and MTHFR seem to be involved and related to migraine susceptibility. This study performed to find the association between SCN1A and MTHFR polymorphism and migraine. Material and Methods Migrainous patient according to the international headache society criteria included in the study. Healthy people without any positive history of periodic headache and no family history of migrainous headache in the family recruited as control group. MTHFR C677T and A1298C polymorphisms and SCN1A gene polymorphisms (rs7601520 and rs2298771) were investigated using PCR-RFLP and direct sequencing method. Results: Seventy-five migrainous patient (18 males and 57 females) and 128 healthy controls (43 males and 85 females) were recruited in the study. MTHFR 677TT was more frequent in migrainous patients (17.1% vs, 3.1%, P<0.05) and were associated with higher risk of migraine (OR= 6.5, CI 95%; 2.03-20.76). 677T allele was also more frequent than 677C (38.0% vs. 25.8) ((P<0.001) (OR= 1.47, CI 95%; 1.10-1.97). 1298A and 1298C allele frequency doesn't differ in cases and controls. Substitution of wild type alleles in 3 out of 4 allele (677TT+1298AC or 1298CC+677TC) was more frequent in migrainous patients (20% vs 3.9%, P<0.0001). We didn't detect any cases with homozygote substitution of wild type allele in both SNPS. Genotypes for rs7601520 were AG, AA and GG in 23 (56.1%), 14 (34.15%) and 4 (9.76%) respectively. Allele frequency were A and G in 51 (62.2%) and 31 (37.8%) respectively. Genotypes for rs2298771 were AG, AA and GG in 26 (57.8%), 15 (33.3 %) and 4 (8.9%) respectively. Allele frequency were A and G in 56 (62.2%) and 34 (37.8%) respectively. Conclusion: It seems MTHFR polymorphism may predict the susceptibility to migraine attach. Genotype and allele frequency of rs7601520 and rs2298771 were in the range of other studies.

859F

Exome sequencing in Charcot-Marie-Tooth disease. *M.A. Gonzalez[1], E. Powel[1], G. Montenegro[1], G. Shengru[1], S. Blanton[1], G. Beecham[1], F. Speziani[1], C. Siskind[6], J. Vance[1], M. Menezes[2], D. Herrmann[3], S. Scherer[4], M. Reilly[5], M. Shy[6], S. Zuchner[1].* 1) John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) The Children's Hospital at Westmead, Sydney, N.S.W; 3) School of Medicine and Dentistry, University of Rochester Medical Center, Rochester, NY; 4) Department of Neurology, The University of Pennsylvania School of Medicine, Philadelphia, PA; 5) MRC Centre for Neuromuscular Diseases, The National Hospital for Neurology and Neurosurgery, London, UK; 6) Department of Neurology and Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI.

Charcot-Marie-Tooth (CMT) disease comprises a large number of genetically distinct forms of inherited peripheral neuropathies. The >40 known genes explain only 20-30% of the axonal forms of CMT. The Inherited Neuropathy Consortium has been formed to improve this situation by applying exome sequencing to dominant CMT2 families in order to identify rare known and novel CMT genes. As a novel and alternative approach, whole exome sequencing has recently been introduced. This method is capable of re-sequencing the near complete set of coding exons of an individual. We performed whole exome sequencing in 15+ CMT2 families that were previously excluded for changes in the gene MFN2. A bioinformatics pipeline was developed and, if applicable, linkage analysis performed. To date, we have produced and analyzed over 40 whole exomes from CMT patients. Depending on the sequence coverage we identified between ~9,000 and 11,300 non-synonymous variants per individual. Special consideration was given to variants in known CMT genes. Further variant filtering for segregation, presence in public databases, and potential functional significance resulted in between zero and several-dozen candidate genes in each family. In one family, we were able to establish significant two-point linkage of LOD>2.6 at a novel rare variant in the mitochondrial tryptophanyl tRNA synthetase gene at chr1:119Mb. In conclusion, we have developed an analysis pipeline for CMT gene identification in exome data. We have learned that exome analysis does not always lead to immediate resolve of a given dominant pedigree. While typically remarkably few genes remain after exome analysis the need for additional follow-up studies became apparent. Such approaches should include molecular studies, but also collaborative screening of additional CMT cases. Preliminary results and late-breaking findings will be presented and we will discuss strategies and pitfalls of the exome sequencing approach.

860F

Whole-exome sequencing in a family with idiopathic generalized epilepsy. *E.R. Londin[1], M.A. Keller[2], A. Ertel[3], K. Delgrosso[3], S. Surrey[4], P. Fortina[3].* 1) Coriell Institute for Medical Research, Camden, NJ; 2) American Red Cross, Philadelphia, PA; 3) Thomas Jefferson University Hospital, Department of Cancer Biology, Cancer Genomics Laboratory, Philadelphia, PA; 4) Cardeza Foundation for Hematologic Research, Department of Medicine, Thomas Jefferson University, Philadelphia PA.

Epilepsy is a heterogeneous group of seizure disorders and represents one of the most common neurological disorders affecting ~3% of the population at some point in their life. Epilepsy is characterized by recurrent, unprovoked episodes of seizures arising from a variety of symptoms; incidences peak in children and the elderly. While symptomatic epilepsies are introduced by outside sources such as brain malformations or trauma, idiopathic epilepsies have no known cause. To date, the study of rare monogenic forms of idiopathic epilepsies have been identified, but identification of genes associated with more common forms has remained elusive. Additionally, genome-wide associations studies of these common epilepsies have failed to identify any significant associations. We present the findings of the first next-generation sequencing project performed to identify potential candidates associated with epilepsy. Whole-exome sequencing was performed on a family consisting of parents and two siblings, one of which has idiopathic epilepsy. The proband has experienced generalized absence seizures since the age of 3 years. Using the Agilent SureSelect 38 Mb All Exome Kit, an average of 4.5 Gb of sequence at an average of 125x read depth per subject was achieved. Over 20,000 genetic variants (SNPs and indels) were identified. The variants were filtered based upon known and unknown function and presence within genes expressed in the brain as well as their presence within either dbSNP or the 1000 Genomes project. While our analysis did not identify novel variants, we identified five previously characterized non-synonymous SNPs in the affected sibling. These variants were present in the affected sibling and one of the parents, but not in the unaffected child. The five variants are within genes encoding neuronally-expressed ion channels not previously associated with epilepsy. We intend to determine the association of these SNPs with epilepsy in a larger study of affected and control subjects. This preliminary study demonstrates that exome sequencing of affected and unaffected family members can be used to identify variants that may be associated with a common form of epilepsy.

861F

Gene expression profiling of both protein-coding and long non coding RNA transcripts from small amounts of total RNA using a single microarray design. *A. Bergstrom Lucas, E. Lin, V. Kulkarni, S. Fulmer-Smentek.* Genomics R&D, Agilent Technologies, 5301 Stevens Creek Blvd., Santa Clara, CA USA 95051.

Recently, thousands of long non-coding RNA (lncRNA) transcripts have been identified in mouse and human cells from genome-wide chromatin-state maps and RNA sequencing. The roles that the lncRNAs play in biology is under debate and vary widely from acting as scaffolds for chromatin remodeling to guiding complexes to the right locations in the genome to enhancing or activating protein-coding gene expression. As more researchers investigate the function of lncRNAs, there is a need for tools that can rapidly and accurately measure lncRNA expression along with mRNA expression. We have previously developed human and mouse SurePrint G3 microarrays comprised of all known protein-coding mRNAs and lncRNAs to enable systematic profiling of lncRNAs and protein-coding genes allowing for simultaneous detection of gene expression from a single sample. To demonstrate the utility of the arrays we used low nanogram amounts of matched tumor and adjacent normal RNA to produce cyanine-labeled cRNA. The labeled cRNA was applied to the arrays to detect differences in coding and non-coding gene expression profiles. Using the GeneSpring GX software we are able to identify differentially expressed lncRNAs and protein-coding RNAs in the tumor and normal samples in less than two days. Comparisons of probe signals from technical replicate samples demonstrated high reproducibility with wide dynamic ranges and high sensitivity. Data from the microarrays correlates well with whole transcriptome sequencing of the same matched tumor/normal samples. Using this approach we show that lncRNA expression coincides with key genes known to regulate biological processes involved in cancer progression and this work demonstrates how profiling mRNA and lncRNA from matched tumor and adjacent normal samples can allow researchers to further define the role of lncRNAs in gene regulation. As further discovery and definition of the structure of lncRNAs continue, new and updated content will be available on these arrays to further enable novel discoveries and understanding of the interaction between non-coding and coding RNAs.

862F

DVWA, osteoarthritis-associated gene, is associated with joint destruction in patients with rheumatoid arthritis. *T. Suzuki[1,2], K. Ikari[1], K. Yano[1], Y. Toyama[2], A. Taniguchi[1], H. Yamanaka[1], S. Shigeki[1].* 1) Inst Rheumatology, Tokyo Women's Med Univ, Shinjuku, Japan; 2) Department of Orthopaedic Surgery, School of Medicine, Keio University, Tokyo, Japan.

Background:To date, a number of osteoarthritis (OA) susceptible genes have been reported. Since OA is characterized by cartilage loss leading to joint damage, it may share the genetic background of the joint destruction with rheumatoid arthritis (RA). The purpose of this study was to evaluate the genetic association of reported OA-susceptible genes and joint damage in Japanese RA patients. Methods:DNA samples of 1504 Japanese patients were collected from the IORRA (Institute of Rheumatology RA cohort) DNA collection. Of the patients, Sharp/ van der Heijde score (SHS) of the hands at 5-year disease duration, which represents joint destruction in RA, could be measured in 628 patients. Following polymorphisms were selected since they were reported to be susceptible to Japanese OA patients; ASPN (an aspartic acid [D] repeat), GDF5 (rs143383), CALM1 (rs12885713) and DVWA (rs7639618) (1-4). The D repeat polymorphism was genotyped using PCR with a fluorescent primer, and the SNPs were genotyped using the TaqMan assay according to the manufacture's instructions. Multiple regression analysis was performed with SHS as a dependent variable, and the number of the risk alleles of OA-susceptible genes and the known joint damage-associated factors as independent variables; the number of HLA-DRB1 alleles encoding the shared epitope (SE), sex, anti-CCP antibody (ACPA), rheumatoid factor and age of onset. We also examined the expression of the gene in RA cartilage tissues using RT-PCR when the association of severity is recognized. Results:Sex (female, P= 1.9x10-5), age of onset (younger, P= 6.4x10-5), ACPA (P= 0.047), the number of SE (P= 0.026) and DVWA (P= 0.023) had showed the impact on radiographic joint damage, while the other tested genes showed no association. DVWA transcripts were expressed in both OA and RA articular cartilage. Conclusions:DVWA was associated with joint destruction in RA patients. References 1. An aspartic acid repeat polymorphism in asporin inhibits chondrogenesis and increases susceptibility to osteoarthritis. Nat Genet.37:138- 44. 2. A functional polymorphism in the 5' UTR of GDF5 is associated with susceptibility to osteoarthritis. Nat Genet. 39:529-33. 3. A functional single nucleotide polymorphism in the core promoter region of CALM1 is associated with hip osteoarthritis in Japanese. Hum Mol Genet. 14:1009-17. 4. Common variants in DVWA on chromosome 3p24.3 are associated with susceptibility to knee osteoarthritis. Nat Genet. 40:994-8.

863F

Meta-analysis of curated public genomic data provides evidence that tibolone induces breast cancer recurrence. *H.S. Wang, S. Weiman, A. Hsu, M. Shekar, I. Kupershmidt.* NextBio, Cupertino, CA.

NextBio's web-based tools and curated public genomic data can be used to investigate therapeutic regimens *in silico*. Here, we present a case study of tibolone, which is prescribed to treat vasomotor symptoms of menopause and has been used to treat vasomotor symptoms associated with breast cancer therapy. Tibolone was thought to interact selectively with estrogen receptors responsible for these symptoms without promoting proliferation and tumor growth. However, a 2009 report in The Lancet showed that tibolone significantly increased the risk of breast cancer recurrence to 15.2% from 10.7% in the placebo group—a discovery that prompted the study's premature termination. We propose that before initiation of clinical investigations, analysis of publicly available genomic data could have predicted this increased risk of recurrence and provided preliminary assessment of alternative treatment options. Our investigation of public genomic data curated by NextBio found positive correlations between tibolone-induced gene expression changes and those seen in breast cancer. We also found that the tibolone-induced gene expression profile resembles that of estradiol hormone replacement therapy (HRT), which has been contraindicated in breast cancer patients. Using NextBio's gene set enrichment analysis tools, we also found that gene expression profiles of tibolone treatment, HRT, and breast cancer were commonly enriched in genes related to the cell cycle, growth signaling, morphological changes, and cancer progression. These enrichment results suggest that tibolone may contribute to disease recurrence by similar mechanisms to HRT. Filtering tibolone expression profiles by ER receptor status in breast cancer revealed correlations with both ER+ and ER- subsets, consistent with literature reports that tibolone also interacts with other androgen receptors. A similar analysis in NextBio for gabapentin, which is also used to treat vasomotor symptoms, shows gene expression changes that are not correlated with breast cancer, HRT, or tibolone signatures, indicating its potential as a safer alternative than tibolone for breast cancer patients. Together, these analyses demonstrate that NextBio's web-based tools and integrative genomics data (including genome wide association studies, epigenetic, microRNA, resequencing, somatic mutation and DNA copy number data), can greatly aid applications such as the prediction of drug efficacy, adverse effects, and alternative therapies.

864F

Comparing methods for mapping *cis* acting polymorphisms using allelic expression ratios. *M. Santibanez-Koref[1], M.D. Teare[2].* 1) Institute of Human Genetics, University of Newcastle, Newcastle upon Tyne, United Kingdom; 2) School of Health and Related Research, University of Sheffield, 30 Regent Street, Sheffield, S1 4DA, U.K.

Genome wide association studies provide increasing evidence that modulation of transcription, in particular through changes acting in *cis*, plays an important role in modifying disease susceptibility. Differences in transcription between the two alleles at an autosomal locus can be used to test the association between candidate polymorphisms and modulation of gene expression in *cis*. This type of approach requires at least one transcribed polymorphism and one candidate polymorphism. In the past five years different methods have been proposed to analyse this type of data. Here we use simulations and real data sets to compare the power of some of these methods. The results show that when it is not possible to determine the phase between the transcribed and potentially cis acting allele there is some advantage in using methods that estimate phased genotype and effect on expression simultaneously. However when the phase can be determined, simple regression models seems preferable because of their simplicity and flexibility. The simulations and the analysis of experimental data suggest that in the majority of situations considered methods that assume a lognormal distribution of the allelic expression ratios are both relatively robust to a range of deviations from this assumption and more powerful than the alternatives that do not make these assumptions.

865F

Association Analysis of Polymorphisms in Insulin Secretion Pathway Genes with T2D in North Indian Punjabi Population. *A.J.S. BHANWER[1], Y.P. SINGH[1], N. KAUL[1], K. MATHAROO[1], R.N.K. BAMEZAI[2].* 1) DEPARTMENT OF HUMAN GENETICS, GURU NANAK DEV UNIVERSITY, AMRITSAR, PUNJAB,INDIA; 2) SMVD UNIVERSITY, KATRA , J&K, INDIA.

Prevalence of T2D has rapidly increased in India and throughout the world during last two decades. The major cause of the disease in India is supposed to be changing life style, increasing stress, less physical activity, dietary pattern and ethnicity. The various clinical and anthropometric parameters have also contributed towards development of the diseases. Thus, environmental factors along with genetic factors influence predisposition to T2D. It is not yet clear after several studies carried out in various populations worldwide that how many confounding factors and which genes are genuinely responsible for development of this complex disease. The GWA studies have also revealed many loci in nuclear genome, most of which confront in the insulin secretion pathway. Present case-control study was initiated to analyse single nucleotide polymorphisms (SNPs) in genes of insulin secretion pathway such as KCNJ11 (rs5219), UCP2 (rs659366), PGC1-((rs8192678), SIRT1 (rs12778366), TCF7L2 (rs7903146), mt-ND3 (rs2853826) with susceptibility to T2D. PCR-RFLP and sequencing procedure was used for genotyping polymorphisms. The association of above mentioned polymorphisms in North Indian Punjabi population was investigated in 242diabetics and 251non diabetic age, sex and economic status matched controls. The participants for the study were recruited with informed consent from north Indian state of Punjab. Binary logistic regression was applied to test the association of risk factors with T2D after adjusting for age, sex and BMI. The polymorphism in UCP2 (rs659366) p=0.02, OR-0.638(0.437-0.933), PGC1-((rs8192678) p=0.001, OR-1.888(1.304-2.733) and mt-ND3 (rs2853826) p=0.00018, OR-0.480(0.327-0.705) showed significantly association under dominant model. Thus, we observed that variants in genes involved in insulin secretion pathway seem to play a role in increasing susceptibility in North Indian Punjabi population. The present investigation also concludes that differential pattern of association of polymorphisms is observed for different populations, suggesting the putative role of ethnicity. Thus, for risk calculation and proper medical intervention, knowledge of the ethnicity and nature of variation in risk factors need serious attention.

866F

A Genomic Region on Chromosome 11q23.3 encompassing BUD13-ZNF259 and APOA5-A4-C3-A1 Gene Cluster Harbors Loci Contributing to Serum Triglyceride Concentrations in Asian Indians. *T.R. Braun[1], L. Been[1], S. Ralhan[2], G.S. Wander[2], J. Chambers[3], J. Kooner[3], D.K. Sanghera[1].* 1) Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2) Cardiology, Hero DMC Heart Institute, Ludhiana, Punjab, India; 3) National Heart and Lung Association, Imperial College London, London, UK.

Recent genome-wide association scans (GWAS) and meta-analysis studies conducted on European populations have identified many genes previously implicated in lipid regulation. Among these genes is the APOA5-A4-C3-A1 gene cluster. Validation of these loci on different global populations is important for their clinical relevance and particularly in developing novel drug targets for treating and preventing dyslipidemia and coronary artery disease (CAD). In an attempt to replicate GWAS findings on a non-European sample, we genotyped two GWAS SNPs (rs964184 and rs12286037) from BUD13-ZNF259 and APOA5-A4-C3-A1 gene clusters in an Asian Indian diabetes cohort comprising 3,781 individuals (2902 from Punjab and 879 from the US) from the Sikh Diabetes Study (SDS). Both SNPs were significantly associated with triglyceride (TG) levels; rs964184 (p=5.94x10-10) and rs12286037 (p=0.003) in this cohort. We further explored ~195 kb region on chromosome 11q23.3 encompassing BUD13-ZNF259 and APOA5-A4-C3-A1 clusters containing 45 SNPs using our preliminary lipid GWAS data from 8,280 individuals. Of these, 6,530 Asian Indians were from the LOLIPOP study (UK) and remaining from the SDS (USA). We identified eight SNPs to be significantly associated with TG in both the SDS and the LOLIPOP cohorts. Seven of these eight variants exhibited a strong association of genome-wide significance (p< 2x10-8) with TG levels in a joint meta-analysis performed on 8,280 individuals and the strongest signal was seen in rs964186 (p=3.06x10-45). Interestingly, other variants showing robust association signals (p< 3x 10-25) with TG in this population have not been identified in previous European studies. Future functional studies should enhance our understanding of clinical relevance of these genes in hyperlipidemia leading to pathogenesis of CAD.

867F

The association between genetic variation in the vitamin D immunomodulatory pathway and susceptibility to severe influenza infection. *S. Malik[1], P. Aurora[1], M.A. Karmali[1], M. Phillips[2], A. McGeer[3], K. Green[3], K. Fonseca[4], M. Graham[5], B. Neupane[6], J. Beyene[6], V. Manning[7], P. Singh[7], M. Loeb[7].* 1) Office of Biotechnology, Genomics, and Population Health, Public Health Agency of Canada, Toronto, Ontario, Canada; 2) Pharmacogenomics Centre, Montreal Heart Institute, Montreal, Quebec, Canada; 3) Mount Sinai Hospital, Toronto, Ontario, Canada; 4) Alberta Provincial Laboratory for Public Health, Calgary, Alberta, Canada; 5) National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada; 6) Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, Ontario, Canada; 7) Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada.

Influenza poses one of the greatest infectious threats to public health. Complications such as pneumonia and hospitalization, highest in those at the extremes of age, have a major impact on the healthcare system. The recent H1N1 outbreak indicated that obese persons, pregnant women and those with Type 2 diabetes were at particular risk for severe influenza, possibly due to the interplay between vitamin D and inflammation. Vitamin D related innate immunity may play an important role in the pathogenesis of influenza by eliciting rapid-acting antiviral defence mechanisms and/or dampening excessive virus-induced inflammation (cytokine storm) which may otherwise lead to severe infection. This study is a national multicenter collaborative effort to identify genetic variants in the vitamin D biosynthetic/immunomodulatory pathway associated with severe influenza. Innate immunity is critical for the early containment of the influenza virus. Recent evidence indicates that innate immunity to infectious agents is modulated by vitamin D, consistent with the observation that influenza infection follows a geographic and seasonal distribution of vitamin D-associated solar ultraviolet radiation. This observation has been substantiated by in vivo, in vitro, and RCT studies. We postulate that genetic variants within the vitamin D biosynthetic/immunomodulatory pathway are associated with severe outcomes of influenza infection. To this end, we have undertaken a candidate gene approach applying a population case-control design (413 cases and 285 controls). All participants are adults of European ancestry and laboratory confirmed carriers of the influenza virus. Cases are those admitted to hospital (severe outcomes); controls, those not admitted (mild outcomes). SNPs along the vitamin biosynthetic/immunomodulatory pathway were examined. We have currently identified three polymorphisms in the vitamin D biosynthetic/immunomodulatory pathway associated with severe influenza, however, the functional consequences of these SNPs is presently unknown. Further work is needed to characterize the role of these variants in the context of severe influenza infection. Results from this study can provide a better understanding into how vitamin D impacts the natural history of influenza infection and, further, can influence public health recommendations for vitamin D supplementation with a view to reduce the burden of severe influenza infection.

868F

Polymorphisms in NFKB1 gene are associated with juvenile rheumatoid arthritis. *J. Ramírez Bello[1,2], O. Mendoza Nava[3], A. Gutierrez-Hernández[4], F. Espinoza Rosales[4], G. Escamilla[4], V. Baca[5], L. Orozco[1,2].* 1) Laboratory of Immunogenomics, National Institute of Genomic Medicine, SSa, DF, Mex; 2) PhD Genomics Science Program, Autonomous University Mexico City, DF, Mex; 3) Faculty of Chemistry, National Autonomous University of Mexico, DF, Mex; 4) Departament of Immunology, National Institute of Pediatric, SSa, DF, Mex; 5) Departament of Pediatric Rheumatology, CMN-Siglo XXI; IMSS.

Juvenile rheumatoid arthritis (JRA) is the most common rheumatic disease in childhood. Over time, evidence has accumulated implicating NFKB1 as a mediator of autoimmunity and potential therapeutic target for treating autoimmune diseases. This transcription factor is a major regulator of immune response, apoptosis and cell-growth control genes. On this way, the aim of this work was to determine whether SNPs located in the NFKB1 gene (NFKB1_1; rs3774933T/C, NFKB1_2; rs3774937T/C, NFKB1_3; rs3774938A/G, NFKB1_4; rs1587213C/T, NFKB1_5; rs1587214C/T) are associated to JRA in Mexican population. This study included 215 patients (127 female and 88 male) with JRA, which fulfilled the American College of Rheumatology (ACR) criteria for the diagnosis of JRA. All patients were <16 years of age at onset of disease. A group of 430 (254 female and 165 male) unrelated healthy controls were included. Genotyping was carried out by 5' exonuclease assay (TaqMan). The association test, Hardy-Weinberg Equilibrium (HWE) and haplotypes, were evaluated using EPIDAT, FINETTI and Haploview softwares, respectively. Both cases and controls were in HWE. Differences between cases and controls were observed when we compared the genotype and allele distributions (NFKB1_1T/C, OR 0.69, p= 0.004; NFKB1_2T/C, OR 0.67, p=.001; NFKB1_3A/G, OR 0.66, p=.0009; NFKB1_4C/T, OR 0.69, p=.004 and NFKB1_5C/T, OR 0.67, p=.001). As expected, the haplotype CCGTT, carrying all minor alleles showed protection to JRA (OR 0.66, p=0.002). Pairwise linkage disequilibrium (LD) between SNPs showed an r2 > 0.90. When we stratified by gender, similar results were obtained in females (data not shown). Analysis in silico showed that these five SNPs could have a biological implication affecting an intronic enhancer. In summary, our data suggest that SNPs with a potential biological function in NFKB1 gene are protector factors to JRA in Mexican population. It is possible that these SNPs can be important in the biological function of NFKB1.

869F

Detection of Sample Contamination and Paralogous Sequence Misalignment Using Allele Balance Metrics Associated with Next Generation Sequencing. *I.B. Stanaway, J.D. Smith, E. Turner, C. Igartua, K. Patterson, S.R. McGee, T. Shaffer, C. Poel, J. Chin, B. Paeper, Q. Yi, K. Sawatzki, B. Munson, I. Robertson, M. Mynsberge, S. Austin, C. Shepard, S. Paquette, M. Tackett, J. Furlong, P. Robertson, D. Siegel, E. Johanson, E. Johnson, E. Phillips, M.J. Rieder, D.A. Nickerson.* Genome Sciences, University of Washington, Seattle, WA.

Recent advances in Next Generation Sequencing (NGS) have allowed the production of massive amounts of read data and the ability to process increasing numbers of samples in parallel. During sample preparation for NGS, there are many steps where low level sample cross-contamination could compromise data quality. We have found that allele balance (AB) is a useful metric for assessing sample contamination and detecting potential sequence paralogs. This metric measures the ratio of the two possible base calls (i.e. alleles) at diploid heterozygous variants. It is defined as the count of the reference allele mapped over a single base position divided by the total counts of bases mapped over that position. For high quality samples, the AB histogram ranges from 0 to 1.0 with a major peak ~0.5 and a second minor peak at ~0.8. We performed Nimblegen exome capture, Illumina sequencing, Burroughs-Wheeler alignment and called variants using the Unified Genotyper from the Genome Analysis Tool Kit (GATK) on 83 human Coriell DNAs. We show that low level sample contamination can be observed from the presence of a third peak in the lower quarter (<0.25) of the AB histogram. The variants falling in this third peak are predominantly common non-reference homozygotes where the reference allele from a second contaminating sample makes the variants appear to be heterozygous with an abnormal allele balance. The area under this peak grows linearly with the fraction of contaminating reads being sequenced. We define the lower limit of detection to be ~2% which allows adequate detection of low level contamination. Analysis of the other peak at ~0.8 shows variants with AB > 0.75 to have an excess of possible matches to other genomic regions. This suggests these are false variants derived from paralogous sequence mapping errors. This property is useful to identify potential novel copy number variants that have diverged in sequence identity from their paralog. Thus, AB is a useful and sensitive metric for assessing the quality of NGS data and subsequent variation calls.

870F

An Integrated Analysis Framework for Detecting Human Genetic Variations from Next Generation Sequencing Data in Tumor Samples. *C. Xiao[1], A. Ward[3], T. Blackwell[2], A. Mnev[1], H. Kang[2], E. Garrison[3], G. Marth[3], G. Abecasis[2], S. Sherry[1].* 1) NIH/NLM/NCBI, 45 Center Drive, Bethesda, MD 20892, USA; 2) Department of Biostatistics, University of Michigan, 1420 Washington Heights, Ann Arbor, MI 48109, USA; 3) Boston College Biology Department, 140 Commonwealth Avenue, Chestnut Hill, Massachusetts 02467, USA.

Tumor mutation spectrum analysis can provide insight into molecular and genetic mechanism of tumorigenesis, thereby improving molecular diagnosis and therapy of cancer diseases. The advancements of high-throughput next-generation sequencing (NGS) technologies have provided researchers with unprecedented opportunities to address biomedical problems in cancer studies, but also imposed huge challenges for downstream analysis due to the volumes and complexities of the data. In order to establish a proper and efficient workflow and facilitate genetic research using NGS technologies, we have developed an integrated analysis framework with multiple read aligners and variant callers to profile genetic mutations from NGS data from various sequencing platforms (including ILLUMINA, LS454, and SOLiD) in a uniform manner. The framework manages parallel-computing resources, checks sample identities, aligns the reads to the reference genome sequences, refines the mapping of placed reads, calls SNPs, INDELs, and SVs, and performs functional annotation. This NIH-based pipeline has been used for detecting genetic variations (approximately 31 million consensus SNPs) from the 1000Genomes main project Phase I data, and currently is expanded to perform mutation profiling for large scale tumor resequencing projects.

871F

Association of Tumor Necrosis Factor Gene Polymorphisms with Chronic Obstructive Pulmonary Disease: A case -control study from India. *N.A. Khan[1,2], N.L. Kamble[1], M.K. Daga[1], S.A. Husain[2].* 1) Medicine, Maulana Azad Medical College, New Delhi, Delhi, India; 2) Department of Biosciences, Jamia Millia Islamia University, New Delhi, India.

Background & Aim: Chronic Obstructive Pulmonary Disease is characterized by a chronic inflammatory process, in which the pro-inflammatory cytokine Tumor Necrosis Factor (TNF) Alpha is considered to play a role. We determined the common functional polymorphisms of TNF-a gene at -308, -863, and -1031 positions and explored the influence of each genetic variant on pulmonary function parameters, body mass index (BMI), serum TNF-a levels, and outcomes among heavy smokers with or without COPD in a case -control study conducted at Maulana Azad Medical College in New Delhi, India. Methods: Stable COPD Patients who were ever smokers and age and packs years smoked matched healthy controls (n= 230 in each group) were recruited. Allele and genotype frequencies of three tag single nucleotide polymorphisms (SNP) of the TNF-a gene promoter Region at -308, -863, and -1031 in all subjects was performed by polymerase chain reaction- restriction fragment length polymorphism technique on genomic DNA obtained from peripheral blood. Serum TNF Alpha level was measured by commercially available ELISA kit. Results: COPD patients had a significantly lower A allele frequency (9.7 vs. 15.1%, OR = 0.6, p = 0.048, false discovery rate q = 0.144) and a significantly lower A carrier genotype frequency (19.3 vs. 30.2%, OR = 0.52, p = 0.042, q = 0.135) than resistant smokers. The -863 CA genotype was associated with a better FEV1/FVC ratio (76 vs. 71.5%, p = 0.032), and higher BMI (25.9 vs. 23.6 kg/m2, p = 0.034). The -308 minor allele (A) had a higher odd ratio (OR) of being associated with COPD (Adjusted for age, sex, pack-yrs: OR 1.9) and was also associated with worse forced expiratory volume in one second/forced vital capacity. In addition, COPD patients with the -1031 C carrier genotype had higher serum TNF-a levels (20.9 vs. 16.2 pg/ml, p = 0.01). BMI (hazard ratio = 0.84, 95% CI = 0.74-0.96, p = 0.008) was the only independent predictor for mortality. Conclusion It is concluded that the TNF-a -863 A and -308A alleles may confer a degree of resistance to the susceptibility COPD among heavy smokers in Indian Population suggesting that tumor necrosis factor Alpha polymorphisms may play a role in the pathogenesis of COPD.

872F

Genome-wide analysis of SNPs impacting methylation and gene expression in *cis*. *T. Pastinen[1,2], B. Ge[2], S. Busche[1,2], T. Kwan[1,2].* 1) Human Genetics, McGill University, Montreal, Quebec, Canada; 2) McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, Canada.

DNA methylation is a frequent epigenetic modification which occurs almost exclusively in the context of cytosine methylation in CpG dinucleotides. It is fundamentally involved in the regulation of many cellular processes,including gene and microRNA expression, cellular differentiation, embryogenesis, and genomic imprinting. Methylation patterns are known to vary not only between cell types but also between individuals. Emerging evidence indicates that a fraction of population variation in methylation is heritable and due to common variants. We have assessed genome-wide methylation patterns in a panel of primary fibroblasts. In parallel, we have measured total gene expression, genome-wide allelic expression, and genetic variation at high density. The panel of 60 unrelated Caucasian individuals (and an additional set of offspring) were measured on Illumina's Infinium HumanMethylation450 BeadChips, which interrogates CpG-methylation at >450K sites. We filtered out CpG probes overlapping SNPs or potential cross-hybridization and then correlated SNPs ±250kb flanking each highly variable (top 25th percentile population variation) CpG-probe with methylation level in a regression test and normalized the methylation values. Approximately 2% of CpG-probes associated with local SNPs at high significance (P<1E-6, FDR<5%). The vast majority of the association signals for differential methylation lie in close proximity (distance <30kb) of measured CpG sites. Genetic variation in *cis*-regulation as determined by allelic expression mapping in these same cells was highly enriched among the SNPs governing differential methylation. Finally, we observe that nearly 100 common disease-associated SNPs are linked with methylation. Our results highlight new levels of diversity of functional variation in non-coding DNA, which may provide novel clues for the mechanistic basis of disease associations.

873F

Nucleosome organisation and chromatin structure in the HapMap cell lines. *D.J. Gaffney[1], G. McVicker[1], Y. Fondufe-Mittendorf[2], A.A Pai[1], R. Pique-Regi[2], J. Degner[1], J. Widom[1], Y. Gilad[2], J. Pritchard[1].* 1) Human Genetics, University of Chicago, Chicago, IL; 2) Department of Molecular Biosciences, Northwestern University, Evanston, IL.

Nucleosomes are the basic unit of eukaryotic chromatin and determine the accessibility of the packaged DNA to protein. Nucleosome organisation, and the factors that control it, are therefore of central importance in understanding how genes are regulated. Here we use MNase digestion of chromatin followed by high-throughput paired-end sequencing to produce a genome-wide map of nucleosome organisation in two HapMap cell lines. We use a computational method to identify the most likely nucleosome phase in regions of active transcription factor binding, and align nucleosomes adjacent to transcription factor binding sites. Our method reveals a clear signature of positioning of nucleosomes surrounding most bound transcription factors, We demonstrate how this signature can be used to estimate the region of open chromatin surrounding a bound transcription factor. Combined with a high-coverage DNase1 hypersensitivity map, our approach allows us to visualize the chromatin structure of enhancers at very high resolution.

874F

Large-scale analysis of heritable *cis*- and *trans*-regulation of gene expression across multiple tissues in twins. *E. Grundberg[1,2], K.S. Small[1,2], A.C. Nica[3], A.K Hedman[4], A. Buil[3], J.T. Bell[2,4], D. Glass[2], TP. Yang[1], A. Barett[5], J. Nisbett[1], A. Wilk[1], M. Travers[5], SY. Shin[1], J.L. Min[4], K.T. Zondervan[4], S. Ring[8], W. McArdle[8], G. Thorleifsson[6], A. Kong[6], U. Thorsteindottir[6,7], C.M. Lindgren[4], N. Soranzo[1,2], K.R. Ahmadi[2], K. Stefansson[6,7], G. Davey-Smith[8], M.I. McCarthy[3,5], P. Deloukas[1], E.T. Dermitzakis[3], T.D. Spector[2], the MuTHER consortium.* 1) Wellcome Trust Sanger Institute, Hinxton, United Kingdom; 2) Department of Twin Research and Genetic Epidemiology, King's College London, London, United Kingdom; 3) Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland; 4) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 5) Oxford Centre for Diabetes, Endocrinology & Metabolism, University of Oxford, Churchill Hospital, Oxford, UK; 6) deCODE genetics, Reykjavik, Iceland; 7) Faculty of Medicine, University of Iceland, Reykjavík, Iceland; 8) MRC Centre for Causal Analyses in Translational Epidemiology (CAiTE), School of Social and Community Medicine, University of Bristol, Bristol, United Kingdom.

Genetic variation in gene expression is an important underlying mechanism in disease susceptibility, but the relative contribution of heritable *cis* and *trans* effects and how it differs across tissues remain largely unknown. Here we use genomic and transcriptomic (IlluminaHT-12) data in LCL, adipose and skin tissue from 856 twins collected as part of the MuTHER study (TwinsUK). We find an abundance of heritable expression traits across tissues (i.e. _40% of adipose transcripts having h^2>0.3). To map the underlying *cis*-effects we carried out eQTL analysis and find that the genetic variance of transcript levels in LCLs are more likely to be explained by a *cis*-SNP than in adipose or skin tissue. For transcripts with h^2>0.3, a single *cis*-SNP explains on average 12% (LCL), 8% (adipose) and 9% (skin) of the genetic variance (h^2>0.3) but the effect sizes are increasing as heritability increases. When studying the 20 most heritable transcripts (h^2>0.6), in most instances (75%) a single *cis*-SNP explains >50% of the genetic variance. Further on-going analysis includes utilizing the twin structure to estimate the total heritable *cis*-effect in order to delineate the contribution of both common and rare variants. Using continuous estimates of tissue-overlap and comparisons of the magnitude of eQTL effects we estimate that 39-58% of *cis*-SNPs act in a tissue-dependent fashion. However, as *cis*-effects seem to account for a minor proportion of the heritability we sought to study the architecture of *trans*-regulation. In general, *trans*-eQTLs seem to be almost exclusively tissue-restricted and contribute small effects. Notably, we find that 9-20% of the *trans*-eQTLs per tissue at P<5E-8 affect clusters of ⁄3 transcripts suggesting presence of master-regulators (MR). These putative MRs display enrichment for low P-values across all transcripts indicating they may regulate additional genes below the threshold. This is supported by estimation of the proportion of true positives (61) where MR are enriched for larger 61 values than non-MR (61_{MR}=0.23 vs. 61_{NMR}=0.14). Replication efforts of *trans*-effects in independent studies ($ALSPAC_{LCL}$: N=936, $Oxford_{LCL}$: N=331, $deCODE_{adipose}$: N=585, $GenCord_{fibroblast}$: N=68) are ongoing but initial findings indicate a 3-fold enrichment for replicated *trans*-eQTLs. In conclusion, we find that the majority of transcripts have complex genetic regulation highlighting the need for continued exploration of the architecture of *cis* and *trans* regulation.

875F

Long-range regulatory elements identified using high-throughput chromosome conformation capture. *YC. Hwang[1], Q. Zheng[3,4], BD. Gregory[1,3,4], LS. Wang[1,2].* 1) Genomics and Computational Biology Graduate Program, University of Pennsylvania, Philadelphia, Pennsylvania, PA; 2) PENN Center for Bioinformatics, University of Pennsylvania, Philadelphia, PA; 3) Department of Biology, University of Pennsylvania, Philadelphia, PA; 4) PENN Genome Frontiers Institute, University of Pennsylvania, Philadelphia, PA.

In mammals, genes are not only regulated by their proximal promoters, but can also be affected by distal and orientation-independent elements such as enhancers. As enhancers can be distal from the genes they regulate, identifying possible enhancer elements is extremely laborious, and more comprehensive methods of detection are only recently being developed. Chromosome conformation capture (3C) is a technique that allows the interaction between DNA regions, both proximal and distal, to be analyzed. Lieberman-Aiden et al. combined 3C with high-throughput sequencing technology (Hi-C) to comprehensively identify the spatial organization of chromatin for human lymphoblastoid and erythroleukemia cell lines. Here, we have used this Hi-C data in conjunction with a de novo suite of algorithms to identify candidate enhancer elements genome-wide. To do this, we use a geometric distribution-based model with bias correcting to identify DNA interacting `hotspots', thereby ensuring that all subsequent analyses are performed with extremely significant datasets. For the lymphoblastoid cell line, we identified 81125 DNA interacting hotspots using this approach. A non-mutually exclusive categorization of these hotspots revealed that 97.2%, 48.2%, and 43.8% of the hotspots are overlapped with transposable elements, tandem repeats, and Refseq genes, respectively. Identification of interaction partners for the hotspots demonstrated that 78447 of the 81125 regions are contacting other hotspots, resulting in a total of 753541 chromosome connections. Of these hotspot-hotspot interactions we identified those where one interaction partner made contact with a gene promoter region, as these are potential enhancer elements. When overlapping this list of candidate enhancers with ENCODE histone modifications, we found that hotspots paired with promoter regions are 3.37 to 4.73 fold more likely to be near any of the three known activating histone modifications (H3K27ac, H3K4me1, and H3K4me3) (chi-square test p-value << 0.01). Meanwhile, we found no discernable enrichment for H3K27me3, a known repressing modification. These findings provide strong evidence that many of our promoter-associated hotspots are bona fide enhancers. In total, we have found that our novel analysis of the Hi-C data comprehensively identifies enhancers in the human genome. Future work includes experimental validation and identifying disease-linked polymorphisms that lie within our newly identified enhancers.

876F

Variation in transcription factor activity inferred by DNase-seq assays provide a trans-acting mechanism for gene expression variation in humans. *R. Pique-Regi[1], J.F. Degner[1], A.A. Pai[1], J.B. Veyrieras[3], D.J. Gaffney[1,4], J.K. Pickrell[1], G.E. Crawford[2], M. Stephens[1], Y. Gilad[1], J.K. Pritchard[1,4].* 1) Department of Human Genetics, University of Chicago; 2) Departments of Pediatrics, Division of Medical Genetics, Duke University; 3) BioMiningLabs, Lyon, France; 4) Howard Hughes Medical Institute, University of Chicago.

Variation in gene regulation plays an important role in evolution and disease. Changes in gene expression can occur at different stages but an important transcriptional mechanism is through DNA-binding proteins (i.e., transcription factors, TF) that recognize specific sequences. DNase-seq is a powerful tool for identifying regions of open chromatin which are often bound by transcription factors. Furthermore, bound transcription factors protect the DNA sequence within a binding site from DNaseI cleavage and produce recognizable ``footprints'' that can also contain information about the binding strength or affinity of the TF. In order to better understand the different genetic and non-genetic mechanisms through which transcription factors affect gene expression in a human population, we used DNaseI sequencing to measure genome-wide chromatin accessibility in 70 Yoruba lymphoblastoid cell lines (LCLs), for which genome-wide genotypes and expression levels from RNA-sequencing are also available. We measured chromatin accessibility in 100bp non-overlapped windows across the genome and extracted a set of 1.5 million locations that varied in chromatin accessibility. We find that inter-individual variation in chromatin accessibility for distinct locations in the region surrounding each gene's transcription start site (TSS) are highly correlated with inter-individual variation in gene expression. These accessible chromatin locations that regulate gene expression contain many distinctive TF ``footprints''. The largest fraction are associated in `cis' to genetic variants that affect both the local chromatin accessibility (caQTL) and gene expression (eQTL). However, about ~2000 chromatin accessibility windows are not significantly associated with any local genetic variation and are regulated in a `trans' mode through genetic variation (trans-eQTL) or environmental changes that may be affecting TF's availability, protein structure or DNA-binding domain. The most intriguing potential trans-signal that we identified is from a SNP (rs8106495) that is a weak eQTL for two neighboring genes (NFKBID and HCST). Our data suggest that this SNP may play a role in regulating chromatin accessibility of a large number of hypersensitive windows that have binding sites for NFKB, a factor which is known to be regulated by NFKBID.

877F

Deciphering the vertebrate regulatory code using short synthetic DNA sequences that drive enhancer activity. *R.P. Smith[1,2], A.K. Holloway[3], S.J. Riesenfeld[3], K.S. Pollard[2,3,4], N. Ahituv[1,2].* 1) Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco; 2) Institute for Human Genetics, University of California, San Francisco; 3) Gladstone Institutes, University of California, San Francisco; 4) Division of Biostatistics, University of California, San Francisco.

A wide variety of clinical and molecular data indicate that noncoding regulatory sequences contribute significantly to human diversity and disease. However, our limited understanding of the vertebrate regulatory code makes it challenging to annotate functional regions and interpret the effects of nucleotide variation within them on gene expression and downstream phenotypes. Most studies seeking to decipher this code have taken a top-down approach, mutating known regulatory elements, experimentally characterizing individual transcription factor binding sites (TFBS) or searching for enriched sequence motifs near co-expressed genes. Here, we have pioneered a novel bottom-up approach, using pseudo de Bruijn sets to design 184 short (15bp) oligonucleotides encompassing all possible 6bp sequences (the shortest average TFBS length, 6-9bp). The regulatory potential of these sequences was tested in zebrafish at 24 and 48 hours post fertilization using a transgenic enhancer assay. Thirty-three sequences produced consistent expression in >50% of embryos, with many expressing in a tissue specific manner. Six of these 15bp sequences, each driving specific expression in a different tissue (somitic muscles, epidermis, forebrain, notochord, heart and spinal cord), were selected for further experiments. Deletion series revealed that in multiple cases, shorter 6-9bp regions within these sequences were responsible for the expression pattern observed. Concatenation of 5 copies of the same 15bp sequence resulted in stronger enhancer activity in some of the constructs, consistent with the observation that homotypic clusters can enhance gene expression. However, in other cases, the expression pattern was abolished, suggesting that the syntax (e.g. spacing, order) of transcription factor binding sites is an important factor in their function. Conservative genome-wide alignments demonstrate that the 15bp sequences appear in the zebrafish genome an average of 128 times, often near genes that function in the same tissues as observed in the enhancer screen. Through this work, we are building and functionally translating a regulatory language from scratch, providing a better annotation of functional elements in the genome and improving our understanding of the regulatory code. These results will also enable researchers from numerous biological fields to engineer regulatory elements that can act as drivers to specific tissues and time points.

878F

Co-localized transcription factor binding sites explain gene expression and disease-associated variants. *D.Y. Wang[1], A. Rendon[2], L. Wernisch[1].* 1) MRC Biostatistics Unit, University of Cambridge, Cambridge, United Kingdom; 2) Dept. of Haematology, University of Cambridge, Cambridge, United Kingdom.

Increasingly more regulatory regions are being cataloged at a genome wide scale by large consortium, such as ENCODE. ChIP-Seq experiments for detecting transcription factor binding sites allow for elucidation of potentially functional regulatory regions. Transcription factor occupancy at a proximal gene locus has been shown to correlate with the gene expression level. Yet, models describing the relationship between the dynamics of multiple transcription factors and gene expression levels have never been introduced for human cell types. Here, we show that information from multiple co-localized transcription factors can be used to explain human cell type-specific gene expression. By mapping combinatorial patterns of binding sites from 11 different transcription factors, we can elucidate K562 and GM12878 specific gene expression. In primary hematopoietic cells that are closely related to the K562 cell line, the amount of gene expression variation explained by the 432 distinct combinatorial patterns can be as high as 48%. In GM12878, 22% of co-localization regions have a different pattern compared to those in K562. The degree of dissimilarity between combinatorial binding patterns in K562 and GM1287 is surprisingly correlated with the amount of target gene expression explained. Generally, the level of gene expression explained is lower for cell types further away in lineage to the cell type from which the transcription factor binding sites are observed. In this study we also find that co-localized transcription factors within 1kb of a target gene's transcription start site provides more information on the gene's expression than distal transcription factors. Nevertheless, we further show that eQTLs can be used to map more distal regulatory regions to target genes. These mapped regulatory regions, in turn, provide an explanation for the variation observed in eQTL results from different cell types. We anticipate that our findings will enable quantitative analysis of cell type-specific regulatory regions, and provide a general framework for the development of combinatorial models for explaining gene expression.

879F

Stress monitoring in the omics era and the future of saliva. *P. De Boever[1,2], N. Saenen[2], D. Santucci[3], G. Vermeir[4], V. Brümmer[5], M. Viaene[6], S. Schneider[5], G. Schoeters[7].* 1) Environmental Risk and Health, VITO, Mol, Belgium; 2) Hasselt University, Centre for Environmental Sciences, Hasselt, Belgium; 3) Istitute Superiore di Sanità, Department of Cell Biology and Neurosciences, Rome, Italy; 4) Openbaar Psychiatrisch Zorgcentrum (OPZ), Geel, Belgium; 5) German Sport University Cologne, Institute of Movement and Neurosciences, Cologne, Germany; 6) Catholic University of Leuven, Department of Occupational and Environmental Medicine, Leuven, Belgium; 7) University of Antwerp, Department of Biomedical Sciences, Antwerp, Belgium.

Environmental and social cues can activate the stress response system and this will influence the balance between the hypothalamus-pituitary-adrenal axis, the sympathetic nervous system and the immune function. These biological systems will stimulate a series of adaptation processes called allostasis. An inefficiently managed adaptation is then referred to as allostatic overload and is associated with an increased risk for getting a disease. We propose the use of space analog environments (such as an Antarctic station, parabolic flight and short arm human centrifuge) as unique settings to study the impact of acute and chronic stress on human physiology, and more specifically mood and mental fitness. These environments, much like actual manned space flight, represent a unique challenge for biological systems and early identification of adverse health effects is of paramount and possibly crucial for mission success. We use a suite of measurements including gene expression analysis, multiplexed immunoassays for protein analysis, and cognitive evaluations to address this issue and to identify biologically relevant warning signals. Furthermore, we are considering countermeasures such as physical exercise and dietary intervention to deal with adverse effects. Many of the biological data are collected from blood samples, but we also explore saliva as a source of a biomolecules. In the context of our studies we have shown that it is possible to collect saliva samples under challenging conditions and to obtain high quality transcriptomics data. The applicability of saliva analysis is further investigated because such a non-invasive and easy sampling procedure would have many advantages over the invasive blood analysis. Our presentation will give an overview of the current status of stress monitoring with several examples relevant for space research. Particular attention will be paid to the use of saliva and the ongoing initiatives to use this matrix to discover stress-relevant signals using transcriptomics and proteomics approaches.

880F

Evoked endotoxemia induces tissue-specific RNA-DNA differences. *J.F. Ferguson[1], Y. Liu[2], B.J. Keating[1], Y. Guo[3], M.P. Reilly[1], M. Li[2].* 1) Dept. Medicine, University of Pennsylvania, Philadelphia, PA; 2) Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania, Philadelphia PA; 3) BGI-Shenzhen, Shenzhen, China.

Inflammation is a key component in the pathophysiology of cardiometabolic disease. We utilize a model of evoked endotoxemia (LPS) to explore inflammatory response. RNA-DNA differences (RDD), or single base sequence differences between RNA and DNA from the same individual, have recently been described. We hypothesized that the occurrence of RDD may be tissue-specific, and may be modulated by acute inflammation. We applied RNA sequencing (RNASeq) to four RNA samples from two tissues (adipose and blood) from a single individual (Caucasian female), before and after evoked endotoxemia (LPS 1ng/kg), a model of inflammatory disease. On average, 10 million 75 bp paired-end reads were generated for each sample. To identify RDDs, we aligned the RNASeq reads to the Gencode mRNA reference sequence (hg18) using Bowtie. We extracted uniquely-mapped reads, requiring ⁄ 20 reads, with a per-base sequence quality score for each of the covered base ⁄ 25, and restricted analysis to known monomorphic sites only. In an initial screen of RDD, we detected 4501 events in adipose pre-LPS (A), 3157 in adipose post-LPS (B), 1733 in blood Pre-LPS (C) and 1168 in blood post-LPS (D). We detected all 12 possible types of base difference between DNA and RNA. After filtering for RDDs present in at least 50% of reads, there were 236 events in A, 254 in B, 117 in C, and 109 in D. We filtered out any RDDs that were found on the boundary between reads, and screened for tissue- and LPS-specific events. We found 8 genes with tissue-specific RDDs. Looking at differential RDDs before and after LPS, we found 16 genes in adipose, and 8 genes in blood. A number of these genes have previously been implicated in disease. We detected RDDs in *MT1E* and *SPTBN1* in adipose at baseline but not after LPS. *MT1E* has previously been implicated in Type 2 Diabetes, while *SPTBN1* has been implicated in cancer. In blood, we detected an RDD in *TYROBP* before LPS but not after; this gene has been shown to be important in immune response, and in macrophage recruitment, thus representing an important biological candidate gene for inflammatory response. Our results are consistent with previous findings of RDDs and may represent a novel mechanism for regulation of genes involved in inflammatory cardiometabolic disease.

881F

Expression changes of xenobiotic efflux genes are associated with ileal inflammation in the pelvic pouch of individuals with ulcerative colitis. *B. Kabakchiev[1,2], A.D. Tyler[1,2], M.S. Silverberg[1,2].* 1) SLRI, Mount Sinai Hospital, Toronto, Ontario, Canada; 2) University of Toronto, Toronto, Ontario, Canada.

Background: As many as 50% of ulcerative colitis (UC) patients who have undergone ileal pouch anal anastomosis (IPAA) develop de-novo inflammation in the ileal pouch within 10 years following surgery. With the use of microarray technology, we sought to investigate what gene expression changes occur in the pelvic pouch after surgery for UC and how these changes vary by outcome. **Methods:** Biopsy specimens from the pouch and afferent limb of the ileum were collected at the time of recruitment and preserved in RNAlater from patients who had undergone IPAA and closure of ileostomy at least one year prior. Based on clinical and endoscopic data at time of enrolment, 40 individuals from this cohort were allocated into 4 outcome groups - no pouchitis (NP), pouchitis (P), Crohn's disease - like phenotype (CDL) and familial adenomatous polyposis controls (FAP). The miRNeasy Kit was used to extract total RNA from all samples. The eluted transcriptomes were analyzed on Affymetrix Human Gene 1.0 ST arrays and the data were background corrected and normalized in Affymetrix Gene Expression Console. An ANOVA model in the statistical package R was used to assess the significance of each gene. Raw p-values were corrected for multiple comparisons by the false discovery rate method. **Results:** The expression levels of 144 gene transcripts were significantly associated with outcome at a corrected p-value cut-off of <0.05. There was also evidence that 117 of these were differentially regulated in the pouch compared to the afferent limb. Approximately 20% of the genes associated with outcome have known xenobiotic activity related to metabolizing and removing various drugs, toxins and other compounds from the cytoplasm of cells. All of these genes exhibited virtually the same expression pattern of up to 4 fold reduction in relative pouch mRNA abundance in the P and CDL groups compared to the NP and FAP groups. A prominent example is the gene ABCB1 also known as multi-drug resistance 1 (MDR1). Previous reports have shown that single nucleotide polymorphisms which result in attenuated ABCB1 expression are associated with UC. **Conclusions:** Transcriptome analysis of UC patients following IPAA showed that P and CDL are associated with significantly repressed drug and toxin efflux activity in the affected tissue. These findings corroborate the hypothesis that impaired barrier function could lead to development of intestinal inflammation.

882F

Expression Profiling of Genes Involved in Transendothelial Leukocyte Migration in HIV-1 Resistant Women in the Pumwani Sex Worker Cohort. *P. Lacap[1], M. Luo[1,2], J. Sainsbury[1], M. Songok[3], F. Plummer[1,2].* 1) Public Health Agency of Canada, Winnipeg, Manitoba, Canada; 2) Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada; 3) Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya.

Objective: A group of sex workers in the Pumwani Sex Worker Cohort, located in Nairobi, Kenya, remains HIV-1 uninfected, as determined by PCR and serology, despite heavy exposure to the virus through sex work. Successful HIV-1 infection is contingent on the virus' ability to infect susceptible leukocytes that migrate to the site of acute infection. Delayed migration of susceptible cells may slow the onset of systemic infection, potentially allowing innate and adaptive immune responses to clear the infection. The aim of this study is to identify genes involved in transendothelial leukocyte migration that are differentially expressed between HIV-1 resistant and susceptible women.

Design: The expression level of 90 genes involved in transendothelial migration were examined in 38 resistant and 37 susceptible women using a custom RT^2 Profiler™ PCR Array (SABiosciences). RNA was isolated from whole blood using the PAXgene Blood RNA Kit (Qiagen). The cDNA was synthesized and amplified using the TransPlex® Complete Whole Transcriptome Amplification Kit (Sigma-Aldrich). The level of gene expression was compared between HIV-1 resistant and susceptible women.

Results: We identified 23 genes, which were differentially expressed in HIV-1 resistant women compared to susceptible women. The following genes were significantly up-regulated in HIV-1 resistant women: RBL2 (p= 0.000498), ITGB1 (p=0.000668), CDK6 (p=0.000677), PIK3CA (p= 0.00249), RASA1 (p=0.00347), DPP4 (p=0.00357), MAPK7 (p=0.00869), MAP4K1 (p=0.0104), MAP3K4 (p=0.0106), MAP2K1 (p=0.0110), CTNNB1 (p=0.0134), SHC1 (p=0.0153), PTPN11 (p=0.0195), RPS6KB1 (p=0.0206), CLTC (p=0.0240), TICAM1 (p=0.0256), TIMP1 (p=0.0307), and VCAN (p= 0.0380). In contrast, the following genes were significantly down-regulated in the HIV-1 resistant group: LEP (p=0.0112), TSC2 (p=0.0135), NFKBIA (p=0.0209), MAPK3 (p=0.0215), and MMP13 (p=0.0478).

Conclusion: The results showed that the expression level of many key genes involved in leukocyte migration in HIV-1 resistant women are different from women who are susceptible to HIV-1 infection. They may represent novel targets for HIV-1 prevention.

883F

Revised Guidelines for RNA Quality Assessment for Diverse Biological Sample Input. *B. Lam, M. Simkin, N. Rghei, Y. Haj-Ahmad.* Norgen Biotek Corp, Thorold, Ontario, Canada.

Determining RNA quality is important prior to performing any downstream expression analysis such as microarrays or RT-qPCR. In many cases, good RNA quality could guarantee the success of the experiment. An RNA sample of poor quality could either lead to a labor-intensive cleanup process or compromise the results of the study. Especially while working with hard-to-obtain samples, starting with high quality, intact RNA could eliminate the burden of subsequent inhibited downstream applications. Traditionally, RNA quality is based on rRNA integrity (28S/18S). With the advancement in technology, additional parameters have been used, including spectrophotometry (260/280 and 260/230 ratios) and RNA Integrity Number (RIN) determined by bioanalyzer. Many of the above parameters and their acceptable values are based on traditional systems such as pure cell lines or tissues from healthy, lab-raised subjects. As scientists begin to study RNA expression in more diverse samples, such traditional standards become hard to achieve due to the nature of the sample input. This is often the case in clinical samples, such as bodily fluid as well as environmental samples where RNA concentration is low. Similarly, it is difficult to isolate a high quality RNA from formalin-fixed and paraffin-embedded (FFPE) tissues, as the RNA is often fragmented and chemically altered. Given the increase in input diversity for RNA extraction, there is a need to re-evaluate the acceptable values for RNA quality for each unique sample type. This study aims to generate a guideline for RNA quality for various inputs, particularly difficult-to-isolate samples including clinical (plasma/serum, blood and FFPE) as well as environmental samples (water, soil or plants). Two common methods (phenol-based and silicon carbide column-based) were employed for extracting total RNA including small RNAs. The various RNA quality assessment parameters were recorded and correlated with downstream application performance including RT-qPCR and microarray. Interestingly, for samples such as plasma/serum and FFPE with no intact large rRNA, RNA samples with RIN values and OD ratios that were significantly lower than commonly accepted still performed well in microRNA RT-PCR or microarray applications. Moreover, by comparing to a serial dilution of a good clean RNA, it was shown that most “out-of-range” RNA quality of clinical or environmental samples was due in part to the sensitivity of the instrument used instead.

884F

Heteroallelic expression and RNA editing using high-throughput RNA sequencing. *J. Li-Pook-Than, R. Chen, G. Mias, L. Jiang, H. Lam, H. Tang, M. Snyder.* Department of Genetics, School of Medicine, Stanford University, Palo Alto, CA.

We are establishing a novel fully comprehensive human genotype-to-phenotype approach of whole -omics profiling analysis (genome, transcriptome, proteome). For the first time, the plethora of information gained from high-throughput genomic sequencing was integrated with transcriptome sequencing (RNAseq -Illumina) of peripheral blood mononuclear cells (PBMCs) in an individual. This was also combined with respective mass spectral proteomic data. We obtained an average of 146 million mapped transcriptomic reads for over 15 time points (including 2 infection states) collected over a year. This abstract focuses on heteroallelic gene expression, alternative spliced forms, as well as the phenomena of RNA editing. To identify RNA variants, we developed the RIT(E)2-seq pipeline (RNA Identifier Tool for Expression and Editing) which compares RNA information with the respective individual's genome (~3.7 million called SNPs). It applies filtering methods to minimize high instances of false positives due to intrinsic platform errors and misalignment to duplicated regions and pseudogenes. This pipeline incorporates several algorithms including TopHat, BWA, SAMtools and Polyphen-2 (Langmead et al, 2009; Li and Durbin, 2009; Li et al, 2009; Adzhubei et al, 2010). Our preliminary data show that of the 22,446 exonic-located genomic SNPs, ~21,700 are expressed across multiple time-points (min. ~5 FPKM): 7,755 are homozygous (~830 missense changes), 13,934 are heterozygous (~1,670 missense changes), ~800 are not present in dbSNP and are candidates for private SNPs. RNA editing is a post-transcriptional event, usually resulting in a deamination process: cytidine to uridine (C-to-U) or adenosine to inosine (A-to-I) conversion. We identify over 2,100 RNA editing candidates (min. 3 time points), including unusual edits that suggest processes unique from deamination. Validation of our RNA data include Sanger sequencing and quantification methods such as digital droplet PCR. So far, 90 missense variants are confirmed with our respective proteomics study. Notable is the importance of such high-depth coverage (total 2.2 billion mapped RNA reads) for the characterization of rare events as editing and low-level heteroallelic expression. This whole -omics approach provides valuable information for correlating variants with phenotype, captures the health state and risk of an individual, as well as gives insights into human diversity studies.

885F

Transcriptome changes following site-specific DNA breaks. *S. Linker, D. Hedges.* HIHG, University of Miami, Miami, FL.

The coordinated response of the genome to double strand break (DSB) DNA lesions is vital for maintaining healthy cellular function. Insufficient repair response can contribute to a number of pathological phenotypes, including cancer. Global expression patterns have been previously analyzed under high-dose DNA damaging conditions, such as ionizing radiation or etoposide exposure. Even though these systems have been useful in studying the response to DSBs, they exaggerate conditions under which natural DSBs occur. In this study, we sought to examine transcription changes in a more natural context by restricting induced double strand breaks to a single genomic location. Using a synthetic zinc finger nuclease (ZFN), we induced a DSB at a unique location in the human genome and examined resulting expression changes. Whole transcriptome data was collected 24 hours after exposure of HeLa cells to the ZFN, which was targeted to the Adeno-associated virus site 1 on chromosome 19. cDNA libraries formed from total RNA were multiplexed, with two transcriptomes (treated and untreated) run per HiSeq2000 lane. Analysis is currently underway, and preliminary Web-Gestalt KEGG pathway analysis of differentially expressed genes suggests significant expression enrichment of genes involved in Parkinson's disease, oxidative phosphorylation, Huntington's disease, and Alzheimer's disease. A majority of the altered genes fall within distinct subclasses of transcription factor targets.

886F

The transcriptome of the Rotterdam Study. *M.J. Peters[1,2], M. Jhamai[1,2], H.J. Westra[3], M. Ganesh[1,2], B. van Ast - Copier[4], F. Rivadeneira[1,2,4], J.M. Vergeer - Drop[4], A. Hofman[4], L. Franke[3], A.G. Uitterlinden[1,2,4], J.B.J. van Meurs[1,2].* 1) Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands; 2) The Netherlands Genomics Initiative - sponsored Netherlands Consortium for Healthy Aging (NGI-NCHA), Rotterdam / Leiden, the Netherlands; 3) University Medical Center Groningen, Department of Genetics, Groningen, the Netherlands; 4) Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands.

Purpose: Gene expression levels might mediate the association between genetic variants and diseases. Yet, expression levels can also be associated with diseases, independently of genetic variation. Within the Rotterdam Study (RS), we examined the overall variability of genome-wide gene expression levels of whole blood samples of 935 unrelated individuals, and assessed its association with 598,821 genotyped SNPs. In addition, we examined which genes are differentially expressed during aging, a strong risk factor for many complex diseases (i.e. osteoporosis, osteoarthritis and cardiovascular disease). Materials & Methods: For 935 randomly selected RS-3 participants, whole blood cells were collected (PAXgene Tubes-Becton Dickinson) and RNA was isolated (PAXgene Blood RNA kit-Qiagen): 891 samples (95.3%) with good quality RNA (RNA Quality Score or RQS ⁄ 7.0), 109 technical duplicates, and 4 control RNAs were amplified, labelled (Ambion TotalPrep RNA), and hybridized to the Illumina Whole-Genome Expression Beadchips (HT-12v4). eQTLs were mapped with AssociationGG (www.genenetwork.nl/associationgg). For differential expression analysis, the genome-wide significance threshold was set at p=1.97x10-6, thereby correcting for the number of tests performed. Association between normalized expression levels and age was analyzed by a linear regression model, adjusted for gender and technical batch effects (plate layout, time between blood donation and RNA isolation, RQS). Summary of the Results: The study sample consisted of 408 men and 471 women, mean age of 59.8 (±8.1). Out of 44,877 probes on the chip, 25,439 probes corresponding to 18,212 genes were significantly expressed in >5% of the samples. Expression levels of the probes were highly reproducible among the technical replicates (average Pearson correlation = 0.98). In total, 6138 expression Quantitative Trait Loci (cis-eQTLs) were detected (FDR<0.05; 25% of the probes have a cis-eQTL-effect). For differential expression analysis, 516 probes corresponding to 471 genes were significantly associated with aging. 38 (8.1%) genes were also found significantly associated by Zeller et al. (2010), which is 3 times more than expected by chance, indicating that our 471 associated hits are enriched for genes previously associated with aging. Most significant genes are VAMP5, IL7R, BCL11A, IRF8, and GRAP, involved in cell cycle progression, recombination, and signalling. Replication of these findings is currently underway.

887F

SNP calling and allele frequency estimation of targeted sequencing of pooled samples. *X. Chen[1], T. Paranjape[2], J. Weidhaas[2], F. Slack[3], H. Zhao[1,4,5].* 1) Program in Computational Biology and Bioinformatics, Yale School of Medicine, New Haven, CT; 2) Department of Therapeutic Radiology, Yale School of Medicine, New Haven, CT; 3) Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT; 4) Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT; 5) Department of Genetics, Yale University School of Medicine, New Haven, CT.

Next generation sequencing has been widely used to study complex hereditary diseases as a powerful alternative to SNP genotyping through its ability to identify both common and rare variants without prior information on SNPs. Pooled sequencing of interested target regions can lower the cost and increase the sample size, thus the power, for disease-associated variant detection. However, compared to individual genomic sequencing which is relatively easy to analyze, pooled target region sequencing is complicated by many additional factors due to target amplification and capturing, formation of pools, and others which all contribute to the variance of the observed data and result in difficulties in SNP calling, allele frequency estimation and association test. Here, we use real pooling data from targeted sequencing of pooled samples with RainDance capture as well as corresponding sequencing of individual samples with NimbleGen capture to motivate a statistical model that encompasses different factors affecting the observed sequencing data. This rigorous model enables us to estimate the variance of the pooling data as a function of sequencing coverage, number of samples in a pool, and minor allele frequency in the sampled population. Equipped with this model and a better understanding of pooling variance, we evaluate the accuracy of allele frequency estimation and the power of association testing. We further propose a model to gain more precise estimation of allele frequencies of SNP positions and more accurate variant detection by incorporating target-capturing and sequencing errors and biases. Our studies show the performance of the target sequencing of pooled samples in disease association tests and provide a method to control the errors and biases in the processes of capturing and sequencing.

888F

Screening individuals with rare variants in large populations: deep re-sequencing the CRP locus. *C. Chen, A. McDavid, O. Kahsai, A. Zebari, C. Carlson.* Fred Hutchingson Cancer Research Center, Seattle, WA.

Common single nucleotide polymorphisms identified in GWAS to date generally account for small effects, leading to the hypothesis that rare variants might also play a functional role in determining phenotypes. Even though technological advances have allowed scientists to sequence many genomes in parallel, identifying individuals carrying rare variants is not a trivial task. One of the challenges is discriminating between sequencing errors and true rare variants. To address this, we have developed an algorithm, which, in combination with a unique pooling strategy, is able to efficiently screen a candidate gene for idiosyncratic variants in large samples.We demonstrate this novel approach by re-sequencing the c-reactive protein locus (CRP) in a sample of 2300 individuals, using just two lanes on an Illumina sequencer. Plasma CRP level is a biomarker predicting future risk of cardiovascular disease. Common variants explain little of the variance in CRP levels among individuals. To investigate whether rare variants at this locus also influence CRP levels, we sequenced a 6kb region spanning CRP extensively in individuals from the CARDIA cohort in 24 separate pools of 96 samples each. Keeping bases with high quality scores in high quality reads, we obtained a coverage depth of 36,000 per position on average across the 24 pools. We calculated a statistic for each position based on the distribution of minor allele frequencies across pools and designated the position to be polymorphic when its value is bigger than the statistic generated from a noise model parameterized from our data. This metric is robust with respect to the number of total number of sequencing pools, the minor allele frequency of the variant, and the number of pools with the variants. In total, we identified 140 polymorphisms, with 46 of these already known in dbGaP. To confirm these variants, we genotyped 63 loci (minor allele frequencies ranging from 0.05% to 25%) in the same population. Treating the genotyping results as a reference, we obtained a 98% sensitivity rate. With this approach, we were able to rapidly and cost-effectively screen for both common and rare variants in a large population. The high sensitivity of this approach enables us to identify variants with a minor allele frequency as low as one copy in 2000 individuals. We believe this efficient algorithm will be useful in replicating rare variants identified in large-scale studies.

889F

Next generation sequencing of centenarian and control genomes. *E.T. Cirulli, M. Zhu, K.V. Shianna, D. Ge, D.B. Goldstein.* CHGV, Duke Univ, Durham, NC.

The factors contributing to exceptionally long life have long been a topic of intense interest. While few clear genetic contributions to exceptionally long life have been identified, whole genome sequencing provides a new opportunity for investigating longevity genetics. Here we report the analysis of 10 centenarian (collected under the Murdock study community registry and biorepository Pro00011196) genomes compared with 50 population controls, all sequenced to an average of 30-40x coverage using next generation sequencing. We focused our analysis on the possibility that centenarians live longer than average because they have fewer rare, damaging mutations than average. We identified different categories of damaging mutations--stop gain, stop loss, nonsynonymous (split by PolyPhen prediction category), splice site, and large deletion--and compared their distributions in cases and controls. We find that for some of these comparisons, there is a lower burden of rare damaging mutations in the centenarians, suggesting that an overall lower burden of damaging genetic variants is a contributor to long life in humans.

890F

MedSavant: a platform for identifying causal variants from disease sequencing studies. *M. Fiume[1], N. Nursimulu1[1], J. Foong[3], M. Manker[3], M. Brudno[1,2,3].* 1) Department of Computer Science, University of Toronto, Toronto, ON., Canada; 2) Donnelly Centre and Banting and Best Department of Medical Research, University of Toronto, ON., Canada; 3) The Center for Applied Genomics, Toronto, ON., Canada.

High Throughput Sequencing (HTS) technologies are providing economical means for deep cataloguing of human variation. Large volumes of genetic variants are being obtained from disease sequencing studies that aim to discover etiology. However, many of the candidate variants found are either not real or have no functional relevance. One of the most challenging problems is in identifying those few genetic variants (among millions predicted per individual) that are actually causal in disease. For this purpose we introduce MedSavant: a software platform for accelerating the identification of disease-causing genetic variants found in population sequencing studies by enabling complex and dynamic querying of patient data. The platform is comprised of a graphical interface and a backend database. The database is designed to store basic patient data, phenotype data, and genotype data for all sequenced individuals. The user interface enables users to dynamically visualize global trends in the data, construct complex queries, and analyze the results. The framework allows one to design queries that return, for example, only variants found in male patients in a specific disease cohort who share a rare point mutation with high predicted confidence. MedSavant also supports filters generated from external datasources, such as whether or not the variation has been discovered before (using dbSNP), is predicted to be damaging (using SIFT), is found in genes having a pertinent function (using GO), or has been associated with a related disease (using OMIM). Furthermore, MedSavant can be integrated with the Savant Genome Browser, for manual inspection of read alignment data supporting the most likely causal variants found in the filtration process. MedSavant will be made available at genomesavant.com/med.

891F

The genetics of endurance: increased frequency of the ACTN3 577X variant in elite endurance Ironman athletes. *R. Grealy[1], C.L.E. Smith[1], T. Chen[1], L. Haseler[2], D. Hiller[3], L.R. Griffiths[1].* 1) Genomics Research Centre, Griffith Health Institute, Griffith University, Gold Coast, QLD, Australia; 2) Heart Foundation Research Centre, School of Physiotherapy and Exercise Science, Griffith University, Gold Coast, QLD, Australia; 3) North Hawaii Community Hospital, Kamuela, Hawaii, USA.

Although human athletic endurance is a complex phenotype influenced by both environmental and genetic factors, genetic factors significantly contribute to endurance performances (with high heritability estimates for a number of endurance traits such as VO2 max and lactic acid anaerobic threshold). In order to examine the genetic components of endurance phenotype, we collected saliva samples, questionnaires, and performance times for 196 elite endurance athletes who participated in the 2008 Kona Ironman championship triathlon. This race is considered one of the most gruelling Ironman competitions in the world, representing athletes with an extremely high level of fitness and endurance. DNA was extracted from saliva and genotyped for the ACTN3 R577X polymorphism using high-resolution melt (HRM) analysis. The ACTN3 gene, coding for the (-actinin-3 protein expressed in fast-twitching muscle fibres, has been investigated as one of the genetic factors that underlie individual endurance ability and several studies have found an association of 577X with endurance, though this is not supported in all populations. One-way ANOVA showed that performance time did not significantly differ between genotype groups, and stepwise linear regression modeling the effect of all variables on finishing time showed that age, sex, and continent of origin were significant predictors of finishing time (age and sex: $p<5E-7$; continent: $p=0.01$). Genotype and allele frequencies obtained (RR 26.5%, RX 50.0%, XX 23.5%, R 51.5%, X 48.5%) were compared with frequencies reported in previous studies, and were found to be not significantly different from Australian, Spanish, and Italian endurance athletes, but were significantly different from Kenyan, Ethiopian, and Finnish endurance athletes ($p<0.01$). As R577X frequencies vary considerably in different ethnic groups (with null allele frequencies ~10% in African populations and ~50% in Eurasian populations) our observed frequencies reflect the predominantly North American, European, and Australian origin (93.9%) of our Ironman athlete sample. Further, obtained genotype and allele frequencies show significantly different frequencies from those reported for Australian and Spanish sprint/power athletes ($p<0.01$), as well as a trend for higher frequencies of XX genotype and X allele in endurance athletes compared with reported general Australian and Spanish population controls, and support a role for the 577X variant in endurance performance.

892F

Inference of modules regulated by eQTLs. *A. Kreimer[1], O. Litvin[3], K. Hao[4], C. Molony[4], D. Pe'er[3], I. Pe'er[2].* 1) Department of Biomedical Informatics, Columbia University, New York 10032, New York; 2) Department of Computer Science, Columbia University, New York, New York 10027, USA; 3) Department of Biological Sciences, Columbia University, New York, New York 10027, USA; 4) Merck Research Labs, 33 Ave. Louis Pasteur, Boston, MA 02115, USA.

Cataloging the association of transcripts to genetic variants in recent years holds the promise for functional dissection of regulatory structure of human transcription. Here, we present a novel approach, which entails detection and analysis of modules of transcripts, each co-associated to a single genetic variant. First, we search pair-wise connections between transcripts whose levels are co-associated to the same SNP. Second, we combine these pairs into modules that share an associated main SNP. We then assign a confidence score to each module. Finally, we find secondary SNPs whose association to transcript levels in a module is conditioned on the main SNP. We applied our method to existing data on genetics of gene expression in the liver. We detect 129,130 pairs of transcripts that are associated to the same SNP in either cascaded or mutually independent manner. These make up 10,354 modules, 518 of which include 10 transcripts or more. The modules are significantly more, larger and denser than found in permuted data. We quantify the confidence in a module as a likelihood score, and prune a subset of 95 distinct modules with FDR<0.02. We show that mild cis effects, underpowered to be detected directly but exposed by our method explain some of the reported modules. We observe similar annotations of modules from two sources of information: the enrichment of a module in gene subsets and locus annotation of the genetic variants. This and further phenotypic analysis provide a validation for our methodology.

893F

A likelihood-based framework for *de novo* mutation detection in families for next-generation sequencing data. *B. Li[1,2], G. Abecasis[1].* 1) Biostatistics, Univ Michigan, Ann Arbor, MI; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN.

Multiple lines of evidence indicate that *de novo* mutation events contribute to disease susceptibility for a variety of human diseases, including neurological disorders such as autism and mental retardation. Next generation sequencing can be used to systematically scan the genome for *de novo* mutation events, potentially improving our ability to understand the molecular basis of these traits. The standard approach to the study of *de novo* mutations is to sequence a small number of related individuals and then identify genotypes which violate Mendelian inheritance. However, due to sequencing errors and allelic drop-out, this naïve approach can lead to very high false positive rates for analysis focused on discovery of *de novo* mutation events. Here, we develop and implement a likelihood-based framework to detect *de novo* mutations by jointly modeling sequence data across a pedigree. Our model explicitly allows for *de novo* mutations and allows us to calculate the posterior probability of a *de novo* event conditional on all available sequence data - making it easier prioritize and contrast evidence for multiple candidate *de novo* events. Our integrated framework for *de novo* mutation detection and variant calling can handle both nuclear families and extended pedigrees. Through simulations we show that, compared to the naïve approach, our framework can achieve markedly improved sensitivity and specificity and that performance further improves as additional individuals are sequenced in each family. In addition, we make recommendations in terms of sequencing depth and choice of different technologies for studies aiming to use next generation sequencing to detect *de novo* events. To illustrate our approach, we apply our method to the two parents-offspring trios sequenced to high coverage by the 1000 Genomes Project and observe high concordance with their findings. For example, using only Illumina data, 39 of 48 of germline mutations in the CEU trio were identified by our method and majority of missing mutations were filtered out based on likelihood ratio due to lower depths. We hope that our work will accelerate continuing efforts to find genetic factors associated with complex diseases.

894F

Genotype Imputation of Metabochip SNPs Using a Study-Specific Reference Panel of 3,924 Haplotypes in African Americans from the Women's Health Initiative. *E.Y. Liu[1], S. Buyske[5,6], A.K. Aragaki[7], U. Peters[7], E. Boerwinkle[8], C. Carlson[7], C. Carty[7], D.C. Crawford[9], J. Haessler[7], L.A. Hindorff[10], L.L. Marchand[11], T.A. Manolio[10], T. Matise[6], W. Wang[1], C. Kooperberg[7], K.E. North[2], Y. Li[3,4].* 1) Department of Computer Science, University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC; 3) Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC; 4) Department of Biostatistics, University of North Carolina at Chapel Hill, Chapel Hill, NC; 5) Department of Statistics, Rutgers University, Piscataway, NJ; 6) Department of Genetics, Rutgers University, Piscataway, NJ; 7) Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 8) Human Genetics Center, University of Texas Health Science Center at Houston, Houston, TX; 9) Department of Molecular Physiology and Biophysics, Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 10) Office of Population Genomics, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 11) Epidemiology Program, University of Hawaii Cancer Center, Honolulu, HI.

Genetic studies of complex traits require large sample sizes. One cost-effective method to increase sample size across multiple platforms is genotype imputation, a standard practice in contemporary genetic studies. Several important imputation-related issues have not been adequately addressed in the literature, including imputation quality for less common (minor allele frequency [MAF] 0.5-5%) and rare (MAF < 0.5%) variants, imputation performance in admixed populations, such as African Americans, and construction of large study-specific reference panels for imputation of SNPs found on region-centric chips with high SNP density but uneven distribution across the genome (e.g., the Metabochip assessing ~200,000 SNPs and including a number of trans-ethnic fine mapping regions). These issues recently became addressable with GWAS follow-up studies that use dense genotyping or sequencing in large samples of non-European individuals. Here, we constructed a study-specific reference panel of 3,924 haplotypes from 1,962 African Americans in the Women's Health Initiative (WHI) genotyped on both the Metabochip (for the Population Architecture using Genomics and Epidemiology [PAGE] study) and the Affymetrix 6.0 GWAS platform (for the SNP Health Association Resource [SHARe] study). Imputation into 6,459 remaining WHI SHARe participants with only Affymetrix 6.0 GWAS genotypes confirmed imputation quality metric Rsq (estimated r^2) as an effective post-imputation filter. We recommend different Rsq thresholds for different MAF categories such that the average Rsq is at least 80% to achieve true r^2 (r^2 between imputed and unobserved true genotypes) of 80% or above. After applying the thresholds, 20.5% (52.0%, 83.6%, 97.5%, 99.9%) of SNPs with MAF 0.1-0.5% (0.5-1%, 1-3%, 3-5%, and >5%) passed the post-imputation filter. The average true r^2 for SNPs passing the filter, by masking and comparing with experimental genotypes, is 79.7%, 83.1%, 89.0%, 92.1%, and 94.7% respectively. These results suggest that, imputation of Metabochip SNPs in an ancestrally diverse population, including less common SNPs with MAF 0.5-5%, is feasible and worthwhile with the availability of a sizable reference panel.

895F

Genetic associations with glycated hemoglobin in glycemic and hematological pathways. *J. Ryu, Y. Kim, C. Lee.* Soongsil University, Seoul, Korea.

Recent genome-wide association studies have identified single nucleotide variants associated with glycosylated hemoglobin in Europeans and Americans. We examined genetic association of the previously identified sequence variants with glycosylated hemoglobin and with glycemic and hematological phenotypes in non-diabetic Koreans. Genetic associations of glycosylated hemoglobin were replicated with sequence variants ($P = 3.77\times10^{-11}$ ~ 3.28×10^{-2}) in GCK, MTNR1B, SLC30A8, ANK1, FN3K, SPTA1, and HFE genes. The sequence variants in GCK, MTNR1B, and SLC30A8 genes were also associated with glycemic traits such as fasting glucose, 1-h glucose after oral glucose tolerance test, and 2-h glucose after oral glucose tolerance test($P < 5.0\times10^{-2}$). On the other hand, the variant in HFE gene was associated only with hematological traits such as hematocrit, MCH, and MCV. This indicates that the sequence variants associated with level of glycosylated hemoglobin were characterized by mechanisms involved in glycemic and hematological phenotypes.

896F

Genetic association between age-related macular degeneration and CASP6-PLA2G12A-CFI locus. *E. Ryu[1], N. Tosakulwong[1], K. Bailey[1], A. Edwards[2].* 1) Health Sciences Research, Mayo Clinic, Rochester, MN; 2) Institute for Molecular Biology, University of Oregon, OR.

Backgrounds: Genetic variation near the 3' end of the gene encoding complement factor I (CFI) on chromosome 4q25 has been associated with the risk of advanced age-related macular degeneration (AMD). Due to lack of obvious functionality of CFI, we expanded this region for better localization of the genetic variation associated with AMD.

Methods: Twenty-two single nucleotide polymorphisms (SNPs) across CFI and three adjacent genes (CCDC109B, CASP6, and PLA2G12A) were genotyped on 2156 AMD cases and 1150 control subjects in a genome-wide association study. Using HapMap II reference panel, 161 imputed SNPs in this region were investigated for their disease association.

Results: Individual SNPs within four genes (CCDC109B, CASP6, PLA2G12A, and CFI) in the chromosome 4q25 locus were associated with AMD. The most significant single SNP association was rs2285714 in the PLA2G12A gene. Conditioning on rs2285714 did not leave any significant association for the remaining SNPs in CCDC109B, CASP6, PLA2G12A, and CFI. Haplotype analyses suggested the best localization between CASP6 and PLA2G12A genes. All AMD subtypes showed similar genetic association.

Conclusions: The genetic variation contributing to AMD in this region best localizes to the CASP6 and PLA2G12A genes and not the CFI gene. However, determination of their roles for AMD development requires further work.

897F

Genome-wide association study of adolescent idiopathic scoliosis in southern China. *Y.Q. Song[1], Y.H. Fan[1], K.M.C. Cheung[2], D. Chan[1], W.Y. Cheung[2], K.S.E. Cheah[1], P. Sham[3], K.D.K. Luk[2].* 1) Dept Biochemistry, Univ Hong Kong, Hong Kong, China; 2) Department of Orthopaedics and Traumatology, Univ Hong Kong, Hong Kong, China; 3) Department of Psychiatry, Univ Hong Kong, Hong Kong, China.

The cause of adolescent idiopathic scoliosis (AIS) is still not known. Although several candidate gene studies and linkage analysis have been done, no causal relationship has yet been established. We report a case-control based genome-wide association study (GWAS) for this trait. The study was undertaken in a set of 196 server cases with a specific AIS phenotype (based on Lenke's classification) in southern China, and in 401 controls without radiological evidence of scoliosis. A Replication set was performed. Two single-nucleotide polymorphisms (SNPs) on one particular chromosome showed marginal significant association (snp1: p=1.32x10E-6, odds ratio=0.52; snp2: p=1.23x10E-5, odds ratio=0.55). Imputation results suggested that three more SNPs in this region showed significant association (snp3: p=2.47x10E-7, odds ratio=0.49; snp4 and snp5: p=1.68x10E-6, odds ratio=0.53). The first replication set confirmed the association and the second replication are being carried out. Despite the small number of cases and controls, the strength of this study is in the use of a specific phenotype and that all controls were mature individuals with radiological confirmation of straight spines. We believe that these factors have contributed to the success of the GWAS. Our findings offer the potential to explore the pathogenesis of AIS with GWAS.

898F

Detection of variants in human cancer genomes using PacBio RS and HiSeq 2000. *K. Stangier, H. Hegele, T. Kurz, Y. Kumar.* GATC Biotech AG, Konstanz, Germany.

The new Pacific Biosciences PacBio RS uses novel technology that enables single molecule, real-time, or SMRT™, detection of biological processes. The ability to resolve single molecules in real time allows the system to observe structural and cell type variation not accessible with other technologies. The Illumina HiSeq 2000 provides the industry's highest sequencing output and quality at fastest data generation rate and can be optimally used to obtain best sequencing quality in re-sequencing projects. Through a combination of different sized insert libraries SNPs, InDels and structural variations can be identified in whole human genomes. In combination with the new Pacific Biosciences PacBio RS real time single molecule sequencer the identified structural variations can be further analyzed in depth. The long reads in the range of Sanger sequencing and so-called strobe reads are perfectly suitable to accurately resolve even large-scale rearrangements. This is especially important in cancer research where structural variations are supposed to play an causative role. We will present data on human cancer genomes showing the usability of the combination of these two technologies. All dataset are produced in our own laboratory. For all analyses, special bioinformatics tools are used. The pipelines are developed in our internal bioinformatics department. High quality results will be achieved when optimally combining several library preparations and Next Gen technologies with state-of-the-art bioinformatics.

899F

Cell Lines Commonly Used as Models for Inflammation Contain Genetic Variation in Toll-Like Receptor Genes. *S.A. Tokarz, J.A. DeValk, B. Pattnaik, D.M. Pillers.* Pediatrics, University of Wisconsin, Madison, WI.

Background: Human-derived cell lines are commonly used in studies to investigate inflammation pathways, including the up-regulation of interleukins and other inflammatory cytokines after exposure to pathogens. Toll-like receptors (TLRs) are a family of transmembrane receptor proteins present in many cell types and serve as the first point of defense in the innate immune system. TLRs are essential in initiating the inflammatory cascade in response to infectious microorganisms. Single nucleotide polymorphisms (SNPs) in genes for TLRs 1, 2 and 4 occur in up to 20% of the population. TLR4 D299G, TLR4 T399I, TLR2 P631H, TLR2 R753Q and TLR1 N248S SNPs have been shown to be associated with a decrease in the innate immune response. Objective: We sought to determine the presence of a subset of common TLR SNPs associated with inflammation in a cohort of cell lines that are used extensively for inflammation studies. Design/Methods: DNA was isolated from the following cell lines: Human Umbilical Vein Endothelial Cells (HUVEC), Human Embryonic Kidney (HEK293), A549 lung epithelial, Human Retinal Pigment Epithelium (HRPE), HeLa, Jurkat lymphocyte cells, MCF-7 human breast adenocarcinoma, A431 human epithelial carcinoma, Y79 human retinoblastoma. Cell line TLR genotype was established using ABI TaqMan SNP Genotyping Assays. The Step OnePlus™ software was used to analyze the intensity of each fluorescent probe assigned to either the wild-type (WT) or natural variant (NV) allele and to produce an amplification plot of the data along with the C_t value. Results: TLR4 D299G, TLR4 T399I and TLR2 P631H SNPs showed heterozygosity for the WT and NV alleles in HeLa cells while the other cell lines were homozygous for the WT alleles. HeLa and HEK293 lines were homozygous for the WT allele for the TLR1 N248S SNP and all other cells were homozygous for the NV allele. All cell lines were homozygous for the WT allele for TLR2 R753Q. Conclusions: HeLa cells are used as a cell model for many inflammation studies, which may not be appropriate considering the heterozygosity of the TLRs in this cell line. The presence of SNPs may impact the normal function of a pathway. With the increasing evidence of the involvement of SNPs in a variety of disease pathologies this study suggests that a new era in cell biology must emerge in which the genetics of the cellular model used in experiments must be scrutinized more thoroughly.

900F

An Exome Variant Server for the NHLBI Exome Sequencing Project (ESP). *Q. Yi, P.D. Robertson, M.J. Rieder, D.A. Nickerson on behalf of the Population Genetics Project Team and the NHLBI ESP Project.* Department of Genome Sciences, University of Washington, Seatle, WA.

The goal of the NHLBI Exome Sequencing Project (ESP) is to discover novel genes and mechanisms contributing to heart, lung and blood disorders through application of next-generation exome sequencing to samples drawn from large cohort and population-based studies. To date, the ESP has produced more than 5,000 exomes from samples with linked phenotype data. From this total set, exome variant data have been summarized from an initial data freeze of 2,500 samples. All exomes were sequenced to high average coverage (>100x depth) and variant calls derived from simultaneous, multi-sample genotyping across all samples To facilitate the distribution of these data to the scientific community, we have developed a web-based data server (NHLBI Exome Variant Server [EVS], snp.gs.washington.edu/popgenSNP). The EVS provides both a full dataset (ESP2500) and filtered (ESP2500P), with the latter being used for the ESP population genetics analysis. The full dataset consists of 2,439 samples and 1,153,118 variant cals. The filtered dataset consists of 2,275 exomes (1351 European and 924 African-American) and 481,712 variant positions from 26 Mb of the exome target. The EVS provides an easy-to-use interface for querying exome variants and sequencing coverage based on specific genes or genomic locations. Using our internally developed analysis pipeline (SeattleSeqAnnotation server - snp.gs.washington.edu/SeattleSeqAnnotation131/), we provide full annotation for every cataloged variant including: dbSNP rs numbers for known variants, minor allele frequency (for both European- and African-American populations), functional context (i.e. missense, synonymous, splice) mapped to multiple gene mRNA accession numbers, related cDNA and protein information, conservation scores based on nucleotide (GERP) and amino acid (Phastcons, Grantham substitution, protein function predictions (e.g. Polyphen) and known clinical implications. This database provides one of the largest, high-coverage exome datasets available, and is extremely useful for estimation of allele frequencies of rare variation (e.g. 0.01-3%) and filtering strategies applied to Mendelian traits. Orthogonal genotyping validation of a sampling of singleton and rare frequency variants is currently in progress. These data are also available publically via dbSNP (build 134) and a large fraction of this genotype data and linked phenotype information are available in dbGaP.

901F

Using induced pluripotent stem cells to uncover the role of DHDDS in retinitis pigmentosa. *D. Dykxhoorn[1, 2], B. DeRosa[1], S. Zuchner[1, 2], G. D'Urso[3], J. Dallman[5], B. Lam[4], J.M. Vance[1, 2], M.A. Pericak-Vance[1, 2].* 1) Hussman Institute for Human Genomics, University of Miami, Miami, FL; 2) John T. Macdonald Foundation Department of Humans Genetics University of Miami, Miami, FL; 3) Department of Molecular and Cellular Pharmacology, University of Miami, Miami, FL; 4) Department of Ophthalmology and the Bascom Palmer Eye Institute, University of Miami, Miami, FL; 5) Department of Biology, University of Miami, Miami, FL.

Retinitis Pigmentosa (RP) is a group of progressive retinal degenerative disorders. These disorders result from the abnormalities of the photoreceptor cells (rods and cones) or the underlying retinal pigment epithelium (RPE). This genetically heterogeneous disease is characterized by a progressive loss of the patient's peripheral vision leading to tunnel vision. Mutations in over 50 genes have been show to be involved in RP etiology. Through whole-exome sequencing, we recently discovered a rare variant in dihydrdolichyl diphosphate synthetase (DHDDS) as being the cause of RP in three affected siblings of a one generation, nonsyndromic family of Ashkenazi Jewish origin. The loss of DHDDS expression in zebrafish resulted in a loss of reaction to light, but not to touch. Histopathological examination of the retina in these zebrafish demonstrated degeneration of the outer segments of photoreceptors, similar to that seen in the human disease. Yeast modeling for DHDDS suggests that the mutant protein has enzymatic activity, but is decreased. This fits with the hypothesis that the patients have enough activity to serve most needs for DHDDS, except the retina, where the metabolic needs for DHDDS are very high. To begin to understand the relationship between this specific mutation and retinal cell functionality in the human eye, induced pluripotent stem cells (iPSCs) were derived from skin fibroblasts derived from the unaffected parents, three affected siblings and the one unaffected sibling. We identified factors that differentiated these iPSCs into photoreceptor and RPE cells to examine the functionality of these cells. These studies will provide valuable insights into the underlying mechanisms responsible for the development of this disease and other RP disorders.

902F

The transcriptional basis of inter-ethnic disparities in glucocorticoid response. *J. Maranville, S. Baxter, J. Torres, A. Di Rienzo.* Department of Human Genetics, University of Chicago, Chicago, IL.

Glucocorticoids (GCs) are steroid hormones that mediate physiological responses to the environment and are widely used as pharmaceutical interventions. Clinical response to GC treatment varies extensively. This poses a serious threat to public health, as a large fraction of patients (~30%), especially those of African descent, show a weak response to treatment. Although little is known about the molecular basis of this variation, transcriptional response is likely to play a key role as GC action is largely mediated by the regulation of gene expression. We previously showed that cis-regulatory polymorphisms contribute to variation within and between populations in GC transcriptional response at some genes. In an effort to characterize the transcriptional basis of variation in GC response, we measured in vitro lymphocyte GC sensitivity (LGS), which is strongly predictive of patient response, and transcriptome-wide response to GCs in peripheral blood mononuclear cells (PBMCs) from African-American and European-American healthy donors (9 donors from each population total, and 6 out of the 9 were profiled for transcriptional response at 8h and 24h post-treatment). We found that transcriptional response after 8hrs treatment at 38 genes was significantly associated with LGS in African-Americans (FDR<0.1), suggesting a transcriptional basis for variation in GC response. We replicated a previous finding of lower LGS in African-Americans (p=0.019). Providing the first glimpse at the molecular basis of this inter-ethnic disparity, we found population differences in the average log fold change in expression at 19 genes (FDR<0.1). A number of these genes have clear relevance for lymphocyte-mediated immune response, such as *GIMAP5*, a regulator of apoptosis in T cells, and linker for activation of T cells family, member 2 (*LAT2*). Interestingly, significant population differences were only seen after 8hrs of treatment and not after 24hrs, suggesting that events early in the GC transcriptional cascade contribute to inter-ethnic disparities. Consistent with clinical and in vitro observations, we also found that the absolute log fold change was generally lower in African-Americans, with 16 of the 19 differentially responding genes showing a weaker response in PBMCs from African-Americans. Collectively, this work suggests a prominent role for transcriptional regulation, and likely regulatory polymorphisms, in variation within and between human populations in GC response.

903F

HLA-DRB1*1501 intensifies the impact of IL-6 promoter polymorphism on the susceptibility to multiple sclerosis in an Iranian population. *M. Shahbazi[1], A. Tahmasebifar[2], H. Ebadi[3], D. Fathi[3].* 1) Medical Cellular and Molecular Research Center,Golestan University Of Medical Science ,Gorgan, Iran.MS-PhD; 2) Medical Cellular and Molecular Research Center,-Golestan University Of Medical Science ,Gorgan, Iran.MD-PhD; 3) Medical Cellular and Molecular Research Center,Golestan University Of Medical Science ,Gorgan, Iran.Neurologist.

BACKGROUND: The multifunctional cytokine interleukin-6 (IL-6) is involved in inflammatory processes in the central nervous system. It is well documented that amount of IL-6 is increased in serum, cerebrospinal fluid and central nervous system lesions of patients with multiple sclerosis. A single nucleotide polymorphism at position -174 in the IL-6 gene promotor appears to influence IL-6 expression. OBJECTIVE: To investigate the possible influence of IL-6/-174 polymorphisms on susceptibility to multiple sclerosis and its integration with HLA-DRB1*1501. Genomic DNA was extracted from whole blood of 345 patients with multiple sclerosis and 426 control subjects. METHOD: The SSP-PCR method was used to determine genotypes and Fisher's exact test was applied to determine differences between groups. RESULTS: HLA-DRB1*1501 was observed more frequently among multiple sclerosis patients compared with healthy subjects (45% and 34%, respectively; OR = 1.6, 95% CI = 1.2-2.2, p = 0.0018). At the IL-6/-174 position, the G allele had higher frequency among multiple sclerosis patients compared with controls (77% and 70%, respectively; OR = 1.4, 95% CI = 1.1-1.8, p = 0.0038). This difference was more significant among HLA-DRB1*1501-positive patients and controls (81% and 67%, respectively; OR = 1.9, 95% CI = 1.5-2.5, p < 0.0001). CONCLUSIONS: Our results have shown that the G allele at the IL-6/-174 promoter polymorphism may be associated with development of multiple sclerosis in this population, and may be strengthened by HLA- DRB1*1501.We suggest more studies to confirm these results in other populations.

904F

Discovery through whole genome sequencing of low frequency sequence variants associating with gout. *P. Sulem[1], DF. Gudbjartsson[1], GB. Walters[1], HT. Helgadottir[1], A. Helgason[1], SA. Gudjonsson[1], C. Zanon[1], S. Besenbacher[1], G. Bjornsdottir[1], OT. Magnusson[1], H. Holm[1], A. Karason[1], T. Rafnar[1], H. Stefansson[1], OA. Andreassen[2], JH. Pedersen[3], AI. Pack[4], MCH. de Visser[5], L. Kiemeney[5,6,7], AJ. Geirsson[8], GI. Eyjolfsson[9], I. Olafsson[10], A. Kong[1], G. Masson[1], H. Jonsson[8,11], U. Thorsteinsdottir[1,11], I. Jonsdottir1[1,11,12], K. Stefansson[1].* 1) Dept Statistics, DeCode Genetics, Reykjavik, Iceland; 2) Division of Mental Health and Addiction, Oslo University Hospital & Institute of Clinical Medicine, Oslo, Norway; 3) Dept of Cardiothoracic Surgery, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; 4) Center for Sleep and Circardian Neurobiology, Division of Sleep Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; 5) Dept of Epidemiology, Biostatistics & HTA, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; 6) Comprehensive Cancer Center IKO, 6501 BG Nijmegen, the Netherlands; 7) Department of Urology, Radboud University Nijmegen Medical Centre, 6500 HB Nijmegen, the Netherlands; 8) Landspitali, The National University Hospital of Iceland, Department of Medicine, Reykjavik, Iceland; 9) Icelandic Medical Center (Laeknastretid) Laboratory in Mjodd (RAM),Reykjavik, Iceland,; 10) Landspitali, The National University Hospital of Iceland, Department of Clinical Biochemistry, Reykjavik, Iceland,; 11) University of Iceland, Faculty of Medicine, Reykjavik, Iceland; 12) Landspitali, The National University Hospital of Iceland, Department of Immunology, Reykjavik, Iceland.

We performed a genome-wide association scan of 16 million SNPs, identified through whole genome sequencing of 457 Icelanders, to search for association with gout and serum uric acid level. Genotypes for these SNPs were imputed into 41,675 chip typed Icelanders and their relatives, for an effective sample size of 968 gout cases and 15,506 individuals with measurements of serum uric acid. A low frequency missense mutation (c.1580C>G) in ALDH16A1 associates with gout (OR=3.12, $P=1.5\times10^{-16}$) and uric acid level (effect=0.36 SD, $P=4.5\times10^{-21}$). This association was confirmed by genotyping of 6,017 Icelanders through Sanger sequencing. The association with gout is stronger among males than females and the variant correlates with an early age at diagnosis. A second low frequency variant associates with gout (OR=1.92, P=0.046) and uric acid level (effect=0.48 SD, $P=4.5\times10^{-16}$). This variant is located at the centromere of chromosome 1, in close proximity to a common variant reported to associate with uric acid level. All previously reported loci were confirmed in our dataset, but their effects are substantially smaller than those of the two novel variants. In addition to the individuals sequenced and used above for the gout project, we plan to whole genome sequenced over thousand subjects during the summer of 2011. We will present the follow up process that we are developping with the purpose of discovering rare and low frequency variants having large effects for common diseases.

905F

Effect of rapamycin treatment on HEK293 cells- a muti-omics approach. *N. Guha, Y. Reddy, S. Rajagopalan, S. Joseph, S. Lateef.* Agilent Technologies India Pvt. Ltd., "C" Block, RMZ Centennial, Plot No. 8A,8B,8C,8D, Doddanakundi Indl Area, ITPL Road, Mahadevapura Post, Bangalore-560037, Karnataka, India.

Biological processes can be examined by collective information available at the genome, transcriptome, proteome and the metabolome levels. Data integration at all levels of omics analysis is required to perform "Systems analysis" which increases the confidence of results obtained from any one methodology. In this study, we performed multi omics analysis using an in vitro model of rapamycin treatment of HEK293 cells. Rapamycin is an immunosuppressant drug that specifically inhibits mTOR activity and cellular hyper-proliferation resulting in G1 growth arrest. HEK293 was harvested 16hrs post treatment of rapamycin or vehicle alone and RNA, miRNA, proteins and metabolites were analyzed. The SurePrint G3 Gene Expression arrays were used to evaluate the expression of both mRNA and large intergenic non-coding RNAs (lincRNAs). miRNA microarray analysis was performed using Human miRNA 8X15K V2 microarray. The complete genomic status of the cell was also interrogated using CGH 1X1M array. Combined gene expression and miRNA analysis was performed using GeneSpring 11.5-MPP which provides comprehensive analytical and visualization tools for different data types. Approximately 10000 mRNAs and 18 miRNAs were differentially expressed between treated and control samples. The target entity list of these miRNAs revealed by target scan showed good overlap with differentially expressed gene list from the gene expression microarray. A number of lincRNAs were found to be differentially expressed upon rapamycin treatment. CGH analysis showed that HEK293 cells possess large amount of aberrations at the genome level. Proteins extracted from HEK293 cells were analyzed on a QTOF coupled to HPLC chip MS system. Statistically significant differential proteins were identified by targeted MS/MS analysis followed by SpectrumMill search. Metabolites were analyzed using 7890 GC coupled to 5975C inert MSD and 1290 Infinity LC system coupled to 6520 QTOF for GC-MS and LC-MS studies, respectively. 200 metabolites were differentially expressed upon rapamycin treatment. The results obtained from the various `omics' was integrated using the pathway analysis tool in GeneSpring 11.5-MPP software enabling visualization of multiple changes occurring in different biological pathways. Rapamycin was found to affect several biological processes including translation, protein folding and stress response. The study demonstrates a workflow solution for multi-omics study in response to drug treatment.

906F

Functional Screening of Novel Drug Candidates by High-throughput Cellular Microarray Using System Biology Strategy. *J. Liu[1], D. Chen[1], D. Liu[1], X. Gao[2], L. Wang[1].* 1) School of Medicine, University of California, Irvine, Irvine, CA, USA; 2) Abbott Vascular Inc., Santa Clara, CA, USA.

Screening a library of small molecules is the first step in the pre-clinical phase of drug discovery process. Due to the tremendous complexity of biochemical circuitry, how a cell would respond to a given small molecule is often unpredictable. By determining which of the thousands of gene products a given small molecule is interacting with would predict biochemical effects. Our goal is to use system biology strategy and automation as means to efficiently screen small molecule library in a high throughput fashion. We therefore set up cellular microarrays as a tool for high-throughput screening of large set of test samples. A yeast deletion set contains over 5,000 individual yeast clones--each with a different gene deleted. Screening small molecules with yeast deletion set in a high throughput fashion is a significant engineer challenge. We have designed a 3 axis platform automation for screening. To test the feasibility of the setup, we use rapamycin which is well characterized. Rapaymycin exerts its action by binding and inhibiting FK binding protein (FKBP) and affects TOR pathway. Wild type yeast clone would not grow due to activation of TOR pathway. To test if we can actually isolate FKBP using this system, we first add rapamycin to a gelatinous solid growth support on a culture plate. All 5,000 yeast deletion clones are robotically "arrayed" onto the solid growth media. Growth size of individual yeast clones are analyzed digitally on day 1, 2 and 3. Several yeast clones are shown to grow preferrential in the presence of rapamycin. Clones which have the most differential growth variation will be candidates. The identities of these clones are checked against the database. The yeast clone with FKBP gene deletion was singled out. We have, therefore, confirmed the feasibility of system biology approach for drug screening using yeast deletion set. Furthermore, we have also isolated several genes from mTOR pathway which is downstream of FKBP as well as other genes with unknown functions associated with mTOR pathway. Thus, by growing numerous cultural clones in the presence of a given small molecule candidate and observe the variations of growth patterns would allow us to determine which genes and biochemical pathways the unknown molecule are interacting with. Miniaturization used in cellular microarrays also increases assay throughput and reduce reagent consumption and the number of cells used. This efficient system will be applicable for a broad range.

907F

Genetics and epigenetics of human gene regulation. *R.M. Myers[1], J. Gertz[1], T.E. Reddy[1], K.E. Varley[1], K.M. Bowling[1], F. Pauli[1], S.L. Parker[1], K.M. Newberry[1], K.S. Kucera[2], H.F. Willard[2], G.K. Marinov[3], A. Mortazavi[3], B.A. Williams[3], B. Wold[3].* 1) 1HudsonAlpha Institute for Biotechnology, Huntsville, AL; 2) Duke University, Institute for Genome Sciences and Policy, Durham, NC; 3) California Institute of Technology, Department of Biology, Pasadena, CA.

Genome-wide studies to identify genetic loci associated with complex diseases frequently implicate mutations in regions of the genome that do not code for proteins. The characterization of non-coding variants is a particularly challenging and increasingly important endeavor. To address this challenge, we have used sequence-based functional genomics measurements to identify alleles that associate with gene regulatory differences in cis. Through a combination of ChIP-seq, RNA-seq and Reduced Representation Bisulfite Sequencing (RRBS), we have discovered allelic biases in gene regulatory events across the genome of a lymphoblastoid cell line. By analyzing SNPs in the RNA-seq data, we were able to determine transcripts that were not equally expressed from each allele. We found significant differential allelic expression of 979 human genes, indicating that 8% of genes are preferentially expressed from a single chromosome. RRBS measurements in the same cells indicated that 7.9% of heterozygous SNPs are associated with differential methylation in cis. By combining allelic differences in expression and DNA methylation, we found that 25% of genes harboring allele-specific methylation near the transcription start site exhibited allele-specific gene expression, which is twice as many as expected by chance. Our results demonstrate that genomic sequence variation can significantly influence DNA methylation. We measured genome-wide differential allelic occupancy of 24 sequence-specific transcription factors in the lymphoblastoid cell line by using ChIP-seq. We identified more than 150,000 transcription factor binding sites, 8% of which overlap heterozygous genomic positions. Using this extensive dataset, we found that transcription factors preferentially bind a single allele at more than 1% of the TF binding sites identified. Differential allelic occupancy of sequence-specific transcription factors predict differential allelic expression, but more weakly than RNA polymerase 2 biases, emphasizing the multi-factor complexity of cis-regulation. Together, these results reveal thousands of functionally associated sequence variants in non-coding portions of the genome. Several of these sequence variants reside in disease implicated loci and provide insight into the mechanisms underlying existing disease related variation.

908F

Rapid sequence-level characterization of transgene insertions reveals significant complexity with ramifications for studies of transgenic animals. *J.C. Jacobsen[1], C. Ernst[1], C. Chiang[1], C. Hanscom[1], A. Heilbut[1], M. Sun[1], S.R. Rudiger[2], C.S. Bawden[2], S.J. Reid[3], R.L.M. Faull[3], R.G. Snell[3], M.E. MacDonald[1], J.F. Gusella[1], M.E. Talkowski[1].* 1) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA., USA; 2) Molecular Biology and Reproductive Technology Laboratories, Livestock and Farming Systems Division, South Australian Research and Development Institute, SA., Australia; 3) The Centre for Brain Research, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand.

Stable expression of transgenes in model organisms or cultured cells is a fundamental genetic tool widely used in biological research, yet for most transgenics, little is known concerning the mechanism of transgene integration, the structural integrity of the introduced transgene, the precise site of integration or the effect of the integration event on the host genome. Using three independent sequencing approaches, we characterized the transgenic sequence and integration site in seven Huntington's disease (HD) animal models: two well-established huntingtin N-terminal fragment mouse models (R6/1 and R6/2, 1.9 kb transgene) and five recently created full-transcript length HD transgenic sheep (11.6 kb transgene). Our analyses precisely characterized the transgene integration site junctions, revealing extensive structural rearrangement complexity that altered both the transgene itself, and the genomic DNA adjacent to the integration site, including duplication, deletion, excision/insertion, inversion and integration of foreign sequence (bacterial DNA). In one model (R6/2), the transgene inserted into a novel, evolutionarily conserved gene, Gm12695, which we find to be up-regulated in mouse brain. These analyses indicate that alterations to the structure and function of transgenes introduced by pronuclear injection occur routinely. They also show that the site of integration and possible resultant rearrangement of the host genome are all factors that can confound interpretation in transgenic model experiments. Our findings argue that it is both feasible and advisable to perform sequence-level characterization of transgene insertions prior to extensive investment in phenotypic characterization of transgenic model organisms.

909F

A genome-wide association study for renal transplant dysfunction. *I. Rebollo Mesa[1], C.S. Franklin[2], J.E. Mollon[1,3], M. Hernandez Fuentes[1], E. Perucha[1], N. Anyanwu[1], P.J. Conlon[4,5], S.H. Sacks[1], N. Soranzo[2], G.M. Lord[1], M.E. Weale[1,3], United Kingdom and Ireland Renal Transplant Consortium and Wellcome Trust Case-Control Consortium 3.* 1) MRC Centre for Transplantation, King's College London, London, United Kingdom; 2) Wellcome Trust Sanger Institute, Hinxton, UK; 3) Department of Medical and Molecular Genetics, King's College London, London, United Kingdom; 4) Beaumont Hospital, Dublin, Ireland; 5) Royal College of Surgeons in Ireland, Dublin, Ireland.

There is a medical imperative to make transplanted kidneys last longer than they currently do. Graft survival varies widely between individuals, and while some of this variation can be attributed to known clinical factors there is much variation left unexplained. We hypothesize that there may be genetic factors in donors, recipients, or interactions between the two which influence graft survival time, and have carried out a donor-recipient genome-wide association study (GWAS) to test this.

Through the large multicentre United Kingdom and Ireland Renal Transplant Consortium, coordinated by King's College London in partnership with the Wellcome Trust Case-Control Consortium 3 and the National Health Service Blood and Transplant database, we have sourced (1) DNA and transplant-related phenotypes from ~2500 (cadaveric) donor-recipient pairs; (2) pre-existing GWAS data from ~600 pairs collected at King's College London, UK; and (3) pre-existing GWAS data from ~340 recipients collected at Beaumont Hospital, Ireland. All samples have been genotyped on Illumina Quad670 or Quad610 chips. Our primary scan fits Cox proportional hazards models for graft survival to donor SNP main effects, recipient SNP main effects and donor * recipient SNP interactions.

Complex correlation structures in the data result from the use of a donor's two kidneys in different recipients, and from the use of repeated serial grafts in the same recipient. We discuss methodological approaches to address this problem, present simulations to show that power and Type I error are expected to be robust for our scans of interest, and discuss the impact of the issues on the results of our primary scans.

910F

Tissue-specific activation of transposons-derived sequences in normal and cancer cells. *G. Bourque[1,2], P.E. Jacques[3], J. Jeyakani[3], X.Y. Woo[3].* 1) Human Genetics, McGill University, Montreal, Quebec, Canada; 2) McGill University and Genome Quebec Innovation Center, Montreal, Quebec, Canada; 3) Genome Institute of Singapore, Singapore.

Next-generation sequencing (NGS) technologies (e.g. ChIP-Seq, RNA-Seq) are now supplanting array-based technologies because of their accuracy, comprehensiveness and cost. Notably, these technologies enable an unbiased look at the functional contributions of the genome including the contributions of repetitive regions. We present results from the analysis of DNaseI hypersensitivity and ChIP-Seq ENCODE datasets in normal, embryonic and cancer cell lines that show that in all cell types between 8% and 12% of the open chromatin regions have been contributed by transposable elements with a significant over-representation of endogenous retroviral (ERV) repeat families. We also show that the same repetitive regions are enriched for relevant transcription factor binding motifs and are observed to be bound in vivo by various regulatory proteins. Interestingly, we further demonstrate that hundreds of these transposons-derived sequences are activated in a cell-type specific manner. We are also able to confirm that genes near repeat instances containing hypersensitive sites in a particular cell type tend to be more differentially expressed. Although we find that most of this tissue-specific activity on transposon-derived sequences is detected in embryonic stem cells and cancer cell lines, a few repeat families are also open in distinct differentiated cell types. For instance, close to 30% of the instances of the LTR2B ERV subfamily are only open in lymphocyte-related and in renal epithelial cells. Given that various repeat families are highly polymorphic across individuals, characterizing their regulatory activity is likely to be critical to understand functional variation and susceptibility to disease. Finally, the activation of specific repeat families in cancer is of particular interest as we show that it is associated with the deregulation of oncogenic gene subnetworks.

911F

Identifying Genome Wide SINE Retrotransposons Contributing to Dog Size Variation. *N. Sutter, M. Tomlinson, J. Homburger, J. Allen, I. Welsh, H. Moghadam.* Clinical Sciences, Cornell University, Ithaca, NY.

Body size variation was very rapidly acquired in dog evolution, perhaps within a few thousand years of domestication. However, it is not known what functional sequence variation enabled this rapid diversification of a complex trait. Gene disruption via retrotransposon integration causes disease in both man and dogs (e.g. narcolepsy, centronuclear myopathy and merle deafness in dogs). Loci segregating retrotransposon insertion and non-insertion are rare in the human genome (~1500 total). In contrast, in the dog genome SINEC_Cf is frequently segregating; Wang and Kirkness identified 10,000 such loci by comparing just the two reference genomes, Boxer and Poodle. SINEs can disrupt gene expression, splicing, chromatin environments and DNA methylation patterns. We hypothesize that gene disruption via SINE integration is frequently the causal mutation for disease susceptibility and traits under selection in the dog such as body size. We have developed a method to create libraries highly enriched for sequences flanking dog SINEs. Using the Illumina genome analyzer we have sequenced seven such libraries. We identified >10,000 new putatively segregating SINEs including hundreds within promoters, exons and splice sites. PCR sizing and capillary sequencing has experimentally validated 32 out of 40 (80%) such candidates for gene disruption. We have identified segregating SINEs associated with body size variation, including the IGF1 SINE we previously discovered ($p<10E-99$) and SINEs integrated in other genes ($p<10E-12$, $p<10E-8$ and $p<10E-5$). The genome-wide pattern of these SINE integrations is currently being investigated.

912F

Uniparental disomy detection in Prader Willi/Angelman syndrome cases utilizing microarray. *D.L. Pickering, B.J. Dave, J.M. Carstens, A. Papugani, A.H. Olney, W.G. Sanger.* Munroe Meyer Institute, University of Nebraska Medical Center, Omaha, NE.

The consequences of uniparental disomy (UPD) are evidenced because of the epigenetic mechanism of genomic imprinting. Due to differential methylation process, certain genes are expressed in a parent-of-origin-specific manner resulting in monoallelic expression of a gene without changing the genetic sequence. Although the majority of Prader Willi syndrome (PW) and Angelman syndrome (AS) are caused by a deletion within chromosome 15q11-q13, UPD and imprinting center mutation are among other etiologies. Diagnostic testing for PW/AS includes chromosome 15 methylation studies in conjunction with or followed by fluorescence in situ hybridization (FISH) or microarray to determine a deletion of 15q11-q13. Because imprinting center mutations are associated with high recurrence risk, it is crucial to assess for the presence of UPD among the non-deletion cases for counseling and management issues. To detect UPD, typically PCR is performed using at least two informative polymorphic markers in both the patient and parents. More recently, whole genome single nucleotide polymorphism (SNP) arrays have been utilized to identify UPD. To examine the feasibility of this technology as a reflex to methylation analysis in PW/AS, we identified 67 (33-PW; 34-AS) methylation-positive cases that were studied in our laboratory in the past decade. Seventeen of these cases (14-PW; 3 AS) were negative for 15q deletion. This subset of patients was investigated utilizing a UPD array with a combination of SNP and oligonucleotide probes distributed throughout the genome. The detection of isodisomy by SNP array does not require parental studies; however heterodisomy studies need a comparative parental analysis. We detected paternal uniparental isodisomy in 66% (2/3) AS cases and maternal uniparental isodisomy was identified in 7% (1/14) PW cases. Seven of 13 (53%) PW studies exhibited segmental loss of heterozygosity (LOH) on chromosome 15 ranging in size from 16-34Mb. Because this was a retrospective study, parental samples were available only in two PW cases and the UPD array confirmed maternal uniparental heterodisomy. As expected, there was a higher incidence of isodisomy among AS cases compared to PW cases. The UPD array thus facilitates isodisomy detection but is best utilized when parental samples are available for identification of heterodisomy and better definition of segmental LOH.

913F

Next Generation Sequencing: Guiding the translation from research to clinical applications. *A.S. Gargis[1], L.V. Kalman[1], M. Berry[2], D.P. Bick[3], D.P. Dimmock[3], T.M. Hambuch[4], F. Lu[2], E. Lyon[5], K.V. Voelkerding[5], B.A. Zehnbauer[1], I.M. Lubin[1].* 1) Centers for Disease Control and Prevention, Atlanta, GA; 2) SeqWright, Inc., Houston, TX; 3) Medical College of Wisconsin, Milwaukee, WI; 4) Illumina Clinical Services, San Diego, CA; 5) ARUP Laboratories, Salt Lake City, UT.

Next-generation sequencing (NGS) has expanded beyond research applications to deliver clinically actionable test results that can effectively inform medical decision making. This new area of clinical testing lacks uniform practices for quality assurance and quality control (QC) that are essential to ensure the analytic validity of test results. Similarly, there is no laboratory practice guidance that is specific for NGS technologies and provides for compliance with existing regulatory and professional standards. To address these shortcomings, a workgroup meeting was convened on April 14-15, 2011 that included 41 invited participants from clinical and research laboratories, clinical care settings, manufacturers, software developers, relevant government agencies, accreditation bodies, and professional organizations. Test validation, quality control, proficiency testing/alternate assessment, and reference materials were key discussion topics. For NGS test validation and QC, accuracy, precision, robustness, and sensitivity were considered in terms of sequence coverage, an attribute not relevant to Sanger sequencing. Orthologous techniques such as Sanger sequencing and analysis by SNP arrays are useful comparators for test validation and QC but have inherent limitations that require validation for the intended application. Analysis of a gene panel, exome, or whole genome requires different approaches for test validation and QC. Meeting participants proposed new strategies to develop biologic, synthetic, and electronic reference materials. Traditional models for proficiency testing and alternate assessment may be impractical for clinical laboratories and new paradigms were considered. Consensus was achieved in several areas to define metrics and processes not currently described that are needed to establish analytic validity and to meet regulatory and professional standards. Outcomes from this meeting included the recognition of technical differences between NGS and Sanger sequencing that affect quality assurance practices and the identification of challenges to implementing NGS as a clinical test with respect to meeting regulatory and professional standards. Metrics and processes needed to assess the analytic validity of NGS results were addressed and participants identified issues that require additional data collection. These findings are being used to develop laboratory practice guidance to ensure the quality of NGS for patient testing.

914F

MiSeq: A rapid low cost sbs sequencing platform. *K. Hall[1], G. Smith[1], J. Betley[1], W. Meuleman[1], J. Weir[1], J. Quick[1], A. Breton[1], M. Wang[2], P. Podlevsky[2], J. Moon[2], B. Crane[2], N. Scheidler[2], R. Yang[2], S. Hong[3], E. Vermaas[3], B. Rabkin[3], M. Siu[3], M. Reed[3].* 1) Illumina Cambridge Ltd., Saffron Walden, Essex, United Kingdom; 2) Illumina Inc. 9885 Towne Centre Drive, San Diego, CA 92121 USA; 3) Illumina Hayward, 25861 Industrial Blvd. Hayward, CA 94545 USA.

Next generation sequencing has revolutionised the ability to collect large amounts of data from genetic material both in terms of depth of coverage and breadth of samples that can be sequenced. As we learn about genetic systems and together with existing applications there is a tremendous demand for a system that exhibits a fast time to answer.

Illumina has designed and produced a new sequencing platform MiSeq that enables extremely rapid sequencing of genetic material. The MiSeq instrument produces up to 1.5Gb from a 2x150 cycle run utilising paired end technology in just over a day. The instrument takes Illumina TruSeq and Nextera libraries and after only 20 minutes of set up time a sequencing run can be initiated that needs no further human intervention. The cluster growth and paired end processes are built into the instrument further simplifying workflow. The primary and secondary analysis including alignment, structural variant calling is also performed on instrument, supporting de novo assemblies of bacterial genomes and producing contig and coverage data. The sequencing is achieved using fast cycle time reversible terminator chemistry derived from the proven robust massively parallel sequencing by synthesis (SBS) chemistry used on Illumina's HiSeq and GA platforms. Depending on application it is possible to achieve sequence data from a library of over 3.4 million tags in less than 4 hours.

This system will have a broad range of applications including clinical sequencing from FFPE samples, amplicons, targeted resequencing and more general application for RNA, bacterial genomes and library quantification.

915F

Development of SureSelect Target Capture Methods for Sequencing on the PacBio *RS*. *S. Happe[1], M. Guadalupe[1], S. Ranade[3], L. Lee[3], J. Barboza[1], B. Novak[2], E. Lin[2], C. Pabon-Pena[2], J. Ong[1], S. Joshi[1], H. Ravi[1], M. Visitacion[2], M. Hamady[2], F. Useche[2], D. Roberts[2], A. Giuffre[1], E. Leproust[2].* 1) Agilent Technologies Inc, Cedar Creek, TX; 2) Agilent Technologies Inc, Santa Clara, CA; 3) Pacific Biosciences, Menlo Park, CA.

Pacific Biosciences' PacBio *RS* is a powerful new technology for single molecule, real-time sequencing, featuring long reads, fast turnaround times, and capabilities for both both *de novo* sequencing and resequencing applications. To determine the efficacy of the Agilent SureSelect™ Target Enrichment System for targeted resequencing on the PacBio *RS*, we developed methods to prepare genomic DNA libraries and analyzed sequencing performance. First, to take advantage of the long read capability, we tested different shearing conditions to create inserts of varying sizes. Second, we modified the Agilent SureSelect^{XT} kit such that unique short adapter sequences were ligated onto sheared DNA, thereby allowing manipulation of the library throughout the target enrichment process. Following hybrid capture with SureSelect biotinylated RNA libraries and appropriate blocking reagents, PacBio SMRTbell™ templates were constructed using the enriched DNA and subsequently sequenced on the PacBio RS. Sequencing results revealed high specificity for capturing targets of interest, excellent uniformity, and deep coverage across the targeted regions. High performance was achieved across different sample types captured with SureSelect libraries of varying size, complexity, and content. Data obtained was used to efficiently identify SNPs and indels, and showed high correlation with previously-determined genotypes. Long insert libraries retained the advantages of long reads while focusing data on regions of interest. These findings illustrate the utility of the SureSelect method for target enrichment on the PacBio *RS* , and provide a path forward for targeted variation discovery and profiling.

916F

Comparison of two new methods to increase the sensitivity of next-generation sequencing (NGS) of short DNA for non-invasive prenatal and cancer diagnostics as well as for ChIP-seq, methyl-seq and other sonicated DNA applications. *E. Kamberov[1], J. Langmore[1], T. Tesmer[1], M. Luo[2], M. Jeong[2], D. Sun[2], W. Lei[2], L. White[2], G. Darlington[2].* 1) Rubicon Genomics, Ann Arbor, MI; 2) Baylor College of Medicine, Houston, TX.

There is an increasing need to sequence picogram quantities of short DNA for diagnostics and research. For example, 1) maternal blood contains small amounts of fetal DNA useful for prenatal diagnostics; 2) cancer patient plasma contains small amounts of tumor DNA useful for cancer diagnostics; and 3) immunoprecipitated DNA and nucleoprotein is used to identify sites of protein-binding and DNA methylation. Unfortunately, conventional NGS preps require ng or µg amounts of DNA.

We tested 2 methods to prepare pg amounts of DNA for NGS, using ChIP and plasma DNA as model systems--the Sigma GenomePlex WGA4 kit and the Rubicon NovaPlex NGS prep kit. H3k4me3 Ab were used to precipitate chromatin fragments from mouse hematopoietic stem cells (HSC). A 2 ng ChIP control was prepared using the Illumina protocol from 300K HSC, and 100-200 pg ChIP experimental samples were prepared from 50K HSC. Multiple GenomePlex experimental samples were amplified using the Sigma WGA4 protocol, followed by NGS adaptation with the Rubicon RetroPlex kit. Multiple NovaPlex samples were directly prepared from experimental ChIP DNA. Multiple NovaPlex samples were prepared from 3 ng human plasma DNA.

The ChIP samples gave similar statistics, with 23M - 38M reads per lane and 71%, 51%, and 58% uniquely mapped reads (control, GenomePlex, NovaPlex). The control had 15,900 significant peaks (p<10e-8), GenomePlex 30,000 peaks, and NovaPlex 15,100 peaks. Although 92% of the NovaPlex peaks overlapped the control, only 41% of the GenomePlex peaks did. The “false positive” GenomePlex peaks were primarily in distal intergenic regions.

NovaPlex plasma NGS samples had 51 base read statistics similar to a 3 µg Illumina prep. The GAIIx gave 30M NovaPlex reads/lane (99% high-quality in read 1 and 89% in read 2, of which 97% mapped to hg19 with <0.2% mismatch with hg19. Paired-end distances were 170 +/- 50), with 2x10e-6 mapped inconsistently due to inversion and <1% due to distance. NovaPlex consistently covered 50% of the genome in a single lane.

NovaPlex enables high-quality sequencing of very small amounts of ChIP and plasma DNA in 2 steps in two hr. Based on these initial successes, stringent testing with smaller amounts of ChIP and patient plasma DNA is being performed in third-party laboratories in order to fully characterize its performance on research and clinical samples.

917F

Cost-effective, high-throughput DNA sequencing libraries appropriate for highly multiplexed target capture. *N. Rohland[1,2], D. Reich[1,2].* 1) Genetics, Harvard Medical School, Boston, MA; 2) Broad Institute of Harvard and MIT, Cambridge, MA.

Improvements in technology have reduced the cost of DNA sequencing to the point that the limiting factor for many experiments is the time and reagent cost of sample preparation. We present an approach in which 192 sequencing libraries can be produced in a single day of technician time, representing an order of magnitude increase in throughput and an order of magnitude decrease in reagent costs. These libraries are effective not only for whole genome sequencing, but also for simultaneously enriching them in pools of at least 95 individually barcoded samples for a subset of the genome using solution hybrid capture without substantial loss in molecular complexity and efficiency of target capture. We illustrate the power and effectiveness of this approach on more than 2,000 samples from a prostate cancer study.

918F

Comparative Analysis of CHO Cell Transcriptional Dynamics under Different Cell Culture Conditions using Next Generation RNA Sequencing Technology. *J.J. Schageman[1], M. Liu[2], S. Wang[2], C. Mueller[1], R.A. Setterquist[1].* 1) Ambion Bioinformatics, Life Technologies Corp., Austin, TX., U.S.A; 2) Bioproduction, Life Technologies Corp.,Frederick, MD, U.S.A.

Next Generation RNA-Sequencing (RNA-Seq) is a methodology for comprehensive measurements of cellular transcription at a scale, accuracy and precision never seen with previous technologies. We conducted a comparative RNA-Seq study of basic cell growth conditions to understand and improve high quality therapeutic protein production in CHO cells. In this study, we examined global changes in gene expression in CHO cells across differing cell subtype and media- specific parameters. Eight CHO RNA samples from different cultures were sequenced on two full slides of a SOLiD™ System resulting in approximately 760 million, 50 base pair sequence reads. These reads were mapped to multiple reference sequences including CHO ESTs, mRNAs and well as mouse chromosomes with annotated genes with cognate functional data. From this, we report estimates of transcript expression levels and use known annotation to infer functional differences that can be associated with changing basic bioproduction growth conditions. These findings may uncover novel genetic mechanisms that could be optimized for improved bioproduction. This analysis of this data set represents the characterization of the CHO transcriptome at an unprecedented depth.

919F

Novel Technologies for Ribosomal RNA (rRNA) Removal (Ribo-Zero™) and Directional RNA-SEQ Library Preparation (ScriptSeq™). *R. Sooknanan, J. Hitchen, A. Khanna, A. Radek.* Epicentre® (an Illumina® company), Madison, WI.

Deep, massively parallel sequencing of cDNA libraries generated from RNA ("RNA-Seq") is rapidly gaining momentum for transcript profiling, discovery of novel transcripts, and identification of alternative splicing events. Current methods for making sequencer-specific di-tagged DNA fragment libraries for RNA-Seq typically comprise preparing rRNA-depleted RNA and either (i) RNA fragmentation, 5' and 3' adaptor-ligation, size selection, cDNA synthesis, and multiple clean-up steps; or (ii) cDNA synthesis followed by cDNA fragmentation, end-polishing, 5' and 3' adaptor-ligation, gel-size selection and multiple clean-up steps. These methods are generally time-consuming and require significant hands-on time. Further, even after removal of rRNA from intact total RNA samples using commercially available kits, 50 % or more of the sequence reads can still be from rRNA, decreasing sequencing depth and coverage of the RNA-Seq libraries. Additionally, these kits are not recommended for fragmented (e.g., FFPE) RNA samples, which further limits RNA-Seq applications. Here, we present RNA-Seq results obtained using a novel "single-pass" rRNA removal technology ("Ribo-Zero™") and a novel and rapid (simple workflow) RNA-Seq library preparation process that utilizes a unique Terminal Tagging technology for preparing directional cDNA libraries ("ScriptSeq™ Technology") for NGS. Directional cDNA libraries with ~98% strandedness can be prepared in about 2.5 hours from either intact or fragmented (e.g., FFPE) rRNA-depleted RNA samples without the need for end-polishing, adaptor-ligation, cDNA fragmentation or gel-size selection. ScriptSeq libraries generated from Ribo-Zero™ treated intact or fragmented total RNA samples for rRNA removal contain <2 % of the sequence reads that map to rRNA sequences (28S, 18S, 5.8S and 5S) while maintaining both coding and non-coding transcripts independent of polyadenylation. This reduction in rRNA sequence reads improves sequence depth and coverage, and increases the percentage of uniquely mapped reads. Further, there is a high correlation (R2=0.9235) between differentially expressed transcripts found in the ScriptSeq™ RNA-Seq libraries and the MAQC qPCR panel of genes.

920F

Post Amplification Ligation Mediated (PALM) barcoding for multiplexed RNA deep sequencing. *F. Van Nieuwerburgh[1], K. Podshivalova[2], E. Ay-Lin Wang[2], L. Schaffer[3], T. Hart[2], D. Deforce[1], D. Salomon[2], S. Head[3], P. Ordoukhanian[3].* 1) Laboratory of Pharmaceutical Biotechnology, Ghent University, Harelbekestraat 72, 9000 Ghent, Belgium; 2) Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10550 N. Torrey Pines Rd, La Jolla, CA, 92037, USA; 3) Next Generation Sequencing Core, The Scripps Research Institute, La Jolla, California, 92037, USA.

Taking advantage of the increasing throughput achieved by second generation sequencing technologies, multiplexing several samples in one analysis can increase experimental throughput while reducing time and cost. We demonstrate a new method for unbiased multiplexed sequencing of microRNA, mRNA and DNA libraries using a novel, efficient and simple barcoding strategy we called Post Amplification Ligation-Mediated (PALM) barcoding. Barcoding is performed as the very last step of a standard Illumina library preparation, eliminating barcode-induced bias caused by PCR amplification of samples with different barcode sequences. As a proof of concept, we sequenced PALM barcoded reference microRNA and mRNA samples and evaluated the quantitative bias in comparison to the same reference samples prepared using a pre-PCR barcoding strategy. For the microRNA samples, we also compared the bias to a barcoding strategy that introduces the barcodes during the PCR step. Results show virtually no bias for barcoded samples prepared by the PALM and the during-PCR barcoding strategy, while samples multiplexed using a pre-PCR barcoding strategy show significant bias. The total number of past-filter reads was comparable between the different barcoding strategies. The number of reads for each of the individually barcoded libraries in the PALM multiplex pool was uniform, showing that none of the PALM barcoded libraries generates more clusters on the flow cell than the other ones. We applied PALM barcoding to search for differentially expressed microRNAs in human T cells activated and/or treated with immunosuppressant drugs. High correlations were found for biological replicates indexed with different barcodes.

921F

***In vivo* analysis of DNA-bound proteins in the UAS_{Gal} region using the novel GENECAPP technology.** *H. Guillen Ahlers[1], M. Zickus[1], A. Musante[2], S. Mirza[1], A. Ludwig-Kubinski[1], M. Zelembaba[1], S. Zhang[1], R. Cole[1], M. Chesnik[1], C-H. Wu[2], Y. Yuan[2], G. Kreitinger[2], M. Scalf[2], M. Levenstein[2], M. Shortreed[2], L. Cirillo[1], L.M. Smith[2], M. Olivier[1].* 1) Wisconsin CEGS, Medical College of Wisconsin, Milwaukee, WI, 53226; 2) Wisconsin CEGS, University of Wisconsin, Madison, WI, 53706.

Numerous proteins mediate DNA stability, control its activity, and regulate transcription of the encoded genetic information. Currently, no technologies exist to examine these protein-DNA interactions in a comprehensive global manner. To overcome this challenge, we developed **G**lobal **E**xo**N**uclease-based **E**nrichment of **C**hromatin-**A**ssociated **P**roteins for **P**roteomics (GENECAPP). While Chromatin Immunoprecipitation (ChIP) characterizes DNA interacting with individual known proteins, GENECAPP, developed at the Wisconsin Center for Excellence in Genomics Science, utilizes oligonucleotide capture to isolate targeted DNA regions in a sequence-specific manner and analyzes captured DNA-associated proteins using mass spectrometry. The Gal upstream activator sequence (UAS_{Gal}) in *S. cerevisiae* was studied using the GENECAPP technology. Streptavidin-coated magnetic beads were used as the solid surface with attached sequence-specific capture oligonucleotides. Cells were lysed and nuclear extracts cross-linked and captured by hybridization after exonuclease-mediated generation of exposed single-stranded overhangs. After trypsin digestion, peptides characteristic of bound proteins were identified by tandem mass spectrometry using an Orbitrap Velos instrument. The analysis identified the majority of the known proteins that bind to the UAS_{Gal} region under repressive conditions. Additional novel proteins were uncovered that have not been previously reported to bind to the DNA sequence. Overall, the GENECAPP technology is evolving into a new powerful tool to identify previously unknown proteins bound to specific DNA regions of interest.

922F

Rapid DNA extraction from human buccal cells for PCR amplification. *J.A. Timbuak[1], A.E. Justice[2], M.H. Crawford[2], P. Williams[3].* 1) Anatomy, Ahmadu Bello University, Zaria, , Kaduna, Nigeria; 2) Department of Anthropology, University of Kansas, Lawrence KS; 3) Evogen Inc., Kansas City, MO.

A variety of methods exist for extraction of human DNA and recent developments in column-based extraction methods have allowed for purified PCR ready DNA with less time at the bench. However, these methods often result in the loss of some DNA on the filter accompanied by laborious protocols. For samples commonly used for anthropological genetics and forensics, such as buccal swabs, the purification of samples using filters is often unnecessary and costly. Evogen One™ (Evogen, Inc.) is a rapid, commercial DNA extraction method that uses a proprietary salting method of extraction. This study examines Evogen One™ to assess the time to extraction of PCR ready samples, sample concentration, and total DNA yield. To further demonstrate the utility of this extraction method, these extractions were compared to QiaAmp® DNA Mini Kit (Qiagen®), a spin column based extraction method. To test the efficacy of Evogen One™ for human buccal cells, 265 samples were collected with a cotton swab from four Nigerian ethnic groups. Evogen One™ uses a proprietary salt, detergent, and heat to lyse the cells. Lysed samples are centrifuged to pellet cellular debris, leaving supernatant containing PCR-ready DNA. A total of 231 samples were extracted using Evogen One, while 34 samples were extracted using QiaAmp® following manufacturer's protocol. Sample concentrations were obtained using a NanoDrop™ 2000c and adjusted for volume to obtain total DNA yield. Total extraction time was less than 25 minutes per 24 samples for Evogen One™ and ranged from approximately 90-120 minutes for QiaAmp®, including lysis steps. Evogen One™ resulted in an average concentration of 271.03 ng/µL (s2= 176.29) and QiaAmp® 31.97 ng/µL (s2= 12.73). Average final volume for Evogen One™ was 61.81 µL (s2= 52.93) and QiaAmp® was 136.32 µL (s2= 23.28). Average total DNA yield of 16.48 µg (s2= 24.57) was obtained for Evogen One™ and 4.32 µg (s2= 1.84) for QiaAmp®. Evogen One™ is a very promising extraction method for human genetics. When compared to column-based methods of extraction, it is more time and cost-effective and reduces lab waste as the total protocol takes place in a single tube. While the final DNA yields are more variable for Evogen One™ than the consistent concentrations of QiaAmp®, Evogen One™ results in greater than 3 times the total DNA yield and offers the researcher more flexibility with regard to purification and dilution of DNA.

923F

Effects of Sample Preparation on Transcriptome Sequencing. *P. Zumbo[1,2,3], CE. Mason[1,2].* 1) Department of Physiology and Biophysics, Weill Medical College, Cornell University, New York, NY 10065, USA; 2) HRH Prince Alwaleed Bin Talal Bin Abdulaziz Alsaud Institute for Computational Biomedicine, Weill Medical College, Cornell University, New York, NY 10065, USA; 3) Epigenomics Core Facility, Weill Medical College, Cornell University, New York, NY 10065, USA.

High-throughput transcriptome sequencing has been herald as a `revolutionary tool for transcriptomics'. But beyond the hype and promise of gene discovery and absolute transcript quantification lies significant library construction biases. Here, we analyzed the effects of sample preparation methodology on transcriptome sequencing, utilizing synthetic RNA spike-ins from the External RNA Control Consortium. We showed that downstream discoveries and determinations are, in part, a function of library construction method, across different modalities of measurement (exon and gene expression, isform detection, novel transcript identification, and variant calling). Specifically, we compared Epicentre ScriptSeq against Illumina TruSeq RNA-seq, amplification versus amplification-free libraries, and ribosomal reduction contra polyadenylate selection. We demonstrated the utility of synthetic RNA spike-ins to correct for some of the biases between different library constructions, enabling more concordant expression measurements. These results can inform future transcriptome studies on an individual basis, and highlight the importance of uniform library methods within a study for comparability, as well as the benefit of multiple methods for fuller characterization of a transcriptome.

924F

DNA binding of Novel Oligoheterocycles. *S.I. Al-Aqeel[1], J.M. Gardiner[2].* 1) Department of Chemistry, Faculty of Science, King Saud University, Riyadh, PO Box 22452, Riyadh 11495, Saudi Arabia; 2) School of Chemistry and Manchester Interdisciplinary Biocentre, The University of Manchester, Manchester M1 7DN,UK.

There has been interest in heteocyclic systems as sequence-selective DNA minor groove binding agents (and some as RNA binding agents), such as dimeric benzimidazoles related to Hoechst33258 (and trimeric equivalents), and oligopyrroles (and benzimidazole-imidazole chimeras). More recently, some examples of symmetrical dimers or linked dimers, have been reported with similar targets, and at least some evidence of structures with potential for targeting G-quadruplexes. Developing new methods to generate novel bis- and oligobenzimidazoles and evaluation as nucleic acid binding agents is thus of interest. This contribution will describe synthesis of several families of novel oligoheterocyclic systems, designed to provide a modular approach to provide a range of new ligands types, categorized based on heterocyclic orientation, separation and various functionality of linkers incorporated, for example including compounds of the generic types shown. Additionally, other series of dimers, trimers and tetramers will be described. Data on evaluation of binding for an array of structures in the above classes to a series of target DNA sequences (containing various length (AT)n sections) will be presented, leading to identification of novel DNA-binding ligands in several structural families with sub-micromolar affinities.

925F

Next generation sequencing applied to Alport syndrome diagnosis. *F. Ariani[1], R. Artuso[1], C. Fallerini[1], L. Dosa[1], F. Scionti[1], M. Clementi[2], G. Garosi[3], L. Massella[4], R. Mancini[1], F. Mari[1], I. Longo[1], M. Bruttini[1], A. Renieri[1].* 1) Medical Genetics, Department of Biotechnology, University of Siena, Italy; 2) Medical Genetics, Pediatrics, University of Padova, Italy; 3) Nephrology, Dialysis and Transplantation, AOU Siena, Italy; 4) Nephrology and Dialysis, Ospedale Pediatrico Bambin Gesù, Roma, Italy.

Alport syndrome is an inherited disorder of type IV collagen, the major collagenous constituent of the basement membrane. Clinically, it presents as a progressive inherited nephropathy characterized by the association of progressive hematuric nephritis with ultrastructural changes of the glomerular basement membrane, high-tone sensorineural hearing loss and ocular lesions. The disease is genetically heterogeneous, but the majority of cases show X-linked dominant inheritance and are caused by mutations in the COL4A5 gene (Xq22). Dominant and recessive autosomal forms have also been reported and are characterized by mutations in COL4A3 and COL4A4 genes (2q36-37). Considering the absence of mutational hot spots and the large size of the genes implicated in Alport syndrome, mutational analyses by standard techniques, such as DHPLC and/or direct Sanger sequencing, represent a very expensive and exhausting molecular testing. To overcome these limitations, we designed a next generation sequencing protocol enabling simultaneous detection of all possible variants in the three genes. We used a method coupling selective amplification to the 454 Roche DNA-sequencing platform (Genome Sequencer Junior System). The application of this technology allowed to identify the second mutation in two patients (p.Ser1147Phe in COL4A3 and p.Arg1682Trp in COL4A4) and to exclude the diagnosis of Alport syndrome in a third patient. In this pilot study, Sanger sequencing was used to evaluate the number of false positive changes and to determine indicative cut-off values (variations detected in <25% of reads) to use in a routine diagnostic setting. Although improvements in accuracy and ease of data analysis will be necessary, our study demonstrates that this system can be used to perform a fast, sensitive and relatively low-cost screening of variations in Alport syndrome genes.

926F

Investigation of High Resolution Melting analysis as a tool for mutation detection. *C.A. Fahey, M. Gill, A.P. Corvin, D.W. Morris.* Trinity College Dublin, Dublin, Ireland.

High Resolution Melting (HRM) analysis using dsDNA-binding dyes and real-time PCR instrumentation is a relatively new and attractive mutation detection technique due to its rapid, high-throughput and sensitive post-PCR analysis. This approach enables investigation of genetic variation including SNP genotyping, DNA methylation, heterozygosity screening and mutation scanning. HRM methodology is based on amplifying a region of interest using primer specific PCR, followed by gradual denaturing of the target region and generation of a melt curve, allowing successful detection of genetic variation in the sequence. In order to test the efficiency of HRM we applied it to exonic regions of the VIPR2 gene. Rare chromosomal duplications at the VIPR2 gene have been found to result in a significant increased risk of schizophrenia (Vacic et al. 2011; PMID: 21346763). Our two-stage approach to testing the HRM method was: (1) Analyse a proportion HapMap CEU samples of known mutation content based on online data including 1,000 Genomes data to optimize performance of the method. (2) Blindly screen remaining HapMap CEU samples for mutations and compare results with online data. Stage 1: Melt curve analysis successfully identified a high proportion of expected mutations in tested HapMap samples. Stage 2: We will report on the specificity and sensitivity of the method when blindly applied to remaining HapMap samples. If the method shows accurate performance, it can be applied to our large sample of schizophrenia cases and control samples. Along with subsequent capillary sequencing, it could potentially identify rare risk variants in patient samples.

927F

Validation of a Next Generation Sequencing Platform in a Clinical Setting. *T.A. Maher[1], M. Zhao[1], R.K. Basran[1,2], A. Milunsky[1,2,3], J.M. Milunsky[1,2,3].* 1) Center for Human Genetics; 2) Department of Pediatrics; 3) Genetics and Genomics, Boston University School of Medicine, Boston, MA.

Next Generation sequencing has now become a reality in the clinical laboratory with the advent of low cost instrumentation and relatively condensed sample processing. Our laboratory sought to validate the Ion Torrent system in a clinical setting. The technology uses a massively parallel array of proprietary semiconductor sensors to perform direct real-time measurement of the hydrogen ions produced during DNA replication. A high-density array of wells on the ion semiconductor chips provides millions of individual reactors. The process of generating sequence is straight-forward and involves four steps as follows: construct a library, prepare the template, generate base calls, and finally data analysis. Data analysis was accomplished using the Softgenetics NextGENe software package. Our validation consisted of ten previously identified mutations through traditional capillary sequencing. The mutations were spread across seven different genes and consisted of point mutations, multiple basepair deletions, an insertion/deletion and multiple point mutations in the same amplicon. The library design consisted of amplicons to generate 100 basepairs of bi-directional sequence according to the Ion protocol. One of the amplicons produced a suboptimal result and was not used. All the amplicons from the different genes were processed and analyzed together. There was sufficient coverage across all the amplicons to identify the mutations. The data was analyzed with NextGENe and all the mutations detected with capillary sequencing were correctly identified. The assay including generating the amplicons through software analysis was accomplished in four days by one technician who was also able to complete additional work in the laboratory. This workflow will easily allow a clinical laboratory to use the technology in multiple ways. One could sequence a panel of disease related genes using the Ion platform or with the availability of bar-coding, sequence multiple patients for the same set of genes simultaneously.

928F

Integration of the REM e Device on the Biomek FX Laboratory Workstation for Efficient High Throughput NGS Bead Enrichment Process. *K.L. Marshall[1], Z. Smith[2], J. Colbourn[2], K. Mockaitis[2], A. Jackson[1].* 1) Automated solutions, Beckman Coulter, Indianapolis, IN., Select a Country; 2) The Center for Genomics and Bioinformatics Indiana University, Bloomington, IN.

Next Generation Sequencing (NGS) is a powerful tool for determining the genetic sequence for subsequent applications such as gene discovery, mutation detection and forensic analysis. Most NGS systems, such as the Roche 454 sequencer, rely on time consuming construction and preparation of genomic libraries, prior to sequencing. The Beckman NGS Suite of methods automates the construction, quantification, normalization, emulsion PCR setup and Bead enrichment portions of the Roche 454 library preparation. Specifically, the Bead Enrichment process, where only 5-15% of monoclonal emPCR beads will be selected, is especially time consuming and prone to error. Beckman Coulter has integrated the Roche REM e device on the Biomek FX, FXP, NX & NXP Workstation Platforms for the automation of the bead enrichment process. This poster provides data on the integration of a single REM e device on the Biomek FX Dual Arm System with Span-8 and Multi-Channel Pipettor. Automation of the Roche protocols reduced hands-on time from 6 hours to 15 minutes. Actual method run times range between 2 ¾ to 3 ¾ hours depending on which protocol is run. The complete walk away automation method for large volume processing (2 - 4 cups) is simple, fast and accurate. Our 2 cup REM e data of Diptera cDNA Library from Indiana University was acceptable using Roche standards, with a 9% post enrichment bead recovery rate and 455 bp median Reads Length. For Laboratory Use Only; not for use in dagnostic procedures Beckman Coulter, they stylized logo, and Biomek are trademarks of Beckman Coulter, Inc. and are registered in the USPTO.

929F

Detection of BRAF v600e to 0.1% Using Unlabeled Probes and High Resolution Melting through Amplification Bias. *M. Poulson, C. Gundry, R. Crisp.* Idaho Technology, Inc., Salt Lake City, UT.

Introduction. High Resolution Melting (HRM) is a set of post-PCR genetic screening techniques to rapidly detect mutations while reducing sample manipulation and time to results. HRM techniques are non destructive, allowing confirmatory sequencing when desired. HRM is highly suited for known mutation screening or targeted genotyping. BRAF v600e is a proto-oncogene mutation linked to many cancers including colorectal, thyroid, and melanomas. Diagnosis is made by taking biopsies of suspected tissue. As biopsies obtain varying amounts of cancerous tissue, a highly sensitive and accurate assay for the v600e mutation is required. This study highlights the ability of allele biased HRM techniques to identify the BRAF v600e mutation in varying wild-type allele backgrounds. Methods. This study was performed using the LightScanner® and the LightScanner®32 (Idaho Technology, Inc.) systems. Unlabeled probe (LunaProbes™) assays use three oligonucleotides, two target-specific primers and a probe, and asymmetric PCR in which the forward and reverse primers are present in unequal concentrations. Genotype differences under the probe are identified as changes in the probe melting profile. The introduction of the probe element can in some cases differentially amplify the mutant allele and increase the mutant allele signal. LunaProbe assays can be carefully optimized to favor the amplification of the mutant allele. In this study, allele blocking techniques were employed to identify the BRAF v600e mutant genotype in a wild-type background. This was done by matching the probe to the wild-type allele and adjusting amplification conditions to preferentially amplify sample-derived DNA containing the BRAF v600e mutation. Varying allele fractions were tested in a wild-type genetic background to determine the lowest detected allele fraction. Results. The LunaProbe assay combined with mutation amplification enhancing techniques were used to achieve extremely high sensitivity detecting the BRAF v600e mutation in a homogenous assay requiring no post-PCR manipulations. The mutant allele amplification bias resulted in detection of BRAF v600e mutation at 0.1% allele fraction. Conclusions. This study shows that HRM in combination with robust assay optimization and precise temperature control can identify the BRAF v600e mutation as low as 0.1% mutant allele fraction. This technique can be applied to many genotyping applications as a means to increase mutation discovery.

930F

Very High Sensitivity Somatic Mutation Detection using Ice COLD-PCR and BLOCker Sequencing. *K. Richardson, P. Eastlake, B. Legendre, G. Wu, R. Lin, Y. Shi, S. Jansen, S. Peterson, K. Echtenkamp, E. Comtois, J. Sommer.* Transgenomic, Inc., Omaha, NE.

Transgenomic has developed very high sensitivity methods for detecting somatic mutations notably in the discipline of cancer therapy where key genetic changes are associated with EGFR antagonists' effectiveness, e.g. in the genes EGFR, K-RAS, PIK3CA, BRAF, p53 and NRAS. The novel techniques of Ice COLD-PCR and BLOCker Sequencing preferentially enrich mutant alleles compared to wild-type alleles. These are not allele-specific techniques; therefore all mutations, both known and unknown, are enriched and identified in a single reaction. Together these methods allow mutations at concentrations as low as 0.01-0.05% in background of wild-type to be confirmed by sequencing. Additionally Ice COLD-PCR and BLOCker Sequencing require no special equipment using only standard DNA thermocyclers and Sanger sequencing equipment. Data will be presented showing how these methods allow DNA sequencing confirmation of somatic mutations in samples such as low tumour-load biopsies, formalin-fixed paraffin-embedded slides, fine-needle aspirates, circulating tumour cells and circulating free tumour DNA in plasma and serum. These methods can also be used to confirm low signal Pyrosequencing and next-generation deep-sequencing results. Using these techniques routine, rapid, simple and inexpensive detection of somatic mutations in (1) cancer patients' plasma or serum or; (2) samples where very limited tumour tissue is available, offers a non-invasive option for cancer biomarker detection as well as monitoring remission, relapse and emergence of resistance mutations post-treatment. Finally Ice COLD-PCR and BLOCker Sequencing can be applied to analysis of mixed viral infections and mitochondrial heteroplasmies.

931F

Identifying diagnostic EGFR mutants by real-time PCR with new DDS probe systems. *D.A. Shafer[1,2], P. Hu[1], D. Xie[3], C.E. Hill[2], M. Rossi[2], M. Bouzyk[3].* 1) GeneTAG Technology, Inc., Atlanta, Georgia; 2) Emory University School of Medicine, Atlanta, Georgia; 3) AKESOgen, Inc., Atlanta, Georgia.

Recent studies have shown that many patients with non small cell lung cancer (NSCLC) are highly responsive to EGFR tyrosine kinase inhibitors (gefitinib and elotinib) and that those responsive patients can be identified by mutations in the EGFR tyrosine kinase domain. The dominant mutations of this type are a single base change in Exon 21 L858R T>G, and a small deletion of 9-24 bp in Exon 19 at codons 746-750. These two mutations account for about 90% of all EGFR mutations in lung cancer. Moreover, another single base mutation of EGFR in exon 20 (T790M) is associated with acquired resistance to gefitinib in patients who were initially responsive to gefitinib. These mutations thus offer a small set of targets that are valuable for cancer diagnostics and pharmacogenomics. The first author has invented new probe technologies for real time PCR that offer high specificity and sensitivity compared to conventional Taqman or TaqmanMGB probes, and these new qPCR technologies have been effectively applied to DNA copies of these cancer related targets. The primary probe system, an internal DDS (DNA Detection Switch) probe, comprises two labeled components - a probe and an antiprobe - that interact together and with the target sequence during real time PCR. The antiprobe provides a signaling function and it also serves as an error checking device to prevent binding and detection of non-matching targets that differ by a single base. This unique mechanism thus enables precise and reliable single base discrimination over a wide range of annealing temperatures (10-20 degrees). Therefore, multiple iDDS probes for different targets can be run together without loss of specificity. The current focus is on testing a set of iDDS probes that can discriminate the L858R mutation and on a related DDS probe set that can detect the Exon 19 deletion site. This collaboration with Emory and AKESOgen is engaged in applying these probe systems to clinical samples from lung cancer patients and controls to validate this methodology for cancer diagnostics. Current progress on this project will be reported.

932F

Single-Day, Highly Multiplexed Library Preparation for Amplicon Sequencing with MiSeq. *A. Tian, E. Guzman, M. Won, W. Chang, D. Pokholok, R. Haigis, A. Iyer, E. Upsall, I. Lewis, S. Norberg, M. Ronaghi, K. Gunderson, R. Shen, C. Lin.* Illumina, Inc. 9885 Towne Centre Dr, San Diego, CA 92121.

Targeted enrichment strategies enable cost-effective analysis of desired genomic regions with next-generation DNA sequencers. We introduce TruSeq™ Custom Amplicon, a new sample preparation method developed and optimized for high-throughput amplicon sequencing on MiSeq™, the personal sequencing system from Illumina. 48 to 384 of user-defined genomic regions can be enriched in a single tube with high specificity (>90% of reads on target) and high uniformity (>85% of targets represented at ⁄ 0.2X mean coverage) in <8 hr (from purified genomic DNA to sequencing-ready library). By comparison, only a dozen or so targets can be amplified in a single tube with multiplex PCR, and hybridization-capture enrichment approaches require 3 days or more from DNA to library. In addition, we have developed a robust and highly scalable sample indexing strategy supporting 96 libraries to be sequenced simultaneously with MiSeq. We also will demonstrate a new library quantitation and normalization approach, which allows 96 libraries to be adjusted to sequencing-ready concentrations in <1 hr without using expensive equipment such as a qPCR thermocycler or Bioanalyzer. These innovations streamline amplicon sequencing workflows, and provide unprecedented amplicon and sample multiplexing. Here we present data demonstrating high-accuracy detection of single-nucleotide polymorphisms (SNP; ⁄ 99.5% concordance with microarray and 1000 Genomes data) and structural variants, some of which are implicated in drug metabolism and cancer biology. Specifically, regions representing mutation hotspots in genes including *TP53*, *KRAS* and *BRAF* were sequenced and the mutations in control samples were accurately identified. Copy numbers of the *GSTM1* and *GSTT1* genes (implicated in the breakdown of drug and carcinogenic compounds) determined by this assay also exhibited excellent concordance with microarray data. Multiple types of genetic variation can now be studied comprehensively in a single rapid assay through this new targeted enrichment approach.

933F

Enhanced genome assembly using single molecule, nanochannel analysis. *J. Sibert, O. Hampton, R. Mohr, S. Billeru, H. Sadowski, J. Finklestein, M. Austin, M. Requa, D. Bozinov, H. Cao.* BioNanomatrix Inc., Philadelphia, PA.

Despite significant advances in short read sequencing technology, de novo genome assemblies using only short read data are generally incomplete due to the complexities found in large genomes. This complexity, which consists of large duplications and repetitive regions, complicates short read assemblies and subsequent comparative genomic analysis. We present a nanochannel array technology that can help resolve these difficulties by providing single molecule analysis of whole genomes at several hundred kilobase scale. The technology provides a visual map of the genome at scales that span repetitive elements and other complexities, allowing more complete short read scaffolding. The system consists of a high resolution imager, disposable nanochannel array chips and software for converting the raw image data into mapped results. The technology is demonstrated here though whole genome mapping of model organisms.

934F

Miniaturize Genotyping Reactions with the Echo® Liquid Handler. *T. Allison, C. Glazer, J. Barco, S. Datwani.* Labcyte, Sunnyvale, CA.

Advances in miniaturization of genotyping assays have tremendously improved reaction throughput, but not without challenges. Efforts to reduce reagent costs have led to higher risks for variability, cross-contamination and additional validation for new detection equipment. Tipless, touchless transfers with the Labcyte Echo® liquid handler eliminate the need for costly disposable tips, wash cycles and greatly simplifies assay development efforts. Precise and accurate drop placement eliminates cross-contamination, while offering the flexibility to transfer from any well of a source plate to any well of a destination plate. This work highlights the ability to transfer nanoliter quantities of PCR reagents for 384-well genotyping analysis utilizing the Roche LightCycler® 480 system. With zero cross-contamination as demonstrated by interleaving positive and negative controls, standard deviations less than 0.25, and CVs less than 1.25%, we demonstrate that the Echo platform ideally suited for miniaturized genotyping assays.

935F

Taking Life Technologies' SOLiD™ Total RNA Seq Kit to the Next Level. *D.M. Batten, N. Hernandez, C. San Jose Hinahon, J. Gu, K. Lea, J. Brockman, J. Schageman, P. Whitley, S. Heater, K. Bramlett.* Life Technologies, Austin, TX.

As next generation sequencing (NGS) technologies continue to evolve and become more readily available to the every day consumer, the need for simple, fast and flexible workflows is a necessary requirement. NGS users are no longer limited to the scientists in the lab but now include an array of consumers and the library preparation methods must be amendable to their equipment and capabilities. Life Technologies' SOLiD™ Total RNA-Seq Kit produces the highest quality RNA-Seq libraries for the NGS market. However, the workflow contains technically challenging steps, is time consuming and is not designed for processing large numbers of samples in parallel. To address these difficulties we introduced beads for purification and size selection, shortened the ligation time and created stable mastermixes to ease the transition onto XYZ platforms. These changes in workflow resulted in over a 82% decrease in the total number of user steps to create a whole transcriptome RNA-Seq library and reduced total bench time from 1.5 days to just over 5 hours. The data is of the same quality and scope as the original SOLiD™ Total RNA-Seq kit. In addition, we have included guidelines with specific details on using lower input RNA into the workflow and new fragmentation methods to allow for more sample flexibility. These changes to the library workflow make it simpler and faster with increased flexibility for the NGS user and demonstrate the evolution of the SOLiD™ Total RNA-Seq kit to generate high quality libraries.

936F

Exome capture of cDNA increases ability to detect splice junctions and low level transcription. *L. Feuk, J. Halvardson, A. Zaghlool.* Dept of Immunology, Genetics & Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden.

RNA sequencing has become a standard method to measure gene expression, identify alternative splicing and to characterize novel transcript. Compared to array based measures of expression, RNA-seq benefits from providing an unbiased view of the genome without the need to select probes representing putative exons. There is also less of a saturation effect in RNA-seq, and therefore a better linear increase from transcripts of low expression to transcripts exhibiting very high levels of expression. However, since there is a limited number of reads in a given sequencing experiment, highly expressed transcripts will use up a very large fraction of the reads, potentially at the expense of transcripts present at low levels. As an alternative way to measure transcription, we have converted RNA to cDNA and then used standard exome capture followed by sequencing. We find that this approach leads to detection of transcripts expressed at very low levels compared to RNA-seq of total RNA from the same samples. We also find that exome capture of cDNA leads to detection of a larger number of splice junctions, as more exons are represented in the sequencing results. Several novel human exons are also identified using this approach, primarily representing alternative splice variants or alternative 5' exons expressed at low levels. Replicate experiments of the same samples indicate that measures of expression are highly consistent between experiments, but that the relative expression between genes is a poorly correlated with total RNA-seq data from the same biological sample. By using data from exome capture of DNA, where we know that the copy number equals two, we find that we can partially normalize for probe hybridization efficiency and increase the correlation with total RNA-seq data. The results are also compared to quantitative PCR data as an independent measure of levels of expression. Our data indicates that sequence capture of cDNA is a useful method for specific applications and provides higher sensitivity than total RNA-seq for detection of low level transcription in human cells.

937F

Comparison of RNA fragmentation methods for RNA-Seq. *J. Gu, K. Lea, S. Heater, J. Schageman, K. Bramlett.* Life Technologies, Austin, TX.

RNA-Seq technology enables global transcript analysis in an unprecedented level of sensitivity, accuracy and complexity. Unfortunately, due to the limitation on the read lengths using current sequencing technologies, RNA/cDNA fragmentation step is indispensable in order to get uniformly distributed reads within the transcripts they represent. The assumptions underlying a number of RNA-Seq analysis approaches have been based on randomness and uniformity of RNA hydrolysis. However, recent data has revealed that both positional and sequence-specific biases exist in sequenced fragments. To evaluate the biases from different fragmentation methods, we constructed libraries from Hela polyA RNA using either enzymatic (RNase III) or chemical/physical fragmentation methods (Mg^{++}, Zn^{++}, heat or sonication) in combination with SOLiD™ Total RNA-Seq kit. Triplicate libraries from each method were sequenced on SOLiD™ V3.5 system with 25-35 million reads obtained from each library. Data analysis indicated similar mapping statistics across methods with the exception of sonication which gave the lowest percentage of mapped reads and detection sensitivity. Spearman correlations on RefSeq were overall high (>0.95) with the highest between heat and sonication methods (0.991). Enzymatic and chemical fragmentation (Mg^{++} and Zn^{++}) methods also showed very high correlation (0.986), which indicated comparable performance for transcript quantification. To evaluate complexity of libraries, percentages of unique genome positions were calculated. Results indicated that libraries from chemical fragmentation using Mg^{++} or Zn^{++} were the most complex ones with more than 60% unique genome positions, while libraries made by sonication were the least complex, with only about 40% unique positions. Further analysis on the nucleotide frequencies at RNA cleavage sites confirmed that chemical fragmentation generated the least bias. Our results indicated that even though the mechanisms of RNA fragmentation were very different for different methods, little impact has been observed on transcript quantification. However, chemical fragmentation using cation is more preferred for exon quantification as well as SNP detection since it provides relatively uniform coverage within the transcripts. To further reduce the biases, it requires not only improvements on fragmentation method, but also development of bioinformatic correction approaches.

938F

Enabling RNA-seq on the Ion torrent PGM. *B. Sanderson, N. Hernandez, D. Batten, J. Cienfuegos, J. Gu, S. Heater, K. Lea, L. Qu, C. San Jose, M. Gonzales, K. Bramlett.* Transcriptome Analysis Group, Ambion, Austin, TX.

The Ion Torrent, Personal Genome Machine (PGM) is a Next Generation Sequencing instrument with immense potential for both research and clinical purposes. As various applications for this platform are introduced and the Ion One Touch automated sample preparation system comes online, RNA whole-transcriptome library construction kits will provide an important addition to the functionality of the instrument. We will report on development work currently being completed which will enable RNA-seq on the PGM. We will also introduce the External RNA Controls Consortium (ERCC) spike-in RNA controls. The ERCCs consist of a blend of 92 RNA transcripts of varying lengths and concentrations which can be deployed to measure experimental sensitivity and ensure RNA library quality control. These RNA library reagents and controls, in conjunction with the PGM instrument capabilities and continuous workflow improvements, will empower users to achieve quality sequencing data that is an accurate representation of RNA content and expression levels in experimental samples.

939F

Detection of Genomic Components in a DNA Mixture using Allele-Specific Real-Time PCR Assays Targeting Insertion/Deletion Polymorphisms. *R. Fang, M. Schumaker, D. Merrill, SN. Liew, MR. Furtado.* Life Technologies 850 Lincoln Centre Drive Foster city CA 94044.

The need to discriminate between genomes of any two individuals is critical in post transplant chimeric research, forensic, and pharmacogenomic samples. The most commonly used methods, such as STR and FISH analysis, are labor intensive and involve multi-step workflows with sensitivity levels >1% (1 copy of allele A in 100 copies of allele B). We have developed a real-time quantitative PCR assay using TaqMan® for these applications. A panel of highly informative bi-allelic insertion/deletion (INDEL) markers were selected and used to design allele-specific real-time PCR assays. The resulting assays comprise an easy-to-use workflow that consistently reach a sensitivity level of <0.1%of minor genotypes. A panel of INDEL markers were chosen with a high minor allele frequency (0.2-0.5) across multiple populations. Allele-specific TaqMan® assays were developed using a propriety TaqMan® assay design algorithm and pipeline. Assays were screened for efficiency and specificity (deletion assay does not amplify DNA containing known insertion etc.) using ninety-one gDNA's consisting of African Americans and Caucasians. Assays meeting specific requirements were first tested on DNA mixtures which consisted of 14, 2-fold serial dilutions of “recipient” DNA in “donor” DNA and vice versa. Dilutions ranged from 1:0 to 1:8192 (250 ng to 15 pg). Twenty-nine INDEL assays were subsequently tested on three CEPH families. Our results show that these assays can sensitively detect a minor genotype in a mixture with high reliability. The maximum sensitivity attained for a single INDEL assay set was 0.006%; (15 pg of minor component in 250 ng of the major component). The polymorphisms selected are also highly genetically informative and comparable with the STR technology. When testing the CEPH samples, the average probability of identification between two individuals in a mixed sample was determined to be 4.0 x 10e-11. In addition, the INDEL assays we sued had genotype ratios conforming to Hardy-Weinberg expectations. In summary, the assay panel we developed can be used for rapid, sensitive, and accurate quantitative measurement of mixed genomic samples and can be effectively used in tissue or cell transplantation research.

940F

LifeScope software; easy analysis of high accuracy next-generation sequencing for the 5500 Genetic Analyzer using ECC chemistry. *F.C.L. Hyland, S. Utiramerur, H. Breu, Y. Lou, C. Yang, M. Sikora, S. Datta, X. Xu, E. Tsung, D. Brinza.* Biological Information Systems, Life Technologies, Foster City, CA 94404.

A substantial barrier to adoption of next-generation sequencing technologies is the challenge of data analysis. In addition to computational, algorithmic, and application needs, the rapidly growing throughput of next-generation sequencers necessitates sample management, reference management, and project management, as well batch analysis of many samples. These needs are addressed by the new LifeScope software. LifeScope™ software provides support for a number of common research workflows, including whole genome sequencing (mapping, SNP and InDel detection, and detection of structural variations, copy number variation and inversions, and annotation); targeted resequencing (mapping, enrichment reporting, SNP and indel detection and annotation); whole transcriptome analysis (customized mapping to genome and exon junctions: reporting of coverage, gene and exon counts, and detection and annotation of exon junctions and gene fusions); small RNA analysis (customized mapping, detection and annotation of known and novel microRNAs, including counts and coverage) ; and mapping for applications such as methylation, ChipSeq, and SAGE. LifeScope software is available via cloud computing, or on local hardware. Rich summary statistics and graphical reporting of results are available for QA and for a fast overview of an experiment.

Emerging high throughput next-generation sequencing technologies provide the ability to sequence genomes at high coverage and low cost. The extremely high accuracy available on the 5500 Genetic Analyzer, enabled by the new Exact Call Chemistry (ECC), and advanced decoding algorithms, makes possible research applications that rely on high accuracy, such as accurate calling of SNPs, insertions and deletions; low frequency variant detection, including discovery of somatic mutations in cancer cells; detection of exon junctions and gene fusions, and de novo assembly. LifeScope software embeds two algorithmic approaches to maximize accuracy with the ECC chemistry and to produce up to 99.99% accurate base sequence. (i) The first is enabled by the redundancy inherent in high coverage color-space data. (ii) The second is a BCJR decoding algorithm for ECC data, using multiple orthogonal readings of the same DNA fragment.

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941F

Low-Cost, High-Throughput DNA Fingerprinting for DNA Quality Control in a Biobank Setting with Fluidigm SNPtype™ Assays. *S.N. Meyers[1], K. Ilic[1], X. Wang[1], G. Sun[1], W. Hwang[1], Y. Yi[1], J.A. Tischfield[2], A.I. Brooks[2], M.A. Unger[1], R.C. Jones[1], J. Wang[1].* 1) Fluidigm Corporation, South San Francisco, CA USA; 2) Rutgers University Cell and DNA Repository, Human Genetics Institute of New Jersey, Department of Genetics, Rutgers University, Piscataway, NJ USA.

Analytical and functional quality control and assurance are of paramount importance to all biorepositories and investigators using biobank samples for a variety of basic science and clinical applications. All archived and distributed samples must be of high quality, suitable for downstream applications and properly annotated. Analytical quality assessment of genomic DNA can quickly be performed by a variety of established methods. However, such methods cannot reveal potential sample contamination or mislabeling issues, nor can they assess sample performance for future PCR-based applications such as genotyping or next-generation sequencing. To this end, we have developed a rapid, reliable, low-cost and high-throughput DNA fingerprinting method for use in functional quality control. This solution pairs the ease and flexibility of Fluidigm Dynamic Array™ Integrated Fluidic Circuits (IFCs) with a new, low-cost genotyping panel of 96 Fluidigm SNPtype™ Assays for the determination of sample contamination, uniqueness, gender and ethnicity. New software features have also been implemented for data management and analysis. Here we describe the use of this product for assessing the quality of human gDNA samples in a biorepository setting and demonstrate data concordance with existing TaqMan® SNP Genotyping Assays. Call rates >99% as well as >99.75% accuracy have been achieved, qualifying this method as a standard quality control procedure for use by all biorepositories and investigators analyzing DNA.

942F

Using whole blood to create induced pluripotent stem cells (iPSCs) for the study of Autism spectrum disorders. *B. DeRosa[1], J.M. Vance[1,2], M.A. Pericak-Vance[1,2], D.M. Dykxhoorn[1,2].* 1) John P. Hussman Institute For Human Genomics, University of Miami Miller School of Medicine, Miami, FL; 2) John T. Macdonald Foundation Department of Humans Genetics, University of Miami Miller School of Medicine, Miami, FL.

Autism spectrum disorder (ASD) refers to a group of neurodevelopmental disorders which are characterized by impaired reciprocal social interaction, repetitive movements, and difficulties in communication. The rapid development of high content genotyping and next generation sequencing platforms has made it possible to identify a variety of genetic variations that are correlated with ASD, including rare variants and copy number variants (CNVs). However, it is often unclear what role these variations are playing in the pathophysiology of autism. This difficulty in understanding the biological role that specific genetic variations are playing in the development of autism is compounded by the lack of appropriate model systems and tissue from patients. iPS technology, experimentally-derived stem cells produced from somatic cells by the enforced expression of a cocktail of transcription factors, could prove to be a powerful approach to uncover the impact that specific genetic variations have in development of key brain cell types . A collection of iPSC lines were developed from a diverse cohort of individuals with ASDs. To avoid the need for skin biopsies, we developed a protocol to derive iPSC lines from peripheral blood mononuclear cells. To investigate the impact that ASD may have on cellular functionality of these developing cells, these pluripotent lines were differentiated into GABAergic and glutamatergic neurons. These cells were investigated for mechanisms involving neurodevelopment, neuron morphology, and synaptic activity.

944F

Changing facial phenotype in Cohen syndrome: towards clues for earlier diagnosis. *S. EL CHEHADEH-DJEBBAR[1], E. BLAIR[2], M. HOLDER[3], A. MONCLA[4], A.M. FRANCES[5], M. RIO[6], F.G. DEBRAY[7], P. RUMP[8], A. MASUREL-PAULET[1], N. GIGOT[9], P. CALLIER[10], L. GUENEAU[11], L. DUPLOMB[11], B. ARAL[9], F. HUET[1], C. THAUVIN-ROBINET[1,11], L. FAIVRE[1,11].* 1) Centre de génétique, CHU de Dijon, Dijon, France; 2) Department of Clinical Genetics, Churchill Hospital, Headington, Oxford, United Kingdom; 3) Service de Génétique, Hôpital Jeanne de Flandres, Université de Lille, Lille, France; 4) Département de Génétique Médicale, Hôpital d'Enfants de la Timone, Marseille, France; 5) Service de Génétique, Hôpital Font-Pré, Toulon, France; 6) Département de Génétique, Hôpital Necker - Enfants Malades, APHP, Paris, France; 7) Service de génétique humaine, CHU Sart Tilman, Liège, Belgique; 8) Department of Genetics, University Medical Center, Groningen, The Netherlands; 9) Laboratoire de Génétique Moléculaire, Plateau Technique de Biologie, CHU Dijon, France; 10) Laboratoire de Cytogénétique, Plateau Technique de Biologie, CHU de Dijon, France; 11) Equipe GAD, Université de Bourgogne, Dijon, France.

Cohen syndrome (CS) is a rare autosomal recessive condition caused by mutations and/or large intragenic rearrangements in the VPS13B gene and characterized by mental retardation, typical facial dysmorphism, childhood hypotonia, post-natal microcephaly, truncal obesity, slender extremities, joint hyperextensibility, myopia, progressive chorioretinal dystrophy and intermittent neutropenia, which are the two hallmarks of this syndrome. Typical facial dysmorphism tends to evolve with age and comprises thick hair and eyebrows with low hairline, downward slanting and wave shaped palpebral fissures with long eyelashes, and a prominent and beak shaped nose with high nasal bridge. The philtrum is usually short and upturned leading to an open mouth expression and appearance of prominent central incisors with a grimacing smile. The diagnosis is generally raised during adolescence, when chorioretinal dystrophy is diagnosed, therefore excluding accurate genetic counselling and reproductive choices. The aim of this work is to analyse facial dysmorphism through ages, in order to try to find clues for earlier diagnosis. Photographs of 18 patients with molecularly proved CS were transmitted by the parents, from birth to school age, and when possible to adulthood. Comparing their facial phenotype at subsequent ages together with literature data, we confirmed that the typical facial characteristics of CS are usually not noticeable before 3 years of age. However infants with CS already share some common facial features that tend to change with advancing age. We also observed older patients presenting with an atypical facial phenotype, making the diagnosis difficult to suggest on clinical examination only. These observations emphasize the importance of neutrophils blood count and ophthalmologic tests, especially electroretinography, in the work-up for children in preschool age presenting with developmental delay, persistent hypotonia and the described facial features, for earlier diagnosis of CS.

945F

ZEB2 missense mutations leading to an unusual presentation of Mowat-Wilson syndrome with moderate intellectual disability. *J. Ghoumid[1,2], A. Briand-Suleau[1,2], L. Drevillon[1,2], L. Goodwin[3], A. Moncla[4], P. Raymond[1], N. Bondurand[2], A. Amiel[5], M. Goossens[1,2], S. Lyonnet[5], D. Mowat[6], I. Giurgea[1,2].* 1) Department of Genetics, Hôpital Henri Mondor, Créteil, France; 2) INSERM U955, IMRB équipe 11, Hôpital Henri Mondor, Créteil, France; 3) Department of Clinical Genetics, Nepean Hospital, Sydney, Australia; 4) Département de Génétique Médicale, Hôpital des enfants de la Timone, Marseille, France; 5) Département de Génétique et INSERM U781, Hôpital Necker Enfants-Malades, Paris, France; 6) 5Department of Clinical Genetics, Sydney Children's Hospital, Sydney, Australia.

Mowat-Wilson syndrome (MWS, MIM#235730) is an intellectual disability-multiple congenital anomalies syndrome characterized by severe intellectual disability, epilepsy, agenesis of the corpus callosum, distinctive facial dysmorphism, heart defects, urogenital malformations and Hirschsprung disease. ZEB2 truncating mutations or deletions are disease causing. SIP1 (Smad Interacting Protein 1), the encoded protein, is a two-handed zinc finger/homeodomain transcriptional factor. Two separate clusters of zinc fingers have been characterized (in N-terminus and in C-terminus), and both must bind for transcriptional regulation. SIP1 target genes are numerous, and the best characterized is E-cadherin. SIP1 is early expressed by several tissues during embryonic development, including the neural crest, neuroepithelium, limb buds. We report two patients with heterozygote missense ZEB2 mutations (c.3164A>G, p.Tyr1055Cys; c.3211T>C; p.Ser1071Pro) localized in the C-terminus zinc finger, and possibly resulting in ADN/protein interactions. We performed in vitro functional tests, using the luciferase reporter system. The p.Ser1071Pro mutation abolished transcriptional repression of SIP1 on the E-Cadherin promoter, whereas, the p.Tyr1055Cys mutation, decrease this repressive activity in one neuroblastoma cell line (SHSY-5Y) only. Functional results correlate with patients' phenotypes, as the patient with the p.Ser1071Pro mutation was more severely affected (more obvious facial dysmorphy and epilepsy) than the patient with p.Tyr1055Cys, which presented only moderate intellectual disability and mild dysmorphism. In conclusion, we report a less severe clinical presentation of MWS with moderate intellectual disability and no malformations ascribed to ZEB2 missense mutations.

946F

Long-term clinical follow-up of a boy with a *de novo* deletion 12p13.33-p13.32 and duplication 12p13.2-p11.1 characterized by microarray-CGH. *A. Iglesias.* Dept Pediatrics, Div Gen, Beth Israel Med Ctr, New York, NY.

Deletions/duplications of chromosome 12p are extremely rare. A patient is presented. A full term boy was born vaginally after an normal pregnancy. Birth weight was 2,880 gr., length 46.5 cm and head circumference (HC) 32 cm. Jaundice, thrombocytopenia, pulmonary hypertension and persistent ductus complicated the newborn period. Except for the neonatal thrombocytopenia, all complications resolved. No major dysmorphic features were noted except beaked nose and apparent hypertelorism. Family history was negative. At one month of age his weight, length and HC were in the 5th percentiles. Folded helices, beaked nose, hypertrichosis in his back and arms, and 7 café-au-lait (CAL)spots larger than 0.5 cm were noted. Development was appropriate. Chromosomes were 46, XY, add (12)(p13.3). Parents were normal. Microarray-CGH showed a *de novo* 2 Mb del 12p13.33-p13.22 and a 23.4 Mb dup 12p13.2-p11.1. Follow-up was done at 4 ½, 7 ½, 12, and 22 months. Progressive delay in milestones, growth retardation and microcephaly developed after the first 3-4 months of life. At 6 months, dysmorphic features included microbrachycephaly, narrow forehead, thin eyebrows, horizontal palpebral fissures, hypertelorism, depressed nasal root, beaked nasal bridge, bulbous nasal tip, anteverted nares, short philtrum, thin upper vermilion, folded helix, left supernumerary nipple, umbilical hernia, hypertrichosis, 7 CAL spots larger than 0.5 cm and muscle hypotonia. No other features of neurofibromatosis type 1 (NF1) were seen. Questionable small testicles were noted lately. Growth and development while on therapies and dietary supplements have being steady, but poor. Currently, he is a 22 month-old boy with growth retardation, microcephaly, developmental delay and dysmorphic features with a de novo complex chromosome 12 aberration. To our knowledge, this is the first reported patient combining a deletion/duplication in chromosome 12p encompassing bands 13.33-13.32 for the deletion and a larger duplication at 13.33-13.11.1. Although phenotypic features share findings previously found in both deletion and duplications 12p, his phenotype seems unique. Moreover, it has been evolving over time and it seems to be currently well established. The presence of the café-au-lait spots has not been reported in previous cases and is intriguing. Continuing his clinical follow-up and testing for NF1 will be done to better assess his phenotype and to better characterize this chromosome aberration.

947F

A Population Study of Prader-Willi Syndrome in Chinese. *S. Lam, H. Luk, T. Tong, K. Lai, I. Lo.* Department of Health, Clinical Genetic Service, Hong Kong, Hong Kong, Hong Kong.

Background: This is a retrospective study of all Chinese Prader-Willi Syndrome (PWS) patients diagnosed at Clinical Genetic Service (CGS), the only genetic referral centre in Hong Kong, from 1995 to 2010. PWS is a complex neurodevelopmental disorder with multisystem involvement. It is caused by haploinsufficiency of paternally expressed genes in the imprinted domain on chromosome 15q11-q13, as a result of mainly three different genetic mechanisms, namely paternal microdelection, maternal uniparental disomy (UPD) and imprinting centre defect. DNA-based methylation analysis of the SNRPN locus by methylation specific polymerase chain reaction (MS-PCR) is the mainstay of genetic test which is positive for over 99% of cases. Fluorescence in-situ hybridization (FISH) is used to confirm microdeletion. Microsatellite analysis is used to confirm maternal UPD. Imprinting centre defect is diagnosed by exclusion. Population studied: A total of 62 cases of PWS were confirmed by methylation study in CGS from 1995 to 2010, 55 of whom were Chinese. The total estimated Chinese births in Hong Kong during this period was 902,224, the incidence of Chinese PWS was hence 1 per 16,404 live births, which is comparable to other parts of the world. Genotype and phenotype analyses: In this cohort of Chinese PWS, 57% was caused by paternal microdeletion, 41% by maternal uniparental disomy and 2% by imprinting center defect. For their clinical features, 89% had severe hypotonia and 77% have significant feeding difficulty during the neonatal period. Early onset hyperphagia and obesity developed in 77% of cases. 51% had moderate- severe intellectual disability. For the 14 adults in this cohort, 14% developed type 2 diabetes mellitus that required therapy. Conclusion: This is the first population study of PWS in Chinese. The incidence, clinical features and pattern of underlying genetic defect is comparable with other western populations. Early accurate diagnosis and multidisciplinary management are important for caring PWS patients. With more understanding of underlying pathophysiology and the use of growth hormone among selected PWS patients, it is expected that their quality of life and life expectancy will be greatly improved.

948F

Deletion of the MBD5 gene is associated with a distinct phenotype. *G.J. Noh, J.M. Graham, Jr.* Medical Genetics Institute, Cedars-Sinai Medical Center, LA, CA.

Epilepsy is one of the most common and genetically intricate neurological disorders in children. Previous studies have shown that chromosomal abnormalities impart susceptibility to epilepsy. Since the introduction of the array CGH, several new deletion and duplication syndrome have been delineated, giving rise to clinically recognizable phenotypes. We report a 2 year old female who initially presented with seizures, developmental delay and dysmorphic features who was found to have a 0.3 Mb deletion at chromosome 2q23.1 encompassing the critical seizure gene, MBD5. Her distinct physical features include bifrontal narrowing with brachycephaly, low anterior hairline, hypotonic facial features with short upturned nose, flat nasal bridge, hypertelorism, tented upper lip with everted lower lip, downturned corners of her mouth, and relatively coarse facial features including thickened tongue. She also had a short neck, brachytelephalangy, clinodactlyly, and hypertrichosis. At 3 ½ years of age, she developed progressive ataxia; and at the age of 4, was able to stand only when holding onto objects when she had starting walking at the age of 2. She also lost vocabulary that she had previously acquired, placing her language skills at the 24 month level. Regression has been reported in one other case of MBD5 deletion. MBD5 is a member of the methyl binding gene family and appears to be responsible for regulating DNA methylation in the central nervous system. Our patient was entirely deleted for this single gene, which suggests that haploinsufficiency of MBD5 is responsible for the distinct phenotype observed. This supports the hypothesis that MBD5 is indeed the critical gene implicated for the findings seen in patients with deletions of chromosome 2q23.1. Further studies are necessary to delineate the role that the MBD5 gene plays in the development of the brain and physical characteristics.

949F

Copy Number Variation Analysis in Cohesion/Condensin genes by a CGH in Cornelia de Lange Syndrome Patients. *D. Pehlivan[1], M. Hullings[2], C.M.B. Carvalho[1], C.G. Gonzaga-Jauregui[1], E. Loy[2], L.G. Jackson[3], I.D. Krantz[2,4], M.A. Deardorff[2,4], J.R. Lupski[1,5,6].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Division of Human Genetics, The Children`s Hospital of Philadelphia, Philadelphia, Pennsylvania, USA; 3) Department of Obstetrics and Gynecology, Drexel University School of Medicine, Philadelphia, Pennsylvania, USA; 4) University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA; 5) Department of Pediatrics, Baylor College of Medicine, Houston, Texas, USA; 6) Texas Children`s Hospital, Houston, Texas, USA.

Cornelia de Lange syndrome (CdLS) is a multisystem malformation syndrome diagnosed primarily on the basis of characteristic facial dysmorphism, intellectual disability/developmental delay, extremity abnormalities and hirsutism. About 60-65% of the cases of CdLS are due to mutation in the NIPBL, SMC1A and SMC3 genes. Since it has been shown that NIPBL is a dosage sensitive gene, we proposed that large genomic rearrangements of cohesion/condensin complex subunit genes may play a role in the molecular etiology of CdLS. We designed an Agilent Custom 8x60K comparative genomic hybridization (CGH) array to analyze copy number variations (CNV) in all cohesion and condensin complex subunit genes; 46 genes and 50 kb up/down stream of those genes have been in our array. To date, array CGH was performed in 162 patients with CdLS, for whom mutations in known CdLS genes were previously negative by sequencing. In seven subjects deletions containing NIPBL exons, ranging in size from 4 kb to 750 kb, were detected (~5%). Breakpoint junctions were obtained in five out of seven patients; which implicated microhomology mediated replicative mechanisms (such as SRS and FoSTeS/MMBIR) as a predominant contributor to these CNVs. Genotype-phenotype correlations suggest patients with larger deletion tend to have more severe growth retardation than those smaller deletions. In two patients we found deletion in NCAPD3 of 3 kb and 6 Mb sizes. Breakpoint analyses showed a 3 kb deletion involving exons 8 to 11; apparently mediated by FoSTeS/MMBIR. Parental studies revealed that the mother carries the same deletion. Another patient was foundto have a 3.3 Mb deletion including the RAD21 gene, in twelve patients deletion and duplication CNVs of exon 2 of SMC4 were identified, and in one patient 10 kb in size deletion of 30 kb upstream of SMC6 and in one patient 3 kb in size deletion of 250 bp downstream of PTTG1 were detected. These findings suggest the identifcation of rare CNV by aCGH may be a cost-effective approach to discover additional CdLS genes. RAD21, NCAPD3, PTTG1, SMC6 and SMC4 are amongst potential candidate genes for the remaining cases. Furthermore, exonic deletion rearrangements of NIPBL are responsible for ~5% of cases. Thus, screening for CNVs may be clinically prudent in mutation negative CdLS cases. Replicative mechanisms, especially FoSTeS/MMBIR, appear to be a prominent underlying mechanism for NIPBL deletion CNVs. Larger deletions convey a more severe phenotype.

950F

Nail-Patella Syndrome (NPS): Of a New Case. *E.J. Ramirez-Lizardo[1,2], S.E. Totsuka Sutto[1], T.A. Garcia Cobian[1], L. Garcia Benavides[1], D. Roman Rojas[3], E.G. Cardona Muñoz[1].* 1) Unidad de Inv Cardiovascular, CUCS Univ de Guadalajara; 2) Instituto Jalisciense de Cirugia Reconstructiva, SSJ; 3) Doctorado Farmacologia CUCS.

Nail-Patella syndrome (NPS; OMIM #161200), also known as Hereditary Osteoonychodysplasia, Turner-Kieser syndrome, Fong disease, is a rare clinical entity autosomal dominant, is due to mutations in the LMX1B gene, is a pleiotropic condition with a classic clinical tetrad of dysplastic finger nails, absent or hypoplastic patellae, elbow dysplasia and iliac horns (bony protuberances of the iliac). In addition abnormalities such as renal dysplasia, muscle weakness, impaired hearing, wadding gate, scapular wining and short stature may be present. The incidence is estimated at 1 in 50,000 live births. We describe a 8-years old female, who was referred to plastic surgery by bilateral antecubital pterygia. She was born after a 40-week gestation, abdominal delivery was normal without complications, psychomotor development was normal. At physical examination showed normal weight and height, enamel abnormalities, dental caries, bilateral antecubital pterygia, fingers nails dysplastic, short Achilles tendon. Radiology evaluation showed hypoplasia patellae and iliac horn. The diagnosis of NPS is based on clinical finding. Molecular genetic testing of LMX1B, the only gene known to be association with NPS, is avaible on clinical basis. The present case show a patient with NPS supported on the clinical data of dysplastic finger nails, hypoplasia patellae, bilateral antecubital pterygia and iliac horn. The history family not reveled similar clinical finding in other relatives, the father was 33 and the mother was 32 years of age at the time of birth so the autosomal dominant inheritance or germline mosaicism cannot be discarded.

951F

Genetic testing of Ras/MAPK pathway syndromes at Tohoku University. *Y. Saito[1], Y. Aoki[1,2], T. Niihori[1], Y. Abe[1], S. Kure[1], H. Ohashi[2], K. Kurosawa[2], N. Okamoto[2], H. Kawame[2], S. Mizuno[2], T. Ogata[2], S. Kuriyama[2], Y. Matsubara[1,2].* 1) Dept Med Genet, Tohoku Univ Sch Med, Sendai, Japan; 2) The Costello/CFC/Noonan Study Group, Japan.

Growing attention to Ras/MAPK pathway syndromes (RASopathies; Costello syndrome, CFC syndrome, and Noonan syndrome) and discoveries of new disease-causing genes in recent years result in sharp increase of requests for genetic testing of these disorders. Molecular diagnosis of these syndromes is often challenging because of their overlapping clinical symptoms and numerous pathogenic genes (PTPN11, SOS1, SHOC2, HRAS, KRAS, BRAF, MEK1/2, NRAS, CBL). Although ultimate solution would be the application of next-generation sequencing, the cost is prohibitively high at the moment. We have been providing genetic testing of Ras/MAPK pathway syndromes since our discovery of HRAS mutations in Costello syndrome (2005) and KRAS/BRAF mutations in CFC syndrome (2006). The tests are offered for free of charge on a research basis, because the cost is not subsidized by the national health insurance service in Japan. To maximize the efficient use of limited resource, we use practical flow charts for the molecular diagnosis of these elusive disorders. At first, detailed clinical information and photographs of the patients are collected and carefully evaluated by a group of experienced clinical dysmorphologists to exclude other anomaly conditions and to select most appropriate set of candidate genes to be analyzed. The “Noonan set” includes selected exons of PTPN11, KRAS, SHOC2 and RAF1 genes. The “Costello/CFC set” includes selected exons of HRAS, BRAF and MEK1/2 genes. When no mutation is identified by either set, all coding exons in SOS1 are analyzed. Mutation-negative cases are re-evaluated by dysmorphologists, analyzed for other missing exons of relevant genes, and subsequently subjected to exome sequencing by next-generation sequencer to search for novel disease-causing genes. We found thorough clinical evaluation of the case is one of the most critical and important steps in efficient genetic testing.

952F

A case of hemiparesis, hemihypoplasia, and athetoid posture with unilateral vision loss - An example of a very rare disorder: familial porencephaly. *H. Ulucan[1], A. Koparir[1], M. Seven[1], E. Yosunkaya[1], G. Guven[1], M. Ozen[1], A. Yuksel[2].* 1) Dept of Medical Genetics, Cerrahpaşa Medical Faculty of Istanbul University, Fatih, Istanbul, Turkey; 2) Dept of Pediatrics, Medical Faculty of Bezm-i Alem University, Istanbul, Turkey.

We describe a 33-year-old female referred to us for unilateral vision loss, suggested to be Leber's optic atrophy by optalmologists.Her medical history and physical examination revealed that she suffered from unilateral hemiparesis, hemihypoplasia,and athetoid posture affecting the right side of her body since infantile period. Her mother described a single occurrence of non-febrile convulsion when she was 13 years old. Family history is remarkable with a maternal grand-mother with hemiplegia,multiple paternal relatives with migraine, a paternal female cousin with mental retardation, and a younger brother deceased from ischemic encephalopathy, and a living sister with mild unilateral hemihypoplasia. Magnetic resonance imaging of the current case showed a cranial cyst, and pulmonary stenosis appeared on echocardiography. Familial porencephaly is a very rare developmental abnormality of the brain, which is mainly characterized by cranial cysts, hydrocephaly, hemiplegia, seizures, and visual defects. We initially considered this disorder in differential diagnosis for the present case. Additionally, hereditary non-progressive athetoid hemiplegia (HHHH), which was reported once in 1977, is a very rare disorder,characterized by hemiparesis, hemihypoplasia, and unilateral atheoid posture. However, in succeeding case reports, HHHH was also interpreted as infantile hemiplegia with porencephaly. Conclusively, we are conducting molecular studies for Leber's optic atrophy and for collagen type IV, alpha-1 gene (COL4A1) mutations, reported to be responsible from familial porencephaly.

953F

Mandibulofacial dysostosis and bowed femora: a new syndrome. *K.N. Weaver[1,2], E.A. Sellars[1,2], P.L. Bender[1], C.B. Gordon[3,4], H.M. Saal[1,2,3].* 1) Div Human Genetics, Cincinnati Children's Hosp Med Ctr, Cincinnati, OH; 2) Department of Pediatrics, Cincinnati Children's Hosp Med Ctr, Cincinnati, OH; 3) Division of Plastic Surgery, Cincinnati Children's Hosp Med Ctr, Cincinnati OH; 4) University of Cincinnati College of Medicine, Cincinnati, OH.

At least five mandibulofacial dysostoses have been described in the literature, with Treacher Collins Syndrome being the best-known of these disorders. Common features to all include variable degrees of maxillary and mandibular hypoplasia, defects of the orbits and eyelids, ear anomalies, and hearing loss. A related group of disorders are the acrofacial dysostoses, which have similar craniofacial anomalies in addition to distal limb defects. We report a patient with severe mandibulofacial dysostosis and bowed femora, which is a combination of features that has not previously been described. The patient was identified prenatally as having craniofacial anomalies including severe micrognathia necessitating delivery via EXIT procedure, with a tracheostomy performed prior to discontinuation of placental circulation. Initial physical examination showed bilateral lower eyelid colobomas, bilateral anotia, maxillary hypoplasia, micrognathia, complete clefting of the secondary palate, and short lower limbs with bowed femora. Colobomas were so severe that there was essentially no lower eyelid present. There were no radial or distal limb anomalies. Facial CT with 3D reconstruction revealed bilateral absence of the mandibular rami and zygomata. Genetic testing has included normal chromosomes, SNP microarray, and TCOF1 sequencing. The family history was negative for deafness, cleft lip, cleft palate, or other birth defects. This patient's features are not consistent with any of the previously described mandibulofacial or acrofacial dysostosis syndromes because of the severity of the craniofacial defects and the presence of femoral abnormalities without pre- or post-axial limb defects. To our knowledge, this is the first reported case of a patient with this constellation of anomalies and therefore it likely represents a new mandibulofacial dysostosis. It is our hope that further cases can be identified to help delineate etiology and natural history.

954F

Birth prevalence rates of osteochondrodysplasias (OCD) in South America (SA): an epidemiologic study in a large population. *C.O.B. Buck[1], I.M. Orioli[2], E.E. Castilla[3,5], J.S. Lopez-Camelo[4], M.G. Dutra[5], D.P. Cavalcanti[1].* 1) Depto. Genética Médica, FCM/Unicamp, Campinas, São Paulo, Brazil; 2) Dept de Genética - Laboratório de Epidemiologia de Malformações, UFRJ, Rio de Janeiro, Rio de Janeiro, Brazil; 3) Dirección de Investigación, CEMIC: Centro de Educación Médica e Investigaciones Clínicas, Buenos Aires, Buenos Aires, Argentina; 4) Dept Genética, IMBICE: Instituto Multidisciplinario de Biologia Celular, La Plata, La Plata, Argentina; 5) Laboratório de Epidemiologia de Malformações Congênitas, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Rio de Janeiro, Brazil.

The osteochondrodysplasias (OCD) birth prevalence rate of 2.0/10,000 is underestimated. This study aimed to assess OCD epidemiology using a large population in SA with the ECLAMC (a case-control, collaborative hospital-based program for birth defects) data. All OCD cases from ECLAMC files born between 2000-2007 were revised and diagnosis ranked in five evidence levels, taking level 1 as gold-standard, having available X-rays or DNA test. EpiInfo™3.5.8 was used for statistical analysis, and significance was defined as p value < 0.05. For comparative analysis all controls born in the same period were used. Prevalence rates are shown by 10,000 births. After excluding 44 from 536 ascertained cases, the prevalence in 1,544,496 births was 3.2 (CI 2.9 - 3.5). Lethal cases were 50% (244/492 - 65 stillbirths plus 179 cases with early neonatal death). Prenatal diagnosis was made in 73% cases. Among the 211 cases from level 1, the main OCD groups according the ISDS were: G-25(OI) - 33% (59% OI-II); G-1(FGFR3) - 29.5% (56% Thanatophoric D); G-2(Collagen 2) - 6.7% (29% Achondrogenesis 2); G-18(Bent bones) - 8% (71% Campomelic D); G-9 (SRP) - 5.7%. The prevalence of the main OCD types were: OI - 0.72 (0.59 - 0.87); Thanatophoric D - 0.47 (0.36 - 0.59); and Achondroplasia - 0.45 (0.34 - 0.56). Paternal age (31.2 yo), parity (2.6) and consanguinity rate (5.4%) were all increased in cases ($p < 0.001$). Birth weight (2,498 g) was lower in cases, however differences were found just for gestational age over 31 weeks ($p < 0,001$). In conclusion, the OCD overall prevalence rate of 3.2 per 10,000 found seems to be more real than the usually cited value of 2.0. This study also indicates a high rate of prenatal OCD diagnosis in SA, confirmed the overall high infant morbimortality, and the association with paternal age. Finally, a high parity and rate of parental consanguinity was observed.

955F

Molecular study of Iranian patients affected with Dystrophic Epidermolysis Bullosa. *M. Mahdavi, N. Hatamnejad, B. Sedaghati khayat, S. Abadpour, A. Yasari mazandarani, S. Matoo, A. Tavakoli tameh, A. Ebrahimi.* Parseh Medical Genetics Counselling Center, tehran iran, tehran, Iran.

Molecular study of Iranian patients affected with Dystrophic Epidermolysis Bullosa Mahdieh sadat Mahdavi; N.Hatamnejad; B.Sedaghati; Sh.Abadpour; A.Yassari; S.Matoo; A.Tavakoli; A.Ebrahimi Parseh Medical Genetics Counselling Center, Floor 7, No.75, Royan Alley,Keshavarz Bolv. Tehran, Iran Tel-Fax: +98 21 88966579,88996889 Coresponding Author:Dr. Ahmad Ebrahimi Molecular Genetics , PhD E-mail: ae35m@yahoo.com Epidermolysis bullosa is a severe skin disorder beginning at birth with several organs involvement. This is a life time onset disease that has a progressive pattern. Therefore molecular diagnosis, provide feasibility of differential diagnosis, help to controlling of the singes, suitable cure, , PND or PGD. The COL7A1 is the only responsible gene in which about 75% of mutations occur in 73-75 exons. Methods: Fifty affected patients recognized by clinical examination and antigen-antibody mapping were selected. Blood samples were collected based on genetic counseling and clinical signs then DNA was extracted. All selected coding regions of COL7A1 (73-75 exons) were amplified by intronic PCR primers and products analyzed by direct sequencing. Results: The results show that only 30% of patients have alterations in these regions of Col7A1. But direct sequencing of PCR products, showed some new sequence variations too. No large deletion was detected. This study revealed that according to heterogeneity of Iranian population the whole gene sequencing is needed. Key words: Epidermolysis, Dystrophic, COL7A1, PCR.

956F

A *de novo* microduplication at 2q24.3 including *SCN2A* and *SCN3A* but not *SCN1A* in a patient with severe epilepsy and profound hypotonia. *M.N. Strecker[1], M. Chadehumbe[2], G. Parsons[3], P. Mark[3], S. Gunn[1], K. Hovanes[1].* 1) CombiMatrix Diagnostics, Irvine, CA; 2) Helen DeVos Children's Hospital, Grand Rapids, MI; 3) Spectrum Health Medical Group, Grand Rapids, MI.

We present a 29 month old female with severe epilepsy, global developmental delay, and profound hypotonia. **CLINICAL HISTORY** Seizures began at one month of age. EEG revealed a modified hypsarrhythmia pattern. Brain MRI demonstrated possible decreased peritrigonal white matter volume. The patient was diagnosed with infantile spasms and placed on levetiracetam and steroids, to which she had no response. She was subsequently switched to topiramate and experienced a 6 month seizure-free interval, at which point she was weaned off of topiramate. Seizures returned at 21 months of age and topiramate was restarted. Since then, she has had good seizure control. **LABORATORY STUDIES:** The patient has profound hypotonia. At 29 months of age, she continues to have significant head lag and is not able to sit unsupported. Previous testing included karyotyping, screening for inborn errors of metabolism and a muscle biopsy to look for evidence of a mitochondriopathy, all of which was normal. A 180K oligonucleotide chromosomal microarray was performed and revealed a 418 kb duplication: 2q24.3(165,602,448-166,020,886)x3, encompassing the sodium channel, voltage-gated, type II subunit alpha (*SCN2A*) and type III alpha (*SCN3A*) genes. Parental microarray studies were normal, indicating a de novo event in the child. **GENOTYPE/PHENOTYPE CORRELATIONS:** *SCN1A* and *SCN2A* have been implicated in a number of seizure disorders. The role of *SCN3A* in relation to seizure disorders is less clear. Functional studies of pathogenic *SCN2A* mutations (such as I1473M) demonstrate altered electrophysiologic channel properties leading to hyperpolarization of the neuron. Since *SCNA2* is highly expressed in the brain and skeletal muscle, we postulate that the duplication of *SCN2A* results in an increased number of sodium channels along the cell surface which leads to ultra-rapid cellular hyperpolarization. In the neuron, this manifests as neuronal hyperexcitability causing epileptiform discharges, and in the striated myocyte, leads to impaired contractile function. The patient's excellent response to topiramate (a sodium channel blocker) supports this hypothesis, however functional studies are needed to further characterize this duplication.

957F

Molecular analysis in hypotonic infants detects high incidence of Genetic diseases. *N. Najera[1], J. Perez[1], E. Barragan[2], N. Garibay[1], T. Martinez[1], L. Gonzalez[1], G. Queipo[1].* 1) Human Genetic, Hospital General de Mexico, Mexico City, Mexico; 2) Neurology, Hospital Infantil de Mexico Federico Gomez.

Neonatal central hypotonia is the lack of spontaneous movement, with or without muscular weakness, and generalized hypotonia during the neonatal period. This condition can be caused by a number of different pathological processes in the brain or defects to any structure in the motor unit. Central hypotonia affects the central nervous system, including the spine, and among its most frequent causes is systemic illness. As part of the central approach to the hypotonic baby, it is important to eliminate syndromic and genetic causes. Some reports have been proposed that 40% of the central hypotonic neonates had Prader Willi the most common genetic cause of obesity however, in children under 2 years of age the diagnosis is especially difficult. It is clear that early diagnosis of PWS o any other genetic entity is crucial to avoid complications and decrease morbidity and life expectancy. Nevertheless, other genetic syndromes have central hypotonia as main clinical manifestation during infancy. So, genetic approach is mandatory during the initial clinical intervention. We present the genetic approach in 30 consecutive pediatric patients with central hypotonia as a major symptom, referred by the neuro-pediatrician. According with the clinical genetic evaluation and presumptive clinical diagnosis, molecular analysis was performed detecting that 70% of the cases had a genetic disorder. We conclude that genetic evaluation is crucial in the central hypotonic clinical approach, proposing that all the central idiopatic hypotonic babies should be evaluated by a clinical genetic professional.

958F

Analysis of 11q22.2-q22.3 chromosomal region for genetic linkage in families with class III malocclusion from Brazil and Colombia. *J. Hartsfield[1], J. Turner[1], G. Falcao-Alencar[1], L. Otero[2], R. Cruz[3], L. Morford[1], D. Fardo[1], S. de Oliveira[4], T. Kluemper[1].* 1) Univ Kentucky, Lexington, KY; 2) Pontificia Universidad Javeriana, Bogotá, Colombia; 3) Private practice, Brasilia, Brazil; 4) Universidade De Brasilia, Brasilia, Brazil.

Objective: Class III malocclusion has an autosomal dominant inheritance with variable expressivity and incomplete penetrance. The purpose of this study was to investigate the potential genetic linkage of a section of chromosome 11 to Class III skeletal malocclusion within two populations from South America: Brazil and Colombia. Methods: Microsatellites DNA polymorphisms, microsatellites D11S1886 and D11S4204 in the 11q22.2-q22.3 chromosomal region, were previously implicated in linkage to Class III in Colombian families (LOD 2.0 and 1.8, respectively) by Frazier-Bowers et al. 2009. Our study focused on the 11q22.2-q22.3 chromosomal region and genotyped another type of DNA polymorphism, single nucleotide polymorphisms (SNPs), near D11S1886 and D11S4204 (SNPs: rs666723, rs578169, rs12416856 and rs1386719). A pedigree for each family was constructed. The affected status of family members was determined by dental charts, cephalometrics, facial and dental photos, and/or dental models. Biosamples were collected from affected and unaffected family members and genomic DNA was isolated for genotyping. SNP analysis was performed using TaqMan Genotyping Assay Kits in the Roche LightCycler480®. The program MENDEL was used to estimate genetic linkage. Results: LOD scores were obtained for all SNPs genotyped, ranging from -1.94 to -7.17. LOD scores for the Brazilian population alone ranged from 0.94 to -3.32, while LOD scores for the Colombian population alone ranged from -1.95 to -3.84. LOD scores with two large Colombia families removed these large families. Removal of the largest or two largest families from the Colombian dataset yielded LOD scores from -0.01 to -2.60 in both cases. Conclusion: Using these markers, in the families included in this study, we did not find any genetic linkage with Class III phenotype. While this does not support the earlier report of linkage in this area in another Colombian population sample, the small number and different type of markers used and the heterogeneity of Class III malocclusion does not preclude this area from further analyses in other samples or genotyping arrays. This study was supported in part by International Centre for Genetic Engineering and Biotechnology grant ICGEB CRP/COL04-03 (LMO), and the E. Preston Hicks Endowed Chair in Orthodontics and Oral Health Research at the University of Kentucky (JKH).

959F

PRRX1 is mutated in an otocephalic newborn infant conceived by consanguineous parents. *D. Kamnasaran[2,3], P.O. Simsek[1].* 1) Clinical Genetics Unit, Department of Pediatrics, Hacettepe University, Ankara, Turkey; 2) Pediatric Research Unit, Centre de recherche du CHUL, Québec, Québec, Canada; 3) Department of Pediatrics, Laval University, Québec, Canada.

Background: Agnathia-otocephaly, is a rare, often sporadic and lethal malformation complex with an estimated incidence less than 1 in 70,000 births, and with etiologies linked to both genetic and teratogenic factors. We previously reported the *PRRX1* gene is mutated in a fetus having agnathia-otocephaly. PRRX1 is a transcription factor with pivotal roles during mandibulofacial development. In this study, we analyzed the *PRRX1* gene for mutations in a new case of a newborn infant with agnathia-otocephaly. **Methods and Results**: The proband was the third child of otherwise clinically normal and healthy parents from a consanguineous first cousin marriage. The family history was unremarkable. Autospy examinations identified the proband with synotia, an extreme hypoplastic oropharynx with a blind-ended and small stoma, hypoplastic and retropositioned tongue, hypoplastic and dysmorphic larynx and epiglottis, agenesis of the trachea-oropharynx connection, and a blind-ended proximal trachea. Furthermore, the external auditory canals were open and atretic, and there was evidence of tracheomalacia, bilateral pulmonary hypoplasia and a secundum type of atrial septal defect. Both the proband's and parents' karyotypes were normal. Molecular genetic testing was undertaken by sequencing all five exons of *PRRX1* using genomic DNA isolated from the peripheral blood lymphocytes. A homozygous G_1004_C recessive mutation was identified in the *PRRX1* transcript isoform 2 of the proband, and was bi-parentally inherited from each parent who were carriers of a heterozygous G_1004_C mutation. Only the homozygous mutation, which causes a A230P missense mutation in the OAR domain of the PRRX1 protein, results in a loss-of-function; a mutation which is unidentified in 100 normal control individuals examined, and human single nucleotide polymorphism databases. **Conclusion:** We identified a rare event which is the first homozygous loss-of-function recessive mutation in *PRRX1* in a newborn infant conceived by consanguineous parents. Findings from this study have important applications in prenatal diagnosis, and to better understand mandibulofacial development in this severe birth defect.

960F

Fragile X Syndrome screening in Korean women of reproductive age. *M. Kim[1], D. Kim[1], J. Yang[1,2], H. Ryu[1,2], S. Park[1].* 1) Laboratory of Medical Genetics, Cheil Medical Research Institute, Cheil General Hospital & Women's Healthcare Center, Kwandong University College of Medicine, Seoul, Korea; 2) Department of Obsterics and Gynecology, Cheil General Hospital & Women's Healthcare Center, Kwandong University College of Medicine, Seoul, Korea.

Fragile X syndrome (FXS) is the most common inherited form of mental retardation after the Down syndrome. FXS is caused by expansion and methylation of a CGG repeat in the 5' untranslated region of FMR1 located Xq27.3. Although several population-based studies have established prevalence of FXS in Caucasians, only a few studies have reported prevalence of FXS in Asians. In Korea, there has been no report of population-based study on FXS except for some studies performed in selected population like mental retardation, autistic group. This study attempts to estimate the FMR1 allele distribution, the frequency of FXS permutation and full mutation in the general population. Screening tests were carried out in 5003 women of reproductive age between September 2003 and December 2010. Our study showed that 70.32% of the women were heterozygous alleles and remains were homozygous alleles for the CGG-repeat size. We found that 9,977 alleles were in the normal range (7-44 CGG repeats) and 19 alleles were in the gray zone (45-54 CGG repeats). Within the normal FMR1 alleles, the most prevalent allele was 27 (37.09%), followed by 28 (31.94%) and 34 (9.08%), respectively. Also 10 carriers were observed (a frequency of 1 in 500); 9 had a permutation and 1 had a full mutation. Prenatal diagnosis for FXS was performed in 11 pregnancies; 5 inherited the maternal normal allele, 5 had a premutation allele, and 1 had a full mutation allele. Two expansions observed when transmitted from mother to offspring. This is the first population-based study to assess the carrier frequency of the fragile X syndrome in the Korean population. Although there may be a founder effect, there is a little difference in our results compared to Caucasians. However, it is believed that these results might be valuable information to understand the fragile X syndrome in Koreans and other Asians.

961F

Intragenic and interspecific alignment of fibrillin-1's calcium-binding EGF-like domains reveals clinically-relevant sequence constraints and improves Marfan syndrome mutation assessment. *B.A. Salisbury, T.E. Callis, L.R. Susswein, J.L. Carr, E.W. Johnson.* Transgenomic, New Haven, CT.

INTRODUCTION: Mutations in the gene *FBN1*, which encodes fibrillin-1, cause Marfan syndrome (MFS) and several other connective tissue disorders. Hundreds of causative mutations have been reported. These mutations can be grouped into various categories, some of which are included among the strong diagnostic indicators for MFS. One such category is disruption to the 47 EGF-like domains, in particular the 43 that are calcium-binding (cbEGF). We sought to identify those aspects of the domains that are most uniform and where mutations are accordingly most likely to be pathogenic. **METHODS:** We aligned the 43 cbEGF domains, which make up most of the fibrillin-1 protein sequence. After determining the most consistent features, we compared the locations of missense variants reported as case mutations at the UMD *FBN1* mutation database with those from 419 ethnically diverse, ostensibly healthy controls that we sequenced. Conservation across chimp, dog, cow, mouse, rat and chicken was also examined. **RESULTS:** The cbEGF motif that we determined for fibrillin-1 is Dx(D/N)ECmCmCx(D/N)xx(G/S)x(Y/F)xCxCmC, where m refers to a variable number of unspecified amino acids and x refers to exactly one. In the previously published motif, the first position is given as D/N and the fourth as E/Q, reflecting variation seen in cbEGF domains found in other proteins. The G/S position and fixed spacing requirements in the middle of the motif are also newly specified. These fixed elements are all almost perfectly conserved across the species. None of the 19 missense variants (18 novel; 1 previously reported as pathogenic) found in our controls altered any of the fixed aspects of these domains, whereas a large fraction of the 367 missense variants from cases in the UMD *FBN1* database did. Further, D>N mutations are found in position 1 in cases but not in position 3 reflecting the extra constraint evident for position 1 versus 3 in the cbEGF domains of fibrillin-1. **CONCLUSIONS:** The highly conserved nature of fibrillin-1 can be leveraged to improve variant classification in MFS genetic testing. By recognizing a larger set of constraints on its sequence, we will be able to ascribe pathogenicity to a larger fraction of otherwise un-characterized variants in clinical testing. Efforts like this are critical for improving the clinical utility of genetic tests that must deal with an appreciable background rate of benign genetic variants that could be confused with pathogenic ones.

962F

FCGR2A, JAK2 or HNF4A variants are associated with ulcerative colitis susceptibility in Koreans. *M. Hong, H. Kim, Y. Jung, S. Yang, K. Song.* University of Ulsan College of Medicine, Seoul, Korea.

Recent genome-wide association studies have identified over forty candidate genes contributing to ulcerative colitis susceptibility. The goal of this study was to test the reported ulcerative colitis susceptibility genes including FCGR2A, SLC26A3, JAK2, and HNF4A in Korean patients with ulcerative colitis and Crohn's disease. Five single nucleotide polymorphisms from 4 loci including FCGR2A, SLC26A3, JAK2, and HNF4A were genotyped in 661patients with ulcerative colitis, 642 patients with Crohn's disease, and 601 healthy controls Statistically significant associations with ulcerative colitis were found at FCGR2A (rs1801274, p = 2.3 x 10-4, OR = 0.70 (95% CI = 0.57-0.84) under the allelic model), the JAK2 locus (rs10975003, p = 6.7 x 10-4, OR = 1.43 (95% CI = 1.16-1.77) under the allelic model), and HNF4A (rs6017342, p = 0.002, OR = 0.66 (95% CI = 0.51-0.85) under the allelic model). The association of FCGR2A was much stronger in female patients with ulcerative colitis (p = 5.7 x 10-6) than in males (p = 0.50). Except rs10975003 from the JAK2 locus, none showed positive association with Crohn's disease. Our data suggest that FCGR2A, JAK2 or HNF4A variants play a role in the pathogenesis of ulcerative colitis in Koreans.

963F

Different Penetrance in cases of Dystrophin deletion. *C. Trujillo[1], M.K. Alwasiyah[2].* 1) Dept Gen, CES Inst, Jeddah, Saudi Arabia; 2) MOH, Jeddah, Saudi Arabia.

We report a family with a deletion of exons 45 to 49 of the dystrophin gene, detectted by MLPA test. The proband is at 18 years of age with complete motor impairment, and using a wheel chair since he was 18 years of age. His brother age 26 is almost completely asymptomatic despite of having the same deletion of exon 45 to 49 detected by MLPA, he relates some claudication when he tries to climb stairs. Muscle biopsy showed typical abnormalities associeted with Duchenne Muscular Dystrophy. We assume that there are some modifier genes related to the clinical phenotype in Muscular Dystrophy. This modifier genes are unkwon at the present.

964F

A novel mutation in the gene LEMD3 in a patient with sub-clinical Osteopoikilosis. *A.R. Couto[1,2], S. Meneses[1,2], M. Soares[1,2], J.P. Pinheiro[1,2], J. Bruges-Armas[1,2].* 1) SEEBMO, Hospital de Santo Espírito, Angra do Heroismo, Azores, Portugal; 2) Institute for Molecular and Cell Biology (IBMC), Universidade do Porto, Portugal.

Osteopoikilosis (OMIM 166700) is a rare autosomal dominant skeletal disorder characterised by the presence of multiple hyperostotic lesions in different parts of the skeleton. This disorder can occur in isolation or in association with other skin and bone dysplasias such as melorheostosis (OMIM 155950). The objective of this study was to identify putative mutations in the gene LEMD3 in affected individuals from a multiple affected Azorean family with sub-clinical osteopoikilosis - with scarce and negligible hyperostotic lesions. The investigated family is a two-generation family of Azorean origin. The proband, a 79 year old female, was identified from patients attending the Rheumatic Diseases Clinic - Hospital de Santo Espírito, Angra do Heroísmo. Three other individuals affected with sub-clinical osteopoikilosis were further identified in this family. All the individuals had a full skeletal radiographic survey. Genomic DNA from the above patients and 40 healthy controls was isolated from peripheral blood and amplified by PCR. The mutated LEMD3 exon and flanking splice sites was sequenced. We identified a novel heterozygous mutation in exon 13 of gene LEMD3. This non-synonymous mutation, a C to T substitute at position 2701bp (cDNA), changes amino acid at 901(1) from Arginine to a Tryptophan, and segregates with the phenotype of all the affected individuals in the Azorean family. None of the healthy controls has this mutation. We here describe a mutation located in the carboxyl-terminal nucleoplasmic region of LEMD3. This region (aminoacids 789-911) is predicted to be an RNA recognition motif-like (RRM-like) protein interaction domain named U2AF homology motif. Further studies will be necessary to investigate the consequence of the mutation in protein function. Our findings suggest that the novel LEMD3 mutation reported here is the cause of the sub-clinical osteopoikilosis.

965F

Absence of *NPY2R* in Saliva Predicts Successful Oral Feeding in Premature Neonates. *J.L. Maron[1, 2], J.A. Dietz[1], M.L. Chen[1], K.L. Johnson[1, 2], D.W. Bianchi[1, 2].* 1) Floating Hospital for Children at Tufts Medical Center, Department of Pediatrics, Boston, MA; 2) Mother Infant Research Institute at Tufts Medical Center, Boston, MA.

Background: Identifying key physiological regulatory genes in neonatal saliva holds great promise for improving our understanding of neonatal biology and advancing clinical care. Currently, only subjective assessment tools exist for determining an infant's readiness to orally feed, placing premature neonates at risk for choking, aspiration, and hypoxia. Previously, we identified a subset of genes, including neuropeptide Y2 receptor (*NPY2R*), through salivary gene expression analyses that were differentially expressed in neonates learning to orally feed. *NPY2R* has been shown by others to modulate feeding behavior, metabolism, and energy homeostasis in humans and mice. Hypothesis: Absence of *NPY2R* in neonatal saliva predicts successful oral feeding in premature neonates and regulates the physiological feeding pattern characteristic of normal postnatal exponential growth. Methods: Salivary samples were collected from 56 preterm and 14 term neonates at various gestational ages (25 4/7 to 41/4/7 weeks) and feeding milestones (no feeds, partial nasogastric feeds, full nasogastric feeds, partial oral feeds, full oral feeds). RNA was extracted from all samples (n=113) using the QIAGEN RNeasy Protect Saliva Mini Kit. Multiplex qRT-PCR was performed for *NPY2R* and three housekeeping genes: *GAPDH*, *YWHAZ*, and *HPRT1*. Presence or absence of *NPY2R* in saliva was determined if *NPY2R* did or did not amplify in the presence of ⁄2 amplified housekeeping genes, respectively. Chi-squared tests were performed between *NPY2R* and feeding status; correlation coefficient was determined for *NPY2R* expression and gestational age. Results: Absence of *NPY2R* expression significantly correlated with feeding status (chi-square p = 0.019). Expression of *NPY2R* was negatively correlated with gestational age (R2= 0.91). Conclusion: Our results support the hypothesis that *NPY2R* is a developmentally regulated gene. Decreased *NPY2R* expression significantly correlates with successful oral feeding in the preterm infant. The salivary transcriptome provides targeted, real-time, functional information about the developing preterm neonate. This work suggests that objective tests, based on gene expression, can be developed for application in newborn care.

966F

Mutations of Connexin Genes 26(GJB2) and 30(GJB6) in a Peruvian Deaf Population. *R.D. CHACON[1,2], R. BADILLO[3], M.R. CORNEJO[1], O. ORTEGA[1], V. MARCA[1], R.J. DESCAILLEAUX[4], J.A. RUBIO[5], P.E. MAZZETTI[1].* 1) Neurogenetics Division, Instituto Nacional de Ciencias Neurologicas, Lima, Lima, Perú; 2) Biology-Genetics School, Universidad Nacional Mayor de San Marcos, Lima, Lima, Perú; 3) Oto-neurosurgery Department, Instituto Nacional de Ciencias Neurologicas, Lima, Lima, Perú; 4) Celular Biology and Genetics, Universidad Nacional Mayor de San Marcos, Lima, Lima,Perú; 5) Otolaryngology, Hospital Nacional Guillermo Almenara Irigoyen, Lima, Lima, Perú.

Hearing loss has a prevalence of 1 per 1000 newborns. Mutations in genes GJB2 and GJB6 cause approximately 50% of the genetic cases. In Latin American populations, 35delG and S199F mutations varies from 17 to 40%, and Δ(GJB6-D13S1830) and Δ(GJB6-D13S1854) mutations varies from 2 to 9% of deaf population. The aim of this study was to estimate the frequencies of 35delG and S199F mutations at GJB2 gene and Δ(GJB6-D13S1830) and Δ(GJB6-D13S1854) mutations at GJB6 gene and, to establish a genotype-phenotype correlation. We evaluated 120 unrelated mestizo patients with non-syndromic sensorineural hearing loss (NSHL), 55 of them with family history and 5 without it. Molecular diagnosis was applied with PCR-RFLP and PCR-MULTIPLEX techniques previously described. In case of S199F mutation, a PCR-RFLP genetic test was developed using the primers F(TCGAGGAGATCAAAACCCAG) and R(GGGAAATGCTAGCGACTGAG) and the enzyme AlwNI (Cail). Pure-tone average (PTA: 05-3 kHz) was obtained after a pure tone audiometry. Positive mutation controls were used in all cases. The S199F, Δ(GJB6-D13S1830) and Δ(GJB6-D13S1854) mutations were not found in this population. The 35delG mutation was found in 12.5% (15/120) of the cases [2.5% homozygous and 10% heterozygous]. In familial cases, 35delG mutation was found in 6 patients (10.9%) while in sporadic cases it was found in 9 cases (13.85%). The allelic frecuency of 35delG mutation was 7.5% (18/240). In the homozygous cases (3), PTA was found under 90 dB (total average: 100.625), with a profound hearing loss phenotype. In the heterozygous cases (12), PTA varies from 72.5 db to 106.25 dB (total average: 87.5) corresponding to severe hearing loss phenotype (6 cases) and profound hearing loss phenotype (6 cases). This findings suggest the presence of other mutation(s) associated to the peruvian population, which receives an important contribution from natives amerindian populations. 35delG homozygous genotype is related to the most severe phenotype, while 35delG heterocygous genotype varies according to the other expected mutation in the same gene or in a different associated gene. We strengthen the importance of genetic diagnosis, specially for 35delG mutation, providing early treatment and genetic counselling of deaf patients and their families.

967F

Molecular analysis of Indian patients with Rett syndrome: Identification of mutations and genotype-phenotype correlation. *D.K. DAS[1], D.A. SANGHAVI[1], R.A. ADHIA[1], V. UDANI[2], A. MAITRA[1].* 1) GENETIC RESEARCH CENTRE, NATIONAL INSTITUTE FOR RES IN REPROD HEALTH, MUMBAI, MUMBAI, INDIA; 2) PD HINDUJA NATIONAL HOSPITAL AND MEDICAL RESEARCH CENTRE, SWATANTRYA VEER SAVARKAR MARG, MAHIM, MUMBAI, INDIA.

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder, primarily affecting females and characterized by regression, epilepsy, stereotypical hand movements, and motor abnormalities. Its prevalence is about 1 in 10,000 female births. Rett Syndrome is caused by mutations within methyl CpG-binding protein 2 (MECP2) gene, Over 200 individual nucleotide changes which cause pathogenic mutations have been reported, however eight most commonly occurring missense and nonsense mutations account for almost 70% of all mutations. Small deletions associated with deletion hotspots in the C-terminal region of MECP2 protein account for an additional 9% of pathogenic mutations Rett Syndrome cases have been reported from India. The phenotype (classical and atypical inclusive) has many differentials. However a genetically based confirmed diagnosis would help in management and counselling. In this study we have analysed MECP2 mutations in 30 Indian sporadic patients diagnosed clinically as having RTT and 5 missense, 3 nonsense, 1 frameshift and 3 silent mutations in MECP2 have been detected. Five mutations (c.806Gdel, p.T158M, p.R255X, p.R168X, p.R270X) were the part of 8 hotspot mutations identified worldwide and are present in classical cases. Four mutations (p.R133C, p.R306C, p.T203M, p.P152R) were identified with atypical cases. We also identified 5 patients with small (7 bp deletion) to large deletion (409 bp deletion) in exon 4 of MECP2 gene. We have also analyzed cyclin dependent kinase like 5 (CDKL5) gene mutation in patients having without mutations and 4 different novel and known mutations in CDKL5 gene were identified. The clincical severity of this disorder also depends on the pattern of X chromosome inactivation (XCI). Therefore, the patterns of X chromosome inactivation were analyzed in all the patients for genotype-phenotype correlation. We found that the skewing pattern of inactivation was correlated with the clinical severity of this disorders. We have also performed multiple ligation dependent probe amplification (MLPA) for the mutation negative patients but no abnormalities have been detected. Our study would help in establishing the panel of mutations prevalent in Indian cases of Rett syndrome.

968F

A panel of genetic tests identifies causative mutation in around 40% of patients affected by bilateral syndromic and non-syndromic congenital limb malformations. *A. Jamsheer[1,2], A. Sowinska[1,2], A. Latos-Bielenska[1,2].* 1) Department of Medical Genetics, University of Medical Sciences in Poznan, Poznan, Poland; 2) Center for Medical Genetics GENESIS, Poznan, Poland.

In recent years, significant number of genes, loci and regulatory elements (RE) involved in human embryonic limb development has been identified. Mutations in those genes, loci and RE result in abnormal genetic programming and lead to various congenital limb malformations (CLMs). The aim of this study was to assess the relative frequencies of known genetic abnormalities in a cohort of Polish probands affected by bilateral CLMs. A 110 probands with bilateral isolated (non-syndromic) or syndromic CLMs were recruited for the study. The probands were clinically evaluated and qualified for genetic testing, based on the tentative diagnosis (karyotype, molecular screening by means of DNA sequencing and/or MLPA). Karyotyping and molecular screening of selected 17 genes and loci known to be involved in pathogenesis of human limb malformations allowed for identification causative mutation in 46 probands (41.8%). Patients diagnosed with the panel of tests comprised 2 probands with apparently balanced chromosomal translocations, 11 probands with preaxial polydactyly type 4 or Greig cephalopolisyndactyly carrying GLI3 mutations, 10 probands with split-hand-foot malformation (SHFM), with 6 out of them carrying TP63 mutation and 4 having 10q24.31-10q24.32 duplication (SHFM3 locus), 4 probands exhibiting synpolydactyly with HOXD13 mutations, 4 with ODDS/syndactyly type III and GJA1 mutations, and 4 with TAR syndrome carrying a deletion in the critical region of 1q21.1. In 2 probands with the diagnosis of Feingold syndrome, MYCN mutations were found; similarly, 2 probands with Bardet-Biedl syndrome had mutations in BBS10, and 2 cases with Townes-Brocks - mutations in SALL1. 4 probands were recognized as having isolated brachydactylies and were subsequently confirmed to carry causative alterations (2 probands manifesting BDB - ROR2 mutation, 1 case of BDC - GDF5 mutation, 1 case of BDE - HOXD13 mutation). In addition, 1 proband manifesting carpal-tarsal coalition syndrome accompanied with symphalangism carried a mutation in NOG. The study shows relative frequencies of specific molecular defects underlying bilateral CLMs and provides suggestions for tests that should be considered in the diagnostic process of patients presenting with bilateral CLMs.

969F

UGT1A1 is a Major Locus Influencing Bilirubin Levels in African Americans. *G. Chen[1], E. Ramos[1], A. Adeyemo[1], D. Shriner[1], J. Zhou[1], A. Doumatey[1], H. Huang[1], N. Gerry[2], A. Herbert[3], A. Beneley[1], H. Xu[1], B. Charles[1], M. Christman[2], C. Rotimi[1].* 1) NHGRI, NIH, Bethersda, MD; 2) Coriell Institute for Medical Research, Camden, NJ 08103 USA; 3) Department of Genetics and Genomics, Boston University School of Medicine, Boston, Massachusetts 02118 USA.

BACKGROUND: Total serum bilirubin, an important byproduct of heme metabolism, has been associated with several clinical outcomes, including cardiovascular disease, diabetes and drug metabolism. Previous genome-wide association studies (GWAS) of serum bilirubin performed in European and East Asian populations reported that variants in the UGT1A1 gene (2q37.1) significantly influence serum total bilirubin levels. These findings have not been replicated in African ancestry populations; thus, we conducted a GWAS in African Americans to attempt to replicate reported findings and to identify novel loci influencing serum total bilirubin levels. METHODS: A total of 619 healthy unrelated individuals who participated in the Howard University Family Study (HUFS) were included in this study. Using a dense panel of over two million genotyped and imputed SNPs, we conducted a GWAS in African Americans for serum total bilirubin level. We assumed an additive genetic model and all statistical models were adjusted for age, sex, and the significant principal component from the sample covariance matrix of genotypes. RESULTS: Heritability for serum total bilirubin was estimated to be 0.42. Thirty-nine SNPs spanning a 78 kb region within the UGT1A1 gene (2q37.1) displayed p-values lower than the pre-determined genome wide significance threshold of 5×10-8. The lowest p-value was 1.7×10-22 for SNP rs887829. Notably, none of the other 38 SNPs in the UGT1A1 gene remained statistically significant in conditional association analyses that adjusted for SNP rs887829. We showed that rs887829 is in tight LD (r2／0.74) with rs10929302 (-3156G > A, UGT1A1*91) located in the phenobarbital response enhancer module that is about 3kb upstream of the (TA)n variant reported to a better predictor of toxicity irinotecan - a cancer drug. We also replicated the reported association between variants in the SEMA3C and bilirubin in this cohort of African Americans. CONCLUSIONS: We observed that UGT1A1 is a major locus influencing total bilirubin levels in African Americans, adding to the evidence that this gene plays an important role in the determination of bilirubin level in human populations with different ancestral backgrounds. Our findings may also contribute to the understanding of the etiology of hyperbilirubinaemia and the pharmacogenomics role of UGT1A1 variants in drug (e.g., irinotecan) metabolism in African Ancestry populations.

970F

A Genome-Wide Association Analysis for Pathological Myopia in Japanese. *I. Nakata[1,4], K. Yamashiro[1], H. Nakanishi[1,4], H. Hayashi[1,4], Y. Kurashige[1,4], M. Miyake[1,4], A. Tsujikawa[1], M. Moriyama[2], K. Ohno-Matsui[2], M. Mochizuki[2], Q. Fan[3], X. Zhou[3], J.L. Xuan[3], SM. Saw[3], T. Kawaguchi[4], R. Yamada[4], F. Matsuda[4], N. Yoshimura[1].* 1) Department of Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan; 2) Department of Ophthalmology and Visual Science, Tokyo Medical and Dental University Graduate School of Medicine, Tokyo, Japan; 3) Department of Epidemiology and Public Health, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; 4) Center for Genomic Medicine/Inserm U.852, Kyoto University Graduate School of Medicine, Kyoto, Japan.

Myopia is one of the most common ocular disorders worldwide, and is in much higher prevalence in Asians than in Caucasians. Pathological myopia, also called high myopia, comprises 1% to 5% of the general population and is one of the leading causes of legal blindness in developed countries. To identify genetic determinants associated with pathological myopia in Japanese, we conducted a genome-wide association study and follow-up replication studies in Chinese and Japanese. In the first stage, we genotyped 505 elderly cases with pathological myopia (axial lengths > 28.0 mm in both eyes) and 3498 general population controls using the Illumina HumanHap 550k and 660K arrays in a Japanese population. We found 767 SNPs which showed P-values smaller than 0.001, and tested them in the dataset of Singaporean Chinese individuals for 222 cases with high myopia, defined by spherical equivalent (SE) ≤ -6.0 diopters (D), and 448 controls (SE between -0.50 and +1.00 D). According to this replication study, 3 SNPs on 3 loci (rs4472734 at PTPN14, rs7634255 on 3q25, and rs6060750 at C20orf152) showed significant associations in high myopia in Chinese (P < 0.05). However, a further replication study in Japanese using additional 638 elderly cases with pathological myopia (axial lengths > 26.0 mm in both eyes) and 1194 general population controls using Taqman SNP assay revealed no significant association for all 3 SNPs (P > 0.05). In the present study, we could not find a susceptibility locus for pathological myopia. Further studies on genetic backgrounds of pathological myopia would lead to understanding pathogenesis of pathological myopia.

971F

Genetic modifiers of the 22q11 microdeletion syndrome: Preliminary results of a GWA study. *G.M. Repetto[1,2], S. McGhee[3], M.L. Guzman[1], N. McLennan[4], M. Palomares[5,6], M. Vasquez[1], G. Lay-Son[1,2,6], A. Ziegler[1].* 1) Dept Gen, Clin Alemana- Univ Desarrollo, Santiago, Chile; 2) Hospital Padre Hurtado, Santiago, Chile; 3) Stanford University, Stanford, CA; 4) UCLA, Los Angeles, CA; 5) Fundación Gantz, Santiago, Chile; 6) Hospital Calvo Mackenna, Santiago, Chile.

Chromosome 22q11 microdeletion syndrome (del22q11) has an estimated frequency of 1/4000 live births. Most patients share a common 3 Mb deletion but despite molecular similarity, the clinical phenotype shows marked variable expressivity. Approximately 50-70% of patients have congenital heart disease, predominantly conotruncal, and 70-80% have palatal abnormalities, including submucous cleft palate and velopharyngeal insufficiency. The cause of the incomplete penetrance of the individual features is unknown. We performed a genome wide association study (GWAS) to search for genetic modifiers of the cardiac and palatal phenotype in Chilean patients with del22q11. DNA samples from 124 patients with del22q11, 67 of them with cardiac anomalies and 87 with palatal anomalies were analyzed with Affymetrix ® v. 6.0 SNP array. Significant differences were found at SNPs in chromosomes 6 and 8, among others, when comparing patients with any cardiac defect to patients normal cardiac anatomy (p 5.8 x 10-7 and 6.1 x 10-7, respectively) and in the same SNPs (p 1.12 x 10-6 and 1.4 x 10-6, respectively) and one in chromosome 2 (p 4.3 x 10-7) when comparing patients with conotruncal heart defects (tetralogy of Fallot and IAA) with patients with normal heart structure. Comparison between patients with or without palatal abnormalities showed evidence of association between SNPs in chromosomes 4 (p 6.8 and 7 x 10-6 for 2 SNPs on this chromosome), 14 (7.6 x 10-6) and X (6.2 x 10-6). Our results suggest that common genetic variants outside of the 22q11 region modify the effects of the deletion, and that these modifiers may be different for the cardiac and the palatal phenotypes, implying that these malformations could involve independent pathways. Funded by Fondecyt-Chile Grant #1100131.

972F

Pathogenic CNVs and causative genes detected by two-stage screening in 647 patients with mental retardation and multiple congenital anomalies of unknown etiology. *S. Hayashi[1], I. Imoto[2], Y. Makita[3], A. Hata[4], J. Inazawa[1].* 1) Dept Molec Cytogenetics, Tokyo Med & Dental Univ, Tokyo, Japan; 2) Dept. Human Genetics and Public Health Graduate School of Medical Science, the University of Tokushima, Japan; 3) Education Center, Asahikawa Medical College, Japan; 4) Department of Public Health, Chiba University Graduate School of Medicine, Japan.

Recently several types of microarrays are available to analyze cases with congenital disorders. We have investigated 647 cases with multiple congenital anomalies and mental retardation (MCA/MR) of unknown etiology for six years by using several types of microarray. First, we performed a two-stage screening by application of two types of in-house bacterial artificial chromosome (BAC)-based arrays in order to detect pathogenic copy number variants (pCNV). In the 1st screening we used `MCG Genome Disorder Array (GDA)' containing BACs covering loci associated with known 30 genomic disorders and subtelomeric regions of all chromosomes as a diagnostic tool, and detected pCNV in 69 of 647 cases (10.7%). In the 2nd screening we used `MCG Whole Genome Array-4500 (WGA)' harboring 4523 BACs throughout human genome and detected pCNV in 57 of 474 cases (12.0%). Based on the accumulation of genotype-phenotype information and bioresources through the screenings, we could show a relation between phenotypes and several pathogenic genes, e.g. BMP4 or UBE2A. Notably, we investigated genotype-phenotype correlation in detail about the CASK gene. We recruited more than ten female patients with MR, microcephaly, disproportionate pontine and cerebellar hypoplasia (MIC-PCH), and demonstrated that all the patients had variable genetic aberrations causing null mutation of CASK. Inspired by these results, we are searching a mutation of another candidate disease-associated gene(s) in a cohort of subjects with resembling phenotypes. Moreover, we are investigating etiologies of the remaining cases by multiple approaches. Currently we are analyzing 387 negative cases of the BAC array screenings by employing SNP array (illumina, HumanOmniExpress) as the 3rd screening in order to detect a small CNV and a partial uniparental disomy affecting phenotypes. We are also re-assessing pCNVs in 55 cases detected by the BAC arrays using high-resolution oligonucleotide array (Roche NimbleGen, Human CGH 2.1M Whole-Genome Tiling Arrays) in order to detect second CNV(s) potentially affecting phenotypes and/or causative genomic structure(s) of the CNV. Here we demonstrate our multiple genome-analyzing approaches to investigate etiology of MCA/MR.

973F

Study of SLC29A3 gene in Rosai-Dorfman histiocytosis associated with hearing impairment. *S. Marlin[1, 2, 3, 4], L. Jonard[5, 2, 3, 4], V. Couloigner[6], S. Pierrot[6], M. Louha[5, 2, 3, 4], B. Neven[7], S. Gherbi[1, 2], R. Couderc[5, 2, 3, 4], A. Fischer[7], E.N. Garabedian[8, 2, 3, 4], F. Denoyelle[8, 2, 3, 4].* 1) Clinical genetics department, Trousseau Hospital, Paris, France; 2) Centre de référence des surdités génétiques, Trousseau Hospital, Paris, France; 3) INSERM, UMRS_587, Institut Pasteur, Paris, France; 4) UMPC, Pierre et Marie Curie University, Paris 6, Paris, France; 5) APHP, Trousseau Hospital, Biochemistry and Molecular Biology department, Paris, France; 6) APHP, Necker Hospital, Pediatric Oto-Rhino-Laryngology department, Paris, France; 7) APHP, Necker Hospital, Immunology department, Paris, France; 8) APHP, Trousseau Hospital, Pediatric Oto-Rhino-Laryngology department, Paris, France.

SLC29A3 is implicated in a syndromic form of genodermatosis: H syndrome. The major features encounters in H syndrome are Hearing loss, Hyperglycaemia, Heart anomalies, Hypertrichosis, Hyperpigmentation, Hepatomegaly and Hypogonadism. More recently, SLC29A3 mutations have been described in families presenting syndromes associating generalised histiocytosis to systemic progressive features: severe camptodactyly, hearing loss, hypogonadism, hepatomegaly, heart defect and skin hyperpigmentation. We have identified a homozygous novel missense SLC29A3 mutation in a patient presenting with only a progressive sensorineural hearing impairment and a single cervical node. The same mutation was revealed in another patient with Rosai-Dorfman histiocytosis, profound hearing impairment, chronic inflammation with pericarditis and ophthalmological disorders. Both patients originated from North Africa and their mutation may be related to a common ancestor. Our results suggest that SLC29A3 should be studied in patients presenting with histiocytosis and hearing impairment.

974F

Correlation between CFTR mutation and the CF phenotypes. *R. Sebro[1,2], H. Levy[3,4].* 1) Institute for Human Genetics, University of California, San Francisco, San Francisco, CA; 2) Department of Radiology and Biomedical Imaging, University of California, San Francisco, CA; 3) Division of Pulmonary and Sleep Medicine, Children's Hospital, Milwaukee, WI; 4) Clinical Research Institute of the Children's Hospital of Wisconsin and Medical College of Wisconsin, Milwaukee, WI.

Cystic fibrosis (CF) is a simple recessive disease with a complex phenotype that can affect the liver, pancreas, sweat glands, gastrointestinal system, vas deferens and lungs. CF is the most common genetic disorder in Caucasians. Morbidity and mortality associated with CF are related to progressive pulmonary function decline, and associated with frequent lung infections. Over 1600 mutations have been identified in the cystic fibrosis membrane transporter receptor (CFTR) gene, however there is difficulty correlating CFTR mutation with phenotype, primarily because all of the mutations except for the DF508 mutation have a prevalence of around 1%. Three mutation scoring schemes were evaluated. The biological classification score is based on the in vitro activity of the mutant CFTR membrane transporter. We hypothesize the in vitro function predicts the in vivo CF phenotype. The Grantham score is based on the degree of chemical dissimilarity between the native and the substituted amino acid. The Sorting Intolerant From Tolerant (SIFT) score uses sequence homology to predict whether an amino acid substitution will affect protein function and potentially be deleterious. SIFT and Grantham scores were calculated for non-synonymous missense mutations. We analyzed cross-sectional data from 108 DF508 compound heterozygotes (1 mutation is the DF508 allele, and the other is a known non-DF508 allele) from a cohort of CF patients at Children's Hospital Boston. Three aspects of the CF phenotype were considered: the pulmonary disease was assessed using the % predicted FEV1, the sweat chloride level, and pancreatic exocrine sufficiency. Linear and logistic regression models were used using age, sex and the mutation classification scores as covariates. Mutations with higher biological classification scores (milder mutations) are associated with more normal sweat chloride levels ($p<0.001$) and pancreatic sufficiency ($p<0.001$). Lower Grantham scores are associated with more normal sweat chloride levels ($p<0.001$) and pancreatic sufficiency ($p=0.01$). Higher SIFT scores are associated with more normal sweat chloride levels ($p<0.001$) and pancreatic sufficiency ($p=0.01$). No association was found between the % predicted FEV1 and either the biological classification ($p=0.08$), Grantham ($p=0.28$) or the SIFT scores ($p=0.62$). Our findings suggest the pulmonary disease may be influenced by modifier genes and environmental factors.

975F

A patient with hemihoplasia, normal growth and *H19* hypomethlation. *L. Zhang[1,2], L. Cohen[1], L. Konczal[1,3].* 1) Dept Human Gen, Case Western Univ SOM, Cleveland, OH; 2) Dept Med, Case Western Univ SOM, Cleveland, OH; 3) Dept Ped, Case Western Univ SOM, Cleveland, OH.

Russell-silver syndrome (RSS) is characterized by severe intrauterine followed by postnatal growth retardation. A significant number of patient with clinically diagnosed RSS have been found to have hypomethylation of *H19* on chromosome 11p15.5. In contrast, the opposite end of the spectrum, Beckwith-Wiedemann syndrome, characterized by large for gestational age at birth and subsequent overgrowth, has been associated with hypermethylation of the *H19* locus, at least in some patients. Both pre- and post-natal growth retardation in RSS is thought to be the result of deficiency of *IGF2*, a paternally expressed growth factor important for fetal and placental growth in utero as well as postnatal growth. Hypomethylation of the 11p15.5 locus results in loss of expression of *IGF2* from the paternal allele and biallelic expression of *H19*. We describe a patient with congenital hemihypoplasia, normal growth both in utero and postnatally and hypomethylation of the *H19* locus on 11p15.5. Our patient is a 10-week-old girl with congenital hemihypoplasia. Her birth weight and height were both between 25-50th percentile. Her current weight (50th percentile), length (25th percentile) and head circumference (75th percentile) are all within normal limits. She has a small triangular face with a relatively broad forehead. Her face, body, upper and lower extremities all show apparent asymmetry with the right side significantly larger than the left. She does not have any other clinical manifestations that are frequently associated with RSS, including 5th finger clinodactyly, cafe-au-lait macules, genitourinary anomalies, feeding difficulty or hypoglycemia. Our case demonstrates that *H19* hypomethylation may be seen in patients with isolated body asymmetry without IUGR or postnatal growth retardation. Further study of the allelic expression pattern of *IGF2* is required to elucidate the mechanism. Close to normal level of methylation may allow appropriate fetal and postnatal growth. Also, more cases with *H19* hypomethylation and a wider spectrum of clinical manifestation may be identified if we expand the testing indication beyond RSS and BWS and include individuals with isolated body/facial asymmetry.

976F

Fetal hemoglobin production cannot be induced by) -thalassemia/ hemoglobin E disease caused by novel 1-bp insertion mutation at codon 35 of *HBB* gene. *O. Trachoo[1], P. Niparuck[2], S. Pingsuthiwong[1], K. Srichan[1], T. Sura[1], B. Phakdeekitcharoen[3].* 1) Division of Medical Genetics and Molecular Medicine, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand; 2) Division of Hematology, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand; 3) Division of Nephrology, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand.

) -thalassemia disease (MIM #141900) is one of the most common public health problems in Southeast Asian countries. The disease is caused by the mutations in *HBB* gene resulting in abnormal) -globin protein synthesis. Clinical manifestations include hemolytic anemia, jaundice, hepatosplenomegaly, failure to thrive, multiple endocrinopathies and hemochromatosis since early childhood. The mutations in *HBB* gene are heterogeneous and hemoglobin E (Hb E, Glu26Lys) is the most prevalent hemoglobinopathy in Thailand. Compound heterozygosity of Hb E and either) ^{0} or) $^+$ mutation gives rise to) -thalassemia/Hb E disease which has diverse clinical phenotypes, from mild to severe form. Mainly, the absence and dysfunction of) -globin trigger the production of . -globin protein comprising fetal hemoglobin (Hb F) which is not normally expressed in adult life. Here we report a middle-age Thai male patient who presented with clinical thalassemia disease, markedly elevated ferritin level and end-stage renal disease. Hb analysis showed the presence of more than 90% Hb E and very low level of Hb F misleading to homozygous Hb E which is a benign condition. Direct DNA sequencing of *HBB* gene revealed the heterozygous Hb E mutation and novel 1-bp G insertion at codon 35 was found. Based on protein prediction analysis, this particular insertion mutation results in frame shift and generating premature stop codon. Therefore, molecular diagnosis of) -thalassemia/ Hb E disease was confirmed. However, Hb F production cannot be induced by) -globin mutation as observed in other common) -thalassemia/Hb E diseases. These genetic and biochemical findings lead to the interest on how Hb F production is affected. Next step to move on includes the works on . -globin expression and . -globin gene sequencing which are hypothesized to modulate Hb F synthesis.

977F

A novel 5-bp deletion in clarin 1 in a family with Usher syndrome. *E. Akoury[1], E. Elie El-Zir[2], A. Mansour[3], A. Megarbané[4], J. Majewski[1,5], R. Slim[1].* 1) Human Genetics, McGill University Health Centre, Montreal , Quebec, Canada; 2) Department of Otorhinolaryngology, Hôpital Sacré-Coeur, Baabda, Lebanon; 3) Department of Ophthalmology, American University of Beirut, Lebanon; 4) Unité de génétique médicale, Faculté de médecine, Université Saint Joseph, Beirut, Lebanon; 5) McGill University and Genome Quebec Innovation Centre, Montreal, Canada.

Background and purpose: Usher syndrome (USH) is an autosomal recessive disorder combining sensorineural hearing loss and retinitis pigmentosa (RP). It is the leading cause of deaf-blindness in humans with a prevalence estimated to range from 2 to 6.2 per 100,000.Our aim was to identify the genetic defect in a Lebanese family with two sibs diagnosed with Usher Syndrome but could not be classified into types I or III because of the intra-familial variability and the absence of progressive deafness. **Materials and Methods:** Exome capture and sequencing was performed on DNA from one affected member using Agilent In Solution Bead Capture, followed by Illumina sequencing. **Results:** This analysis revealed the presence of a novel homozygous 5-bp deletion, in the exon 2 of the Clarin 1 (CLRN1), a known gene responsible for Usher syndrome type III. The deletion is inherited from both parents and segregates with the disease phenotype in the family. The 5-bp deletion, c.301_305delGTCAT, p.Val101SerfsX27 was found within a region of 10.3 Mb of homozygosity on chromosome 3 and is predicted to lead to a frameshift in the second transmembrane domain and result in protein truncation after 27 amino acids. Sequencing all the coding regions of the CLRN1 gene in the proband did not reveal any other mutation or variant sustaining therefore that this 5-bp deletion is responsible for the Usher phenotype in this family. **Conclusion:** Here we describe a novel deletion in CLRN1 in a Lebanese family with Usher syndrome. Our data support previously reported intra-familial variability in the clinical features of Usher syndrome type I and III and highlight the importance of next-generation sequencing in mutation analysis.

978F

Evidence of DLL1 as a Susceptibility Locus in Indian Visceral Leishmaniasis. *S. Mehrotra[1], M. Fakiola[2,3], A. Mishra[1], P. Tiwari[1], M. Sudarshan[1], S. Jamieson[3], D. Selvi Rani[4], S. Sundar[1], J. M Blackwell[2,3].* 1) Department of Medicine, Institute of Medical Sciences, Banaras Hindu University Varanasi, India; 2) Cambridge Institute for Medical Research and Department of Medicine, University of Cambridge School of Clinical Medicine, Cambridge, UK; 3) Telethon Institute for Child Health Research, Centre for Child Health Research, The University of Western Australia, Subiaco, Western Australia, Australia; 4) Centre for Cellular and Molecular Biology, Hyderabad, India.

Previous genome wide linkage studies in Sudanese and Brazilian populations identified chromosome 6q27 as a strong candidate region containing a gene(s) regulating susceptibility to visceral leishmaniasis (VL). The region contains genes involved in the host immune response, amongst which the most compelling candidate susceptibility gene for VL is DLL1 (Delta Like Protein 1), a member of the notch signalling pathway that directs naïve T helper differentiation towards Th1, Th2 or regulatory T cell lineages. This population-based study investigated the role of the 6q27 genes (PHF10, C6orf70, DLL1, FAM120B, PSMB1, TBP) in Indian VL by genotyping (Sequenom) twenty-one single nucleotide polymorphisms in 941 VL cases and 992 ethnically-matched controls. Logistic regression analyses using additive and genotypic models show significant associations with variants at DLL1 (rs1884190) and FAM120B (rs9366198, rs9460106, rs2103816). FAM120B_rs9460106 (intronic) was the most associated SNP (OR=1.22; 95%CI=1.07-1.39; P=0.0027), but the associated variants all fall within a strong linkage disequilibrium block. Quantitative gene expression was analysed in 19 paired pre- (Day 0), and post- (Day 30) treatment splenic aspirate samples from VL patients receiving antileishmanial drug therapy. DLL1 showed significantly (P value less than 0.0001) higher expression in Day 0 samples compared to Day 30. These genetic and functional studies in an endemic Indian population provide strong evidence for DLL1 acting as a susceptibility locus for VL in India.

979F

Mutations in the WNT10A gene are the most frequent cause of isolated hypodontia. *M.H. van den Boogaard[1], M. Créton[2], A.Y. Bronkhorst[1], A.H. van der Hout[3], M. Cune[4,5], J.K. Ploos van Amstel[1].* 1) Department of Medical Genetics, University Medical Center Utrecht, Utrecht, Utrecht, Netherlands; 2) Department of Oral and Maxillofacial Surgery, Prosthodontics and Special Dental Care, University Medical Centre Utrecht, The Netherlands; 3) Department of Genetics, University Medical Center Groningen, The Netherlands, Groningen; 4) Section of Oral Function, Academic Centre for Oral Health, University Medical Center Groningen, The Netherlands; 5) Department of Oral-Maxillofacial Surgery, Prosthodontics and Special Dental Care, St. Antonius Hospital Nieuwegein, The Netherlands.

Ectodermal Dysplasia (ED) is a clinically and genetically heterogeneous disorder. ED features involve the abnormal development of at least two of the ectodermal structures namely teeth, hair, nails and sweat glands. Genes that are associated with ED are e.g. EDA1, EDAR, EDARADD and WNT10A. Mutations in the WNT10A gene have been identified to be associated with several forms of ectodermal dysplasias including odonto-onycho-dermal dysplasia (OODD). Hypodontia is a common feature in these ectodermal dysplasia forms, which makes WNT10A a good candidate as a cause of isolated hypodontia. Recently, indeed a family with isolated hypodontia has been described that is caused by missense mutations in the WNT10A gene. Other genes that are known to cause isolated hypodontia are MSX1, PAX9, AXIN2 and IRF6. To get insight in the genetic heterogeneity of isolated hypodontia, we tested a panel of 36 probands that show variable severity of tooth agenesis. The probands all had agenesis of six or more teeth (excluding the third molars), with a range of 6 - 28 teeth. There was a positive family history for tooth agenesis in ~ 70% of the patients. The genes that have been analyzed were MSX1, PAX9, IRF6, AXIN2 and WNT10A. The gene tests involve the direct sequencing of the exons and their flanking sequences that were amplified by PCR. Twenty probands (56%) showed alterations in the WNT10A gene: 8 probands were homozygous, 3 probands were compound heterozygous and 9 probands were heterozygous for a single WNT10A mutation. Heterozygosity for a mutation in the PAX9 gene was identified in 3 patients. One patient showed a nonsense mutation in the AXIN2 gene. We also tested patients with hypodontia and an additional feature (hypodontia plus) e.g. 1 patient had clefting with tooth agenesis outside the cleft region. In these patients also WNT10A mutations could be identified. The WNT10A mutation p.Phe228Ile (48% of the alleles) was identified in 8 out of 352 control chromosomes (2.3%). The hypodontia status of the controls is not known. Strikingly, this WNT10A allele frequency fits with the incidence of tooth agenesis in the general population (~5%). We conclude that mutations in the WNT10A gene are by far the most frequent cause of isolated hypodontia. Thus, should be implemented in the DNA diagnostics of isolated hypodontia. Furthermore, there is an indication for a genotype-phenotype relationship for WNT10A mutation carriers depending on type of mutation and zygosity.

980F

Haplotypes of the 3'untranslated region of the HLA-G gene are associated with TD1. *R. de Albuquerque[1, 4], C. Leal[2], E. Castelli[3], N. Lucena-Silva[4], D. Rassi[2], C. Mendes-Junior[5], E. Donadi[2].* 1) Departamento de Genetica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo,Ribeirão Preto, São Paulo, Brazil; 2) Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo,Ribeirão Preto, São Paulo, Brazil; 3) Departamento de Biologia Geral, Universidade Federal de Goiás,Goiás,Brazil; 4) Departamento de Imunologia, Centro de Pesquisa Aggeu Magalhães, Fundação Oswaldo Cruz, Recife, Pernambuco, Brazil; 5) Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil.

The TD1 (Type 1 Diabetes) is a multifactorial disease in which the autoimmune destruction of pancreatic cells is mediated by humoral and cellular mechanisms. Various subpopulations of lymphocytes are involved in this process, however, the CD8 + T lymphocytes appear to be the cells responsible for early insulitis. Genetic susceptibility to TD1 has been primarily attributed to MHC genes: a recent study mapping the MHC region identified the HLA-G region as an independent susceptibility gene for the disease. A major function of HLA-G is the inhibition of Natural Killer cells (NK) and cytotoxic T cells (CTL), by interaction with inhibitory receptors found in these cells. The HLA-G gene presents a 3' untranslated region (3'UTR) that contains at least eight polymorphisms, three of them related with several posttranscriptional regulatory elements. Study the 3'UTR of the HLA-G gene in 110 patients with TD1 and 110 controls from the same geographical region of the patients. DNA was extracted from peripheral blood cells by the salting out procedure. The 3'UTR fragments were amplified using the polymerase chain reaction (PCR) and were directly sequenced in an ABI310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The presence of significant associations between the SNPs detected at the 3'UTR of the HLA-G gene was evaluated by means of a likelihood ratio test of linkage disequilibrium (LD), using the ARLEQUIN version 3.1 program. The PHASE method and expectation-maximization (EM) algorithm were used to infer 3'UTR haplotypes. For the eight polymorphic sites (14bp Del/Ins, +3003T/C, +3010C/G, +3027C/A, +3035C/T, +3142C/G, +3187A/G and +3196C/G), only the +3010 genotype CC was significantly associated with protection against the disease, being less frequent in patients compared with the control group ($p = 0.0461$; OR = 0.5139). In a recent study, our group has identified the +3010C/G site as a putative target for microRNAs, suggesting a possible posttranscriptional control of this site in HLA-G expression. Regarding 3'UTR haplotypes, we have observed a significant increase in the frequency of haplotype UTR-6 among patients, conferring susceptibility to TD1 ($p = 0.0034$, OR = 3.068), and a significant decrease of haplotype UTR-3, suggesting a protective role to TD1 ($p = 0.0082$, OR = 0.3547). In conclusion, the present study revealed significant associations between 3'UTR polymorphisms and haplotypes with Type 1 Diabetes susceptibility.

981F

SNPs located in regulatory regions of TPM1 and TPM2 affect gene expression and are associated with clubfoot. *K.S. Weymouth[1,2], S.H. Blanton[3], S. Richards[4], M.J. Bamshad[5], A.E. Beck[5], A. Savill[6], M.B. Dobbs[7,8], C.A. Gurnett[7,8], J.T. Hecht[1,2].* 1) Dept Pediatrics, Univ Texas Medical Sch, Houston, TX; 2) Graduate School of Biomedical Sciences, Houston, TX; 3) University of Miami Miller School of Medicine, Miami, FL; 4) Texas Scottish Rite Hospital, Dallas,TX; 5) University of Washington, Seattle,WA; 6) University of Cardiff, Wales, UK; 7) Washington University School of Medicine, Saint Louis, MO; 8) Saint Louis Shriners Hospital for Children, Saint Louis, MO.

Isolated, nonsyndromic clubfoot is a common birth defect affecting 135,000 newborns worldwide each year. Clubfoot is characterized by an inward posturing of the foot in a rigid, downward position. Calf muscles in the affected leg(s) are underdeveloped and remain small even after corrective treatment. Clubfoot is a complex birth defect and is posited to be caused by perturbation in multiple genes. However, the genetic variation contributing to this birth defect is largely unknown. To identify the genetic variation(s), we interrogated fifteen muscle contraction genes in our family-based discovery clubfoot dataset composed of multiplex and simplex families. Single SNP, haplotype and gene interaction analyses identified 3 associated SNPs (rs4075583, rs3809565 and rs3825973) in tropomyosin 1 (TPM1, fast-twitch muscles) and 2 (rs2025126 and rs2145925) in tropomyosin 2 (TPM2, slow-twitch muscles genes). All of these SNPs were located in potential regulatory regions. In silico analysis predicted that all the associated regulatory SNPs in TPM1 and TPM2 could alter DNA binding depending on whether the ancestral or alternate allele was present. EMSA (gel shift) and luciferase assays were performed in differentiated and undifferentiated muscle cell nuclear extracts and muscle cells, respectively. EMSA analysis showed that the alternate allele of rs4075583/TPM1 and rs2025126/TPM2 eliminate a DNA binding site, while two DNA binding sites are created when the alternate allele of rs2145925/TPM2 is present. No effect on DNA binding was seen for rs3809565/TPM1 and rs3825973/TPM1. Importantly, we find that the alternative allele of rs4075583 causes decreased gene expression. Studies to assess the effect of rs4075583/TPM1, rs2025126/TPM2 and rs2145925/TPM2 on gene expression are on-going. For clubfoot, we show that regulatory variants affect DNA binding sites and gene function in two different but related muscle-specific biologically relevant genes. These results support the multifactorial etiologic model for clubfoot and suggest dysregulation of gene expression as a mechanism underlying clubfoot. These exciting and novel findings provide important insight about a potential cause of clubfoot.

982F

Protein Biomarker Development in Niemann-Pick Disease, type C. *S.M. Cologna[1], X-S. Jiang[1], P.S. Backlund[2], N.M. Yanjanin[1], C.A. Wassif[1], A.L. Yergey[2], F.D. Porter[1].* 1) Program in Developmental Endocrinology and Genetics, NICHD, NIH, DHHS Bethesda, MD; 2) Biomedical Mass Spectrometry Facility NICHD, NIH, DHHS Bethesda, MD.

Niemann-Pick Disease, type C (NPC) is a genetic, neurodegenerative, lipid storage and transport disorder. As a result of lipid accumulation found in NPC, a cascade of pathological events occurs including oxidative stress, apoptosis and neuroinflammation. The phenotypic spectrum of NPC is broad, typical clinical presentations include ataxia, liver dysfunction and seizures among others. There is no FDA approved therapy for NPC therefore we sought to identify differentially expressed proteins in an attempt to (i)develop therapies to target the pathological cascade and (ii)identify protein markers that can be used as tools when testing therapeutic interventions. Protein lysates were obtained from female *Npc1*$^{+/+}$ and *Npc1*$^{-/-}$ mice cerebella ($n \checkmark 4$) at 1, 3 and 5 weeks post birth. Protein pools were subjected to 2D-gel electrophoresis in triplicate and silver stained. Differentially expressed spots were excised, digested with trypsin and analyzed using MALDI-TOF/TOF and/or LC-ESI-MS/MS. MS/MS data was submitted to Mascot and validated using Scaffold. Protein identifications at the 99% confidence level required two peptides (95% confidence level). In total, 109 spots were differentially expressed relative to controls ($R>1.5/<0.67$, $p<0.05$). One novel finding was the altered expression of fatty acid binding protein (FABP) family members in the cerebellum of mutant mice. FABPs are responsible for fatty acid transport and metabolism. We identified three differentially expressed FABPs: FABP3, FABP5 and FABP7 which were over-expressed in the mutant relative to control. Interestingly, increased cerebrospinal fluid (CSF) FABP3 expression has been observed in Alzheimer disease, a disorder with pathological overlap with NPC. FABP5 has been associated with inflammation while FABP7 has been proposed as a biomarker for brain injury. We used Western blot to validate the FABP3 expression. To translate our findings to the clinic, increased FABP3 expression levels were validated in CSF from NPC patients relative to controls using ELISA. The concomitant increase found in both studies suggests that FABP3 may be a candidate protein marker to study. Furthermore, NPC patients taking off-label miglustat, a glycosphingolipid inhibitor, show lower CSF FABP3 levels, relative to age-matched controls and FABP3 levels decreased significantly after initiation of the therapy. We are currently studying the function of these FABPs in NPC and exploring other differentially expressed proteins.

983F

Classification of severity of cleft lip and palate in patients attending the genetic consultation in Operation Smile. Colombia. *J. Martinez[1], A. Venegas[3], I. Briceno[2,3].* 1) Bioscience, Grupo de Genetica Humana, Universidad de La Sabana, Chia, Colombia; 2) Biocience, Grupo de Genetica Humana, Universidad de La Sabana, Chia, Colombia; 3) Grupo de genetica Humana, Universidad Javeriana, Bogota, Colombia.

The Cleft Lip-Palate (FLP) is one of the most common congenital malformations and is caused by an alteration in the fusion of the tissues that give rise to the upper lip and palate during embryonic development. According to etiology, the timing of embryonic development that occur and its epidemiological characteristics, the FLP are classified in Non sindromics and Sindromics, into four groups: pre-palatal clefts or primary palate, fissures of secondary palate (which can affect the hard palate and / or soft), mixed (with involvement of the lip and palate) and minor fissures of rare occurrence. The manifestation of these malformations is conditioned by genetic, environmental and nutritional factors. The global frequency of these malformations is 1 for every 1,200 live births. Colombia is a multiethnic country, with the population distributed in different regions, from dry deserts, mountains and rainforest. Currently there are no studies to determine precisely the extent of these malformations in Colombian population. This study was performed in 1100 patients who attended the genetic consultation under the program “Operation Smile Colombia”, in different regions of the country, which underwent a full genetic evaluation and classification of each of the presented malformations The results indicate that of the 1100 patients, 1019 are non-syndromic and 81 are syndromes, these syndromes have heart defects 19, 24 have Aarskog syndrome and the remaining 38 have other syndromes. , the results are the closest to the impact of this anomaly in the Colombian population.

984F

Momo syndrome: broadening the clinical spectrum and delineating natural history. *M.L.M. CASTRO[1], J.G.C. MEIRA[3], L.R. MARTELLI[1,2], M.J. RODOVALHO-DORIQUI[7], V.E.F. FERRAZ[1,2], C.H. GRANGEIRO[1], W.A.R. BARATELA[1], C.G. PICANçO[1], R.F.N. BAREIRO[5], J.B. VOLPON[4,5], A.C. SANTOS[6], M.H.N. BARBOSA[6], D.P. PAIXÃO[1], R.T. AKAMINE[1], A.X. ACOSTA[10], D.G. MELO[8], M.P. FOSS[9], J.M. PINA-NETO[1,2].* 1) SETOR DE GENETICA MEDICA DO HOSPITAL DAS CLINICAS -FMRP/USP/ RIBEIRÃO PRETO/SP/BRASIL; 2) DEPARTAMENTO DE GENETICA FMRP/USP/RIBEIRÃO PRETO/SP/BRASIL; 3) DEPARTAMENTO DE GENETICA E BIOLOGIA EVOLUTIVA DO INSTITUTO DE BIOCIENCIAS.USP-SAO PAULO/BRASIL; 4) DEPARTAMENTO DE BIOMECANICA MEDICINA E REABILITAçÃO DO APARELHO LOCOMOTOR /FMRP/USP/ BRASIL; 5) SETOR DE ORTOPEDIA DO HOSPITAL DAS CLINICAS-FMRP/USP /RIBEIRÃO PRETO/SP/BRASIL; 6) DEPARTAMENTO DE CLINICA MEDICA-DIVISAO DE RADIOLOGIA-FMRP/USP/RIBEIRÃO PRETO/SP/BRASIL; 7) SERVIçO DE REFERENCIA EM TRIAGEM NEONATAL DO MARANHAO/BRASIL; 8) FACULDADE DE MEDICINA/UFSCar/ SÃO CARLOS/SP/BRASIL; 9) FACULDADE DE FILOSOFIA, CIENCIAS E LETRAS.DEPARTAMENTO DE PSICOLOGIA-FMRP/USP/RIBEIRÃO PRETO/SP/BRASIL; 10) SERVIçO DE GENÉTICA MÉDICA E LABORATÓRIO DE GENÉTICA HUMANA E MUTAGÊNESE DA FACULDADE DE BIOLOGIA/UFBA/SALVADOR/BA/BRASIL.

MOMO syndrome is a rare mental retardation /multiple anomalies syndrome of unknown etiology, characterized by macrosomia, obesity, macrocephaly, and ocular abnormalities. We report a new case in an adult patient to describe the natural history of this entity.This patient is a 23 years old, brazilian boy, third child of healthy non-consanguineous parents. Gestation was uneventful and delivery was at term. Birth weight of 3550g and length 53cm.The boy had developmental delay and needed special education. Obesity was present since childhood, and recently he appeared to be hyperactive and aggressive. At 8 years he presented with delayed neuropsychomotor development, hyperactivity, and macrosomia. Height was 137cm(90th-97th), weight 47,3kg (>97th), and OFC 54,5cm(98th centile), internipple distance 20cm (>97th).Craniofacial features included coarse facies, macrocephaly, high forehead with plain supraorbital ridges, deep set eyes, strabismus, synophrys, hypertelorism, overfolded horizontal ear helix, large nose with broad nasal root, short philtrum, cupid bow mouth, irregular teeth, prognatism, and short neck. Limited supination and pronation of the forearm as well as limited flexion of the knees and elbows, and flat feet were present.Patient developed obstructive sleep apnea at 18 years old. At current physical exam he was 169,8cm (25th-50th) tall, weighted 113 kg (>97th), OFC 59cm (>98th) and kept the same dysmorphic features and mental retardation. Eye exam showed high myopia, retina and vitreous alterations. So far, he hasn't had increased intraocular pressure, nevertheless he is still at risk . Mild to moderate conductive hearing loss on the right ear was detected. Orthopaedic evaluation showed gait abnormalities, shoulder, hip and knees contractures. Macrocrania and advanced bone age were seen on X-rays. Echocardiography was normal. Brain MRI revealed ventricular, cortical sulci, cisterns and fissures dilatation, and craniofacial disproportion with increased extra-cranial soft tissues. Citogenetic analysis was normal. Southern Blotting analysis for methylation 15q11-13 showed biparental pattern. Microsatellites segregation analysis for 1p36 region excluded microdeletions. Molecular analysis for FRAXE and FRAXA (Xq27.3 q28) were normal. At this point, the patient is one of the eldest already reported having MOMO syndrome. Therefore we aimed to extend the clinical spectrum, and provide a possible natural history for this condition.

985F

Past and present investigations in a family with Christianson syndrome and SLC9A6 gene defect. *S.G.M. Frints[1,2], C.E. van Roozendaal[2,3], V.M. Kalscheuer[4], W.O. Renier[5], D. Tserpelis[2], E.E. Smeets[1,2], A.P. Stegmann[1,6], R. Blok[2,3], I.P. Krapels[1,2], C.T.R.M. Schrander-Stumpel[1,2], H.H. Ropers[4], C.E.M. de Die-Smulders[1,2].* 1) Department of Clinical Genetics, Maastricht University Medical Center+, azM, Maastricht, the Netherlands; 2) Research Institute for Oncology and Developmental Biology, GROW, Maastricht University, Maastricht, The Netherlands; 3) Clinical Genomics, Department of Clinical Genetics, Maastricht University Medical Center+, azM, Maastricht, the Netherlands; 4) Max Planck Institute for Molecular Genetics, Department Human Molecular Genetics, Ihnestrasse 73, D-14195 Berlin, Germany; 5) Department of Neurology, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands; 6) Cytogenetic Laboratory, Department of Clinical Genetics, Maastricht University Medical Center+, azM, Maastricht, the Netherlands.

Christianson syndrome (OMIM 300243) is a rare syndromic form of X-linked intellectual disability disorder (XLID) which resembles clinically Angelman syndrome at young age. Affected men have profound cognitive dysfunction without or with nearly no speech. They show spasticity, ataxia and facial grimacing. They are relatively microcephalic and have cerebellar a-/hypoplasia. In adulthood, they develop a lean body and present with constant drooling. One-third of the carrier women have learning disabilities, dyslexia with or without additional clinical features. The underlying SLC9A6 (OMIM 300231) gene defect has been reported in families with Christianson syndrome. We present a new family with Christianson syndrome with additional brain pathology and molecular investigations. Clinically, classical features of Christianson syndrome were present in affected men. Open brain biopsy, performed in the past, in an affected man showed distinct pathological second and poorly differentiated third and fourth cerebral cortical layer using the Golgi-Cox staining. Cerebrospinal fluid investigations were normal, as well as other metabolic and infection parameters in blood. Carrier women had mild learning problems and were relatively microcephalic. Through next generation sequencing, the X-exome was systematically screened in this family. A SLC9A6 c.1481delG, p.Gly526fsX non-recurrent truncating mutation was identified which segregated with the clinical phenotype. Missense mutations in other causal XLID genes included GDI1 c.154-3C>T and SHROOM2 c.3608A>G (p.Asn1203Ser). Both mutations could, but FTSJ1 c.-87-10 (without splice effect after in silico analysis) variance could not be excluded through segregation analysis in this family. FTSJ1 expression analysis to investigate eventual modifying effect on the clinical phenotype in this family might be needed. We conclude, that through past and present investigations, the clinical phenotype of Christianson syndrome has further been delineated and that possible modifying effects of other XLID genes might interfere with the clinical phenotype or might explain inter and intra familial variability.

986F

A patient with Allan-Herndon-Dudley syndrome due to a complete deletion of exon 1 of the *MCT8* (*SLC16A2*) gene. *B. García de Teresa[1], A. González del Angel[2], ML. Ruíz Reyes[3], R. Calzada Leon[3], B. Perez Enriquez[3], MA. Alcántara Ortigoza[1].* 1) Laboratorio Biología Molecular, Instituto Nacional de Pediatría, Mexico City, México; 2) Subdirección de Investigación Médica, Dirección de Investigación, Instituto Nacional de Pediatría, Mexico City, México; 3) Servicio de Endocrinología, Instituto Nacional de Pediatría, Mexico City, México.

In 1944, Allan, Herndon and Dudley described a severe form of X-linked mental retardation with a particular thyroid hormone profile that was later known as AHD syndrome. Years later, mutations in *MCT8* (*SLC16A2*), a gene that codes for a thyroid hormone transporter, were found to cause this syndrome. Since then, affected males from 47 families carrying an hemizygous mutation in *MCT8* have been described. Case report: 30 month old patient, second son of a non-consanguineous couple, delivered by cesarean section at 39 weeks due to a history of polyhidramnios. The patient's neonatal screening for hypothyroidism was reported normal. At 3 months of age, his mother noticed somnolence, a wide anterior fontanel and difficult feeding, which required a gastrostomy. A recent physical exam confirmed a wide anterior fontanel, and revealed facial dysmorphism (long lashes, depressed nasal bridge, telecantus, bulbous nose, anteverted nares, prominent upper lip) and central hypotonia. The patient's psychomotor development is severely affected, he has not achieved cephalic support and is unable to speak. Thyroid tests show a particular profile: high T3, T4 in the lower limit of normal range, and normal TSH. Despite appropriate thyroid hormone replacement for over 18 months, no improvement of the condition has been documented. Upon the suspicion of AHD syndrome, molecular study of *MCT8* was performed by amplifying each of its six exons, following the method described by Schwartz et al. 2005.

The genomic DNA sample of this patient only allowed the amplification of five exons of *MCT8*. Despite many attempts, the expected 773 pb product of exon 1 was never obtained. The carrier status of the mother is being tested in a gene dosage assay. Conclusion: The results are consistent with a deletion that completely spans exon 1 of *MCT8*. To our knowledge, this would be the 48th family in which a *MCT8* mutation has been identified. Of all mutations, 52% are point mutations, 31% small in/dels and the remaining 17% large deletions. The complete deletion of exon 1 has been found in 4 different families, the rest are private mutations. This recurrence of the complete exon 1 deletion could be indicative of a genomic region prone to deletions. The loss of exon 1 predicts a complete inactivation of the MCT8 transporter and is associated with a severe phenotype where independent walk or speech are never attained. Our patient presents this severe phenotype.

987F

MEHMO syndrome: report of a male patient. *D. Gul[1], S. Vurucu[2], R. Akin[2].* 1) Dept Medical Genetics, GATA Medical Faculty, Ankara, Ankara, Turkey; 2) Dept Pediatric Neurology, GATA Medical Faculty, Ankara, Turkey.

The MEHMO syndrome (MIM 300 148) is the acronymic designation for a complex of mental retardation, epileptic seizures, hypogonadism and hypogenitalism, microcephaly, and obesity. In 1998, Steinmuller et al described the syndrome in a live male of the five affected males. To date four reports have been published and all seem inherited in X-linked fashion. We here report a 6-month-old male with MEHMO syndrome. A male, the first child of non-consanguineous parents, was delivered vaginally at 34 weeks' gestataion. The prenatal history was unremarkable. Her birth weight, length and head circumference had been 2 080 g, 46 cm and 31 cm. The family history was negative. He was referred because of motor retardation, epilepsy, and obesity at the age of 6 months. On admission, his height was 61.3 cm (10th centile), weight 11.800 g (> 97th centile) and OFC 40 cm (<3rd centile). He had motor retardation and several epileptic seizures. Microcephaly and severe obesity were also noted. Facial anomalies were evident: narrow bifrontal diameter, short nose, full cheeks, wide philtrum, and downturned corners of the mouth. The testes were hypoplastic, whereas the penis appeared small in size. Fundoscobic examination revealed a pale optic disc. Serum cholesterol and LDL-cholesterol were both elevated. On the Denver Developmental Screening Test (DDST) he was assessed abnormal. Cranial CT shows cerebral hemisphere atrophy in the temporal, frontal and parietal areas. Electroencephalography showed fronto-central epileptic discharges. Cytogenetic study using high resolution banding technique revealed 46,XY karyotype. PWS was excluded on FISH. Cerebellar atropy and hypercholesterolemia are the unusual findings in this patient.

988F

A contiguous 6.6 Mb region of homozygosity on chromosome 10 spanning the bands p15.1-p14 is associated with unusual obesity, dysmorphic features, and mild intellectual disability. *P.P. Koty[1], J.N. Hellwege[2], T. Jewett[1], M. Mortenson[3], G.A. Hawkins[4], D.W. Bowden[5], N.D. Palmer[5].* 1) Department of Pediatrics, Section on Medical Genetics, Wake Forest Baptist Health, Winston-Salem, NC; 2) Wake Forest University School of Medicine, Molecular Genetics and Genomics Program, Winston-Salem, NC; 3) North Carolina Department of Health and Human Services, Division of Public Health, Raleigh, NC; 4) Center for Genomics and Personalized Medicine Research, Wake Forest Baptist Health, Winston-Salem, NC; 5) Department of Biochemistry, Wake Forest Baptist Health, Winston-Salem, NC.

Here we report a 12 year old female with central obesity, very prominent striae overlying the abdomen and medial upper arms, a “buffalo hump”, and acanthosis nigricans who is mildly intellectually disabled. In addition, she has macrocephaly (as does her mother) together with telecanthus, ocular hypertelorism, large ears, and slightly tight Achilles tendons. Chromosome analysis revealed a 46,XX karyotype. Cytogenomic microarray analysis, which included zygosity analysis, revealed no copy number variants of interest but did show a contiguous 6.6 Mb region of homozygosity on chromosome 10 spanning the bands p15.1-p14. This region contains 29 known genes including several potential candidate genes for the patient's clinical presentation. Following IRB approval and informed consent, subsequent whole exome sequencing of this region confirmed the homozygosity and revealed rare variants, as compared to known variants in the HapMap and dbSNP databases, within several of the candidate genes. The first rare variant results in a missense amino acid substitution in *AKR1C4. AKR1C4* is one of four soluble human 3 (-hydroxysteroid dehydrogenase (HSD) isoforms that are members of the aldo-keto reductase (AKR) superfamily. Two other isoforms, *AKR1C2* and *AKR1C3*, have been implicated in the regulation of androgen availability in adipose tissue and dysregulation may result in abdominal obesity. Thus, this homozygotic missense variant within *AKR1C4* may be causative of the unusual abdominal obesity identified in our patient. Two other genes within this region, *NET1* and *RBM17*, have rare splice-site variants. Variants within *NET1*, which encodes a noradrenaline transporter, have been associated with attention-deficit/hyperactivity disorder (ADHD) in females, while *RBM17* has been shown to promote neurotoxity. Either or both of these variations may explain our patient's mild intellectual disability. Finally, two rare variants causing missense mutations within *ITIH5*, a gene that encodes a member of a family of structurally related plasma serine protease inhibitors involved in extracellular matrix stabilization, were identified. Variants within this gene may be unrelated to our patient's clinical presentation or may be involved in her dysmorphic features. Our data suggest several candidate genes that, at least partially, may explain our patient's clinical presentation, but additional investigation is needed for further validation.

989F

Outcomes of clinical genetic evaluation of 186 patients referred for intellectual disability or global developmental delays of unknown cause. *J. Moeschler[1], M. McClain[2], L. Burke[3], M.B. Dinulos[1], R. Smith[4], W. Smith[4], P. Miller[5].* 1) Dept Pediatrics, Medical Genetics, Dartmouth-Hitchcock Med Ctr, Lebanon, NH; 2) New England Genetics Collaborative, IOD/UCED, University of New Hampshire, Durham NH; 3) Department of Pediatrics and the Vermont Regional Genetics Center, Burlington, VT; 4) Dept of Pediatrics, Genetics Division, Maine Medical Ctr, Portland ME; 5) University of New Hampshire, IHPP, NEGC, Durham, NH.

The New England Genetics Collaborative includes a regional effort to improve the medical genetic evaluation process and outcomes for patients referred for global developmental delays (GDD) or intellectual disability (ID). The purpose is to improve outcomes for such patients by optimizing the diagnostic process. Diagnostic testing protocol was not set but guided by the clinician decision and existing published protocols. This presentation reports on results of all 186 consecutive patients referred to participating centers from March 2009 through May 2011 referred for intellectual disability (ID) or global developmental delay (GDD). 75 (36%) were referred for that alone; the remained included ID/GDD plus one or more other indications, e.g., dysmorphic features, malformations, micro- or macrocephaly (from a list 13 other indications). Patients ranged in age from <1 year to 23 years; median age 5 years. Male-to-female ratio was 60:40 (112 males; 74 females). Disability was mild (IQ 50-57) in 62%; moderate (25-49) in 34%; and, severe (<25) in 5%. Diagnoses were established in 76 (41%) after evaluation, with the following categorization: chromosomal conditions in13 patients; cytogenomic or microarray abnormalities in 28; X-linked genetic disorders in 13; monogenic conditions (not X-linked) in 10; known syndromes (and not another category) in 7; and, multifactorial in 5. 20 patients had more than 1 visit; in 4 (25%) the diagnosis was established only after a second visit. Among patients referred with GDD/ID and autism (46), outcomes differed significantly compared to those without this co-occurring presentation: significantly fewer with known cytogenomic (microarray) disorders ($p<0.01$); multiple minor anomalies ($p<0.001$); dysmorphic features ($p<0.05$); significantly more males ($p<0.01$); and significantly fewer with known diagnosis ($p<0.0001$). Of the 46 with autism, 10 (22%) resulted in a specific diagnosis with no one category of test predicting the outcome. In 117 patients the diagnosis remained unknown following the evaluation. Of these 38 (32%) had co-occurring autism; 30 (26%) were those with dysmorphic features (“multiple minor anomalies”); 16 (14%) were “non-dysmorphic” with no major or minor anomalies and no neurologic signs or symptoms, i.e., non-syndromic GDD/ID; and 11 (9%) had micro- or macrocephaly. The medical decision-making was key in process and outcomes. Cytogenomic conditions comprised the largest proportion of known diagnoses.

990F

Extremely high prevalence of primary microcephaly in Bushehrian population (a southern province of Iran): novel STIL and ASPM mutations. *E. Papari, M. Bastami, M. Hoseini, SS. Abedini, I. Bahman, L. Nori_vahid, F. Behjati, K. Kahrizi, H. Najmabadi.* Genetics Research Center, University of Social Welfare and Rehabilitation Siences, Kodakyar Street, Daneshjo Ave, Tehran, Iran;.

Autosomal recessive primary microcephaly (MCPH) is a rare condition characterized by mild to severe Mental Retardation and head circumference equal or more than three SDs below the population mean based on sex and age without any further major neurological findings. Up to now, seven genes (Microcephalin, WDR62, CDK5RAP2, CEP152, ASPM, CENPJ and STIL) have been known to cause this disorder. In this study, we distinguished primary microcephaly in 14 families out of 55 Bushehrian families (25.45%) mostly with two or more mentally retarded individuals. Genotyping carried out using appropriate microsatellite marketers for 7 MCPH loci. Linkage was established in two families; one for MCPH5 and the other to MCPH7. The remaining 12 (85.71%) families did not showed linkage to any of the known MCPH loci. Further analysis by DNA sequencing in the two linked families revealed a homozygous (c.4849C>T) truncating mutation in the ASPM gene, and a homozygous missense mutation (c.2392T>G) in STIL gene, respectively . In present study we could only detect mutation in two out 14 families with MCPH (14.29%) by using linkage analysis and direct sequencing methods. The high prevalence of MCPH in Bushehr Province (southern Iran, with a long coastline onto the Persian Gulf) and the fact that we could only identify the causative mutation in a couple of families shows the need for more follow up in the rest of the families; therefore, homozygosity mapping, exon enrichment and next-generation sequencing (NGS) for the remaining families are currently under way to determine the responsible genes for the remaining families.

991F

Searching for copy number changes in nonsyndromic X-linked intellectual disability. *E.G. UTINE[1], P.O. KIPER[1], Y. ALANAY[1], G. HALILOGLU[2], D. AKTAS[3], K. BODUROGLU[1,3], E. TUNCBILEK[1,3], M. ALIKAŞIFOGLU[3].* 1) Hacettepe University, Department of Pediatrics, Genetics Unit, Hacettepe University, Ankara, Turkey; 2) Hacettepe University, Department of Pediatrics, Neurology Unit, Hacettepe University, Ankara, Turkey; 3) Hacettepe University, Department of Medical Genetics, Hacettepe University, Ankara, Turkey.

Intellectual disability (ID) has a prevalence of 2-3% with 0.3% of the population being severely retarded. Etiology is heterogeneous, owing to numerous genetic and environmental factors. Underlying etiology remains undetermined in 75-80% of mildly disabled patients and 20-50% of those severely disabled. Twelve% of all ID is thought to be X-linked (XLID). This study covers copy number analysis of some of the known XLID genes, using multiplex ligation-dependent probe amplification (MLPA) in 100 nonsyndromic patients. One of the patients was found to have duplication in all exons of MECP2 gene, and a pair of brothers had duplication in the fifth exon of TM4SF2/TSPAN7 gene. Affymetrix® 6.0 whole-genome SNP microarray confirmed the duplication in MECP2 and showed duplication of exons 2-7 in TM4SF2/TSPAN7, respectively. MECP2 duplication has recently been recognized as a syndromic cause of XLID in males, whereas duplications in TM4SF2/TSPAN7 are yet to be determined as a cause of XLID. Being an efficient, rapid, easy-to-perform, easy-to-interpret and cost-effective method of copy number analysis of specific DNA sequences, MLPA presents wide clinical utility and may be included in diagnostic work-up of ID, particularly when microarrays are unavailable as a first line approach.

992F

47 XX iso 18p Syndrome. *A. venegas, i. briceno.* Medicine, Universidad Javeriana, Bogota, Colombia.

CASE REPORT: Female patient (19 years old), with moderate mental retardation in the fine and coarse movements, language and coordination areas. She presented severe gastroesophageal reflux disease during her first year of age. Her mother suffered pre-eclampsia during her pregnancy. First signs of developmental delay became evident at three months of age because of floppiness, walked at 22 months and although her first words were at 12 months, her vocabulary is scarce and has difficulty in constructing sentences. No consanguinity or relevant family history. Physical examination showed height 1.53 cm., weight 57 kg hypoplasia of 4th and 5th toes from right leg, kyphoscoliosis and lumbar lordosis . Kariotype showed: 47XX + mar. Microarray analysis showed: 47XX iso (18p) (p11.21-pter). Brain nuclear magnetic resonance and tomography: Normal Echocardiogram: Normal.

993F

The first case of Sjögren-Larsson syndrome with a mutation in ALDH3A2 in Korea. *K.Y. Koo, J.Y Yang, C.H. Lee, J.S. Lee.* Department of Clinical Genetics, Yonsei University, College of Medicine, Seoul 120-752, Korea.

Sjögren-Larsson syndrome (SLS, OMIM #270200) is a rare autosomal recessive disorder characterized by ichthyosis, mental retardation, and spastic diplegia or tetraplegia. It is caused by mutations in the ALDH3A2 gene that encodes fatty aldehyde dehydrogenase (FALDH, OMIM *609523, EC 1.2.1.3), a microsomal enzyme that oxidizes fatty aldehyde to fatty acid. Secondary accumulation of abnormal lipid in the membrane of the skin and brain is thought to cause the pathogenesis of cutaneous and neurologic symptoms of SLS. The prevalence of SLS has been estimated as 0.4 per 100,000 or lower in Sweden, and over 200 cases have been reported worldwide since first reported by Sjögren. However, there is currently no report in the Korean population and we present the first cases of SLS in Korea. The patient manifested generalized ichthyosis predominantly on the posterior neck, both axillary areas, and hands since the age of one month and subsequent neurological delay since the age of 9 months with spastic diplegia. By this typical triad, we could diagnose SLS with mutation analysis of the ALDH3A2 gene.

994F

Genetic Heterogeneity of Mabry syndrome. *M.D. Thompson[1], T. Rosioli[2], M.N. Nezarati[3,8], E. Sweeney[4], P. Meinecke[5], D. Horn[6], P.M. Krawitz[7], R. Londono-Mendoza[3], H. van Bokhoven[2], D. Andrade[9], A. Munnich[10], H.B. Brunner[2], D.E. Cole[1].* 1) Laboratory Medicine & Pathobiology, Banting Inst, Univ Toronto, Toronto, ON, Canada; 2) 2Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; 3) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, ON, Canada; 4) Royal Liverpool Children's Hospital, Liverpool, United Kingdom; 5) Medizinische Genetik, Altonaer Kinderkrankenhaus, Hamburg, Germany; 6) Institut für Medizinische Genetik, Charité Universitätsmedizin Berlin, Berlin, Germany; 7) Berlin-Brandenburg Center for Regenerative Therapies; 8) Department of Genetics, North York General Hospital, Toronto, ON, Canada; 9) L'Hopital NECKER, Paris, France; 10) Toronto Western Hospital, Toronto, ON, Canada.

Mabry syndrome (hyperphosphatasia with developmental disability) was first described by Mabry et al.(OMIM#239300) in 1970. At first considered rare, improved syndrome identification has led to recruitment of more than twenty families world-wide. Salient features of the disorder include: characteristic facial dysmorphology (hypertelorism, a broad nasal bridge and a tented mouth); variable shortening of middle and distal phalanges; and neural abnormalities on biopsy (plaques disrupting Schwann cells). Like many infantile metabolic storage disorders, seizures associated with Mabry syndrome have an onset in the first year of life. Persistently elevated serum alkaline phosphatase (ALP) levels are now known to be associated with abnormalities of the phosphatidylinositol glycan (PIG) anchor, which has been found disrupted in ten of these families. Lysosomal storage material detected in patients with Mabry syndrome has been putatively identified as glycolipid in nature - possibly resulting from the improper formation of the PIG anchor. Since mutations of the PIGV gene have not been identified in all patients with a clinical diagnosis of Mabry syndrome, we describe further molecular work on genetically heterogeneous cohort. Since there are more than 15 genes integral to PIG anchor synthesis, the disorder may result from disruptions to other genes in the PIG anchor biosynthesis pathway. This work will assist in elucidating the inborn errors of metabolism that underlie the spectrum of abnormalities found in Mabry syndrome.

995F

Effect of NSD1 Mutations on the DNA Methylome in Patients with Sotos Syndrome. *S. Choufani[1], J. Hailey[1], Y.A. Chen[1], D. Grafodatskaya[1], R. Mendoza[1], S. Bowdin[1], W. Reardon[2], C. Cytrynbaum[1], R. Weksberg[1].* 1) The Hospital for Sick Children, Toronto, On, Canada; 2) Our Lady's Hospital for Sick Children, Dublin, Ireland.

Sotos syndrome (OMIM 117550), is a genetic overgrowth and malformation disorder associated with a variety of neurodevelopmental problems including intellectual and behavioral issues. More than 75% of individuals with Sotos syndrome are haploinsufficient for NSD1 (nuclear receptor-binding SET domain protein 1), a histone lysine methyltransferase important for multiple aspects of normal development. Recently, NSD1 was shown to bind near various promoter elements and regulate multiple genes via interactions with H3K36 methylation and RNA polymerase II suggesting a potential role of NSD1 in regulating transcription initiation via epigenetic mechanisms such as DNA methylation. The goal of this project is to define the impact of loss of NSD1 function on the DNA methylome. The identification of such DNA methylation alterations will elucidate the molecular pathophysiology of Sotos syndrome and identify potential molecular targets for therapeutic intervention. Sotos syndrome patients were recruited at the Division of Clinical and Metabolic Genetics at the Hospital for Sick Children. Bisulfite modified blood DNA from 8 NSD1 mutation- positive cases were investigated by genome-wide profiling of DNA methylation using the Illumina Human Infinium 27K Microarray covering the promoter of over 14000 genes. Differential methylation analysis identified large scale significant differences between Sotos cases and age/sex/tissue -matched controls (n=12). We report for the first time a significant loss of methylation identified in 493 probes (mapping to the promoter of 443 genes).This group of genes was enriched in anatomical structural morphogenesis, ectoderm development, and carbohydrate transport processes. These epigenetic alterations are likely to explain the developmental features of Sotos syndrome such as malformations, developmental delay/mental retardation. These data demonstrate how crosstalk between deficiency of a histone methyltransferease results in alterations in promoter DNA methylation and how the molecular pathophysiology of a genetic syndrome can be defined by a specific epigenetic signature.

996F

46,XX male associated with a de novo duplication of the SOX3 gene. *R. Babul-Hirji[1], D. Wherrett[2], D. Bagli[3], D. Chitayat[1,4].* 1) Div of Clin and Metab Genetics, Hosp Sick Children, University of Toronto, Toronto, ON, Canada; 2) Division of Endocrinology, Hosp Sick Children, University of Toronto; 3) Division of Urology, Hosp Sick Children, University of Toronto; 4) The Prenatal Diagnosis and Medical Genetics Program, Dept of Obst and Gyne, Mount Sinai Hospital, University of Toronto, Toronto, ON.

Sex in mammals is determined genetically by the sex chromosome complement (XY males and XX females) and phenotypically by the development of gender-specific anatomy, physiology, and behavior. Complete or partial mismatch between the genetic and phenotypic sex result in disorders of sexual development (DSDs). The incidence of complete sex reversal (46,XY females and 46,XX males) is about 1 in 20,000 births. As not all cases of 46,XX male sex reversal involve the SRY gene, other gene(s) or mechanisms are thought to be involved in this disorder. One of the genes recently found to be involved in sex determination is the SOX3 gene. We report a newborn male with an undervirilized male phenotype including bifid scrotum, penoscrotal hypospadias and chordee with a normal phallic length, width and erectile tissue and testes palpable in the labioscrotal folds. His karyotype was 46,XX, FISH for SRY was negative and microarray analysis showed a de novo 0.494 Mb copy number gain in chromosome region Xq27.1. This region contains approximately 4 genes including the SOX3 gene. Sox3 gain-of-function in the developing gonad was found to induce testis development by functioning as a surrogate for SRY. Sutton et al., (2011) reported 3 patients with XX male sex reversal associated with a unique genomic rearrangement of the SOX3 regulatory region. Our case supports the theory that rearrangements of SOX3 cause XX male sex reversal in humans.

997F

XY disorder of sexual development (DSD) associated with microdeletion of chromosome 9p33.3 including the *NR5A1*(SF-1) gene. *L.M. Blanchard[1], K. Desai[1], S. Nimkarn[2], T. Brandt[1], L. Edelmann[1], L. Mehta[1].* 1) Dept. of Genetics & Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) Dept. of Pediatrics, Weill Cornell Medical College, NY.

A 6 1/2 -year-old female with mild developmental delay and minor dysmorphisms presented for re-evaluation due to a new twin pregnancy in her mother. Karyotype at age 3, done for developmental delays, was reported to be 46,XY. Facial features included prominent forehead, long palpebral fissures, slightly cupped ears, malar flattening, full mouth, and 3-4 small café-au-lait macules over the trunk. She was phenotypically female with normal height (-0.064 SDS for females) and normal prepubertal female external genitalia. Pelvic sonogram did not identify a uterus or gonads. Adrenal hormone production after ACTH stimulation was normal. Gonadal testosterone showed poor response to hCG stimulation but normal T to DHT ratio. AntiMüllerian hormone, FSH, LH, inhibin B were appropriate for age. Sequencing of the *AR*, *SRD5A2*, *SRY*, *NR5A1* and *WT1* genes did not detect any pathogenic mutations. At age 6 1/2, she was receiving special education services for communication and articulation problems. Array CGH revealed a novel 1.54 Mb interstitial 9q33.3 deletion. FISH studies on the proband, her mother and cultured chorionic villi from the mother's ongoing twin pregnancy (both fetuses 46,XY) confirmed that the deletion had occurred de novo. The deleted region encompassed 11 genes including *NR5A1*, a transcription factor implicated in testicular and adrenal development. XY patients with heterozygous mutations in *NR5A1* have been reported with variable degrees of undervirilization (from micropenis, underdeveloped testes, genital ambiguity to complete female external genitalia), with presence or absence of Mullerian structures. Adrenal insufficiency was a less consistent finding. Women (XX) with *NR5A1* mutations are mostly ascertained through family studies, and do not have DSD; a single XX patient with adrenal insufficiency is reported. There are two reports of patients with deletions of *NR5A1*: one with genitopatellar syndrome due to a 3 Mb contiguous gene deletion, and another with 970 kb microdeletion and XY DSD. The exact role of *NR5A1* in sexual differentiation and adrenal function remains unclear. This patient further expands the literature, and illustrates the value of array CGH studies in patients presenting with unusual DSDs.

998F

Further Clinical Delineation of BPES associated with microdeletions encompassing *FOXL2*. *K. Enomoto[1,3], Y. Sugawara[2], N. Furuya[3], M. Adachi[4], S. Mizuno[5], Y. Yamanouchi[6], M. Masuno[6], T. Kondo[7], S. Doi[1], S. Mizutani[2], K. Kurosawa[3].* 1) Department of Pediatrics, Perinatal and Maternal Medicine, Tokyo Medical and Dental University Graduate School, Tokyo, Japan; 2) Department of Pediatrics and Developmental Biology, Tokyo Medical and Dental University Graduate School, Tokyo, Japan; 3) Division of Medical Genetics, Kanagawa Children's Medical Center, Yokohama, Japan; 4) Division of Endocrinology and Metabolism, Kanagawa Children's Medical Center, Yokohama, Japan; 5) Department of Pediatrics, Aichi Prefectural Colony Central Hospital, Kasugai, Aichi, Japan; 6) Kawasaki University of Medical Welfare, Kurashiki, Okayama, Japan; 7) Institute for The Handicapped, Mutsumi Home, Misakaeno-sono, Nagasaki, Japan.

Blepharophimosis Ptosis Epicanthus Inversus Syndrome (BPES; MIM# 110100) is a clinically well recognizable autosomal dominant disorder, characterized by the complex eyelid malformation consisting of the literal triad and telecanthus with or without ovarian dysfunction caused by FOXL2 mutations. Individuals with BPES and an intragenic disease-causing mutation, so-called classical BPES, generally have normal intelligence and few extraocular dysmorphic features. In contrast, patients with genomic rearrangements ranging from partial or total FOXL2 deletions to cytogenetically visible microdeletions encompassing neighboring genes frequently present mental retardation, growth failure, and evident extraocular dysmorphic features. In this study, to refine the genotype-phenotype correlations and to add novel insights to clinical diagnosis, we present three new BPES cases associated with microdeletions encompassing FOXL2. Genome-wide microarray-based copy number screening using Agilent SurePrint G3 arrays showed that the deletion intervals varied between 13.7 to 21.6 Mb. Patient 1 is a 10-year-old boy, who presents typical dysmorphic features of BPES, post-natal growth failure, intellectual disability, cerebellar hypoplasia and left hemidiaphragmatic eventration. As diaphragmatic eventration had been misdiagnosed as diaphragmatic hernia in prenatal period, he underwent some excess therapies and examinations in neonate. Patient 2 is a 1-year-old boy, who also presents typical facial features and left hemidiaphragmatic eventration apart from feeding difficulty and growth failure with mental retardation. It was necessary for him to have home oxygen therapy resulted by respiratory disorder with micrognathia complicated by laryngomalacia, and surgical canthoplasty to prevent deprivation amblyopia owing to severe periocular triad. Patient 3 is a 13-year-old girl, who has mild dysmorphic features consistent with BPES and mild eventration of the left diaphragm. She suffers from complex congenital heart disease consisting of truncus arteriosus with left pulmonary arterial defect and right pulmonary arterial malformation leading to significant pulmonary hypertension. She also presents pre-natal growth failure without post-natal catch-up and intellectual disability. Our observations indicate that the cytogenetic rearrangement involving FOXL2 is a recognizable syndrome associated with BPES and cardinal features in the skeletal and diaphragm.

999F

A novel de novo microduplication of 1q21.3-q22 in a patient with growth retardation and developmental delay. *S. Gunn[1], M.N. Strecker[1], P. Mark[2], K. Hovanes[1].* 1) CombiMatrix Diagnostics, Irvine, CA; 2) Spectrum Health Medical Group, Grand Rapids, MI.

We present a 3 year 2 month old male with poor growth and developmental delay. He was the product of a triplet conception which resulted in an *in utero* demise of a female fetus and survival of the two males, born at 29 weeks. Medical history is significant for severe gastroesophageal reflux disease, nystagmus and sleep apnea. Height, weight and head circumference are all <3rd centile. He walked at 24 months and spoke his first word at 30 months. He is generally non-dysmorphic. The twin brother of our patient has been diagnosed with autism spectrum disorder, tachycardia and asthma. A 105K oligonucleotide microarray revealed a duplication of approximately 2.49 Mb: 1q21.3q22(151,137,486-153,630,210)x3, encompassing 93 genes. Confirmation was performed by BAC array. Parental testing was normal, indicating that this is a *de novo* event in the child. To our knowledge, this particular duplication has not been reported previously. A female with a microdeletion of 1.4 Mb overlapping the duplicated region in our patient has been reported as having moderate mental retardation, poor appetite and growth, a high arched palate, and persistent fetal pads. Two patients with larger duplications (10.9Mb and 11.4Mb) overlapping this region have been reported in DECIPHER. This duplicated region overlaps with many genes implicated in OMIM disorders as well as genes expressed in neurons. Autosomal dominant disease genes include: *LOR* (Vohwinkel syndrome and ichthyosis), *CHRNB2* (nocturnal frontal lobe epilepsy), *TPM3* (infantile nemaline myopathy), *ADAR* (dyschromatosis symmetrica hereditaria). Autosomal recessive disease genes include: *PKLR* (hereditary hemolytic anemia due to pyruvate kinase deficiency), *GBA* (Gaucher disease types I), *DPM3* (congenital disorder of glycosylation type I) and *HAX1* (severe congenital neutropenia). Neuronally expressed genes include: *EFNA1*, *EFNA3* and *EFNA4* (members of ephrin gene family which is involved in mediating developmental events, especially in the nervous system) and *KCNN3* which encodes an integral membrane protein that forms a voltage-independent calcium-activated channel involved in regulation of neuronal excitability. It is difficult to know how much the patient's prematurity affects the phenotype, however, follow-up of this patient and classification of other patients with similar and/or overlapping duplications may further elucidate genotype-phenotype correlations and identify the dosage-sensitive genes in the region.

1000F

Copy number alterations in VACTERL association detected by chromosomal microarray analysis. *SY. Shin[1, 2], EJ. Seo[1, 2], JO. Lee[2], M. Hong[2], KJ. Kim[2], AR.E. Kim[3], HW. Yoo[2,3], JK. Ko[2,3], YH. Kim[2,3], IS. Park[2,3].* 1) Dept. of Laboratory Medicine, University of Ulsan College of Medicine and Asan medical Center, Seoul, Korea; 2) Genome Research Center for Birth defects and Genetic disorders, Asan Medical Center, Seoul, Korea; 3) Dept. of Pediatrics, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea.

VACTERL association is a non-random clustering of congenital anomalies: vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies and limb malformations. Although it is relatively common, the underlying genetic mechanisms are not well known yet. In the present study, we performed high resolution 244K or 180K oligonucleotide microarray to find genetic copy number alterations. Fourteen cases (M:F ratio, 1:1) presenting three or more diagnostic signs for VACTERL association were subjected. All the cases had various cardiac defects. Anal atresia was observed in 13 cases (92.9%), renal anomalies in 10 cases (71.4%), vertebral anomaly in 8 cases (57.1%), limb anomaly in 7 cases (50.0%) and tracheo-esophageal fistula in 4 cases (28.6%). Four cases showed single umbilical artery (28.6%). Probably pathogenic copy number alterations were found in three cases (21.4%): deletions at 6q25.3-q27 (10.7 Mb) and 18q12.1 (42 Kb) in two cases, respectively, and gain at 8q24.22-q24.3 (12.0 Mb) with deletion at 7q36.1-q36.3 (9.7 Mb) in another case. The deletion region at 6q25.3-q27 contains genes related to the anatomical structure morphogenesis (MAS1, T, KIF25), and cell proliferation or apoptosis (RPS6KA2, FGFR1OP, PDCD2). The small deleted region at 18q12.1 contains DSC2 which has been reported in familial arrhythmogenic right ventricular dysplasia and cardiomyopathy (OMIM #610476). The case who had gain at 8q24.22-q24.3 and deletion at 7q36.1-q36.3 by der(7)t(7;8)(q36.1;q24.22)-mat presented bilateral colobomas and Currarino's triad overlapping to VACTERL association. Intriguingly, MNX1 reported in a family with Currarino's triad and SHH related to holosencephaly, polydactyly, cleft lip/palate and colobomatous microphthalmia, are located in 7q36. Two cases with large-scale copy number alterations had other multiple malformations besides four signs of VACTERL association, which seems to be caused by contiguous gene syndrome. Case with small deletion at 18q12.1 revealed five signs including limb anomaly of left thumb hypoplasia, suggesting that a further validation is needed for the role of the deleted gene in VACTERL association. The remaining cases without evidence of copy number alterations could be applied to whole-genome or exome sequencing to identify clinically relevant variants.

1001F

Intragenic *De Novo* Deletion of *COL11A1* in a Patient with Stickler/ Marshall Syndrome. *M.L. Wiggins, B.J. Dave, D.L. Pickering, J.M. Stevens, D.M. Golden, J.M. Carstens, D.L. Bishay, E.T. Rush, R. Lutz, W.G. Sanger.* Dept Human Genetics, Univ Nebraska Medical Ctr, Omaha, NE.

Current customized high resolution microarray platforms allow for the detection of novel intragenic copy number alterations that are not detectable and hence cannot be confirmed by FISH, typically the `gold standard' for microarray verification studies. The detection of these very small deletion/ duplications often times present a challenge for the laboratory to establish the clinical relevance and phenotypic correlation is essential for the interpretation. We present a 21-month-old female patient referred for microarray analysis after an evaluation with a clinical geneticist who noted strabismus, cataracts, “ear issues,” a flattened nasal bridge, a small upturned nose and a modestly hypoplastic midface. She was previously examined at birth for chromosome analysis and FISH due to dysmorphic features and multiple congenital anomalies. The laboratory results had revealed a normal 46,XX karyotype and normal aneuploidy FISH results at that time. The current microarray analysis, using a customized oligonucleotide (180K) array that targets genes and exons therein, revealed a 5Kb interstitial deletion within the *COL11A1* target gene region at 1p21.1 implicated in Stickler and Marshall Syndromes. These syndromes exhibit considerable phenotypic overlap and are speculated to represent one phenotypic spectrum. Due to its small size, FISH confirmation was not possible, however, the deletion was confirmed by quantitative PCR (qPCR) utilizing pre-designed copy number assays both within and outside the minimum deletion interval. Parental microarray studies showed the deletion to be a *de novo* event in this patient. Genotype-Phenotype correlations helped in categorizing the microarray result as abnormal. Development of microarray with exon coverage has enabled the detection of intragenic deletions and duplications throughout the human genome. The present study exemplifies that genotype-phenotype correlations along with parental studies are essential for the interpretation of small intragenic copy number changes observed by high-resolution gene arrays. Additionally, confirmatory methods other than FISH are necessary to verify some results detected by high resolution microarray studies.

1002F

Analysis of clinical phenotype and inheritance in 50 nuclear pedigrees carrying mitochondrial DNA m. 3243A>G mutation. *Y. MA[1], F. FAN[2], Y. CAO[3], Y. YANG[3], J. WANG[3], L. ZOU[4], Y. ZHANG[1], S. WANG[1], S. ZHU[5], Y. QI[1].* 1) Central Laboratory Dept, Peking University First Hospital, Beijing, China; 2) Neurology Dept, Beijing Children's Hospital, Beijing, China; 3) Pediatrics Dept, Peking University First Hospital, Beijing, China; 4) Pediatrics Dept, General Hospital of People's Liberation Army, Beijing, China; 5) Statistics Dept, Peking University First Hospital, Beijing, China.

m.3243A>G mutation in mitochondrial DNA (mtDNA) is the most frequent heteroplasmic mtDNA mutation. Individuals carrying m.3243A>G mutation may manifest either as syndromic or nonsyndromic mitochondrial disorders, while others may be asymptomatic. The mechanism underlying the diversity of its clinical presentations is not fully understood. To understand the factors that affect the inheritance of mutant mitochondria from mothers to their descendents, we analyzed clinical presentations and history, characteristics of inheritance, and mitochondria related complications in 50 Chinese families. We recruited 50 patients carrying m.3243A>G mutation of Chinese Han ethnicity and treated in our hospitals, as well as their 50 mothers and fathers during the period from 2005 through 2007. Informed consent was obtained from the patients or patient guardians and their related family members. The study protocols were approved by the Medical Ethics Committee of Peking University First Hospital. The symptoms, disease history, inheritance, mitochondria related complications and m.3243A>G mutation load of 50 probands and their parents were analyzed. In the probands, myopathy, seizures, hirsutism, headache, cognitive impairment, weight loss and short stature were the most common clinical features, and which occur at the same time; and the lactic acid, pyruvate and MRI were abnormal in the most probands. Normal phenotype was found in most mothers, and myopathy, weight loss and short stature were their most common clinical features. m.3243A>G mutation was detected in 50 probands and 45 mothers. In the probands, the mutation ratio in blood was threefold higher and the ratio in urine was twofold higher than those of their mothers. m.3243A>G mutation load in mothers' blood correlated positively with that in offspring's blood, the correlation coefficient r=0.438, P<0.01. In conclusion, the phenotype of patients carrying m.3243A>G mutatiom is variant, the symptoms and results of laboratory in probands were more severity than that in mothers, which might due to the higher m.3243A>G mutation ratio in their tissues. m.3243A>G mutation load in mothers' blood may have a positive correlation with the mutation load in blood a in their children.

1003F

Relatively high frequency of patients with Aarskogg syndrome in a group of patients with cleft lip and palate from Operation Smile Foundation. Colombia. *I. Briceno[1 y 3], A. Venegas[3], J. Martinez[2].* 1) Bioscience, U Niversidad de La Sabana, Chia, Colombia; 2) Grupo de genetica humana, universidad de La Sabana, chia. Colombia; 3) Grupo de Genetica Humana, Universidad Javeriana, Bogotá. Colombia.

Description: Aarskogg syndrome is a very rare congenital disorder characterized by structural abnormalities, growth retardation, wide facial features, short hands and feet, genital abnormalities and mental retardation. Only about 100 cases have been reported with this syndrome in the literature. The most common features are: hypertelorism, ptosis, short hands with short fingers, wide ears, small and very flexible joints, flat feet with wide bulbous toes, abnormal scrotum with extension around the base of the penis, inguinal hernia, and criptorchidea. A rare feature is Cleft lip and palate. The gene responsible is in the short arm of chromosome X (Xp11.21) Case Report: During the medical missions of Operation Smile, Colombia, the Human Genetics group from University of La Sabana, examined 500 patients with cleft lip and palate, syndromic and nonsyndromic, including 6 cases also showing Aarskog syndrome features. The high frequency observed, considering that is a very rare syndrome, suggests a founder effect in Colombian population-.

1004F

Genome-Wide Association Study Identifies Three Loci Associated with Pyloric Stenosis. *B. Feenstra[1], F. Geller[1], C. Krogh[1], M.V. Hollegaard[2], S. Gørtz[1], H.A. Boyd[1], J.C. Murray[3], D.M. Hougaard[2], M. Melbye[1].* 1) Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark; 2) Section of Neonatal Screening and Hormones, Statens Serum Institut, Copenhagen, Denmark; 3) Pediatrics, University of Iowa, Iowa City, Iowa.

Pyloric stenosis (PS) is a severe condition of infancy in which hypertrophy of the pyloric sphincter smooth muscle leads to obstruction of the gastric-outlet. Symptoms, including projectile vomiting, weight loss and dehydration, typically appear between 2 and 8 weeks after birth. Family and cohort studies have established that PS is highly heritable, but the genetic basis of the condition is poorly understood. To identify sequence variants associated with PS, we carried out the first genome-wide association study of the condition on 1,001 surgery-confirmed cases and 2,401 controls with samples drawn from the Danish National Biobank. The 6 most strongly associated SNPs were tested in a replication set of 796 cases and 876 controls, and three SNPs reached genome-wide significance (OR=1.61, P=1.5 x 10^{-17}; OR=1.42, P=1.5 x 10^{-15}; and OR=1.41, P=4.3 x 10^{-12}). Two SNPs represent independent signals (r^2<0.0001) close to the muscleblind-like 1 gene (*MBNL1*), which plays a central role in the regulation of splicing transitions occurring shortly after birth. The third variant is located near the cardiac transcription factor gene *NKX2-5*, which is known for its role in development of cardiac muscle tissue, but also is important for embryonic gut development. In addition to the genetic association signal near *NKX2-5*, we found epidemiological evidence suggesting commonalities in the etiology of PS and congenital heart defect (CHD). Using a cohort of all persons born in Denmark in 1977-2008, the incidence rate ratio of PS given a CHD diagnosis was 2.61 (95% confidence interval 2.05-3.27).

1005F

A novel PAX9 mutation caused oligodontia in a Japanese family. *M. KIDA[1], T. KIKUIRI[2], T. ARIGA[1].* 1) Department of Pediatrics,, Hokkaido University Graduate School of Medicine, Sappro, Japan; 2) Department of Pediatric dentistry, Hokkaido University Graduate School of Dental Medicine.

<Introduction> Agenesis of permanent teeth is defined by two terms; hypodontia (an absence of fewer than six teeth) and oligodontia (an absence of six or more teeth). Agenesis of permanent teeth occurs in 7.1~7.8% of the Danish schoolchildren, whereas the prevalence of oligodontia is very rare at only 0.16%. We had an opportunity to study a Japanese family with nonsyndoromic oligodontia including molecular genetic defects. We performed mutation analysis of the *PAX9* responsible for nonsyndoromic oligodontia in the family, and found a novel mutation.

<Method> Genomic DNA isolated from PBMCs from all the family members in the table below, as well as that from control individuals (n=50), were analyzed. We performed the mutation analysis in the *PAX9* gene. PCR amplified spanning the entire coding region of the *PAX9* using the originally designed primers. For the mutation screening in the patients, PCR products were analyzed by a single-strand conformation polymorphism (SSCP) method. Coding exons of the *PAX9*, together with their flanking introns, were PCR-amplified and then directly sequenced.

Number of missinng teeth

Proband : 16	Proband's brother : 19	Proband's mother : 16	Proband's father : 0

<Result> The number of missing teeth was described in the table above. Proband had fusion teeth consisting of the left lower central incisor and lateral incisor; the mother and brother had peg-shaped upper lateral incisors. Mutation analysis revealed a novel heterozygous single deletion (c.591delC, S197fsX211) within exon 2 of the *PAX9* gene. The mutation was detected only in the patients in the family, not detected in the other family members or control individuals.

<Conclusion> We have identified the novel mutation (c.591delC, S197fsX211) of the *PAX9* in a Japanese family with nonsyndoromic oligodontia. Interestingly, the number of missing teeth was varied among the affected family members, however, the patterns of missing teeth in maxilla was symmetrical in all affected family members.

1006F

Cafe Variome: a novel platform for the routine exchange of genetic variation data. *O. Lancaster[1], R.K. Hastings[1], R. Dalgleish[1], D. Atlan[2], G.A. Thorisson[1], R.C. Free[1], A. Webb[1], J. Muilu[3], M. Byrne[3], A. Blavier[4], F. Wolinski[4], P.E.M. Taschner[5], A.J. Brookes[1].* 1) Department of Genetics, University of Leicester, Leicester, LE1 7RH, United Kingdom; 2) Phenosystems SA, Belgium; 3) Institute for Molecular Medicine, Finland; 4) Interactive Biosoftware, France; 5) Leiden University Medical Center, Netherlands.

Diagnostics laboratories assess DNA samples from many patients with various inherited disorders, and so produce a great wealth of data on the genetic basis of disease. Unfortunately, those data are not usually shared with others. To address this gross deficiency, a novel system has been constructed that aims to facilitate the automated transfer of diagnostic laboratory data to the wider community, via the internet-based Cafe Variome. Diagnostic laboratories are not reluctant to release their data. Instead, the obstacles are merely practical: First, diagnostic laboratory personnel do not have time nor funding to manually submit data to internet depositories such as Locus Specific databases (LSDBs). Second, diagnostic laboratories would receive no recognition or reward for releasing their data, giving them little incentive to even try. The Cafe Variome approach takes account of the real-world obstacles and the needs of diverse LSDBs and aims to minimize the effort required to publish variant data by: a) Endowing data analysis tools used by diagnostic laboratories with a `data submission' function that automatically pushes processed data onto the internet b) Offering manual support to laboratories to move their variant datasets into Cafe Variome (legacy data and new data in batches or in real time) c) Producing a single internet depot (café) to receive these data and make them available for download by diverse third parties. Cafe Variome is not a database, but a depot that mediates data flows between data uploaders and data downloaders and subsequently allows any interested parties to announce and discover a comprehensive listing of observed neutral and disease-causing variants in patients and unaffected individuals. The development of Cafe Variome (based at the University of Leicester) involves the cooperation of diagnostic software companies PhenoSystems (Gensearch) and Interactive Biosoftware (Alamut) as well as academic partners in the Bioinformatics Support Group at Leiden University Medical Centre (LUMC) whose Mutalyzer data-validation tool will allow us to feedback data-inconsistencies to submitters, at the Institute for Molecular Medicine Finland whose development of a standardized variant exchange format (VarioML) allows the system to use just one format for exchange and at the NGRL Manchester who have extensive expertise in diagnostic databases through their development of DMuDB. http://www.cafevariome.org.

1007F

***MLL2* mutation spectrum and clinical phenotype in patients with Kabuki syndrome.** *N.J. Lench, A.N. Barrett, C. Gerveshi, N. Trump, R. Palmer, L. Jenkins, R.H. Scott.* NE Thames Regional Genetics Service, Great Ormond Street Hospital for Children, London, WC1N 3BH, United Kingdom.

Background: Kabuki syndrome (KS) is a rare disorder characterised by a recognisable facial dysmorphism, mild to moderate mental retardation, growth retardation, skeletal and cardiac anomalies and immunological defects. Mono-allelic loss of function mutations have been identified in the *MLL2* gene, a member of the SET domain family of histone methyltransferases that account for approximately 66% of Kabuki syndrome cases. Here we describe the *MLL2* mutation spectrum and clinical profile of 60 patients ascertained from the UK with a clinical diagnosis of Kabuki syndrome. **Methods:** A total of 83 DNA amplicons encompassing the 54 coding exons and intron-exon boundaries of *MLL2* were analysed by bidirectional Sanger sequencing in 60 probands. Where available, parental DNA samples were obtained and sequenced to determine if putative mutations had arisen *de novo* or were stably inherited. In order to screen for large intragenic insertions and deletions in mutation negative patients, we designed a Nimblegen 135k custom microarray encompassing the entire genomic region of the *MLL2* gene. Standardised clinical information was collected on all patients and, where available, facial photographs were also obtained for central review. **Results:** DNA sequence analysis identified *MLL2* mutations in 40 patients (~66%). The mutations were distributed across the entire gene and included frameshift, non-sense, mis-sense and splice site changes. 2 mutations have been reported previously, the remainder are novel. Targeted chromosomal microarray analysis did not identify any obvious copy number changes. Phenotypic features associated with *MLL2* mutations included the characteristic facial features of Kabuki syndrome and growth retardation. **Conclusions:** Our data support other published studies confirming that *MLL2* point mutations are the major cause of Kabuki syndrome, accounting for approximately 66% of cases. However, we were unable to detect mutations in the remaining 33% of patients suggesting that there are other, as yet unidentified causative genes. These findings highlight the importance of *MLL2* mutation screening in patients with Kabuki syndrome and this is now part of the diagnostic portfolio of the NE Thames Regional Genetics Service.

1008F

Multiple or high flow cerebral arteriovenous shunts in children: prospective angiographic and genetic analysis of 26 consecutive cases. *A. Ozanne[1], M. Eyries[2], J.P. Pelage[3], T. Chinet[3], J.H. Blondel[3], F. Coulet[2], J. Roume[3], L. Gouya[3], I. Bourgault-Villada[3], S. Blivet[3], D. Corbazan[3], C. Fagnou[3], G. Lesur[3], M.C. Waill[2], P. Lasjaunias[1], G. Saliou[1], D. Ducreux[3], B. Raffestin[3], P. Lacombe[3].* 1) Neuroradiology, CHU Kremlin-Bicetre, Le Kremlin-Bicêtre, France; 2) Oncogenetics and Angiogenetics laboratory, Pitié-Salpétrière Hospital, AP-HP, Paris, France; 3) HHT pluridisciplinary clinics, Hôpital Ambroise Pare, AP-HP, Boulogne, France.

Hereditary hemorrhagic telangiectasia (HHT) is a heterogeneous vascular disease that can present with a variety of clinical manifestations. The neurovascular complications of this disease, especially in children, may be potentially devastating. The clinical diagnosis of HHT is determined by using the following Curaçao diagnosis criteria : -Epistaxes -Telangiectasias : multiple, at characteristic sites (lips, oral cavity, fingers and nose) - Visceral vascular lesions: gastrointestinal telangiectasias (with or without bleeding) and arterio-venous malformations (pulmonary, hepatic, cerebral and spinal arteriovenous fistula) - Family history : a first-degree relative with HHT. However, Curaçao criteria may not be all present in the pediatric population, and genetic analysis is recommended in case of central nervous system (CNS) high flow arteriovenous fistula (single or multiple). Mutations of the Endoglin (ENG) and Activin A receptor type II-like kinase-1 (ACVRL1) genes are known to be the major genetic factors of HHT. Recently, RASA1 mutations were described in CM-AVM (capillary malformation-arteriovenous malformation) and an association between RASA1 mutations and spinal arteriovenous anomalies was reported. We selected 26 consecutive cases of high flow cerebral arteriovenous fistula (CAVF) presenting at the pediatric age among patients refered for endovascular treatment of CNS arteriovenous shunt in Bicêtre. We excluded single nidus or micro arteriovenous malformation (AVM), and spinal arteriovenous shunt. Angiogramms were analysed, and the possible multiplicity of AV shunts was carefully sought. Genetic variation of HHT1 (Endoglin), HHT2 (ACVRL1) and RASA1 genes was assayed by PCR and sequencing of the entire coding sequence and intronic junctions of the 3 genes. Multiplex ligation-dependent probe amplification (MLPA) was used to screen for large rearrangements of the ACVRL1 and Endoglin genes. Mutations in Endoglin, ACVRL1, and RASA1 genes were found both in single and multiple CAVF. The rate of single versus multiple CAVF seems predominantly associated to RASA1 mutations than HHT1 or HHT2 mutations. The rate of positive genetic analysis in this cohort of pediatric arteriovenous high-flow fistula is 57%. This study emphasizes the importance of the RASA1 gene in pediatric CAVF.

1009F

One extra expanded allele in Huntington patient do not affect the phenotype. *S. Dadgar[1], M. Dehghan[1], O. Aryani[1], M. Houshmand[1, 2].* 1) Genetic Department of Special Medical Center, , No.10, Ostad Nejat allahi Ave, Enghelab St., Tehran, Iran; 2) National Institute of Genetic Engineering & Biotechnology, Pazhohesh Blv. 17th Km Tehran-Karaj Highway, Tehran, Iran.

Huntington disease (HD) is a progressive disorder of motor, cognitive, and psychiatric disturbances. The mean age of onset is 35 to 44 years and the median survival time is 15 to 18 years after onset. Huntingtin gene is the only gene associated with Huntington disease. A trinucleotide CAG repeat expansion is the only cause of the disease. HD is inherited in an autosomal dominant manner. The range of normal alleles are 26 or fewer CAG repeats whereas the range of intermediate alleles and HD-causing alleles are 27-39 and 39-121 CAG repeats, respectively. Anticipation increases disease severity or decreases age of onset observed in successive generations which is known to occur in HD. In the current study, 86 patients were investigated for HD gene by PCR-based method. The results were including 29 patients with one expanding allele (39-53 repeats), five patients with an intermediate allele, one patient with two expanding alleles (40-50 repeats) and one patient with both intermediate and expanding alleles (36 and 42 repeats, respectively). Although we expected that the patients who had two expanding alleles to represent more severe phenotype compared to those with one expanding allele, they both showed the same phenotype. Other overlapping diseases which are most Huntington-Like disease, must be investigated for the rest 50 negative cases.

1010F

A male infant of Leigh syndrome complicated with Fukuyama congenital muscular dystrophy. *H. Kondo[1], C. Tabata[1], A. Yamada[1], K. Hayashi[1], M. Kotani[1], K. Tanda[1], M. Kihara[1], Z. Kizaki[1], K. Murayama[2], A. Otake[3].* 1) Pediatrics, Kyoto First Red Cross Hospital, Kyoto, Japan; 2) Metabolism,Chiba Children's Hospital,Chiba,Japan; 3) Pediatrics,Saitama Medical University Hospital,Saitama,Japan.

Introduction: Fukuyama congenital muscular dystrophy (FCMD) is an autosomal recessive disorder, characterized by hypotonia, symmetric generalized muscle weakness, and CNS migration disturbances that result in changes consistent with cobblestone lissencephaly with cerebral and cerebellar cortical dysplasia. Leigh syndrome (LS) is a progressive neurodegenerative disorder with motor and intellectual developmental delay, signs and symptoms of brain stem and/or basal ganglia involvement, and raised lactate levels in blood and/or cerebrospinal fluid. The disease is caused by either mitochondrial or nuclear gene mutations. We report the first case of LS complicated with FCMD. Case report: A 15 month-old Japanese boy as the third child of the non-consanguineous healthy parents. The older brother died of sudden cardiac dysfunction at 4 months of age. This case was born without any complications, but the serum concentration of creatine kinase was extremely high at the 1st day of age. He suffered from lactic acidosis, hyperglycemia and acute heart failure at 17th day of age. Subsequently he presented with consciousness disturbance, muscle weakness, respiratory failure, hypertrophic cardiomyopathy and hydrocephalus. MRI images showed pachygyria in the frontal region and bilateral symmetrical lesions in the brainstem. A homozygous mutation of the FCMD gene was detected by PCR analysis. The activity levels of mitochondrial respiratory chain complex I+II were decreased in his skin fibroblasts. We diagnosed the case as LS and FCMD by clinical findings, neuroimaging, laboratory data and molecular genetic testing. Conclusion: The comorbidity of LS and FCMD has not been previously reported. We suspected that he had both LS and FCMD not by a contiguous gene syndrome but by chance.

1011F

Molecular analysis and Genetic counseling in Tuberous Sclerosis. *P. Balakrishnan, R.D. Puri, U. Kotecha, S. Kohli, R. Saxena, S. Bijarnia, I.C. Verma.* Center of Medical Genetics, Sir Gangaram Hospital, New Delhi, Delhi, India 11 0060.

We report our experience of molecular diagnosis and counseling in patients of Tuberous Sclerosis attending the genetic clinic. There were twenty three subjects for whom deletion / duplication analysis by MLPA and sequencing of TSC 1 and TSC2 genes was performed between February 2010 and May 2011. The indications for molecular testing in these patients were evaluation during pregnancy for prenatal diagnosis in 6 families and preconception counseling in 9. Mutations have been identified in 13/23 (15 families), of which 04 had deletions identified by MLPA and 09 mutations identified by sequencing of TSC2 gene. There were 09 deletions and 04 point mutations in TSC2 gene. No mutations were identified in TSC1 gene. One novel mutation was present. Of the twenty three subjects, no mutations were identified in 10 probands. Prenatal diagnosis was performed in 06 families. In one family where the mother and two children were symptomatic, prenatal diagnosis was performed by linkage analysis and the fetus was not affected. In the rest of the cases, the mutation identified in the family was evaluated in the fetus. Four pregnancies were not affected. In this cohort, three probands had a positive family history, but in two of them, diagnosis in the parent was established after the current clinical and molecular evaluation. One family decided to continue pregnancy of an affected fetus. This was in view of mild manifestations in the husband and first daughter. However after birth the child developed seizures and has significant neuro-developmental delay. Based on our experience we have formulated a protocol for evaluation of families with tuberous sclerosis. In view of the costs involved in diagnosis, we first perform deletion / duplication of TSC1 and TSC2 by MLPA, followed by sequencing of TSC2 gene. If no mutations are identified so far, we carry out the sequencing analysis of TSC1 gene. Ensuring cost effectiveness of genetic testing is of critical importance in developing economies like India as they are not covered by State or insurance.

1012F

Whole-exome sequencing combined with linkage analysis identifies BSCL2 mutations in two Korean Charcot-Marie-Tooth families. *S.K. Koo[1], M.H. Park[1], H.M. Woo[1], B.O. Choi[2], K.W. Chung[3].* 1) Division of Cardiovascular and Rare Diseases, Center for Biomedical Sciences, National Institute of Health, Cheongwon-Gun, Korea; 2) Department of Neurology, Ewha Womans University School of Medicine, Seoul, Korea; 3) Department of Biological Science and Research Center for Biotechnology, Kongju National University, Gongju, Chungnam, Korea.

Charcot-Marie-Tooth (CMT) is a clinically and genetically heterogeneous disorder of the peripheral neuropathy. To identify the causative mutations in two Korean CMT families (FC51 and FC305) with autosomal dominant transmission, we conducted genome-wide linkage analysis using Axiom Genome-Wide Human Array and whole-exome sequencing with two individuals in each family. In the whole exome analysis, two patients of FC305 were used for the identification of the sharing variants, while one patient with a normal relative of FC51 was applied for the exclusion. We found that evidences of linkage in each family mapped to a region on chromosome 11p11-11q13.3 with LOD scores of 2.7 (FC51) and 3.3 (FC305), respectively. In this linkage region, we identified 147 variants (FC51) and 287 and 266 variants (FC305) after filtering the non-functional changes (intronic or synonymous varitnats). Integrating the results from the two individuals within the family, we identified the causative mutations of BSCL2 in each family, which were previously shown to be associated with distal hereditary motor neuropathy (silver syndrome). Our study shows that the power of exome sequencing with linkage analysis could be efficiently applied to the diagnosis in a dominant-trait disease.

1013F

Differences in fractures and activity in children with NF1. *E.K. Schorry[1], J.K. George-Abraham[1], M.B. Rieley[3], D.A. Stevenson[2], D.H. Viskochil[2], L.J. Martin[1], H.J. Kalkwarf[1], R.J. Hopkin[1], A.M. Stevens[2], S. Allen[2].* 1) Div Human Gen, Cincinnati Childrens Hosp, Cincinnati, OH; 2) University of Utah, Salt Lake City, UT; 3) Bangor, ME.

Neurofibromatosis 1 (NF1) is an autosomal dominant genetic disorder which can affect multiple different organ systems. Osseous abnormalities occur in at least 30% of patients with NF1, and there is growing evidence of a generalized bone dysplasia associated with NF1. Mild osteopenia has been well documented in both adults and children with NF1, although the significance of this in terms of fracture risk has not yet been determined. We undertook a retrospective study to determine incidence, types, and location of fractures in children with NF1, ages 5-20 years, using a standardized questionnaire. We surveyed 256 individuals with NF1 from two multidisciplinary clinics, and compared to 178 controls of similar ages and gender. Data collected included numbers and location of fractures, dietary calcium intake, and physical activity levels. There were no significant differences in age, gender, or dietary calcium intake between the two groups. Interestingly, we found no significant difference in rate of ever having a fracture in the NF1 group (24.1%) versus the control group (23.7%). However, there were significant differences in location of bone fractured. Control patients were most likely to have fractures of the wrist and arm (54%), which are the most commonly fractured bones in the pediatric population; and rarely had fractures of the leg and ankle (5.3%). In contrast, NF1 patients (excluding those with known long bone dysplasia) were less likely to fracture wrist and arm (32%), and more likely to have fractures of the leg and ankle (15.5%). One NF1 patient with known osteoporosis had vertebral compression fractures, a fracture type not seen in any control patients. Physical activity scores, measured as MET/week, were significantly lower in the NF1 group compared to controls ($p<.001$). Based on these data, we speculate that NF1 patients, even those lacking a known dysplastic bone or tibial bowing, may have an inherent weakness in bones of the lower extremities, leading to increased fracture rate in those locations. Although overall rate of ever fracturing was not different between the 2 groups, the much lower physical activity level in the NF1 group may have prevented them from having a higher fracture rate. This study may lead to better understanding of the osseous dysplasia underlying NF1.

1014F

Interstitial duplication 15q11-q13 syndrome: language impairment, autism, abnormal EEG patterns and mild facial anomalies. *N. Urraca[1], J. Cleary[2], E. Pivnick[3], K. McVicar[3], V. Brewer[3], R. Thibert[4], LT. Reiter[1,3].* 1) Department of Neurology, UTHSC, Memphis, TN; 2) Department of Speech and Language Pathology, University of Memphis, Memphis, TN; 3) Department of Pediatrics, UTHSC, Memphis, TN; 4) Department of Neurology, Mass General Hospital, Boston, MA.

Chromosome 15 has a number of low copy repeats that predispose regions to deletion/duplication events mediated by non-allelic homologous recombination. The 15q11-q13 region also has a cluster of genes preferentially expressed from one parental allele. It is estimated that ~3-5% of all autism cases may be the result of this 15q copy number variant (CNV). Most cases are de novo and maternal in origin. There are two major types of interstitial duplications on 15q: class I with breakpoints at BP1 (proximal) to BP3 (distal) and class II with breakpoints from BP2 (proximal) to BP3. In this study we performed an in depth phenotype analysis of individuals with interstitial 15q duplications and determined if maternal duplication is required for the diagnosis of autism spectrum disorder (ASD). A Clinical Geneticist and a Pediatric Neurologist blind to parental origin and breakpoints evaluated the subjects. We used neuropsychological, language and ASD diagnostic tools for phenotypic analysis. Methylation Sensitive High Resolution Melting (MS-HRM) analysis of the maternally methylated SNRPN locus was used to determine the parent of origin of the duplication. Fifteen subjects have been recruited: 11 maternal and 4 paternal cases. Twelve are de novo and 3 familial cases with one pair of siblings. Four of 11 maternal cases were Class I and 4 of 11 Class II while the paternal cases 2/4 Class I vs. II. Most subjects had mild nasal labial fold asymmetry and a rounded short nasal tip. EEG report: 9/15 had excessive 18-22 Hz beta activities throughout the waking record. This EEG pattern appears to be specific to the duplication and not the parent of origin or presence of ASD. Nine maternal and 2 paternal subjects scored as ASD using ADOS/ADI-R analysis. Most subjects had a low-moderate adaptive functioning score on the Vineland II test with no differences among groups. All subjects performed below age corrected average for receptive language but we found a significant difference ($pval<0.03$) between maternal class I and maternal class II subjects. In conclusion, the majority of int dup(15) cases ascertained were maternal in origin, most likely due to the coincidence of ASD with maternal duplication. The size of the duplication did not correlate with the severity of the phenotype. Subjects had mild facial anomalies. Receptive language impairment was evident, but will require more subjects to be accurately described.

1015F

Exome sequencing identifies a DYNC1H1 mutation in a large pedigree with dominant axonal Charcot-Marie-Tooth disease. *M.N. Weedon[1], R. Caswell[1], R. Hastings[2], W. Xie[1], K. Paszkiewicz[3], T. Antoniadi[4], M. Williams[4], C. King[2], L. Greenhalgh[2], R. Newbury-Ecob[2], S. Ellard[1].* 1) Peninsula Medical Sch, Exeter, United Kingdom; 2) Clinical Genetics Department, University Hospitals Bristol NHS Foundation Trust, St Michaels Hospital, Bristol, United Kingdom; 3) Exeter Sequencing Service, Biosciences, College of Life & Environmental Sciences, University of Exeter, Exeter, United Kingdom; 4) Bristol Genetics Laboratory, Pathology Sciences, Southmead Hospital, North Bristol NHS Trust, Bristol, United Kingdom.

Charcot-Marie-Tooth disease is characterised by length-dependent axonal degeneration with distal sensory loss and weakness, deep tendon reflex abnormalities and skeletal deformities. It is caused by mutations in over 40 genes. We investigated a four generation family with 23 members affected with the axonal form (type 2) where the common causes had been excluded by Sanger sequencing.Exonic sequences from 3 affected individuals separated by 8 meioses were enriched using Agilent's SureSelect kit and sequencing performed on an Illumina GAII. Variants were filtered to exclude variants in the dbSNP131, 1000 genomes and our in-house exome databases.

We identified 177, 192 and 199 novel heterozygous variants in the three individuals. Only one variant was shared by all three and testing of 6 additional affected family members showed co-segregation with a maximum LOD score of 3.6. The shared DYNC1H1 gene variant is a missense substitution, p.His306Arg, at a highly conserved residue within the homodimerisation domain of cytoplasmic dynein heavy chain 1. Three mouse models with different mutations within this domain have previously been reported with age-related progressive loss of muscle bulk and locomotor ability.

This is the first report in humans of a dynein gene mutation linked to neuropathy. Cytoplasmic dynein is a large multi-subunit motor protein complex with a key role in retrograde axonal transport in neurons. Our results demonstrate the power of exome sequencing for genetic diagnosis and highlight the importance of dynein and retrograde axonal transport in neuronal function in humans.

1016F

Tay Syndrome: a rare syndrome diagnosed in two brothers. *F.T. de Lima[1], T.E. Sfakianakis[1], E. Perrone[1], M.F.F. Soares[2], V.F.A. Meloni[1].* 1) Morphology and Genetics, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil; 2) Department of Imaging Diagnosis, Universidade Federal de São Paulo, São Paulo, SP, Brazil.

Trichothiodystrophy (TTD) is a distinct group of autosomal recessive disorders with a wide range of phenotypic expression, unified by the presence of sulfur-deficient brittle hair. Patients with TTD have abnormal production of transcription factor IIH (TFIIH), active in basal transcription and nucleotide excision repair, due to mutations in genes encoding 3 subunits of TFIIH—ERCC2(XPD), ERCC3(XPB), and GTF2H5(TTDA). Associated findings may include short stature, intellectual disability, photosensitivity, ichthyosis, nail dystrophy, cataracts and immunodeficiency. Generalized dysmyelination is the most common neuroimaging abnormality in TTD. Some other nonspecific abnormalities may include hipomyelination, cortical heterotopias, partial agenesis of the corpus callosum, perimedullary fibrosis of the spinal cord, and intracranial calcifications. It has also radiological evidence of progressive central osteosclerosis. The association of TTD with uncommon features is far rarer and it is known by different names, such as Tay syndrome or PIBIDS (OMIM #601675), an acronym standing for Photosensitivity, Ichthyosis, Brittle hair, Intellectual impairment, Decreased fertility, and Short stature. We describe two brothers showing ichthyosis, nail dystrophy, short stature, and intellectual disability. Other clinical features presented in these patients were low birth weight, growth deficiency, microcephaly, low-set and posterior rotated ears, dental abnormalities and cataracts. The clinical picture prompted the diagnosis of TTD and, for the presence of additional symptoms, within the broader spectrum of Tay syndrome. They also had radiographic evidence of progressive central osteosclerosis, distal osteoporosis, and marked increase in density of several bones. There were MR imaging findings of dysmyelination and intracranial calcifications too. Similar cases with this combination of signs and symptoms have been described in literature, but little is known about the natural history of this disease. These further cases described here provide clear evidence that TTD should be included among the wide range of differential diagnosis of the affected pediatric population, as long as they carry this group of specific signs and symptoms, which are the presence of diffuse dysmyelination in conjunction with documented hair and skin abnormalities and the presence of central osteosclerosis. Key Words: Tay Syndrome - Trichothiodystrophy - Dysmyelination - Central Osteosclerosis.

1017F

Trajectories of Cognitive-Behavioral Development in Children with Wolf-Hirschhorn Syndrome. *GS. Fisch[1], JC. Carey[2], A. Battaglia[3], R. Falk[4], R. Simensen[5].* 1) NYU College of Dentistry Dept. Epidemiology & Health Promotion New York, NY; 2) University of Utah Dept. of Pediatrics Salt Lake City, UT; 3) Dept. Child Neuropsychiatry Postgraduate Medical School University of Pisa, Pisa, Italy; 4) Dept. of Pediatrics Cedar Sinai Medical Center Los Angeles, CA; 5) Greenwood Genetics Center Greenwood, SC.

Norms for neurocognitive and neurobehavioral development established early in the 20th century were used to detect infants with developmental delays. However, after a child is identified as delayed, it is important to establish trajectories of development in those youngsters, and for several reasons. First, trajectories aid clinicians and caregivers what to expect as individuals in this cohort ages. Second, they facilitate research examining brain-behavior relationships. Third, if specific genetic disorders are involved, to determine genotype-brain-phenotype relationships. Previously, we and others have examined trajectories of development in children with the fragile X mutation (FMR-1), Williams-Beuren syndrome (WBS), and NF1. In our researches, we found different trajectories for each of these disorders. The purpose of our current research is to examine trajectories in children with subtelomeric deletions. Specifically, in this report, we tested 20 children with deletion 4p16, Wolf-Hirschhorn syndrome (WHS), and retested 15 two years later. Their ages at initial testing ranged 4-20 years. They were tested at 9 sites in the US and Europe, using a comprehensive neuro-psychological battery. Our assessment battery consisted of 5 standardized measures: cognitive ability (Stanford-Binet 4th Ed: SBFE), adaptive behavior (Vineland Adaptive Behavior Scale: VABS), emotionality and temperament (Child Behavior Checklist: CBCL), attentiveness/ hyperactivity (Conners Parent Rating Scale: CPRS), and autism (Child Autism Rating Scale: CARS). Most of our participants tested at or near the floor value of the SBFE and VABS. Therefore, we chose to examine this cohort using raw scores from these 2 tests, and to compare them with raw score trajectories of age-matched children diagnosed with FMR-1 or WBS. Results show that children WHS are able to complete fewer items on the SBFE, have fewer Daily Living Skills as measured by the VABS, have a flatter trajectory than children with either FMR-1 or WHS; but, socialization levels and trajectories are comparable among the 3 genetic disorders.

1018F

Variability of phenotype in monozygotic twins with Charcot Marie Tooth type 4C (CMT4C). *P. Laššuthová[1], D. Šišková[2], P. Seeman[1].* 1) Dept of Child Neurology, The Motol University Hospital 2nd Medical School, Prague, Czech Republic; 2) Dept of Child Neurology, Thomayer University Hospital, Prague, Czech Republic.

Charcot-Marie-Tooth (CMT) type 4C neuropathy (CMT4C) is characterized by autosomal recessive (AR) inheritance, demyelinating neuropathy and early onset spine deformities. CMT4C is caused by mutations in the SH3TC2 gene. Prevalent mutation in Czech population and also in several other populations is a nonsense mutation p.Arg954Stop. CMT4C seems to be the most frequent type of AR CMT and one of the most frequent of all CMT types among Czech patients. Scoliosis is a typical presenting symptom in most of the CMT4C patients. We report a striking variability in the phenotype of two monozygotic twins - brothers - who are both affected by CMT4C and both carry the same mutation p.Arg954Stop in the SH3TC2 gene in homozygous state. Despite this, each of them has had a different course of the disease. Neither parents nor other family members are affected. Mother and father are heterozygous carriers of the p.Arg954Stop. One of the boys has scoliosis since the age of 4, which is in contrast to the other brother who has no scoliosis at the age of 11 years. The last time we have examined them was when they were 11 years old. Patient 1 plays football and rides a bicycle. However, his ability to run is worsened. He has foot deformities. Muscular atrophy was only very mild. No scoliosis was visible. In patient 2, first symptoms were evident at the age of 4 with scoliosis. From this time on the disease has been slowly progressive. At the age of 11 the patient has prominent foot deformities and pronounced scoliosis. Whether exogenic factors, epigenetics or even modifier genes have contributed to phenotypic differences remains to be explained. Supported by IGA MZ CR NT 1521-4.

1019F

Molecular testing enhances characterization of EVC phenotypes. *L. Mehta[1], H.Q. Rana[1], M. Galdzicka[2], E. Ginns[2].* 1) Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) Molecular Diagnostics Laboratory, UMass Medical School, UMass Memorial Medical Center, Worcester, MA.

Weyers Acrofacial Dystosis (WAD) and Ellis van Creveld syndrome (EVC) are allelic conditions caused by mutations in the EVC and EVC2 genes. Historically, the WAD phenotype is described as a milder condition than EVC, with short stature, postaxial polydactyly and nail and dental anomalies segregating in an autosomal dominant pattern, while classic EVC is autosomal recessive, with congenital heart defects in 60% of patients. However, a wide range of severity is known for EVC. We describe a patient who appeared clinically to have a WAD phenotype, but on molecular testing was found to have compound heterozygous mutations in EVC2. The 40 year old man reported a history of bony postaxial polydactyly of his hands for which surgery was performed at 1.5 years of age. He has had dental problems with peg teeth. Height was 160 cm (3rd centile, SDS -2.67). Limbs were short with normal trunk. He had hypoplastic nails on both hands and feet. Echocardiogram was normal. Family history was negative for similar problems, with parents being of normal heights. Sequencing of the EVC and EVC2 genes revealed two mutations in the EVC2 gene, Q755X and IVS21+2T>C. Each parent was heterozygous for one of the mutations. These mutations have been reported in patients with EVC in association with a second mutation. Neither mutation has been reported to cause WAD phenotype. Mutation Q755X results in a premature stop codon in exon 14 (c.2263C>T) and produces a truncated protein. The intron 21 change has previously been described in two cases of EVC and is predicted to cause a splicing error. While the initial consideration in our patient was for WAD, the molecular testing supports a diagnosis of mild EVC. This case highlights the phenotypic continuum of EVC and WAD, both disorders caused by mutations in EVC and EVC2. Since mild EVC and WAD cannot be distinguished on a clinical basis, in a “simplex” case with negative family history and no consanguinity, molecular testing is critical for accurate genetic counseling.

1020F

Genetic Effects on the Craniofacial Morphology of Unaffected Parents of Children with Oral Clefts. *L.M. Moreno[1], S. Miller[1], D. Defay[1], S. Weinberg[2], M.L. Marazita[2], J.C. Murray[3], G.L. Wehby[4].* 1) Dows Inst Dental Research, Univ Iowa, Iowa City, IA; 2) University of Pittsburgh, Pittsburgh, PA; 3) Department of Pediatrics, University of Iowa, IA; 4) College of Public Health, University of Iowa, IA.

Purpose: Nonsyndromic cleft lip and palate (NSCL/P) is a common birth defect caused by genetic and environmental factors and their interactions. Besides overt clefts of the lip and palate, other subphenotypic characteristics such as distinct craniofacial morphology can be present in NSCL/P individuals and their close relatives. Most of the genetic effects that account for this diversity of NSCL/P phenotypes are yet to be identified. The purpose of this study was to identify features of distinct craniofacial morphology in unaffected parents of children with NSCL/P and control parents of unaffected children and to investigate whether candidate genes for NSCL/P and left-right asymmetry exert effects on these features using a multivariate regression model. Methods: Facial 3D images of 123 unaffected parents (39 males and 84 females) and 57 control parents (23 males and 34 females) were analyzed with 24 common anthropometric landmarks. 3D coordinates were used to derive nine linear distances and were scaled for size using Procrustes methods. Procrustes residuals were analyzed using principal component analyses. All analyses were performed for males and females separately. DNA samples were genotyped for 13 SNPs on 4 clefting candidate genes/loci (IRF6, FOXE1, 8q24, 20q12) and 4 left-right asymmetry genes (LEFTY1, LEFTY2, SNAI1, ISL1). Results: Preliminary results indicate that male cases have significantly (uncorrected $p<0.05$) narrower and more protrusive faces than controls. Female cases have significantly (uncorrected $p<0.05$) larger philtrum widths, lower face height, lower face protrusion and smaller facial sizes than controls. In males, SNP rs1443434 near FOXE1 explains about 24% of the difference in facial width and lower face protrusion ($p=0.02$) between case and control parents. In females, rs37585249 near FOXE1 explains about 7% of the difference in lower face height ($p= 0.04$) and SNP rs360059 near LEFTY1 explains about 7% of the difference in lower face protrusion between case and control parents ($p=0.03$). Conclusions: Identifying genotype-phenotype correlations between distinct craniofacial morphology features and genes involved in craniofacial development is essential for understanding the genetic etiology of NSCL/P. Support:DE016148, CDC5R01DD000295, AAOF 2008 OFDFA.

1021F

Association of CFH and HTRA1/ARMS2 Polymorphisms with Early-Age related Maculopathy. *H. Chen[1], J.H. Chen[1], Y. Yang[1], P.O.S. Tam[2], M. Zhang[1], C.P. Pang[2].* 1) Joint Shantou International Eye Center, Shantou, Guangdong, China; 2) Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong Special Administration Region, China.

Purpose: The polymorphisms of complement factor H (CFH) and HtrA serine peptidase 1 (HTRA1)/Age-related maculopathy susceptibility protein 2 (ARMS2) have been reported to be associated with age-related macular degeneration (AMD). However, the genetics of early age-related maculopathy (ARM) less investigated and remains unclear. In the current study we investigated the association of CFH and HTRA1/ARMS2 polymorphisms with early ARM in a Chinese Han cohort. Methods: The current study cohort contained 157 early ARM patients and 161 controls recruited in Nan'ao, an island in southern China. CFH single nucleotide polymorphisms (SNP) rs1061170 and rs800292 and HTRA1/ARMS2 SNPs rs10490924 and rs11200638 were genotyped using Taqman genotyping assays. Logistic regression implemented by the R statistical language was used for association analysis. Results: None of the 4 SNPs deviated from Hardy Weinberg Equilibrium ($P > 0.05$). For association with early ARM, none of the SNPs showed any statistical significance in single SNP association analysis, with allele odds ratio ranging from 1.03 to 1.15 ($P > 0.05$). Neither haplotypes of CFH or HTRA1/ARMS2 were significantly associated with early ARM ($P > 0.05$). Conclusions The association of HTRA1/ARMS2 and CFH polymorphisms in early ARM was not detected in our cohort. The odds ratios close to 1 might indicate the effects of AMD-associated gene in early ARM could be much lower compared to those in AMD. These preliminary findings thus warrant further studies.

1022F

Clinical genetics resources at NCBI: ClinVar and ISCA support evidence-based interpretation of human variation. *J. Paschall, D.R. Maglott, J. Lee, L. Phan, D.M. Church, G. Riley, M. Ward, D. Shao, R. Maiti, R. Tully, S. Chitipiralla, J. Garner, S. Stefanov, M. Feolo, S.T. Sherry, J.M. Ostell.* NCBI, NIH/NCBI/NLM, Bethesda, MD.

The interpretation of genetic variants observed during clinical genetic testing is increasingly a limiting factor in translating genomic technology advances into medical practice. Current practice is often insular, because records of variation, supporting clinical and experimental evidence, and current interpretations are not captured in a structured format or available from a shared public resource. Patient safety as well as practicality and efficiency demand a community effort of integrating data so evidence based methods of interpreting clinical variation can be facilitated. We are working with members of the testing community to establish such a shared framework. **The ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar)** was established to provide structured, archived, versioned records of asserted relationships among observed variations, phenotype, and supporting evidence. ClinVar is part of the infrastructure of NIH's Genetic Testing Registry (GTR, http://www.ncbi.nlm.nih.gov/gtr), but also has a wider scope. Review of analytical validity is supported by capturing methods of identifying the variation. Types of evidence include observations in affected or unaffected individuals, animal models, in vitro assays, and in silico predictions. We are actively working to ensure that submitted data can be exchanged with other groups seeking an integrated and cross-validated clinical-grade global knowledgebase. Some of the data in ClinVar are provided from another project hosted at NCBI, the International Standard Cytogenetic Arrays consortium (ISCA), which is focused on accumulating observations and applying evidence-based standards in the area of cytogenetic clinical testing. The ISCA effort incorporates tools to identify regions of conflicting interpretation to direct expert review, as well as establishing evidence-based guidelines where the current weight of evidence is judged by experts to support such conclusions. Data in ClinVar are integrated with other resources such as OMIM ®, PubMed, Gene, dbSNP, dbVar, and RefSeq. In addition to the web site, ClinVar can be accessed via ftp and standard NCBI tools such as E-utilities API, RSS feeds, and queries registered in MyNCBI. We expect to provide reports that can be incorporated into existing clinical interpretation analysis workflows. We strong encourage feedback and participation from the clinical genetics community, please contact us at (clinvar@ncbi.nlm.nih.gov).

1023F

Telegenetics Clinics in the United States: Successes, Challenges, and Future Directions. *S. Au[1], H. Andersson[2], L. Creel[3], S. Kubendran[4].* 1) Genetics Program, Hawaii Department of Health, 741 Sunset Avenue, Honolulu, HI 96816; 2) Hayward Genetics Center SL-31, Tulane University Medical Center, New Orleans, LA 70112; 3) Mountain States Genetics Regional Collaborative Center, 8501 North MoPac, Suite 300, Austin, Texas 78759; 4) KU School of Medicine-Wichita, 1010 N. Kansas, Wichita, KS 67214.

In much of the U.S., there is a shortage of genetic specialists, and rural and isolated populations are often underserved. Telemedicine offers a cost-effective solution to this problem by increasing access to genetic services for families living far from genetic centers. It is being increasingly used by many specialties as an innovative way of delivering clinical services and decreasing travel time for both the patient and the provider. However, the utilization of telemedicine in clinical genetic services has been limited, and there is a general lack of knowledge of telemedicine as a successful service delivery model among genetics professionals. With health care reform working to incorporate telemedicine into routine care including appropriate reimbursement, the genetics community must proactively take advantage of the benefits and promise of telemedicine. Funded through the Health Resources and Services Administration, the seven Genetics and Newborn Screening Service Collaboratives (RCs) are in various stages of using telegenetics to increase access to genetic evaluation, counseling, and management, particularly in rural and underserved communities. This session will include discussions of programs in the Heartland, Southeast, Mountain, and Western States RCs. The Heartland RC holds regularly scheduled telegenetics clinics located in AR, KS, and MO that provide services within these states and across state lines. The Southeast RC provides clinical telegenetics services from the genetic specialists directly to an in-patient hospital floor and also to a community pediatric clinic. The Southeast RC also hosts monthly telegenetics regional grand rounds for continuing genetic specialist education. The Mountain States RC provides genetic counseling services for oncology and pediatric hearing loss patients through telegenetics. The Western States has telegenetics activities within states (OR and WA), between states (OR to ID), and across the waters of the Pacific (Honolulu and Hawaii neighbor islands). The panel discussion will: 1) illustrate the successes and challenges faced by a small medical specialty in adapting to telehealth, 2) address gaps in knowledge and performance by providing concrete steps on how to establish and maintain a telegenetics program, and 3) demonstrate telegenetics as an innovative service delivery model that could be emulated in other underserved regions in country.

1024F

A rare DNA variant in a cis-overlapping motif in an IRF6 enhancer element is associated with Van der Woude Syndrome. *W. Fakhouri[1], F. Rahimov[2], H. Zhou[3], T. Du[1], E. Kouwenhoven[3], H. van Bokhoven[3 and 4], J. Murray[2], B. Schutte[1 and 5].* 1) Microbiology and Molecular Gen, Michigan State University, Lansing, MI; 2) Department of Pediatrics, University of Iowa, Iowa City, Iowa; 3) Department of Human Genetics, Nijmegen Centre for Molecular Life Sciences; 4) Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition, and Behavior, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 5) Department of Human Development, Michigan State University, Lansing, MI.

Cleft lip and palate (CLP) is one of the most common birth defects in humans with an incidence rate of 1/700 live births. DNA variation in Interferon Regulatory Factor 6 (IRF6) causes Van der Woude syndrome (VWS), an autosomal dominant form of CLP, and contributes risk for orofacial clefting, including a common DNA variant rs642961. Rs642961 is located in a multi-species conserved sequence (MSC) that is 9.7 kb upstream from the IRF6 transcriptional start site. The MCS9.7 element was shown to possess enhancer activity that mimicked the expression of endogenous Irf6 in oral epithelium and periderm, but not in medial edge epithelium of the palatal shelves. In order to identify possible etiologic DNA variants, we sequenced MCS9.7 in DNA samples obtained from individuals with VWS. We screened 48 DNA samples for which no disease-causing mutation was detected in IRF6 exons. We observed one new DNA variant. The DNA variant is an A insertion that is predicted to disrupt the DNA binding for both p63 and for bHLH transcription factors. Interestingly, the predicted binding sites for these two families of trans factors overlap completely. For the large family of bHLH trans factors, we focused on four members whose expression pattern appeared to overlap with Irf6. Using a DNA binding assay, we observed that this DNA variant abrogated binding by p63 and reduced the binding affinity for the bHLH trans factors. In a transient transactivation assay, we observed strong enhancer activity by the MCS9.7 element. This activation was highly dependent on p63, and the activation was abrogated by the A insertion mutation. In conclusion, these data are consistent with the hypothesis that the rare DNA variant at the cis-overlapping site in MCS9.7 is etiologic for VWS, and supports the rationale for additional mutation screening of the MCS9.7 enhancer element in patients with cleft lip and palate.

1025F

Histopathologic changes associated with development of retrosternal CDH in *Frem1*-deficient mice. *T.F. Beck[1], M.J. Wat[1], B.J. Kim[1], H. Zaveri[1], O. Shchelochkov[2], M. Justice[1], B. Lee[1], D.A. Scott[1].* 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pediatrics, University of Iowa, Iowa City, IA.

Congenital diaphragmatic hernia (CDH) is a life-threatening sporadic birth defect that affects approximately 1 in 4000 newborns. Terminal deletions of chromosome 9p have been identified in several patients with CDH. However, the gene(s) that contribute to the development of CDH in these patients have yet to be identified. In a recessive ENU mutagenesis screen we identified a mouse strain that was homozygous for a truncating mutation (L826X) in *Frem1*—a basement membrane protein encoding gene located on chromosome 9p22.3. A portion of *Frem1*-deficient mice develop retrosternal CDH. Penetrance of this phenotype is highly dependent on strain background, providing evidence for the existence of one or more genetic modifiers. Several other genes have been implicated in the development of retrosternal CDH in mice including *Slit3*—which encodes another extracellular matrix protein—and *Gata4*—which encodes a transcription factor that has also been implicated in the development of CDH associated with 8p23.1 deletions in humans. We have shown that both of these genes interact genetically with *Frem1* in other developmental processes. Analyses of *Slit3* and *Gata4* mouse models of CDH suggest that a number of histopathalogic changes in the anterior diaphragm may contribute to an increased risk of developing CDH. These changes include decreased cell proliferation, increased cell death, decreased diaphragm thickness and disorganized collagen fibrils in the central tendon. We are presently working to determine if similar changes are present in Frem1-deficient mice. Preliminary results of phospho-histone H3 immunostaining revealed a trend towards decreased cell proliferation in the anterior and central diaphragm of *Frem1*$^{L826X/L826X}$ mice compared to littermate controls. A similar pattern has been reported in *Slit3* mice, suggesting that the extracellular matrix plays an important role in regulating cell proliferation in the developing diaphragm. We are also working to determine the expression pattern of Frem1 and associated genes in the developing diaphragm. These studies will help us understand the mechanisms by which *Frem1* deficiency causes retrosternal CDH in mice and may provide insight into the causes of similar hernias in humans.

1026F

Infantile systemic hyalinosis presenting as multiple joint pain. *B. Chung[1], E. Lausch[2], A. Superti-Furga[3], T.Y. Tan[1,4].* 1) Pediatrics & Adol Med, University of Hong Kong, Hong Kong, ON, China; 2) Centre for Pediatrics and Adolescent Medicine, University Hospital Mathildenstr., Freiburg, Germany; 3) Centre Hospitalier Universitaire Vaudois, University of Lausanne, Switzerland; 4) Genetic Health Services Victoria, Murdoch Children's Research Institute, Department of Paediatrics, University of Melbourne, Australia.

Background: Infantile systemic hyalinosis (ISH) is a rare autosomal recessive disorder characterized by abnormal hyaline deposits in the papillary dermis and other tissues. It presents in early infancy with severe pain with movement, progressive joint contractures, thickened skin and hyperpigmented macules over bony prominence. Gingival hypertrophy, skin nodules, perianal masses are common but late findings. We report a female infant with ISH and subsequently confirmed to have known pathogenic mutations in the ANTXR2 gene. Clinical information: The proband was the 3rd child of a Pakistani couple who were first cousins. Her elder sister passed away at 8 months with unknown cause. She was born full term by NSD with normal growth parameters and Apgar scores. Newborn examination was normal. She presented with decreased limb movement at 3 months. Clinical assessment showed that limb movement was limited by severe pain; her ears were simple but prominent and there were hyperpigmentation over the finger knuckles and ankles. Skeletal survey showed metaphyseal/submetaphyseal widening. Bone marrow examination excluded myeloproliferative disorders. The absence of fever and skin rash, normal ophthalmologic examination and normal levels of serum inflammatory markers made the diagnosis of Chronic infantile neurolological, cutaneous and articular (CINCA) syndrome unlikely. As limb pain and the skin findings could be the only findings in the early stage of ISH, genetic testing was offered to the parents with genetic counseling. A known pathogenic homozygous mutation, c.[652T>C] was found in the ANTXR2 gene causing an amino acid substitution in codon 218, p.C218R, confirming the diagnosis of ISH. The parents were carriers of the same mutation. Conclusion: ISH is more commonly reported in ethnic groups with consanguineous marriage. This patient developed protein-losing enteropathy at 5 months, perianal masses at 6months and mild gingival hypertrophy at 8 months. The prognosis of patients with ISH was poor and they usually die at infancy with recurrent infections and malnutrition.

1027F

A patient with Van den Ende-Gupta syndrome: Molecular analysis and comparison of clinical features with previously reported patients. *G.C. Gowans, K.C. Platky, K.E. Jackson, P.L. Brock, J.J. Wetherbee-Landis, J.H. Hersh, K.M. Goodin, A. Asamoah.* Genetics Unit, WCEC, Pediatrics, University of Louisville, Louisville, KY.

Mutations in the SCARF2 gene (MIM 613619) have recently been identified (AJHG, 87:553-559.) in some patients with the rarely reported Van den Ende-Gupta syndrome (MIM 600920). Van den Ende-Gupta syndrome is characterized by distinctive craniofacial and skeletal manifestations including blepharophimosis, malar and maxillary hypoplasia, a convex nasal ridge, an everted lower lip, arachnodactyly, and camptodactyly with mild bowing of the long bones. The physical growth and cognitive development is normal in the approximately 25 previously described patients. We describe the craniofacial and skeletal features of a now 5 year and 3 month old Laotian female child and compare these features to the previously reported patients. We also describe the analysis of our patient's SCARF2 genes.

1028F

Increased bone density, short stature and bowed lower limbs in an inbred kindred: a probable new genetic skeletal disorder. *G.F. Leal[1], R. Lyra[2], E.O. Silva[1].* 1) Medical Genetics Service, Instituto de Medicina Integral Fernando Figueira, Recife, Pernambuco, Brazil; 2) Endocrinology Service, Universidade de Pernambuco, Recife, Pernambuco, Brazil.

The 2010 Revision of the Nosology and Classification of Genetic Skeletal Disorders includes 456 entities placed in 40 groups defined by clinical and radiographic features and molecular pathogenesis. Two groups are characterized by increased bone density, and together they comprise forty recognized diagnostic conditions. We have ascertained a consanguineous family presenting a clinical picture that do not completely fit with any of these conditions. The genealogical data from seven generations show 13 affected persons (eight females and five males) distributed in eight sibships, and born to consanguineous parents. The ages and heights of the living affected individuals (four females and one male) varied, respectively, between 17-45 years and 128-149 cm. In addition to the short stature, all the patients presented bowing of the lower extremities involving femora, tibiae, and fibulae. There was no evidence of hearing loss, visual impairment or any other neurological problems, and no history of bone fractures. Head circumference and intelligence were normal in all patients. The radiological studies of the complete skeleton performed in two of them showed generalized cortical hyperostosis affecting all bones. The thickening and sclerosis were particularly marked in skull, clavicles, pelvis, and long bones. Bowing of femora, tibiae, and fibulae, and coxa vara were also observed. Biochemical exams were performed in two patients: serum levels for alkaline phosphatase, phosphorus and calcium were respectively elevated, decreased and normal. Serum parathyroid and thyroid hormones were normal in the only patient investigated. The radiographic features of van Buchem disease and sclerosteosis overlap to some extent to those seen in our patients, however short stature and bowing of lower limbs are not seen in these disorders. Individuals with van Buchem disease or sclerosteosis are of normal or tall stature, have a large mandible, and may present facial paralysis, deafness and optic atrophy due to progressive cranial nerve palsies. Osseous or skin syndactyly, and absent or hypoplastic phalanges have also been found in sclerosteosis. Considering that our patients present only short stature and bowing of lower limbs in addition to the increased bone density, we suggest that their phenotype constitute a new genetic skeletal disorder.

1029F

Oculodentodigital dysplasia in a family with multiple malformations of hands and feet. *G.L. Yamamoto[1], M.C. Moreira[1], I. Gomy[1], A.C. Pereira[2], C.A. Kim[1], D.R. Bertola[1].* 1) Genetica Medica ICR-FMUSP, Universidade de São Paulo, São Paulo, São Paulo, Brazil; 2) Laboratório de Biologia e Cardiologia Molecular, InCor, Faculdade de Medicina da Universidade de São Paulo, São Paulo, São Paulo, Brazil.

Malformations of hands and feet, such as polydactyly and syndactyly, are easily identified, but commonly ignored in clinical practice. These findings are mainly autosomal dominant traits with variable penetrance. However, some forms of presentation may be suggestive of specific diseases such as the fourth and fifth fingers syndactyly, that must address to the possibility of oculodentodigital dysplasia (ODDD). ODDD is an autosomal dominant disease associated to the gap junction protein alfa 1 (GJA1) gene, with high penetrance and pleiotropic phenotype that includes typical facial appearance with epicantic folds, prominent columella, hypoplastic alae nasi and oculodentodigital anomalies (microphtalmia, microcornea, hypoplastic or carious teeth and complete syndactyly of fourth and fifth fingers). Sparse curly hair, progressive spastic paraparesis and palmoplantar keratoderma are also described. We report a two-month-old female infant, born of unrelated healthy parents, with unremarkable pre-natal and neonatal history. At initial evaluation, large anterior fontanel, short palpebral fissures, hypoplastic alae nasi, inverted nipples, bilateral complete syndactyly of fourth and fifth fingers and partial syndactyly of third and fourth toes were noted. Her parents had normal hands and feet and her sister had post-axial polydactyly in left hand. In the paternal family history, there were cases of fingers and toes syndactyly, whereas multiple maternal relatives presented post-axial polydactyly in hands. There were no abnormalities upon cardiac, abdominal and ophthalmological evaluations. X-ray showed left hand synostosis of the distal phalanges of fourth and fifth fingers and agenesis of middle phalanges of toes. Parents' X-ray was normal. GJA1 gene sequencing of the proband revealed the pathogenic p.R202H heterozygous mutation, confirming the hypothesis of ODDD. Although there were no hand abnormalities in the proband's parents, the family history is suggestive of autosomal dominant ODDD and polydactyly with incomplete penetrance. Therefore, the ongoing molecular analysis of the parents and affected relatives is important to confirm the diagnosis and for an accurate genetic counseling.

1030F

Two sibs with mulibrey nanism reported from Japan. *H. Yoshihashi[1], S. Oomori[2], S. Satoh[2], C. Torii[3], K. Kosaki[3].* 1) Div Med Gen, Tokyo Metropolitan Children's Med Cente, Tokyo, Japan; 2) Div of Pediatr, Saitama City Hospital, Saitama, Japan; 3) Dep of Pediatr, Keio University, School of Medicine, Tokyo, Japan.

Mulibrey (MUscle-LIver-BRain-EYe) nanism (MUL ; MIM #253250) is a rare autosomal recessive disorder caused by *TRIM37* (located on 17q22-q23), encoding cytoskeletal adaptor protein. To date, at least 130 patients are known worldwide, most of them from Finland, including sporadic cases reported from different ethnic groups. MUL is characterized by prenatal onset growth failure, distinctive face, fibrous dysplasia of long bones, hepatomegaly, pericardial constriction, and no psychomotor delay. Some of them are compatible with Silver-Russel syndrome (SRS; MIM #180860) phenotype. Here, we present the first affected two sibs from Japan, whose parents are not consanguineous. Subject1: 3y8m-male. At 39 weeks of gestation, he was delivered without any critical problems during the perinatal period, but including IUGR (1662g,-3.3SD), atrial septal defect (ASD), hypospadias, and undescended testis. At the age of 21 months, the poorly-distensible cardiac membrane made it impossible to correct ASD with surgery. At 3 years old, he had no developmental delay and needed to be treated with GH therapy. However, he died as a result of fungous pneumonia at the age of 44 months. Subject2: 1y6m-female. In pregnancy, the fetal screening echo showed short limbs and IUGR. At 40 weeks of gestation, she was born by induced delivery due to growth arrest. Birth weight was 2244g (-2.5SD), length45.5m (-2.2SD), and head circumference 32.0cm (-1.0SD). There were no severe perinatal involvements, except for TTN. At the age of 18 months, she presented with failure to thrive and short stature(68.5cm, -3.3SD), including distinctive face (relative macrocephaly, broad forehead, low depressed nasal bridge), rhizomelia, and gracility of long bones. The features led to the clinical diagnosis of MUL. The direct DNA sequencing of *TRIM37* gene revealed novel compound heterozygous mutations in the exon 11 (c.874_877delAGAG p.Arg292Gln fsX18) and the exon 12 (c.1016C>G p.Ser339Cys). Conclusion: MUL is not well clinically-recognized syndrome in Japan. The evaluations of the bone X-P survey and echocardiographic study will help in distinguishing MUL from SRS and three M syndrome type1(3M1; MIM #273750). The genetic counseling may fall into a “pitfall” about estimating the recurrence risk for patients diagnosed with SRS, especially not caused by aberrant methylation status.

1031F

Novel mutations in *ADAMTSL4* in non consanguineous isolated ectopia lentis. *A. Chandra[1, 2], JA. Aragon-Martin[1], DG. Charteris[2], A. Saggar[3], AH. Child[1], G. Arno[1].* 1) Cardiac & Vascular Sciences, St George's University of London, London, United Kingdom; 2) Vitreoretinal Research Unit, Moorfields Eye Hospital, City Road, London, United Kingdom; 3) Clinical Genetics Unit, St George's University of London, London, United Kingdom.

Introduction: Mutations in *ADAMTSL4* are associated with the ophthalmic clinical condition of autosomal recessive isolated ectopia lentis. We have previously described six distinct mutations, with one recurrent mutation (c.767_786del20) identified in exon 6 being found most prevalently by different research groups throughout Europe. Herein we describe further probands with *ADAMTSL4* mutations. Methods: Known syndromes associated with ectopia lentis, particularly Marfan Syndrome, were excluded in affected probands. DNA underwent PCR and Sanger sequencing using optimised conditions and primers previously described. Results: Three probands were analysed. All patients had bilateral ectopia lentis. Family histories suggested possible recessive inheritance. There was no history of consanguinity in any of the families. Two probands, both white Caucasian were found to be homozygous for c.767_786del20 mutation on exon 6. The third proband was found to be compound heterozygous for two mutations. This included the c.767_786del20 (p.Gln256ProfsX38), leading to a frameshift resulting in a premature termination codon in exon 6. The proband also had a novel heterozygous deletion c.237delC (p.Pro80ArgfsX53) in exon 5 leading to a frameshift resulting in a premature termination codon in exon 5. Discussions: We confirm the importance of the c767_786del20 mutation in autosomal recessive ectopia lentis. Its prevalence in other reports may suggest it originates from within Europe. This supposition is further supported by the racial origin of our probands. Our novel mutation results in a premature termination codon. The majority of the mutations detected in *ADAMTSL4* seem to result in protein truncation, if the resulting protein survives nonsense-mediated decay (NMD). If, as we suspect, the truncation mutations result in mRNA that undergoes NMD, this would suggest that a null phenotype is associated with disease and the role of ADAMTS-like 4 is critical to ciliary zonule function.

1032F

Small size Xq28 duplication with unusual Filamin A (FLNA) gene amplification associated with mental retardation, severe behavioural inhibition, and high intensity signals of periventricular white matter. *F. BILAN[1], N. BAHI-BUISSON[2], B. MANIERE[1], P. DUMAINE[1], A. KITZIS[1], B. GILBERT-DUSSARDIER[1].* 1) Medical Genetics, CHU , Poitiers, France; 2) Neuropediatrie, CHU Necker-Enfants Malades Paris, France.

A 14-years old boy had development anomalies, behaviour disorders and epilepsy. At birth, after an uneventful pregnancy, he had some signs of overgrowth (birth weight: 4170g, birth height: 53cm and head circumference: 37cm). Then, growth was in the mean range but he kept up a macrocephaly at +3SD. He had normal motor development with walking at age of 14 months. His mental development was marked by language delay and moderate mental disability leading to specialized education. The main feature of his behaviour was an unusually severe shyness and inhibition making him very uncomfortable in his social life. He had some dysmorphic features: slight hypertelorism, straight eyebrows, bulbous nose tip, slight microretrognathia, brachymetatarsia of both 5th toes. Generalized epilepsy was also reported. Cerebral MRI showed mild subcortical atrophy with cerebellar hypoplasia, and T2/FLAIR hypersignal in periventricular white matter. His mother had also been in a specialized educative course and a maternal aunt was said to have a mild mental retardation. Index patient DNA was subjected to array-CGH analysis using 105K microarray (Agilent technologies). We highlighted a previously reported 250 kbp Xq28 duplication (Vandewalle et al., 2009) involving 17 genes in which 6 were reported as causing disease genes in the OMIM database. This chromosomal anomaly was then confirmed by several MP/LC (Multiplex PCR/ Liquid Chromatography) in the patient. In a second step it was also studied in his large kindred for genetic counselling purpose. We show that Xq28 duplication was inherited from his mildly affected mother in whom the chromosomal rearrangement occurred de novo. Surprisingly, we show that FLNA encoding filamin A was de novo duplicated within the large Xq28 duplication in the index patient. This result differs from Vandewalle et al. and shows that GDI gene alone is not sufficient to explain mental retardation observed in such duplication. Moreover, the maternal grand-mother who does not harbour this 250 kbp duplication, presented a non expected increase of the copy number of IKBKG gene and pseudogene as compared to healthy subjects and five of her six children. This anomaly was transmitted to the mildly affected aunt of the index patient. Taken together, these data strengthen that Xq28 region is prone to recombine and that IKBKG gene and pseudogene located within Xq28 region might play a crucial role in this chromosomal rearrangement.

1033F

A novel Xq28 duplication syndrome distal to *MECP2* accounts for 10% of male patients with combined callosal and cerebellar anomalies. *S.L. Christian[1], S. Sajan[2], C.T. Sullivan[1], L. Fernandez[3], S. Esmeeli[3], E. Rider[3], P.G. Wheeler[4], K. Silver[5], D. Mei[6], E. Parrini[6], R. Guerrini[6], J.T. Glessner[7], H. Hakonarson[7], E.H. Sherr[3], W.B. Dobyns[1].* 1) Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA; 2) Division of Medical Genetics and Department of Genome Sciences, University of Washington, Seattle, WA; 3) Department of Neurology, University of California, San Francisco, CA; 4) Division of Genetics, Nemours Children's Clinic, Orlando, FL; 5) Division of Pediatric Neurology, University of Chicago, Chicago, IL; 6) Pediatric Hospital A. Meyer, University of Firenze, Firenze, ITALY; 7) Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA.

Although duplications of Xq28 that contain *MECP2* have been documented in more than 50 patients, only a few have been reported with duplications of the more distal ~300 kb region (Vandewalle et al., 2009), and even fewer with details of brain imaging. We genotyped a total of 340 patients with agenesis of the corpus callosum (ACC) or cerebellar hypoplasia (CBLH), including 67 with both, on the Illumina HumanHap 610 Quad SNP arrays. Our initial copy number variation (CNV) bioinformatics analysis discovered small overlapping duplications of Xq28 distal to *MECP2* in 2 males with combined ACC and CBLH. We next manually curated all the CNV in these subjects and found 2 additional males with the same duplication. All duplications were present in only male patients consistent with an X-linked disorder. We thus detected this duplication in 2% (4/214) of all male subjects with ACC or CBLH, and in 10% (4/42) of male subjects with combined ACC and CBLH. We also obtained breakpoint data for one previously reported boy with a small visible dup Xq28 (Dobyns et al., 1997). The phenotype in all 5 males plus 2 affected male siblings consists of severe intellectual disability and seizures with brain imaging findings of ACC, CBLH and brainstem hypoplasia. In addition 3/7 had periventricular nodular heterotopia. The arrays and fine mapping by qPCR showed that the SRO extends from *FLNA* through *CTAG2* (chrX:153,576,900-153,816,423 hg19), a region containing 19 RefSeq genes but not *MECP2*. Mutations of *FLNA* have been associated with X linked periventricular nodular heterotopia and oto-palato-digital syndrome, while mutations of *GDI1* have been associated with XLMR. Quantitative PCR was performed to determine the copy number and detected internal triplications in 3 of 4 males tested. The phenotype did not differ in the two boys with larger duplications that included *MECP2*. However, the brothers with the largest internal triplication had a more severe phenotype than the others. Finally, the CNV were often inherited, as 3 mothers and 1 grandmother also carried extra copies of Xq28. In summary, we have defined a novel CNV syndrome that may account for up to 10% of male children with combined ACC and CBLH.

1034F

Long Term Survival in TARP Syndrome and Confirmation of *RBM10* as the Disease Causing Gene. *K.W. Gripp[1], E. Hopkins[1], J.J. Johnston[2], C. Krause[2], W.B. Dobyns[3], L.G. Biesecker[2].* 1) Division of Medical Genetics, A. I. duPont Hospital for Children, Wilmington, DE; 2) Genetic Disease Research Branch, NHGRI, NIH, Bethesda, MD; 3) Seattle Children's Research Institute, Seattle, WA.

TARP syndrome (talipes, ASD, Robin sequence, and persistence of left superior vena cava LSVC), is X-linked with pre- or postnatal lethality in males. Exon resequencing in 2 families identified *RBM10* as disease causing gene. Clinical report: Pt. 1 was born preterm with U-shaped cleft palate, ASD and persistent LSVC, facial dysmorphia and cryptorchidism. He had severe respiratory distress and at age 3 ½ years remains ventilator-dependent at night and tube-fed. Brain anomalies include partial ACC, dysplastic caudate, and megacisterna magna. He has hypotonia, sensorineural deafness, optic atrophy and cortical visual impairment. Atrial flutter required ablation. Results: A novel *RBM10* frameshift variant was found in Pt. 1 and his mother was heterozygous. Table 1: Pt. 1's findings, compared to previously diagnosed TARP syndrome individuals

	Talipes	ASD	Cleft	Persist. LSVC	Infant Death	Cryptorchidism	Low-set ears
Pt. 1	-	+	+	+	-	+	+
Gorlin 1970	6/7	3/7	4/6	4/7	7/7	+	+
Johnston 2010	2/3	1/1	3/3	0/0	3/3	+	+
Combined N=11	8/11 (73%)	5/9 (100%)	8/10 (80%)	5/8 (63%)	10/11 (91%)	3/3 (100%)	(100%)

Discussion: We confirm *RBM10* as pathogenic for TARP. This first TARP patient with long term survival expands the phenotype to include brain anomalies; hypotonia and developmental delay; deafness and optic atrophy; arrhythmia and characteristic facial features. Maternal heterozygosity underscores the importance of accurate diagnosis and counseling.

1035F

Functional analysis of novel missense alleles in Arylsulfatase E and identification of potential phenocopies in patients with X-linked recessive brachytelephalangic chondrodysplasia punctata (CDPX1). *C. Matos-Miranda[1], G. Nimmo[1], S. Bale[2], B. Williams[2], N. Braverman[1].* 1) Montreal Children's Hospital Research Institute, Dept. of Human Genetics, McGill University, Montreal, QC Canada; 2) GeneDx, Gaithersburg, Maryland, USA.

Inherited deficiency of Arylsulfatase E (ARSE) causes CDPX1, which features chondrodysplasia punctata, nasomaxillary hypoplasia and brachytelephalangy. There is a spectrum of clinical severity in affected males. Diagnosis requires molecular analysis due to the absence of a biochemical assay for endogenous ARSE. Yet, a significant number of patients with CDPX1 phenotypes do not have identifiable ARSE mutations and are thought to represent phenocopies due to fetal vitamin K deficiency inhibiting a normal ARSE enzyme. From 2008-2010, assisted by referring physicians and the Collaboration Education and Test Translation program (CETT) for CDPX1, ARSE mutation analysis was performed in 41 male probands with CDPX1 phenotype and clinical data was collected to determine genotype-phenotype correlations and to identify maternal effects in mutation negative patients. 20 novel ARSE alleles were studied for activity using a transient COS1 expression system and the fluorogenic artificial substrate, 4-MU sulfate. Initial studies showed absence of ARSE activity for most alleles, but potential residual activity for alleles located towards the C-terminus. Here we optimized the ARSE assay to include enzymatic time courses and report negligible activity for these C-terminal alleles. We also found that the product 4-MU was relatively unstable over time at neutral ph, which is required for this assay. In conclusion, our results showed that (1) frequency of all ARSE mutations identified in male probands with CDPX1 phenotype is ~ 56% (17/30), using prospective data from CETT), (2) all studied missense alleles had negligible ARSE activity suggesting they are pathological, (3) since the mutations are located throughout the protein and similar phenotypes are observed whether a gene deletion or missense alleles is present, genotype-phenotype correlations are unlikely, (4) maternal effects in mutation negative patients could not be established because the clinical data collections were incomplete, and also suggest the possibility of undetected ARSE mutations or genetic heterogeneity, and (5) the fluorescent product (4MU) at neutral pH adds variability to the ARSE assay results. Future focus on further evaluation of mutation negative patients, as well as development of an endogenous ARSE assay is warranted.

1036F

Ehlers-Danlos syndrome and bilateral periventricular heterotopia due to the novel mutation of p.G132V in the CHD1 in FLNA: An infantile case presented with respiratory distress due to tracheomalacia. *Y. Watanabe[1], J. Okada[1], K. Kimura[1], Y. Okamatsu[1], T. Oya[1], M. Yoshino[1], H. Yagi[2], M. Sato[2].* 1) Dept Pediatrics, Kurume Univ, Kurume, Japan; 2) Dept Morphological and Physiological Sciences, University of Fukui, Fukui, Japan.

Background: Bilateral periventricular heterotopia is known to be caused by mutations in FLNA. Other disease phenotypes with FLNA mutations include Frontometaphyseal dysplasia (FMD), Otopalatodigital syndrome (OPD type 1 and 2), Melnick-Needles syndrome (MNS), Cardiac valvular dystrophy, Ehlers-Danlos syndrome and bilateral periventricular heterotopia (BPNH-EDS). Three cases of BPNH-EDS was first reported in 2005 and approximately 10 cases of this rare genetic condition have been reported. Mutations in the one of the two calponin homology domains, CHD1, have been identified in the majority of the cases. Since many of the patients were diagnosed in their adulthood, clinical features seen in infantile cases have not been well documented. The case presented in this report describes clinical features of an infant with BPNH-EDS due to the novel mutation in FLNA. **Case Report:** The patient was a full term 3028 g baby girl, born by NSVD to a 28 year-old G2P1-2Ab0 Japanese woman who is in good health. Prenatal care was reportedly unremarkable. Apgar scores were 91 and 105. Parents noted her as a newborn baby to be floppy compared to her elder brother. At age 4 Mo, she had seizures and developed RS virus pneumonia requiring assisted ventilation for 28 days. BPNH was diagnosed by MRI. At 6 Mo, she was readmitted to a hospital for persistent wheezing and failure to thrive. After 7 Mo, she has been in chronic use of an assisted ventilator due to frequent episodes of respiratory distress. Tracheomalacia as well as generalized increased skin elasticity were noted. FLNA mutation analysis revealed the novel heterozygous mutation of p.G132Vconfirming the diagnosis of BPNH-EDS. The patient had a lung volume reduction surgery (LVRS) for chronic pneumonia at age 1 years and is stable and growing well. Physical Examination at age months was remarkable for generalized loose elastic skin, hypertelorism, frontal bossing, tracheomalasia, hyperextensible major and minor joints. No digital/toe anomalies noted. Hearing was normal. **Discussion:** FLNA mutations consist of two types: loss of function mutations and gain of function mutations. Periventricular heterotopia is believed to be due to the former mechanism. OPD, FMD, and MNS are thought to be due to the latter mechanism. The presented case showed clinical features of both mechanisms. Tracheomalacia is rare in patients with FLNA mutations although two sibling cases with OPD have been reported.

1037F

Phenotype and Genotype in 22 Slovene Patients with Incontinentia Pigmenti. *K. Writzl, N. Teran, B. Peterlin.* Institute of Medical Genetics, Ljubljana, Slovenia.

Incontinentia pigmenti (IP) (OMIM 308300) is a rare genodermatosis that segregates as an X-linked dominant disorder and is almost always lethal prenatally in male. The disorder demonstrates high penetrance, but its phenotypic expression is highly variable. In affected females it causes abnormalities of the skin, nails, teeth, hair, eyes and central nervous system. In 2000 the mutations in IKBKG gene were found to be causative of IP. Here we report the phenotype and genotype of 22 female IP patients from 16 different families from Slovenian approximately two million population. In 9 families (11 patients) an IKBKG mutation was found. Mutations included a common deletion of exons 4-10 (in 7 families; 8 patients) and two frameshift mutations (in 2 families; 3 patients). No genotype-phenotype correlation could be found. All patients with confirmed mutation but only 55% of patients without mutations were reported to have stage I skin abnormalities. Neurologic findings including cognitive delay and seizures were present in two patients only; both had IKBKG mutation. Lower limb asymmetry was found in four patients; two with and two without mutation. One family had three members with characteristic skin lesions, hypodontia and dystrophic nails. No mutation was found using target mutation analysis for common deletion and sequence analysis of IKBKG, but linkage analysis confirmed co-segregation of Xq28 region markers with the disease phenotype. We conclude that IKBKG mutations were found in about 70% of IP patients with documented stage I skin lesions. The low mutation detection rate is probably partly due to the lack of strict diagnostic criteria for IP but also to the fact that not all IKBKG mutations can be detected using analysis for common deletion and sequence analysis of IKBKG.

1038F

Amplicon Resequencing of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene. *L. Joe[3], R. Achmann[1], F. Stellmer[1], S. Zhan[1], L. Argyrioul[1], R. Greither[2], R. Dixon[2], A. Rico[2], A. Sartori[2], N. Fong[3], R. Padilla[3], R. Fish[3], N. Patel[3], J. Berena[3], T. Burcham[3], B. Auber[1].* 1) MVZ genteQ GmbH, Lornsenstr. 4, 22767 Hamburg, Germany;; 2) Life Technologies GmbH, Frankfurter Str. 129B, 64293 Darmstadt, Germany;; 3) Life Technologies, 850 Lincoln Centre Drive, Foster City, CA. 94404 USA.

Cystic Fibrosis (CF) is a common autosomal recessive disorder caused by mutations in the Cystic fibrosis transmembrane conductance regulator (CFTR) gene. Depending on ethnical and phenotypic background there can be great mutational heterogeneity. More than 1800 CFTR mutations are known. Commercial screening kits consider only a subset of relatively frequent mutations covering around 80% of mutations causing classic CF. However, only about 50 to 60% of mutant alleles in the CFTR genes of individuals with atypical forms of CF like congenital aplasia of the vas deferens (CBAVD) are interrogated. Resequencing of the entire coding sequence of the gene would increase the sensitivity of mutation detection, but Sanger sequencing is still costly. Alternatively, Next-Generation Sequencing can be cost effective, especially when samples are multiplexed. We describe a pilot study comprising 20 samples (17 with known and 3 control samples from healthy individuals with unknown CFTR gene mutational status), using a Multiplicom multiplexing assay for the CFTR gene, sample barcoding and subsequent Next-Generation Sequencing (NGS) with both the SOLiD™ 4 and 5500xl Genetic Analyzer Systems. We also executed a custom protocol for Ion Torrent's semiconductor-based Personal Genome Machine (PGMTM) System that enabled the sequencing of established (larger) amplicons. Finally, we performed Sanger sequencing and compared the data from all four systems.

1039F

Whole genome sequencing in a patient with aortic aneurysm: when gene panels are not sufficient. *C. Skrzynia[1], C. Schmitt[2], J. Berg[1].* 1) Genetics Department The University of North Carolina at Chapel Hill Chapel Hill, NC; 2) The Renaissance Computing Institute Chapel Hill, NC.

As the cost of whole genome sequencing (WGS) decreases, its ability to detect variation in all genes as well as genomic rearrangements, will make it highly attractive as a potential universal diagnostic test. The advantages of WGS are particularly evident in conditions with genetic heterogeneity, in which the cost of comprehensive genetic testing exceeds the cost of WGS. Furthermore, clinically available testing may not include all genes known to cause a given disorder. We identified a proband with aortic root aneurysm at age 33. Her sister died at age 41 due to dissection of a 7.1cm aortic root aneurysm. Several family members had features consistent with connective tissue disorders but no specific diagnosis had been established. Our proband did not have physical findings of Marfan syndrome but had joint hypermobility. In order to determine a specific diagnosis, we obtained extensive testing of genes known to cause familial thoracic aortic aneurysm and dissection (FBN1, ACTA2, TGFBR1, TGFBR2, and MYH11) and genes associated with forms of Ehlers-Danlos syndrome (COL3A1, COL5A1 and COL5A2). This testing was negative. We investigated the possibility of WGS in order to identify mutations in genes not yet available for clinical testing (for example, MYLK) or genomic rearrangements. During extensive pre-test counseling, we reviewed possible outcomes of the diagnostic evaluation and the potential for discovery of incidental findings. We organized the consent using a system of “bins” that categorize incidental findings according to clinical actionability. WGS was obtained through Illumina's clinical sequencing program, and the patient's insurance provider reimbursed the cost of the WGS testing in full. To our knowledge, this is one of the first cases of insurance coverage for WGS. WGS data is in the process of being analyzed. The diagnostic analysis will evaluate all genes associated with aortic root or thoracic aortic aneurysms, and results will be divulged in a formal genetic counseling session. Clinically actionable incidental findings, if any, will be revealed at that time. Other non-actionable incidental findings will be categorized according to the binning scheme we have developed, and the patient will be given the ability to select the categories of information she wishes to receive. We will present aspects of the medical and genetic counseling ramifications of WGS in this typical patient seen in our Adult Genetics Clinic.

1040F

Pre-test genetic counseling for whole genome sequencing: Development of a protocol and evaluation of participant experience. *K. Trzupek[1], A. Trivedi[1], K. Ormond[2], R. Sutphen[1,3].* 1) Informed Medical Decisions, Inc, St Petersburg, FL; 2) Dept of Genetics, Stanford University School of Medicine, Stanford, CA; 3) University of South Florida College of Medicine, Tampa, FL.

Background The broad spectrum and quantity of data yielded by whole genome sequencing (WGS) presents distinct challenges for the delivery of genetic counseling (GC). We developed a pre-test GC protocol for WGS that is currently being utilized as part of a research study providing WGS to faculty members at Stanford University. Methods “Genetically educated” faculty were offered the opportunity to participate in a WGS study with the option of clinical annotation; pre- and post-WGS GC are required for participation. Potential participants attend information sessions about the study, and schedule a pre-test GC appointment if interested. Following GC, participants can elect to pursue WGS; those who elect clinical annotation receive results via post-test GC. Participants are also invited to take part in ongoing behavioral surveys. Results The pre-test GC session is divided into three sections: 1) personal/family history review and risk assessment, 2) WGS technical capabilities and limitations for detecting a wide variety of genetic conditions, and 3) psychosocial benefits and risks. The counseling protocol evolved following feedback from the initial participants. At their request, additional information was included regarding the technical aspects and limitations of the sequencing methodology. Virtually all of the participants categorize themselves as “information seekers,” interested in receiving all available health information, and did not anticipate significant personal emotional risks. However, most participants recognize the sensitive nature of predictive genetic information for others, and engaged in discussing the psychosocial impact of WGS as it may relate to their family members. These familial implications became a focal point of the psychosocial counseling. Conclusion There is very limited experience to date with GC for WGS, particularly among asymptomatic individuals who are expected to comprise the majority of tested individuals in the future. Although targeted to a scientifically knowledgeable group, this pre-test GC protocol has implications for broader populations. Our protocol is evolving as an iterative process incorporating lessons learned in order to balance general information about the scope of findings from WGS with comprehensive personalized risk information to enhance client understanding, ensure true informed consent and optimize client preparedness for receiving and sharing WGS results.

1041F

Screening of LRTOMT gene (DFNB63 locus) in Iranian patients with autosomal recessive nonsyndromic hearing loss. *N. yazdanpanahi[2], S. Ashoori[1], E. Farrokhi[1], H. taghizade[1], R. heshmatifar[1], SH. Shahbazi[1], M. Hashemzade[1].* 1) Cellular and molecular research center, Shahrekord Univ of Med. Sci. Iran, shahrekord, Islamic Republic of Iran; 2) Islamic Azad University , Falavarjan branch . Iran,isfahan, Islamic Republic of Iran, Ph.D student.

Background: Prelingual hearing loss occurs in 1 in 1000 newborns and is inherited in more than 60 percent cases. Approximately more than 80 percent cases are nonsyndromic. Nonsyndromic hearing loss is so heterogene and more than 100 loci are known to be related to it. Objectives: To determine LRTOMT gene mutation profile in Iranian patients. Methods: 200 patients with nonsyndromic hearing loss from different provinces of Iran were recruited for this study. DNA was extracted using Phenol-Chloroform method. PCR reactions were done, then DNA sequencing of coding region was done. It's supposed to study all 10 exons of this gene. Three exons (1,3 , 5 and 9) have been studied up to now. Our work is in progress. Results: These four sequenced exons (1,3 , 5 and 9) were studied using Chromas software and no mutation was detected in these three exons. Conclusions: It seems that this gene has no critical role in hearing loss in these studied populations. But for a more accurate result, all of the exons must be studied.

1042F

Association of ADAM33 gene polymorphisms and their haplotypes with asthma in an Indian population. *P. TRIPATHI[1], S. AWASTHI[2], R. PRASAD[3], N. HUSAIN[4], S. GANESH[5].* 1) Pediatrics, Chhtrapati Shahuji Maharaj Medical University, Lucknow, UP., India; 2) Chhtrapati Shahuji Maharaj Medical University, Lucknow, UP., India; 3) Pulmonary Medicine, Chhtrapati Shahuji Maharaj Medical University, Lucknow, UP., India; 4) Pathology, Chhtrapati Shahuji Maharaj Medical University, Lucknow, UP., India; 5) Biological Sciences and Bioengineering, IIT, Kanpur, India.

Abstract: Background and purpose: A disintegrin and metalloprotease 33 (ADAM33), was the first identified asthma-susceptible gene by positional cloning. Presently, there is a need to study the association of all most common SNPs of ADAM33 with asthma and its severity. Therefore aims of present study were; (1) To assess association of ADAM33 gene polymorphisms(V2 C/T, T2 A/G, T1 A/G, Q-1 A/G, BC+1 A/G and S1 A/G) with asthma and its severity in all subjects as well as in children and adults separately . (2) To assess the distribution of ADAM33 gene polymorphisms haplotypes and association with risk of asthma. Methods: This was a case-control study, conducted after approval of institutional ethics committee and patients enrolled after obtaining informed consent of participants/ Guardians. Included were those aged between 1 to 50 years, in two groups; 1) children (age less than or equal to 19 years) and 2) adults (age greater than 19 years). Diagnosis and severity of asthma were made according to the Global Initiative for Asthma criteria (GINA) guidelines. Controls were age and sex matched non-relatives of cases without any respiratory disease. Genotyping was done by PCR-RFLP method. Results: From august 2007 to September 2009, total of 390 non- asthmatic controls (mean age 22.9years±14.5, 76.2% females) and 386 cases (mean age18.7years±15.9, 68.4% females) of asthma were recruited , of which 95 (24.6%) had mild intermittent, 235 (60.9%) had mild persistent and 56 (14.5%) had moderate persistent asthma. In all subjects as well as in children and adults separately, we found statistically significant association of SNPs T1, T2 and S1 with asthma (p value less than 0.05). Additionally association of SNP V2 was observed with asthma in only adults. SNPs T2, T1 and S1 showed statistically significant association with severity subgroups of asthma as compared with controls. However after analyzing children and adults separately, only T2 SNP was associated with severity subgroups of asthma in adults and T1 and S1 were associated with severity subgroups of asthma in children. The GTGGGG haplotype was found to be associated with asthma [OR=4.40; 95%CI=2.93-6.65; P value less than 0.0001]. Conclusion: There is an association of ADAM33 SNPs (T1, S1, T2 and V2) with asthma in Indian population.

1043F

Consensus: A novel framework for evaluation of uncertain gene variants in laboratory test reporting. *D.K. Crockett[1], P.G. Ridge[1], A.R. Wilson[1], E. Lyon[1], M.S. Williams[3], S.P. Narus[2], J.C. Facelli[2], J.A. Mitchell[2].* 1) University of Utah School of Medicine, Pathology, ARUP Laboratories, Salt Lake City, UT; 2) University of Utah School of Medicine, Biomedical Informatics, Salt Lake City, UT; 3) Intermountain Healthcare Clinical Genetics Institute, Salt Lake City, UT.

Purpose: As electronic medical records incorporate genetic sequence information, gene variant classification is critical to inform clinicians on the most appropriate course of treatment. Proposed guidelines have recommended classification terminology and definitions for improving laboratory gene variant reporting. A standardized framework however, does not yet exist for quantitative evaluation of disease association for uncertain gene variants in an objective manner.

Methods: Gene-specific prediction (PSAAP) was trained using clinically curated gene-disease data from the RET proto-oncogene. This predictor output was implemented into a Consensus framework, including a weighted metric of complementary prediction algorithms (SIFT, PolyPhen, PMUT and MutPred). Consensus score “reference intervals” were also calculated from known disease outcomes (benign, n=46; pathogenic, n=51). Prediction metrics for RET gene variants of uncertain significance (n=45) were then evaluated in this novel framework.

Results: Where authoritative collections of disease association-gene variants exist, the Consensus model yields highly accurate evaluation of uncertain or novel gene variants against the backdrop of calculated reference intervals from known benign and pathogenic gene variants. Algorithm limitations may include lack of representative variant in a training data set or nucleotide level splice variation. Where confirming evidence for a gene variant is lacking, visualization of the Consensus output is also proposed for augmenting diagnostic decisions.

Conclusions: Accurate interpretation of gene testing is a key component in customizing patient therapy. A reliable and objective phenotype classification framework with a quantitative metric for evaluation of novel or uncertain gene variants may supplement limited clinical information and assist in improving prediction algorithms as gene variant knowledge increases.

1044F

Association of Human Leukocyte Antigen Polymorphisms and Chronic Hepatitis B Infection Outcome. *A. Fejzullahu[1], L. Doganay[2], I. Tuncer[2], F. Enc[2], O. Ozturk[2], Y. Colak[2], G. Dinler[1,3].* 1) Molecular Biology & Genetics and Biotechnology Graduate Program, Institute of Science and Technology, Istanbul Technical University, Istanbul, Turkey; 2) Ministry of Health, Department of Gastroenterology, Goztepe Teaching and Research Hospital, Istanbul, Turkey; 3) Department of Molecular Biology and Genetics, Faculty of Science and Letters, Istanbul Technical University, Istanbul, Turkey.

Chronic hepatitis B virus (HBV) infection is a global health problem with an estimated 350 million people chronically infected worldwide. Considerable evidence suggests that the progression of hepatitis B virus infection is influenced not only by the viral genotype and the level of viremia but also host genetic factors play a significant role in the pathogenesis and clinical outcome of chronic HBV infection in several ethnic groups. It is mentioned that both cellular and humoral responses are required for viral clearance. Hence, the aim of this study is to investigate the association between the polymorphisms of HLA-DRB1 alleles and viral hepatitis B in Turkish population. In this study 324 patients were included; 63.4% male, 36.4% female and, where 23.5% of patients being HBe antigen positive, 23.8% having cirrhosis. The median age was 44.4 years, median follow up time was 86.5 months. HLA-DRB1 alleles in 324 patients with chronic hepatitis B were analyzed using the polymerase chain reaction/sequence specific primer (PCR/SSP) technique. In our group of patients, DRB1 alleles were not related to active or inactive disease, or interferon treatment response or virologic breakthroughs observed during nucleoside analog treatments. On the other hand our data showed that, patients who had DRB1*07 allele had lower probability to have cirrhosis (likelihood ratio chi square:4.17, p:0.04). In this case, DRB1*07 allele may be one of the host factors, which influences immune response to HBV, by self-limiting cirrhosis, one of the main complications of chronic HBV. Additional study is needed to validate these findings and to further explore the genetic pathogenesis of HBV infection.

1045F

Role of Population Counseling and Screening for Thalassemia Prevantion in India. *A. Nair, L. Sharma, J. Hatti, G. Mulgund, S. Aneeb, Q. Hassan.* Kamineni Hospital, Hyderabad, India.

Thalassemia which originated in the Mediterranean region is an inherited autosomal recessive blood disorder with an incidence of 10,000 to 12,000 thalassemic children born in India per year. Globally, 4.5% of the population has thalassemia minor and in India 1 out of every 25 individuals is a carrier. Despite the fact that India is a culturally diverse nation, arranged marriages between fixed endogenous populations are the norm. In the South Indian region this is compounded by the fact that consanguineous marriages are quite common. These facts increase the prevalence of recessive disorders like Thalasemia. There is no comprehensive governmental program for thalassemia prevention in India and several NGO's especially the Thalassemia and Sickle Cell Society of India are struggling to provide blood transfusion and chelating therapy at affordable costs to patients. However, they are not equipped for appropriate counseling. The present study highlights the role of genetic counselors in population screening and counseling to prevent thalassemia in our population. Andhra Pradesh(AP), is a state in South India, with 23 districts having a population of approximately 8 million, where consanguinity is seen in 48% of families. A study was carried out among 100 thalassemic families of AP to assess if consanguinity plays a major role in the high incidence of this pathology . 54% of the children were observed to be born out of consanguineous marriages. This shows that a high number of thalassemic children are born to non consanguineous couples. This can be attributed to the fact that most of the marriagesin the state are arranged within same communities. Therefore, just focusing on discouraging consanguineous marriages may not be the appropriate method of thalassemia prevention. But, since most of the marriages are still arranged in India, premarital testing of target group, consisting of females eligible for marriage (age range 16-30 years) and males (age18-35 years) by hemogram and followed by HPLC/Hb Electrophoresis would be useful. This program will help in identifying carriers, who would require a focused counseling regarding planning a suitable marriage and subsequent healthy offsprings.

1046F

Importance of Appropriate Genetic Counseling in Families to Prevent Thalassemia in India. *L. Sharma.* Kamineni Hospital, Hyderabad, India.

With over 35 million carriers of the abnormal gene for thalassemia and approximately 10,000 to 12,000 thalassemic children born every year, beta thalassemia is a genetic disorder of major concern in India. One hundred families registered with the Thalassemia and Sickle Cell Society, Hyderabad, India, were interviewed to evaluate information received during counselling and their understanding of the disease. 32% of the patients were <5 years, 34% between 5-10 years and 34% >10 years of age. 32% families had multiple affected members. The mean age of diagnosis was ± 6 months. 78% families were counselled by a general paediatrician. Most of them received information about the disorder from other families at the clinic, this was regarding recurrence risk, costs of the tests, treatment and measures for prevention, which was often incorrect. Social and religious beliefs also influenced their understanding. 69% of the families had misconceptions regarding various aspects: (i) 9% patients were aware of recurrence risks, (ii) 30% about prenatal testing, and (iii) 17% about extended family testing, 65% of these did not get the testing done because of social stigmatisation of carriers. Our study highlights the need for genetic counselling services by trained counsellors in the various centres which provide services to families of thalassemic patients. Due to the lack of trained genetic counsellors in India, patients do not receive the required counseling. To address this deficit adequately, there is an urgent need to develop training programs in genetic counselling. Continuing medical education in genetics for paediatricians is also sorely needed in India, to ensure that they understand the role of genetics in prevention of the disorder and the importance of referring patients for genetic counselling. Apart from this to increase public awareness about thalassemia various forms of media (audio, TV and print), educational print material for patient and genetic counselling are essential for making the thalassemia prevention program a success in India.

1047F

Next Generation Sequencing Panel for the Evaluation of Syndromal Autism. *C.D. Collins[1], E. Chin[1], S. Bhide[1], S. McGee[2], A. Tanner[1], D. Rhodenizer[1], J. Jones[2], M. Basehore[2], M. Friez[2], M. Hegde[1].* 1) Emory Genetics Laboratory, Department of Human Genetics, Emory University, Atlanta, GA; 2) Greenwood Genetic Center, Molecular Diagnostic Laboratory, Greenwood, SC.

Autism spectrum disorders (ASD) are a clinically heterogeneous group of neurodevelopmental disorders defined by a complex behavioral phenotype. Currently, identified genetic causes of ASD can be classified into cytogenetically visible chromosome abnormalities (~5%), copy number variants (10 - 20%), and single gene disorders (~5%). Individually, single gene causes of autism, which are frequently tested for on a gene-by-gene basis, typically make up <1% of the total etiology of autism. The implementation of Next Generation Sequencing (NGS) technology in the clinical laboratory has allowed for large-scale panel testing of many genes simultaneously at a reduced cost and turn-around time. As an initial effort to address autism using this technology, we have developed a NGS panel that is designed to target genes associated with genetic syndromes that include autism as a significant clinical feature. This panel also includes additional genes that have been previously associated with autism, along with a smaller number of well-characterized genes associated with conditions involved in the differential diagnosis of Rett syndrome and/or Angelman syndrome. In total, 62 genes have been incorporated into this panel which is intended to ultimately serve as a diagnostic tool for the evaluation of syndromal autism. Initial validation of the testing platform included 24 blinded positive control samples that were independently analyzed by bioinformatic personnel at both Emory Genetics Laboratory and the Greenwood Genetic Center. This preliminary evaluation detected 98% of the previously known mutations. Assay improvements are currently in progress to improve detection and overall sequence coverage. Additional probands from an undiagnosed autism cohort have also been studied and lessons learned will be presented. In conclusion, the use of NGS technology will add another dimension to the clinical evaluation of syndromal autism at a fraction of the cost and turn-around time of traditional Sanger sequencing.

1048F

Adaptive functioning and health-related quality of life of patients with Mucopolysaccharidosis type II (Hunter syndrome). *W. Packman[1,2], M.C. Needham[1], S. Packman[2].* 1) Palo Alto University, Palo Alto, CA; 2) University of California, San Francisco, San Francisco, CA.

We investigated the adaptive functioning and health-related quality of life (HRQOL) of patients with Mucopolysaccharidosis type II (MPS II; Hunter syndrome) through interviews with parents/caregivers of 73 patients. To investigate adaptive functioning, the Vineland Adaptive Behavior Scales (2nd Ed.) Parent/Caregiver Rating Scale (Vineland-II) was administered and the results were compared to the normative sample. On the Vineland-II, the parents/caregivers of MPS II patients reported scores significantly lower than the normative sample in the domains of communication, daily living skills, socialization, and motor skills, as well as for each subdomain of the measure. To examine health-related quality of life, the PedsQL Quality of Life, Parent Proxy Report was administered to 65 parents/caregivers of patients with MPS II; the results were compared to a healthy sample and an oncology sample. On the PedsQL, the reported HRQOL of the MPS II patients was significantly lower than the reported HRQOL of healthy children for the following scales: physical health, psychosocial health (including emotional functioning, social functioning, and school functioning), and the total score. Furthermore, statistically significant differences were found on all aforementioned PedsQL scales when comparing the scores reported by parents/caregivers of MPS II patients to the scores reported by parents of oncology patients. These findings demonstrate that individuals with MPS II have significant limitations in their adaptive functioning, as well as poorer HRQOL than healthy individuals and oncology patients in terms of physical, emotional, social, and school functioning. Professionals and caregivers involved in the lives of MPS II patients should be aware of, and attentive to, the limitations for MPS II patients within the realms of adaptive behavior and HRQOL. Additionally, it is important for those involved in the patients' lives to seek opportunities to improve the HRQOL of these patients.

1049F

Results and experiences involving microarray-based comparative genomic hybridization (aCGH) in an inner-city setting. *E. Pereira[1], A. Singh[2], J. Samanich[1], P. Levy[1], R. Marion[1], B. Morrow[3], K.H. Ramesh[2], L. Cannizzaro[2], Q. Pan[2], R. Naeem[2].* 1) Division of Genetics, Department of Pediatrics, Children's Hospital at Montefiore, Bronx, NY; 2) Department of Pathology, Montefiore Medical Center, Bronx, NY; 3) Department of Genetics, Albert Einstein College of Medicine, Bronx, NY.

Microarray-based comparative genomic hybridization (aCGH) allows for a high-resolution evaluation of DNA copy number alterations. In 2010, the International Standard Cytogenomic Arrays (ISCA) Consortium and American College of Medical Genetics (ACMG) recommended using aCGH as the first tier genetic test for patients with developmental disabilities including autism spectrum disorders (DD ± ASD) and multiple congenital anomalies (MCAs). Using an Agilent custom design 44k array, we describe our aCGH testing experience from October 2007 to December 2010 on 623 consecutive patients at Montefiore Medical Center, which serves a large, underserved, inner-city population in the Bronx, NY. Testing indications were mainly for DD ± ASD (35%), MCAs (22%), neurological issues (13%), and suspected known genetic syndromes (10%). The majority of our patients were of Caribbean Hispanic (Dominican/Puerto Rican), Caucasian, and multiracial background, with higher rates of Caribbean Hispanic (29%) and multiracial (15%) patients than included in previous studies. A total of 160 patients (25.7%) had aCGH testing with reportable copy number variations (CNVs). Patients tested because of DDs ± ASD had a higher percentage of abnormal aCGH results than previously reported in previous studies (25% vs 17% respectively). We had similar rates of CNVs as reported in the literature for congenital anomalies (26%) and neurological abnormalities (24%). A limitation in interpreting abnormal results is the lack CNV data for the Caribbean Hispanic community. This is a significant short-coming in currently available published data and needs to be studied in more individuals before any generalizations can be made. Another limitation in interpreting abnormal aCGHs in our community stems from unavailable parental samples. Of the 132 abnormal aCGH results in which parental blood samples were requested, 47 (36%) had parental testing; only 15 patients (11%) had both parents sampled. In the Bronx where nearly one-third of households are single parent (maternal) homes, paternal sampling poses a challenge. From our experience, we recommend that pretest counseling emphasize the possible need of parental testing for abnormal results.

1050F

An Iranian cohort suspected to MELASE-MERRF with a known muatation (A5814) in tRNA Cys gene. *H. Aryan[1], O. Aryani[1], N. Farzan[1], M. Houshmand[1,2].* 1) Special Medical Center, Tehran, Iran; 2) National institute for genetic engineering and biotechnology, Tehran, Iran.

Background: The mitochondrial encephalomyopathies are a heterogeneous group of disorders associated with structural, biochemical, or genetic abnormalities of mitochondria. The most severely affected organs are those with high oxidative metabolism, such as brain, skeletal and cardiac muscle, and kidney. Another pathogenic mutation is the 5814T_C mutation reported independently by three groups to be associated with MELAS syndrome and with progressive external ophthalmoplegia in the first two cases. Materials and Methods: The proband was a 6-year-old boy, born at term by normal delivery. In the eighth months of life, the mother noted that he had muscular weakness. She controlled his head size at 2 years of age; he did not show any growth in his head, sat without support at 4, but could never stand without support or walk. He had multiple brief seizures, characterized by loss of consciousness and followed by generalized jerks. Genomic DNA was extracted from fresh blood samples of all patients using QIAGEN DNA Kit. All 22tRNA of mitochondrial DNA were amplified by PCR using specific pairs of primers which were designed in this laboratory and sequenced. Result: Sequence analysis of both proband's blood mt-DNA and his mother including all 22tRNA confirmed the presence of the homoplasmic T5814C mutation. Conclusion: PCR- sequencing of the 22tRNA genes showed the T5814C mutation in tRNA Cys previously described in association with a MELAS-like phenotype. However, our results showed that this substitution found not only in the patient but also in his mother both as homoplasmic change. This case suggests more consideration on this reported mutation.

1051F

Automatic annotation of mitochondrial variants from high throughput next generation sequencing in a clinical molecular diagnostic laboratory. *D.CY. Chen, V.W. Zhang, J. Wang, LJ. Wong.* Molecular and Human Genetic, Baylor College of Medicine, Houston, TX.

Mitochondrial diseases are clinically and genetically heterogeneous, with variable penetrance, expressivity, and different age of onset. The spectrum of mitochondrial DNA (mtDNA) mutations can range from point mutations, small insertion/deletion to large insertion/deletions. Point mutations are the major type of mutations responsible for mitochondrial diseases. With the introduction of next-generation sequencing (NGS) technology for molecular analysis of the mtDNA and the enormous amount of sequence data output, there is an urgent need to streamline the analysis workflow for efficient results output and patient's report with a clear molecular diagnosis.

Publically available mtDNA SNP databases; Mitomap and mtDB, were used in conjunction with our own database (MitoLink) of >4000 SNPs with family and clinical information collected through the past 5 years from more than 5,000 families. Due to the incompleteness and occasional disagreement among the public mtDNA SNP databases, the incorporation of clinical and genetic information from our MitoLink database is essential in making more accurate diagnosis.

The development of a bioinformatic software pipeline that encompasses publically available databases mitomap and mtDB; Sift and Polyphen2 for amino acid prediction; and the in-house built MitoLink database, facilitates the annotation of huge amount of sequence results from NGS quickly, efficiently, and accurately. MitoLink is a superb database which contains >4000 mtDNA variants with genetic and clinical information for the interpretation of the clinical significance in the complex mitochondrial disorders. In conclusion, the integration of MitoLink database into the analytical pipeline, allows accurate and comprehensive interpretation of the sequencing results, user friendly output of the complicate NGS results, and efficient clinical reporting.

1052F

Comprehensive molecular analyses of mitochondrial genome by next-generation sequencing. *H. Cui, V.W. Zhang, L.J. Wang.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Recent statistics suggest that 1 in 200 individuals develop mitochondrial diseases that are clinically and genetically heterogeneous, with variable penetrance, expressivity, and different age of onset. Disease causing mutations, such as point mutations and large deletions, in the mitochondrial genome often exist in a heteroplasmic state. Current molecular analyses require multiple different and complementary methods, including Sanger sequencing, quantitative PCR (qPCR), Southern blot or array CGH for the detection and quantification of point mutations, larger mtDNAs, and mtDNA depletion analysis. These procedures are labor intensive, time consuming, and costly.

We have developed and validated a clinically applicable “deep” sequencing technique by the application of next generation sequencing (NGS) to the clinical diagnosis (1). Our results demonstrated uniform coverage of each of the 16,569 bases of the mitochondrial genome at over 20,000 folds. Among the 36 samples that we have validated, all of them show 100% specificity and 99.9% sensitivity with quantitative base calls when compared to results from Sanger sequencing. We are also able to detect low levels of heteroplasmy, which were below the limit of detection by Sanger sequencing, in several family studies. Moreover, this approach allows detection of small and large deletions with exact breakpoints and deletion mutant heteroplasmy. We have successfully captured several large deletions including one 8 kb deletion at 90% heteroplasmy level. Proper qualitative and quantitative controls were analyzed along with each sample for quality assurance in a clinical setting. Our results demonstrated the superior sensitivity and specificity of base calling when compared to the gold standard Sanger sequencing, the importance of developing a mathematical formula for performance evaluation, and the incorporation of internal controls for quality assurance. In conclusion, the one-step “deep” sequencing approach offers a comprehensive qualitative and quantitative molecular analysis of mitochondrial diseases in a timely, accurate, and cost-effective manner.

Reference: 1. Zhang, V.W.*, H. Cui*, L.J. Wong, (2011). “Comprehensive one-step molecular analyses of mitochondrial genome by next-generation sequencing.” Manuscript in preparation. (*These two authors contribute equally to the paper.).

1053F

Identification of three Novel Mutations within the FVIII Gene in Hemophilia A. *N.J. Faridi[1], N. Husain[1], I. Siddiqi[2].* 1) *Pathology, Ram Manohar Lohia Institute of Medical Sciences Lu, Luvknow, U.P., Uttar Pradesh, India; 2) # Molecular and Structural Biology Division, Central Drug Research Institute, Lucknow, India.

Background: Hemophilia A is a bleeding disorder caused by heterogeneous mutations of the FVIII gene. Direct mutation detection approach allows definitive diagnosis and allows carrier detection in sporadic cases as well as incomplete families. The current study is being done to detect small, novel mutations and Intron 1 inversion in the Hemophilia A gene in Indian cases with hemophilia and to predict structural and functional alterations in the gene using modeling Design: All 26 exonic regions including exon 14 were amplified by PCR in 23 cases with mild and moderate hemophilia A and screened with single stranded conformational polymorphism (SSCP) for mutations in FVIII gene. We have also screened 150 severe cases for intron 1 inversion mutations. DNA Sequencing was done in cases showing shift using ABI-XL3130 Genetic Analyzer (Applied Biosystem, USA) and sequences analyzed with Set-Scape Software. Structural change in F8 gene was predicted using modeling with Sybyl 7.1(Tripos) and GROMACS 4.0.5. Result: A total eight different Disease causing mutations were found in Cases with hemophilia A, three of which were described for the first time. We found intron 1 inversion in four cases with hemophilia A. Protein modeling to elicit the structural changes in FVIII protein due to these mutations was also done. Conclusion: We report three novel mutations not previously reported at the Hemophilia mutation database (HAMSTeRS). Small mutation detection can be achieved using SSCP-sequencing protocols and DNA modeling helps predict the structural and functional changes in the protein structure. The outcome of this study would enable us to give an accurate diagnosis in affected cases with hemophilia a by direct mutation analysis.

1054F

Optimizing genetic counseling services for prenatal patients in a subspecialty Neurogenetics clinic: Four years of experience with this patient population. *C.L. Goldsmith, M. Cloutier, K.M. Boycott.* Genetics, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada.

Prenatal patients in Canada have traditionally been cared for in the context of a Prenatal Program servicing a wide-spectrum of genetic issues. A survey of Neurogenetics clinics in Canada did not reveal any clinic that provides prenatal genetic counseling in the context of a Neurogenetics clinic. We established a Neurogenetics specialty clinic in 2007 which serves pediatric and adult patients with neurological disorders or individuals with a family history of a neurological disease. One neurogeneticist and two genetic counselors saw all the patients in the clinic, allowing this small group of providers to develop expertise with the particular needs of this patient population. Since its inception four years ago, the prenatal aspect of our service has provided care to 56 patients. Our prenatal cohort includes pregnant patients who had a personal or family history of a neurological disease, as well as those with a non-aneuploidy-associated fetal brain abnormality. The majority of patients were referred for a family history of a pediatric onset neurogenetic condition (30/56). These included spinal muscular atrophy, Duchenne or Becker or other muscular dystrophies, (congenital) myotonic dystrophy, and hereditary seizure disorders. The next largest group was women referred because of a current, previous or family history of a non-aneuploidy-associated fetal brain abnormality (14/56). These included prenatal ultrasound detection of lissencephaly, Dandy Walker malformation, and cerebellar vermis hypoplasia. Follow-up of these complex patients often included fetal MRI, chromosomal microarray analysis, and post-delivery examination and investigation. Other patients in this group were referred for a family history of a fetal brain abnormality, such as a family history of Joubert syndrome or aqueductal stenosis. Referrals because of a personal or family history of an adult onset neurological disorder (6/56) included four for Huntington disease. Three of these were for women whose fetuses at 50% risk and all proceeded with prenatal diagnosis for HD. A consult with our bioethicist was required for one of these cases, highlighting the complex issues that can arise when discussing prenatal diagnosis for adult-onset neurogenetic diseases. The clinic model has been highly successful; uniquely providing optimal prenatal services for this group of patients with a focus on continuity of care in the context of a specialized Neurogenetics clinical setting.

1055F

Mitochondrial dysfunction in Mexican patients with Parkinson's disease. *E. Martinez[1], G. Dorazco[2], E. Lara[1], Y. Serrano[1].* 1) Univ de Guadalajara, Guadalajara, Mexico; 2) Hospital General de Zona No. 7. IMSS.

BACKGROUND: Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease, affecting approximately 1% of population aged over 60 years. Thus far, the molecular mechanisms underlying the pathogenesis of PD still remain unknown. But the majority of patients with PD sporadic show the presence of reactive oxygen species, and free radical stress play important roles in the pathogenesis of PD. On the other hand, have been found the presence of a defect of complex I activity in platelets, complex IV in brain and lymphocytes in PD patients. Also, has been found mutations in some complex I and IV of the mitochondrial respiratory chain. OBJECTIVES: Determine the frequency of T8993G mutation of mitochondrial ATPase6 gene, and their correlation with ATP synthase enzymatic activity, in PD patients and a control group. MATERIAL AND METHODS: In order to explore if mutation T8993G and the hydrolytic activity of the ATPase correlate with the PD, were studied 14 patients and the same amount of controls, matched-aged and gender. The mtDNA extraction was according to the Miller method and the activity enzymatic to the Summer method. RESULTS: The obtained data revealed that the hydrolytic activity of ATP synthase was significantly increased in patients with AD (14.3 ± 1.4 nmol PO4 min-1(mg protein)-1, n = 14) in comparison with the control group (10.0 ± 0.6 nmol PO4 min-1 (mg protein)-1, n = 14). The mutation T8993G was observed in 57.14% (8/14) of the PD patients, whereas in the control group it was not identified. CONCLUSION: These results probably suggest that a weak activity functional of the enzyme can be explained because of the T8993G mutation in ATPase6 gene affect the proton channel for enzymatic activity.

1056F

Study of Charcot-Marie-Tooth in Iran: Novel types? *E. Mohammadi Pargoo[1], O. Aryani[2], H. Tonekaboni[3], P. Yaghmaei[1], M. Houshmand[2,4].* 1) Science & research branch of I, Science & research branch of Islamic Azad Universi, tehran, Iran; 2) Medical Genetics Department, Special Medical Center, Tehran, Iran; 3) Mofid Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Islamic Republic of Iran; 4) National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran.

Charcot-Marie-Tooth (CMT) disease is the commonest neurogenetic disorder with phenotypic and genotyping heterogeneity. Both the phenotypic features and disease severity can either be consistent or vary widely both within and among families. The affected individual typically has distal muscle weakness and atrophy often associated with mild to moderate sensory loss, diminished or absent deep tendon reflexes, high-arched feet and skeletal deformities such as pes cavus .The CMT hereditary neuropathies are categorized by mode inheritance and causative gene or chromosomal locus. We usually classified autosomal-dominant inherited CMT patients with demyelinating form, severely reduced nerve conduction velocities (NCVs) in type 1 (CMT1) and axonal form in type 2 (CMT2). X-linked inheritance pattern classified in CMTX and autosomal recessive classified in CMT4 type. All the patients (167), according to electro-clinical, neurologic Ex. and pedigree were investigated for: PMP22, GDAP1, GJB32, EGR2, MPZ and MFN2 genes by PCR-Sequencing method for all the exons and exon-intron boundaries. In order to show that these found novel mutations are pathogenetic 50 normal controls were sequenced for all of these genes for whom no such mutations were found. We are going to discuss about clinical and molecular finding in our patients. Key Note: The problem of genetic diagnosis of CMT is: a) Many genes involving for same phenotype b) Many phenotypes caused by same gene. So batteries of genetic diagnosis need to clarify the cause of CMT by investigation of other associated genes with the disease.

1057F

A revised diagnostic algorithm for analysis of Zellweger spectrum disorders peroxisome assembly defects. *G.S. Charames[1,2], B.A. Karczeski[1,2], G.R. Cutting[1,2,3], S.J. Steinberg[1,4,5].* 1) DNA Diagnostic Lab, Johns Hopkins University, Baltimore, MD; 2) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 3) Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD; 4) Department of Neurogenetics, Kennedy Krieger Institute, Baltimore, MD; 5) Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD.

Zellweger spectrum disorder (ZSD), comprising Zellweger syndrome, neonatal adrenoleukodystrophy and infantile Refsum disease, are autosomal recessive multi-system developmental disorders impairing brain, skeletal, hepatic and renal development. ZSD in humans are caused by defects in proteins encoded by 12 PEX genes. Complementation studies performed at Kennedy Krieger suggested that *PEX1* defects accounted for about 70% of ZSDs. However our pilot analysis of 91 patients indicated that only about 58% of patients had *PEX1* gene defects (Steinberg *et al* 2004; Yik *et al* 2009) and this was recently confirmed by a much larger study in 600 ZSD patients (Ebberink *et al* 2010). We developed an algorithm for PEX gene sequencing -The PEX Gene Screen- to identify the putative genetic defect (Steinberg *et al* 2004) based on sequencing the exons of the six PEX genes -*PEX1, PEX2, PEX6, PEX10, PEX12,* & *PEX26*- where mutations were most commonly reported in ZSD patients previously documented in the literature. The high proportion of *PEX1* cases is due to several common mutations found amongst exons 13, 15 and 18. In contrast, the second most common defective ZSD gene, *PEX6*, accounts for 10-16% of patients (Steinberg *et al* 2004; Yik *et al* 2009; Ebberink *et al* 2010), but does not have common, recurrent mutations across large populations. To help in the optimization of our PEX Gene Screen algorithm, we sequenced the entire coding region of *PEX1, PEX2, PEX6, PEX10, PEX12* and *PEX26* in 11 patients who were negative for the common PEX1 mutations in exons 13, 15 and 18. We identified two clearly pathogenic mutations in 9 patients: 4 *PEX6* defects, 2 *PEX1* defects, 2 *PEX2* defects, and 1 *PEX12* defect. In two patients we identified a single *PEX1* or *PEX6* mutation and further studies are underway in these cases. Although the number of patients included in this follow up study is small, it supports modifying our PEX Gene Screen algorithm to sequence the entirety of *PEX6* prior to looking for mutations in the other five most common gene defects in ZSD in those negative for the common *PEX1* mutations.

1058F

IL28B SNP rs12979860 genotyping: Allele frequencies in a diagnostic testing population. *M. Mikula, A. Buller Burckle, W. Sun, C. Strom.* Dept Molecular Genetics, Quest Diagnostics Nichols Inst, SanJuanCapistrano, CA.

Introduction: Knowledge of the genotype of patients infected with HCV aids in the clinical decision to initiate treatment with pegylated interferon-(/ribavirin (PegIFN/RBV). The importance of this prediction is that the treatment has limited efficacy and is poorly tolerated in certain patients, and the course of treatment is long (48 weeks). Genotyping of the SNP IL28B rs12979860 helps predict therapeutic response.The CC genotype in this SNP is associated with as much as a two-fold greater sustained virological response (SVR) to this treatment in European, African-American, and Hispanic populations compared to CT or TT genotypes. The purpose of this study was to determine the frequency of the genotypes in a mixed ethnic population. Methods: We detected “C” and `T” alleles at SNP rs12979860 in 608 de-identified samples on a real-time PCR platform using an allelic discrimination method. Results: We observed 159 C/C homozygotes (26%), 321 C/T heterozygotes (53%), and 128 T/T homozygotes (21%). The observed frequency of the “C” allele in our diagnostic testing population was 0.53. Our results show good fit with Hardy-Weinberg analysis (chi squared 1.931, P = 0.38) even though our sample population contains a mix of ethnicities known to have different allele frequencies. Conclusion: Nearly half of the individuals tested by this assay are predicted to have a clinically significant result with 26% of the individuals (C/C) predicted to have a SVR and 21% (TT) predicted to have only half as much response to PegIFN/RBV.

1059F

FAMILY MEMBERS OF PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA: HHT CENTER PARIS EXPERIENCE IN 343 RELATIVES FROM 116 FAMILIES WITH GENOTYPING. *J. ROUME - BAUDRIER[1], M. EYRIS[3], I. BOURGAULT - VILLADA[1], J.H. BLONDEL[1], S. BLIVET[1], D. COBARZAN[1], J.P. PELAGE[1], A. OZANNE[2], C. FAGNOU[1], G. LESUR[1], L. CRIVAT[1], F. COULET[3], P. LASJAUNIAS[2], B. RAFFESTIN[1], L. GOUYA[1], T. CHINET[1], P. LACOMBE[1], F. SOUBRIER[3].* 1) Haemorragic Hereditary Telangiectasia Center PARIS PARIS V University CHU Ambroise Pare, AP-HP, Boulogne ,92100 France; 2) Neuroradiology National Rare Diseases Center: Neurovascular Malformations in Children. CHU Bicêtre Hospital- Paris Sud University AP-HP 78 rue du Général Leclerc Le Kremlin-Bicêtre 94275 cedex France; 3) Oncogenetic and angiogenetic Molecular Laboratory Groupe Hopitalier Pitié-Salpétrière Paris VI University AP-HP 47/83 bd de l'hôpital 75651 PARIS Cedex 13 France.

Hereditary Hemorrhagic Telangiectasia (HHT) is a heterogeneous vascular disease that can present with a variety of clinical manifestations. The visceral vascular lesions of this disease may be potentially devastating. The clinical diagnosis of HHT is determined by using the four following Curaçao diagnosis criteria: Epistaxes(1),Telangiectasias multiple(2)at characteristic sites,Visceral vascular lesions(3): gastrointestinal telangiectasias and arterio-venous malformations (pulmonary, hepatic, cerebral and spinal arterio-venous fistula)and family history(4). Mutations of the Endoglin (ENG) and Activin A receptor type II-like kinase-1 (ACVRL1) genes are known to be the major genetic factors of HHT. **OBJECTIVE**: To assess the clinical and genetic characteristics of family members of patients with hereditary hemorrhagic telangiectasia (HHT).**SETTING**: HHT Center in Paris. **PARTICIPANTS**: 343 members family of 116 genotyping HHT patients screened from January 2004, through December 2010. **INTERVENTIONS**: volunteers previously well informed directly by means of the educational course of the proband about the intrafamilial variability of symptoms and potential severe visceral complications; screened during one half a day by mean of a stepped multidisciplinary clinical screening protocol based upon Curaçao criteria and genetic screening systematically proposed. **MAIN OUTCOME MEASURES**: Age, sex, prevalence of cutaneo mucous telangiectases, visceral ArterioVenous Malformations(AVMs), genetic characteristics and proposed therapeutics. **RESULTS**: 182 relatives were found with manifestations of HHT. Below twenty years old:34%, between twenty and forty:31%, over forty: 35%. Sex Ratio:0.75. All four Curaçao criteria were present for 123 cases, three criteria for 30 cases, two criteria for 18 cases and 11 cases (6%) were asymptomatic with only the familial criteria and found by mean of the genetic focused screening. 108 were relatives of the 66 HHT1 probands. 41 of them were referred for embolization of large pulmonary arterio venous malformations (PAVMs); 74 were of the 50 HHT2 probands, one of them, proposed for cerebral AVMs embolization. Antibioprophylaxy was proposed for 123 patients with small PAVMs. **CONCLUSIONS**: 68% of HHT family members present visceral AVMs which can lead to life-threatening events. They should be encouraged to engage in a screening program, from earliest age, since the prevalence of potentially serious localizations is higher than previously thought.

1060F

Predictive testing for adult-onset neuromuscular diseases. *Y. Sato, Y. Ikegami, N. Minami, N. Okamoto, M. Mori, M. Murata, Y. Goto.* National Center of Neurology and Psychiatry, Kodaira, Japan.

Molecular genetics research has led to the identification of an increasing number of disease-associated genes. Genetic testing is now available to confirm the diagnosis for symptomatic individuals and to assess whether their families at risk have the gene mutation. Their lives may be affected or controlled by test results. Predictive genetic testing for adult-onset neuromuscular diseases is presently offered in a few medical centers in Japan. National guidelines suggest that these testing should be performed carefully under specific conditions after genetic counseling. We developed an original protocol for predictive testing program in 2009. Our staff includes board-certified medical geneticist, genetic counselor, neurologist, psychiatrist and molecular geneticist. Medical geneticist and genetic counselor assess the client's knowledge of disease, obtain the family history, consider the suitability for genetic analysis, explore the motivations for testing and determine the consequences of testing. Guidance for client's families is suggested as needed. Neurologist and psychiatrist provide specialized screening. Our program comprises at least four pre-test sessions. Genetic testing is performed in our laboratory under jurisdiction of the institutional review board. After the disclosure of test results, several post-test sessions are scheduled. During the period from April 2002 to March 2011, a total of 268 clients visited our genetic counseling unit, approximately sixteen percent of them concerned about predictive testing. We provided genetic counseling on predictive testing for eleven clients in the past year. Seven of them entered testing program, and three had the genetic testing. While two got negative results, one tested positive for Huntington's disease. We continually keep in contact with them. Our predictive testing program seems to provide appropriate care for clients up to now. The ease in access to the testing will likely increase the demand for services related to predictive testing. Every testing requires a case-by-case consideration. We may need to modify the program to comply with client's situation and medical infrastructure peculiar to Japan. A substantial number of appropriate genetic centers for predictive testing are required in Japan.

1061F

The combination of (((anti 3.7 triplication and) -thalassemia in an Iranian family. *f. moosavi, z. kaini moghaddam, a. Valaei, s. Zeinali, m. Karimipoor.* Molecular Medicine Dept., Biotechnology Research Center, Pasteur Institute of Iran.

Introduction: The pathophysiology and clinical severity of) -thalassemia are associated with the degree of (/non(-chain imbalance. A triplicated (-globin gene locus can exacerbate the effects of (-chain excess caused by a defective) -globin gene, although this combination in different individuals results in variable phenotypes. In the present study we report the molecular analysis of an Iranian subject with a thalassemia intermedia phenotype, heterozygous for) -thalassemia. Methods: Complete blood counts were measured with a Sysmex KX-21 cell counter. DNA extraction from peripheral blood leukocytes was performed by salting out method. Mutation analysis of the) -globin gene was detected by ARMS-PCR and multiplex-PCR was used to detect alpha triplication (((anti3.7). Multiplex ligation-dependent probe amplification (MLPA) technique was performed for confirmation the results of multiplex PCR. Results: The mother of index case was a carrier of) -thalassemia with IVSII-I mutation in heterozygous form and her husband was hematologically normal with no mutation in) -globin gene. The propositus, a 2.5 years old child presented a transfusion dependent thalassemia intermedia phenotype. Multiplex PCR detected the presence of extra (-globin gene in the patient and her mother but her father was normal. MLPA results indicated that there is a large triplication from probe 1 (upstream HS-40) to probe 26 (3.7 Kb downstream HbA) in their (-globin gene cluster. Conclusion: The clinical and hematological picture of) -thalassemia heterozygotes with a triplicated (-globin gene arrangement is variable, ranging from an asymptomatic presentation to a mild to moderate thalassemia intermedia phenotype and it depends on the size of the additional part within the (-globin gene cluster. This finding has important implications for genetic counseling and prenatal diagnosis programs. This family may be at risk for another child with severe thalassemia intermedia. The genetic and phenotypic characteristics of the patients described here indicate the need to consider the possibility of a triplicated (-gene allele in patients with heterozygosis for) -thalassemia who show an unexpected severe phenotype.

1062F

"THE MERRY-GO-ROUND OF DISCOVERY": DIAGNOSIS OF FRAGILE X SYNDROME AND DUCHENNE MUSCULAR DYSTROHPY. *A.D. Archibald[1], B.J. McClaren[1,2], S.H. Wong[1,2], M.N. Cotter[1], A. Kornberg[1,2,3], A.M. Jaques[1], S. Wake[2,4], J. Cohen[5,6], S.A. Melcalfe[1,2].* 1) Murdoch Childrens Research Institute, Melbourne, Victoria, Australia; 2) Department of Paediatrics, The University of Melbourne, Melbourne, Victoria, Australia; 3) The Royal Childrens Hospital, Melbourne, Victoria, Australia; 4) Genetic Health Services Victoria, Melbourne, Victoria, Australia; 5) Fragile X Alliance Inc, Melbourne, Victoria, Australia; 6) Centre for Developmental Disability Health Victoria, Monash University, Melbourne, Victoria, Australia.

Genetic conditions presenting as developmental delay early in childhood can often be difficult to diagnose due to non-specific symptoms and lack of awareness of these conditions amongst the community and health professionals. Fragile X syndrome (FXS) and Duchenne muscular dystrophy (DMD) are both X-linked conditions with variable initial presentations and ages of diagnosis. We investigated the experience of obtaining diagnoses of FXS and DMD in Victoria and Tasmania, Australia using a mixed methods approach to: 1) identify the age at which diagnoses of FXS and DMD occur and, 2) to explore the experiences of parents of children with these conditions regarding the diagnosis.An audit of laboratory and clinical records for all DNA tests ordered in Victoria and Tasmania for FXS (2000-2009) and DMD (2005-2010) was undertaken. Semi-structured interviews were conducted with relatives of individuals with FXS and parents of boys with DMD. Analysis of records revealed that the median age at testing for individuals with a FMR1 full mutation was 15 years (n=133 of 16,845 records) and was 4.6 years for boys with DMD(n=77 of 448 records); this age has not changed substantially over the time period investigated. Clinical indications for testing and referring health practitioner influenced age at testing with paediatricians being the predominant healthcare practitioners ordering the tests. Forty four interviews were conducted (FXS = 29; DMD = 15). In the vast majority of families, there was a delay between when initial concerns about the child's development were noticed by parents and when the diagnosis was received; all found this to be a very difficult time. For the parents of boys with DMD, this delay ranged from 6 months to almost 5 years. Families described taking a proactive and persistent approach to searching for a cause for their child's developmental delay and consulting multiple healthcare providers before genetic testing was undertaken. Initial emotional reactions following the diagnosis were shock and grief which, over time, gave way to relief at having an explanation for the child's difficulties.These results provide a valuable insight into diagnoses of FXS and DMD and indicate the process of diagnosing these conditions could be improved; new strategies for diagnosis should be considered. Based on these data we are currently developing a model of offering population screening for genetic causes of developmental delay, when the child is about 1 year of age.

1063F

The Role of Social Comparisons in Quality of Life following a Prostate Cancer Diagnosis. *S.S. Kalia[1], A.C. Madeo[2], D. Roter[3], L.A.H. Erby[3], T.O. Blank[4].* 1) Boston University School of Medicine, Boston, MA; 2) National Human Genome Research Institute, NIH, Bethesda, MD; 3) Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 4) Human Development and Family Studies, University of Connecticut, Storrs, CT.

Introduction: Many prostate cancer patients grapple with uncertainty regarding the best treatment option. Comparing oneself with others is one way of coping with uncertainty. Limited research has shown that such social comparisons help prostate cancer patients to regulate their emotions. The objective of this study was to investigate relationships between social comparisons and quality of life among men who had been diagnosed with localized prostate cancer and participated in support groups. Hypothesis: The theoretical model for this study was based on Social Comparison Theory and the Transactional Model of Stress and Coping. We hypothesized that positive social comparisons would be positively associated with quality of life. Methods: Participants (186) were recruited from prostate cancer support organizations and provided responses through online and paper surveys. Quality of life was assessed with the Quality of Life Index-Cancer version. The Identification/Contrast measure was used to assess social comparisons on Likert scales with total scores between 3-15. Results: Participants reported comparing themselves to support group members, relatives, friends, and colleagues. Overall, participants reported making more positive (mean=10.9, SD=2.4) than negative (mean=6.2, SD=2.5) social comparisons ($p<0.0001$). Open-ended responses included positive comparisons such as, "I have been able to reorient and control my life, better than I think they will" and negative comparisons such as, "[I am] frustrated about how they got where they are... I'm at the end of the bell curve." Positive comparisons were positively correlated with quality of life ($r=0.351$, $p<0.001$). After controlling for covariates, the use of positive comparisons was a predictor of better quality of life () =0.170, p=0.002), and the use of negative comparisons was a predictor of poorer quality of life () =-0.259, $p<0.001$). Conclusions: The data indicated that men in the study population compare themselves to others and that their comparisons tend to reflect positively on their own situation. One's interpretation of social comparisons as positive or negative may be a determinant of one's quality of life. These findings are relevant to clinicians who help patients in making treatment decisions and adapting to a cancer diagnosis. Future research could investigate the impact on quality of life of interventions that help individuals to modify their social comparisons toward more positive interpretations.

1064F

Development of preventive strategy for healthy adults with family history of type 2 diabetes: Challenges in Japan. *M. Nishigaki[1], A. Ota[1,3], K. Kobayashi[1], T. Shibayama[1], E. Sato[1], Y. Tokunaga[1], S. Kosaka[1], N. Seki[2], M. Yokoyama[2], T. Yokomura[2], Y. Fujikawa[4], T. Shimomura[4], T. Ota[4], C. Matsuzaki[4], S. Taguchi[4], J. Nishida[5], K. Kazuma[1].* 1) Adult Nursing, University of Tokyo, Japan; 2) Social Insurance Funabashi Central Hospital, Chiba, Japan; 3) Fukuyama Transporting Shibuya Longevity Health Foundation; 4) Brain Attack Center Ota Momorial Hospital, Hiroshima, Japan; 5) Social Insurance Chuo General Hospital, Tokyo, Japan.

Drastic progression in genome analysis enables genome wide search for candidate genes for type 2 diabetes. However, the recent studies have shown that genotype would add slightly more information to predictive models which consists of common risk factors, including family history. Thus, family history is still an important tool for identification of high-risk populations in this post genomic era.

We have performed continuous work for developing preventive strategy based on family history of type 2 diabetes since 2004. Step1: collecting expert opinion; Step2: investigating subject's health belief and genetic risk perception; Step3,4: development and feasibility assessment of lifestyle intervention, and genetic counseling tool for type 2 diabetes; and Step5: Randomized controlled trial at medical check-up institution to investigate the effect of lifestyle intervention and genetic counseling on diabetes prevention.

Step1 study had showed that diabetes specialist thought that preventive intervention for relatives of type2 diabetes patients might be effective but there were several barriers to practice: lack of time and human resource, hard to contact the subjects, and less motivation to preventive behavior in relatives. Step2 study had revealed that half of relatives already took action for prevention while they had less knowledge about concrete method for prevention. This study also revealed that they tended to underestimate the effect of environmental risk factors while overestimate the effect of genetic risk factors. Then Step3 study developed genetic counseling tool(six-page booklet). This tool was aimed to facilitate adequate understanding about genetic-environmental interaction in diabetes onset. Simultaneously, Step4 established feasible preventive intervention via mail focused on education about concrete preventive method. These studies had revealed their possible effect and efficiency. On the other hands, these feasibility studies had also revealed difficulty of recruiting subjects into prevention program via patients. Based on these studies, now we have been running Step5 study at medical check-up institution in order to make face-to-face contact with relatives who have family history of type2 diabetes.

1065F

Characterizing the Role and Skills of the Research Genetic Counselor Over Time. *A. Rupchock[1], H. Zierhut[2], E. Heise[3], E. Burkett[4], A. Dressen[1], S. Hahn[1].* 1) Hussman Institute for Human Genomics, Miami, FL; 2) University of Minnesota Medical Center, Hugo, MN; 3) Duke University, Durham, NC; 4) Legacy Center for Maternal Fetal Medicine, Emanuel Hospital, Portland, OR.

According to the 1994 Professional Status Survey conducted by the NSGC only 2% of genetic counselors (GCs) reported a primary role in research (> 0.5FTE). By 2000, this increased to 31% and remains steady. Despite its prevalence, this role has not been well characterized over time. One objective of this study was to delineate the characteristics and roles of research counselors. A web-based survey was administered in 2003 and repeated in 2010. Participants were recruited via e-mail through the NSGC listserv and relevant special interest groups. The survey was taken by 110 and 129 GCs, respectively, with a current or past primary role in research. Most demographics were largely unchanged. Those with >10 years of experience increased from 7% to 19%, and average age increased from 34 to 36 years. The most common types of studies remain unchanged, but fewer have been involved in gene/mutation identification studies (79% vs. 66%) and more have been involved in translational/genomic medicine (23% vs. 30%). While the majority have still been a study coordinator and/or provided genetic counseling to study participants, the characteristics and roles appear to be evolving. Those who served as principal investigator or co-investigator increased, from 34% to 47%, with 18% of 2010 respondents having been a PI. Those pursuing or holding an advanced degree increased from 5% to 11%. Those who submitted their own grant also increased, with funding of at least one grant increasing from 60% to 73%. Those involved in focus groups increased from 15% to 22% with higher level skills also increasing. Designing the focus group guide increased from 53% to 89% and analyzing the data increased from 53% to 79%. For those involved in qualitative interview studies, study design increased from 50% to 74% and creating the guide increased from 56% to 76%. From the 2010 data, PIs/Co-Is are more likely to have submitted a grant, have more experience with statistical methods, and are more likely to be involved in focus group research, qualitative interviewing, and scale based research than those who have not been a PI or Co-I ($p<0.05$). These remain significant when adjusted for years of experience. Elucidating the characteristics of the research GC will expand the definition and roles of a GC, contribute to the identification of new and emerging opportunities for the profession, and may guide curriculum development among GC training programs.

1066F

Lack of lung function prediction value for genetically determined ancestry in French Canadian population. *J. Berube[1], M. Lamontagne[1], M. Laviolette[1], Y. Bossé[1,2], The Merck-Laval-UBC-Groningen Lung eQTL consortium.* 1) Centre de recherche Institut universitaire de cardiologie et de pneumologie de Québec, Laval University, Quebec, Canada; 2) Department of Molecular Medicine, Laval University, Quebec, Canada.

A recent report suggests that genetically determined ancestry improves predicted lung function measurements in African Americans. In this study, we test whether genetic ancestry derived from genome-wide genotyping data is useful to predict lung function in a French Canadian population. A total of 426 subjects of French Canadian origin were genotyped for nearly 1.2 million SNPs using the Illumina Human1M BeadChip. 420 of them passed genotyping quality control filters. All these subjects underwent lung cancer surgery. Patients with concomitant bronchiectasis, infection, lymphoma, lymphangioma, mesothelioma, and organizing pneumonia were excluded. The genotypes of the 403 remaining subjects were filtered to eliminate SNPs in linkage disequilibrium ($r^2 \nearrow 0.1$) using the LD-based SNP pruning option in PLINK. We used genotypes from unrelated HapMap CEU and YRI populations as reference sets for European and African ancestry, respectively. This resulted in a final set of 66,749 autosomal SNPs in linkage equilibrium and shared across the different populations. This set of SNPs was used to calculate genetic ancestry using the ADMIXTURE program and fixing two ancestral populations (K = 2). Low variability in genetically determined European ancestry was observed in this French Canadian population (European ancestry range = 93.8 to 99.6%). We used linear regression, stratified by gender, to evaluate the association between lung function and European ancestry. None of the lung function measurements were significantly associated with the percentage of European ancestry. Two models were compared to assess whether genetic ancestry influences pulmonary function predictions. The first, labeled as the standard model, included age, age squared and height squared. The second one included all the same variables but also the individuals' percentage of European ancestry. Mean absolute differences of 6 ± 5 ml and 14 ± 13 ml were observed between the two models for men and women, respectively. These results suggest that genetically determined ancestry do not change the predicted lung function measurements in this French Canadian population. Further studies using different source of European populations and wider admixed populations will be required to evaluate the clinical potential of ancestry-based lung function prediction models.

1067F

Gene-gene and gene-environment interactions in risk prediction. *H. Aschard[1], J. Chen[2], P. Kraft[1].* 1) Department of Epidemiology, Harvard School of Public Health, Boston, MA; 2) Department of Biostatistics, University of Pennsylvania, Philadelphia, PA.

Background. Genome-wide association scans have identified scores of common genetic variants associated with the risk of multifactorial diseases in the past few years. However their impact in discrimination and risk prediction beyond traditional risk factors has been shown to be limited. Therefore it has been suggested that the identification of gene-gene (GxG) and gene-environment (GxE) interactions would allow more accurate predictions of disease and facilitate prevention compared to risk-assessment models that include only the marginal effects of the known risk factors.

Method. In this study we conducted a simulation study to explore the potential improvement of discrimination if GxG and GxE interactions exist and we know them. The disease status of three common complex diseases, Breast Cancer (BRCA), Type 2 Diabetes (T2D) and Rheumatoid Arthritis (RA), were generated across a broad range of hypothetical GxG and GxE interaction effects. The interaction effects were defined such that the final marginal effects of known genetic risk variants and known clinical and environmental risk factors were equal to their previously reported estimated effects.

Results. The addition of the simulated GxG and GxE interaction effects in risk models shows overall very modest improvement in the discrimination ability as measured by the C-statistic (<5%). Moreover this improvement was dramatically decreasing with the increase discrimination ability of the marginal models (when no interaction was simulated): the average C-statistics of the models including only the marginal effects of the genetic and non-genetic risk factors were 0.62, 0.71 and 0.79 for BRCA, RA and T2D respectively; when adding ten low to large interactions the corresponding increases in C-statistic were 3.9%, 2.1% and 1.5% on average. Our simulations also show that few strong interactions are more likely to improve discrimination than a large number of small interactions.

Conclusion. The inclusion of GxG and GxE interaction effects in risk prediction models as usually described in the literature, is unlikely to improve significantly the discrimination ability of these models. If such interactions exist, other strategies should be explored to leverage this information.

1068F

X-Linked retinitis pigmentosa in a three-generation family of females and a female-sporadic case accounted for by single heterozygosity for RPGR mutations. *J. KAPLAN[1,2], N. DELPHIN[2], S. GOBIN[2], J.-P. BONNEFONT[1,2], J.-L. DUFIER[3], O. ROCHE[3], A. MUNNICH[1,2], J.-M. ROZET[1,2].* 1) Genetics and epigenetics of metabolic, sensorineural diseases and birth defetcs, INSERM U781-Paris Descartes University & Institute of genetic diseases IMAGINE, Necker - Enfants Malades University Hospital, Paris, France; 2) Genetics Department, Necker - Enfants Malades University Hospital, Paris, France; 3) Ophthalmology Department-Paris Descartes University, Necker - Enfants Malades University Hospital, Paris, France.

Purpose: To confirm unexpected X-linked dominance of RPGR mutations in a three-generation family of females and a female-sporadic case. The X-linked inheritance was suspected on a basis of a variable disease expression through the three generations along with existence of a unilateral high myopia in the oldest patient as well as in the female sporadic case. Patients and Methods: In Family 1, a 46 year-old woman was seen for a progressive decrease in visual acuity. Upon ophthalmological examination her fundus displayed diffuse “tiger-stripe” striations resulting from a diffuse retinal pigment epithelium atrophy revealing the underlying choroïdal vessels. Her sister and two brothers as well as her 5 nephews and 4 nieces had no known visual deficiency. Conversely, her 68 year-old mother presented since infancy with unilateral amblyopia and her fundus presented the same “tiger-stripe” striations. Finally, while her 24 year-old son had no visual problem, her 21 year-old daughter presented a moderate and well corrected myopia but her fundus was similar to that of her mother and grand-mother. In Family 2, a 39 year-old woman came to the consultation for history of night blindness since her 20s and progressive constriction of her visual field leading to an unambiguous diagnosis of RP. She displayed high asymmetric myopia and low visual acuity. Her parents, her three sibs and her three young children were healthy. The RPGR gene, including the ORF15 exon, was screened for mutation using direct sequencing in the two families. Results: The genetic screening of the RPGR gene evidenced truncating mutations in the ORF15 exon: a 4 bp deletion (c.3007-3010delGGAG; p.ORF15+Gly418fsX85) segregating with the disease in Family 1 and a 1 bp deletion (c.478delA, p.ORF15Glu249fsX503) in Family 2. In Family 1, the youngest patient asked for prenatal diagnosis in case of pregnancy of a male fetus. In Family 2, the proband had three healthy young children (7 year-old girl, 5 and 3 year-old boys). The girl and the youngest boy were found to carry the mutation. Conclusion: The existence of unusual retinal manifestations in women with no related affected males does not rule out X-linked transmission especially when highly asymmetric or even unilateral myopia and variable expressivity or incomplete penetrance are noted. In addition, the identification of RPGR mutations in females, including sporadic cases, suggests that the X-linked inheritance of RP is likely underestimated.

1069F

Stargardt disease: clinical utility and sensitivity of a next-generation sequencing panel. *S.M. Sperber[1], B.J. Williams[1], D. Blain[2,3], K. Goetz[3], V. Ndifor[3,4], M. Reeves[3,4], S. Vitez[3,4], X. Wang[3], S.J. Tumminia[5], N. Smaoui[1].* 1) GeneDx, Gaithersburg, MD; 2) MedStar Research Institute, Hyattsville, MD, USA; 3) 3Ophthalmic Genetics and Visual Function Branch, National Eye Institute/NIH, Bethesda, MD, USA; 4) 4BioSearch/Team Placement Service Incorporated, Rockville, MD, USA; 5) OD, National Eye Institute/NIH, Bethesda, MD, USA.

Background: Stargardt disease (SD) is the most common form of macular dystrophy found in patients under the age of 20, exhibiting a frequency of 1/10,000. Exemplified by decreased central vision and a bronzing of the macula with yellow flecks, SD progresses to blindness with some preservation of the peripheral vision. SD is inherited in an autosomal recessive manner and is caused by mutations in the ATP-binding cassette transporter (*ABCA4*) gene. Other types of macular dystrophy are inherited in an autosomal dominant form and include the *RDS*/*PERIPHERIN2* and *ELOVL4* genes. Here we present data assessing the clinical utility and sensitivity of genetic testing using a Stargardt next-generation sequencing panel.

Methodology: In our CLIA certified diagnostic laboratory, consecutive samples of 45 patients with clinically identified Stargardt disease were analyzed using the Illumina HiSeq platform to perform massively-parallel sequencing of an *ABCA4*, *RDS* and *ELOVL4* gene panel totaling 57 amplicons. PCR followed by conventional dideoxy capillary sequencing was used to confirm all mutations and variants of unknown significance.

Results: Seventy-six percent of patient samples harbored mutations or novel sequence variants of potential pathogenicity. The majority of patients carried mutations in ABCA4 (32/34). Mutations in the *RDS* gene were identified in two patients, and none were found in the *ELOVL4* gene. Of the total variants analyzed, 28% were novel (14/50). Thirty percent of the patients with identified *ABCA4* mutations had only a single heterozygous mutation or variant of unknown significance.

Conclusion: Our results suggest that diagnostic testing for suspected Stargardt disease with a sequencing panel for the *ABCA4* and *RDS* genes is sensitive and efficient. Five percent of the patients harbored mutations in the RDS gene, suggesting inclusion of the gene in genetic counseling sessions of this disorder. No mutation was found in a quarter of the samples, indicating that an expansion of the number of genes within the panel would increase its sensitivity.

1070F

Exome sequencing in phenotypic facioscapulohumeral muscular dystrophy (FSHD2). *A. Leidenroth[1], H.S. Sorte[3], P. Lunt[2], R. Lyle[3], J.E. Hewitt[1].* 1) The University of Nottingham, Nottingham, United Kingdom; 2) University Hospitals Bristol NHS Foundation Trust, Bristol, United Kingdom; 3) The Norwegian High-Throughput Sequencing Centre, Dept. of Medical Genetics, Ullevål, Oslo University Hospital, Norway.

Most cases of facioscapulohumeral muscular dystrophy (FSHD1) are caused by the contraction of the 3.3kb macrosatellite repeat array D4Z4 on the chromosome 4 subtelomere. The current model of the underlying molecular disease mechanism is that the dystrophic defect results from contraction-dependent epigenetic changes at D4Z4. In turn, these allow aberrant transcription of the DUX4 retrogene, which is located within each repeat unit. DUX4 transcripts have recently been shown to be stabilized by a polymorphic polyadenylation signal at the distal end of D4Z4. This poly-A site is in linkage disequilibrium with markers proximal to the array, variants of which segregate with disease status. Between 5% and 10% of patients are diagnosed with the FSHD phenotype but do not have a contracted D4Z4 array (phenotypic FSHD or FSHD2). However, they do show the same epigenetic changes of histones and DNA methylation at D4Z4 as FSHD1 patients. Specifically, this includes the loss of histone 3 lysine 9 tri-methylation and hypomethylation at certain restriction enzyme sites (FseI, BsaAI and CpoI). The defect that triggers these epigenetic changes and the muscle phenotype in these patients is still unknown, and we intend to use exome sequencing to identify it. We have characterized the methylation status of a panel of FSHD2 patients by methylation-sensitive restriction digest assays combined with non-radioactive Southern blotting. For this, we developed a modification of the published protocol for D4Z4 methylation analysis for use with digoxigenin labeled probes. This analysis showed the proximal D4Z4 repeat to be hypomethylated in FSHD2 patients, confirming previous studies. For the exome sequencing we are using Agilent SureSelect 50Mb human exome capture combined with high-throughput analysis on the Illumina HiSeq2000. What makes phenotypic FSHD interesting is the fact that the molecular disease mechanisms of FSHD1 are still only poorly understood. While the case for a DUX4 involvement is convincing, we still do not understand how it affects downstream targets to cause a muscle phenotype. If we can identify the genetic mutation in FSHD2, pathway analysis could help explain the molecular link between these two related but distinct disorders. A better understanding of FSHD2 will help us develop therapeutic strategies for the majority of FSHD cases. We would like to thank The Muscular Dystrophy Campaign UK for funding.

1071F

Candidate gene and whole exome sequencing to identify novel muscular dystrophy mutations. *K.K. McDonald[1,2], E.E. Davis[3,4], J. Stajich[1], J. Rimmler[1], B. Rusnak[1], K. Crooks[1], N. Katsanis[3,5], A.E. Ashley-Koch[1,2], M.A. Hauser[1,2].* 1) Center for Human Genetics, Duke University, Durham, NC; 2) Department of Medicine, Duke University, Durham, NC; 3) Center for Human Disease Modeling, Duke University Medical Center, Durham, NC; 4) Department of Pediatrics, Duke University Medical Center, Durham, NC; 5) Department of Cell Biology, Duke University Medical Center, Durham, NC.

Muscular dystrophies are devastating diseases affecting 1 in 3,000 individuals worldwide, and there are no cures for affected individuals and no preventative therapies for individuals who are known to be at risk. There has been great progress in the identification of genetic mutations that cause some forms of muscular dystrophy; however, genetic heterogeneity is the rule rather than the exception. Consequently, many orphan dystrophies remain to be characterized and are a considerable burden to those affected individuals and their families. Several strategies can be utilized to identify new mutations and to confirm that they impair muscle function. Our group has identified a novel mutation in an orphan dystrophy family as a result of candidate gene sequencing of linkage peaks, and we have begun the analysis of second generation sequencing data to identify a causative mutation in another family. Linkage analysis for a family segregating a scapuloperoneal muscular dystrophy (SPMD) phenotype produced a peak on chromosome 14. Sequencing revealed a novel deletion of a lysine residue at amino acid position 1786 in MYH7 that segregated with affection status in the family and was not present in 382 control chromosomes. The deletion is consistent with observed skeletal muscle and cardiovascular phenotypes. We have also performed whole exome sequencing to identify variants causing disease in an autosomal dominant limb-girdle muscular dystrophy (LGMD) family. Preliminary analysis of these data has not revealed a previously identified muscular dystrophy mutation in these individuals. However, 98 novel, non-synonymous coding SNPs have been identified which are not present in two control individuals and are heterozygous in 3 cases. Seven splice site mutations were identified which met the same criteria. Segregation analysis in the full family is underway. Possible disease-causing SNPs will be sequenced in at least 500 control chromosomes. To show causality of any suspected muscular dystrophy mutations identified through sequencing, testing in a zebrafish model will be performed. As a proof of principle for this assay, zebrafish embryos were injected with mRNA containing the myotilin T57I mutation. These injections resulted in myofiber defects consistent with both the T57I mouse model and also LGMD patients harboring this mutation, demonstrating the utility of a developmental vertebrate model to assay late-onset muscular dystrophy alleles.

1072F

Genetic causes of primary lymphedema. *A. Mendola[1], A. Ghalamkarpour[1], C. Debauche[2], N. Van Regemorter[3], Y. Sznajer[4], D. Thomas[5], N. Revencu[1,4], Y. Gillerot[4], K. Devriendt[6], E. Legius[6], G. Pierquin[7], R. Hennekam[8], G.A. Diaz[9], J.B. Mulliken[10], L.M. Boon[1,11], M. Vikkula[1], The Lymphedema Research Group.* 1) Human Molecular Genetics, de Duve Institute Université catholique de Louvain, Brussels, Belgium; 2) Department of Neonatology, Cliniques Universitaires Saint-Luc, Brussels, Belgium; 3) Centre de Génétique ULB, Hôpital Erasme, Brussels, Belgium; 4) Center for Human Genetics, Cliniques Universitaires St Luc, Brussels, Belgium; 5) Unité diagnostic anténatal, Hôpitaux Iris Sud, Brussels, Belgium; 6) Center for Human Genetics, University Hospitals Leuven, Leuven, Belgium; 7) Center for Human Genetics, University Hospital Center-CHU Sart-Tilman, University of Liège, Liège, Belgium; 8) Department of Paediatrics, AMC, Amsterdam, The Netherlands and Clinical and Molecular Genetics Unit, The Netherlands; 9) Departments of Genetics & Genomic Sciences, Mount Sinai School of Medicine, New York, USA; 10) Vascular Anomalies Center, Plastic Surgery, Children's Hospital, Harvard Medical School, NY, USA; 11) Centre for Vascular Anomalies, Cliniques Universitaires St Luc, Brussels, Belgium.

Lymphedema is caused by a dysfunction of the lymphatic vessels, leading to disabling swelling occurring mostly on the extremities. There exist two major categories: primary (idiopathic) and secondary (acquired) lymphedema. Familial lymphedema usually segregates in an autosomal dominant or recessive manner. It can also occur in association with other clinical problems. In our lab, the screening of 237 primary lymphedema samples led to the discovery of different mutations in the five genes associated with lymphedema. VEGFR3, a class III receptor tyrosine kinase, is predominantly expressed in lymphatic endothelial cells in adults. Mutations in the gene cause primary congenital lymphedema or Nonne-Milroy disease. We found 10 mutations, 7 unpublished and 3 known, in 11 patients. Mutations in the transcription factor genes FOXC2 and SOX18 have been reported in lymphedema-distichiasis syndrome and hypotrichosis-lymphedema-telangiectasia syndrome, respectively. In our series, 9 patients had a mutation in one of these genes. We also screened two more recently identified genes: CCBE1 and PTPN14. CCBE1 (collagen and calcium binding EGF domain 1) was screened in 158 lymphedema cases. Mutations were identified only in patients with Hennekam syndrome. The PTPN14 gene, encoding a non-receptor tyrosine phosphatase, was reported to encompass an exon 7 deletion in one consanguineous family, in which affected individuals also present choanal atresia. We screened 188 samples for mutations by high resolution melting and sequencing. Only one mutation was found. In addition, intronic changes were identified, the disease-causing nature of which is unknown. We conclude that mutations in these genes are rare causes of primary lymphedema, limited to specific (syndromic) forms. The identification of the genetic causes of primary lymphedema nevertheless allows for more precise diagnoses, follow-up, and treatment, and enables the generation of disease models for future development of novel therapeutic approaches. Moreover, patients without a mutation in the known genes represent a valuable resource in the search for other genes causing primary lymphedema.

1073F

Mutations in Gene X cause enchondroma formation in Ollier disease and Maffucci syndrome. *T. Pansuriya[1], J. van Oosterwijk[1], R. van Eijk[1], M. van Ruler[1], S. Verbeke[1], D. Meijer[1], K.H. Nord[2], S. Daugaard[3], L. Sangiorgi[4], B. Toker[5], B. Liegl-Atzwanger[6], M. San-Julian[7], R. Sciot[8], L.G. Kindblom[9], K. Szuhai[1], J.V.M.G. Bovee[1].* 1) Pathology, Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands; 2) Lund University Hospital, Lund, Sweden; 3) University Hospital of Copenhagen, Denmark; 4) Rizzoli Orthopedic Institute, Bologna, Italy; 5) Istanbul University Medical School, Turkey; 6) Medical University of Graz, Austria; 7) University Clinic of Navarra, Spain; 8) University of Leuven, Leuven, Belgium; 9) Royal Orthopaedic Hospital, Birmingham, United Kingdom.

In Ollier disease and Maffucci syndrome, patients have multiple enchondromas in their skeleton without (Ollier) or with (Maffucci) haemangiomas. Enchondromas are benign cartilage forming tumours. Malignant transformation of enchondromas into chondrosarcoma occurs in more than 30% of the cases. Point mutations in *PTH1R* have been reported in about 8% of Ollier patients whereas they are absent in Maffucci syndrome. In our series all four reported point mutations in *PTH1R* were absent. Genome-wide analysis and expression arrays were performed previously and no large genomic aberrations were found. In this study, we have selected three candidate genes, for which sequencing was performed using 56 tumours of 40 patients. We found somatic heterozygous mutations at a single codon position in one of the three candidate genes. Mutations were found in 95% of the enchondromas related to Ollier disease and Maffucci syndrome. Using immunohistochemistry for the mutant protein, we observed a mixture of cells expressing mutated and non mutated proteins within the tumour, suggesting presence of mosaicism in the enchondromas like in other type of benign bone tumours. In addition, in normal cells (normal bone) surrounding the tumour we also observed a very low frequency of mutated cells suggesting germ line mosaicism in these patients. The frequency of mutations decreases with increasing histological grade in chondrosarcomas. This result confirms that this mutation is an early event in enchondromagenesis while another hit is needed for progression towards chondrosarcomas. We speculate that this hit not necessarily occurs in the mutated cells but can also occur in the wild type cells of the benign enchondroma precursor. In conclusion, we studied the largest series of patients with these two rare and non hereditary skeletal disorders and found a causative mutation in gene X present in 95% of enchondromas and our results suggest germ line mosaicism in patients with non hereditary Ollier disease and Maffucci syndrome.

1074F

The Molecular Genetic Basis of Pentosuria: Solving Garrod's Fourth Inborn Error of Metabolism. *S.B. Pierce[1,2], C.H. Spurrell[2], J.B. Mandell[1], M.C. King[1,2], A.G. Motulsky[1,2].* 1) Medicine (Medical Genetics), University of Washington, Seattle, WA; 2) Genome Sciences, University of Washington, Seattle, WA.

In 1908, Archibald Garrod introduced the concept of “inborn errors of metabolism,” citing albinism, alkaptonuria, cystinuria and pentosuria, each of which he postulated to be caused by recessive inheritance of a single mutation leading to deficiency of a specific enzyme. His concept supported the link between Mendelian factors, which had been rediscovered not long before, and enzyme chemistry, also then in its infancy. Of the four traits described by Garrod, the mutations responsible for the first three conditions have long since been solved. In contrast, the molecular genetic basis of pentosuria has remained a mystery. Pentosuria is characterized by high concentrations in urine of the pentose sugar xylulose and is associated with deficiency of the enzyme L-xylulose reductase. The trait is completely benign, but in the early and mid-20th century was important because it was confused with diabetes. Persons with pentosuria were often inappropriately treated with insulin, leading to severe reactions. As more specific diagnostic methods for diabetes were developed, persons with pentosuria were no longer at risk of being inappropriately diagnosed and treated as diabetic. Pentosuria is found almost exclusively among persons of Ashkenazi Jewish (AJ) ancestry. Families with pentosuria were studied for more than 30 years by Margaret Lasker, who demonstrated that inheritance was autosomal recessive and that the phenotype had no impact on longevity. In the 1960s, Mrs. Lasker bequeathed her records and data to AGM. This year, in the course of exome sequencing DNA from a control of AJ ancestry, we discovered by serendipity a frameshift (c.598delC) in *DCXR*, which encodes L-xylulose reductase. From the records of the families, we searched for surviving probands and adult children, finding informative members of 15 families, all of whom agreed to participate. In the families, we confirmed *DCXR c.598delC* and discovered a second mutation, *DCXR c.55(+1)G>A*, which leads to aberrant splicing and an unstable protein. All probands are homozygous or compound heterozygous for one or both of the mutations. Consistent with this, high levels of xylulose were detected in probands' serum. In 1240 AJ controls, the allele frequency of *DCXR c.598delC* was 0.0121 and of *DCXR c.55(+1)G>A* was 0.0040. Proband genotypes are as expected under Hardy-Weinberg equilibrium. Archibald Garrod and Margaret Lasker were entirely correct.

1075F

Exome sequencing in a consanguineous family segregating familiar juvenile polyarthritis. *R. Rabionet[1], J.I. Aróstegui[2], R. Medino[3], C. Tornador[1], D. Comas[4], E. González[2], S. Ossowski[1], J. Yagüe[2], X. Estivill[1].* 1) Genes and Disease, Center for Genomic Regulation and UPF, Barcelona, Spain; 2) Inflammatory Disease Unit, Immunology Service, Hospital Clínic i Provincial de Barcelona, Barcelona, Spain; 3) Pediatric Rheumatology Service, Hospital La Paz, Madrid, Spain; 4) Institut de Biologia Evolutiva (CSIC-UPF), Barcelona, Spain.

In the last year, exome sequencing has given a new spin to the genetics of rare Mendelian disorders. This new technology allows the analysis of diseases that were very difficult to tackle with traditional linkage and homozygosity mapping, especially those rare disorders with a small number of affected cases. We have applied exome sequencing to the analysis of a consanguineous family from Morocco carrying a rare recessive articular disorder. This pedigree consists of a consanguineous couple (first cousins) with three affected and two unaffected siblings, affected of a juvenile form of arthritis, with an onset at 2-4 y.o. Patients presented an erosive and symmetric polyarthtitis, and were seronegative for AntiNuclearAntibodies (ANA), Rheumatoid Factor (RF) and HLA-B27. Patients developed severe dysplasia and loss of walking ability before adulthood. The three affected siblings and one unaffected brother were genotyped with the Illumina 370K duo array, in search of regions of homozygosity. This produced four genomic regions, located in Chr3:4552500 - 10646218, Chr6:4707143 - 6584424, Chr6:106443904 - 123266646 and Chr13: 42419227 - 70194040. WISP3, a gene related to progressive pseudorheumatoid dysplasia, was located within the candidate regions. Sanger sequencing analysis did not identify variants in this gene. It was then decided to perform exome sequencing of two affected and one unaffected samples. Sequencing data was analyzed through our in-house developed pipeline for variant discovery. This lead to the identification of one novel (not in dbSNP, nor in 1000 genomes) nonsynonymous variant in each of the four homozygosity regions. These variants were then genotyped in 300 control samples from the Moroccan population, and all but one were absent in the control dataset. Additional characterization is ongoing in order to determine which of the remaining three variants leads to this damaging phenotype.

1076F

Discovery of defects in two central checkpoints of protein glycosylation associated with a broad spectrum of disorders. *S. Rust[1], L.C. Tegtmeyer[2], S. Moormann[2], E. Schrapers[2], A. Witten[1], J. Reunert[2], T. Marquardt[2].* 1) Leibniz-Inst f Arteriosclerosis Res, Albert-Schweitzer-Campus 1, Building Domagkstrasse 3, Münster, Germany; 2) Klinik für Kinder- und Jugendmedizin, Universitätsklinikum, Albert-Schweitzer-Campus 1, Building A13, 48149 Münster, Germany.

A large amount of proteins is N-glycosylated during synthesis at the endoplasmatic reticulum (ER). A preformed complex sugar moiety is transferred just when the respective Asn-residue of the growing peptide chain enters the ER-lumen. The sugar is then modified to produce the final glycoprotein. Protein glycosylation has many biological functions including basic functions in protein folding, protein interactions, stabilization, and regulated degradation of proteins. Genetic defects that disturb the glycosylation process are known as congential disorders of glycosylation (CDG), and defects typically are visible as reduced glycosylation of serum transferrin even in children. A large number of enzymes and transmembrane-transporters is involved in glycosylation. Typically CDG-defects are inherited as recessive traits. The aim of this study was to elucidate the genetic defects in two new CDG-families. In both families the parents were first cousins. Thus, we obtained candidate gene lists by homozygosity mapping (Illumina 660W SNP chips) and identified the defects by sequencing (incl. whole exome Illumina next generation sequencing). Interestingly in both cases the defective genes were previously not considered as CDG candidates and surprisingly turned out to be central checkpoints in protein glycosylation. One is involved in a gatekeeper position at the translocation pore through which the ribosome delivers the growing peptide chains of all new N-glycoproteins and the defect was lifethreatening. Our functional data show that the defective protein is essential to allow N-glycosylation of proteins, showing some site preferences. The other gene is a central player of sugar metabolism. It affects efficiency of glycogen usage and glycolysis, as well as synthesis of sugar-precursors for N-glycosylation. Defects in the same gene were discovered in a number of additional CDG-families. Hence, there is a broad range of traits associated with defects in this gene, including dilatative cardiomyopathy and liver fibrosis and affecting glycogen usage in muscles. As the metabolic intermediates are easily detected by small modifications of existing neonatal screening programs, this condition is suitable for predictive testing. Furthermore, since different affected metabolic intermediates may be restricted or substituted via diet we believe that already safety tested preventive treatment options are available.

1077F

Mutations in NOTCH2 cause Hajdu Cheney syndrome and serpentine fibula polycystic kidney disease. *M.A. Simpson[1], M.D. Irving[1,2], M.J. Gray[3], E. Asilmaz[1], D. Dafou[1], F.V. Elmslie[4], S. Mansour[4], S.E. Holder[5], C.E. Brain[6], B.K. Burton[7], K.H. Kim[7], R.M. Pauli[8], S. Aftimos[9], H. Stewart[10], C.A. Kim[11], M. Holder-Espinasse[12], W.M. Drake[13], S.P. Robertson[3], R.C. Trembath[1].* 1) Division of Genetics and Molecular Medicine, King's College London School of Medicine, Guy's Hospital, London, SE1 9RT, United Kingdom; 2) Clinical Genetics, Guy's and St Thomas' NHS Foundation Trust, Guy's Hospital, London, SE1 9RT, United Kingdom; 3) Department of Paediatrics, Dunedin School of Medicine, Dunedin, 9054, New Zealand; 4) SW Thames Regional Genetics Service, St George's, University of London, London, SW17 0RE, United Kingdom; 5) North West Thames Regional Genetics Service, NWLH NHS Trust, Harrow, HA1 3UJ, United Kingdom; 6) Department of Paediatric Endocrinology, Great Ormond Street Hospital For Children NHS Trust, London, WC1N 3JH, United Kingdom; 7) Department of Pediatrics and Division of Genetics, Northwestern University Feinberg School of Medicine, Children's Memorial Hospital, Chicago, IL, USA; 8) Department of Medical Genetics, University of Wisconsin-Madison, Madison, Wisconsin, USA; 9) Northern Regional Genetics Service, Auckland, New Zealand; 10) Department of Clinical Genetics, Churchill Hospital, Old Road, Headington, Oxford OX3 7LJ; 11) Genetics Unit, Instituto da Crianca do Hospitaldas Clínicas - FMUSP, Säo Paulo, Brazil; 12) Service de Génétique Clinique, Hôpital Jeanne de Flandre, Lille, 59037, France; 13) Department of Endocrinology, St Bartholomew's Hospital, London, EC1A 7BE, United Kingdom.

Hajdu-Cheney syndrome (HCS; OMIM 102500) is characterised by progressive focal bone destruction including acro-osteolysis and generalised osteoporosis together leading to characteristic radiographic abnormalities. To search for disease alleles in HCS we undertook whole exome sequencing of three unrelated affected individuals of European origin. Comparison of the exome variant profiles were undertaken using a model of a rare autosomal dominant disorder, requiring at least one previously unobserved heterozygous nonsynonymous or splice site substitution or a coding insertion or deletion in the same gene in all three individuals, a process that highlighted NOTCH2 as the only candidate gene matching these criteria. The three NOTCH2 variants are all located in exon 34, the terminal exon, of the 11.4kb transcript of this gene. We subsequently identified a series of novel heterozygous NOTCH2 genetic variants in 11 additional HCS kindreds with Sanger sequencing. All identified mutant alleles are predicted to lead to premature termination of the protein product before the complete translation of the PEST domain. We demonstrate that the level of expression of HCS alleles was comparable to that of wild-type alleles and total abundance of NOTCH2 transcript was equivalent to wild-type controls, suggesting the mutant transcripts evade the nonsense mediated decay pathway. Indeed, we were able to detect a protein of the size of the predicted truncated NOTCH2 intracellular domain in primary skin fibroblasts form an affected individual. These findings suggest that HCS alleles generate a mature NOTCH2 protein product which contains a disrupted or absent proteolytic PEST sequence the absence of which has previously been shown to increase Notch signalling. There is significant phenotypic overlap between HCS and serpentine fibula polycystic kidney disease (SFPCKD; MIM600330) a rare skeletal dysplasia with pronounced bowing of the long bones, polycystic kidneys and characteristic dysmorphic facies. One HCS subject in whom we had identified mutations had previously been reported as a SFPCKD case. We have now identified a NOTCH2 mutation in a second individual with SFPCKD suggesting that this disease forms part of the phenotypic spectrum of HCS and should no longer be classified as a distinct disease entity.

1078F

Chondrodysplasia, joint dislocations, brachydactyly and cleft palate caused by recessive mutations in *IMPAD1*, the gene coding for the Golgi-resident nucleotide phosphatase, gPAPP: a novel distinct phenotype associated with a proteoglycan sulfation defect. *A. Superti-Furga[1], E. Lausch[2], S. Unger[3], C. Gilissen[4], A. Rossi[5], U. Knoll[6], S. Nampoothiri[7], M. Nair[8], M. Del Rosario[4], H. Venselaar[9], J. Spranger[2], B. Campos-Xavier[1], H.G. Brunner[4], L. Bonafé[1], J.A. Veltman[4], B. Zabel[2], L. Vissers[4].* 1) Dept Pediatrics, University of Lausanne, 1011 Lausanne, Switzerland; 2) Div Pediatric Genetics, Centre for Pediatrics and Adolescent Medicine, Univ Hospital Freiburg, 79106 Freiburg, Germany; 3) Medical Genetics Service, University of Lausanne, 1011 Lausanne, Switzerland; 4) Dept Human Genetics, Nijmegen Ctr for Molecular Life Sciences and Inst Genetic and Metabolic Disorders, Radboud University Nijmegen Medical Ctr, 6500 HB Nijmegen, The Netherlands; 5) Department of Biochemistry, University of Pavia, 27100 Pavia, Italy; 6) Ctr for Prenatal Diagnosis, 10719 Berlin, Germany; 7) Amrita Institute of Med Sciences and Res Ctr, Cochin, Kerala, India; 8) Dept of Pediatrics, Medical College, Calicut, Kerala, India; 9) Ctr for Molecular and Biomolecular Informatics, Nijmegen Ctr for Molecular Life Sciences, Radboud University Nijmegen Medical Ctr, 6500 HB Nijmegen, The Netherlands.

We studied three individuals from two consanguineous families with a distinct condition characterized by short stature, chondrodysplasia with brachydactyly, congenital joint dislocations, cleft palate and facial dysmorphism. The differential diagnosis had included diastrophic dysplasia, Desbuquois dysplasia and recessive Larsen syndrome, but no mutation had been identified in *DTDST*, *CANT1* or *CHST3*. Whole exome sequencing revealed a single gene, *IMPAD1*, in which the three affected individuals had homozygous missense mutations. *IMPAD1* had been originally believed to code for an inositol phosphatase (hence the name) but was subsequently shown by Frederick et al (PNAS 105:11605-11612, 2008) to code for gPAPP, a Golgi-resident nucleotide phosphatase that hydrolyzes phosphoadenosine-phosphate (PAP), the byproduct of sulfotransferase reactions, to AMP. The mutations in our patients affected residues in or adjacent to the phosphatase active site and are predicted to impair enzyme activity. We then found homozygosity for a premature termination codon in *IMPAD1* in a fourth, unrelated newborn with the same phenotype. *Impad1* inactivation in mice has previously been shown to produce chondrodysplasia with abnormal joint formation and impaired proteoglycan sulfation (Frederick et al, 2008). The human chondrodysplasia associated with gPAPP deficiency joins a growing number of skeleto-articular conditions associated with defective synthesis of sulfated proteoglycans, highlighting the importance of proteoglycans in the development of skeletal elements and joints.

1079F

Exome sequencing a single proband reveals that mutations in the *MTHFD1* gene are responsible for a novel inborn error of folate metabolism. *D. Watkins[1], J. Ganesh[2], J.A. Schwartzentruber[3], L. Dempsey Nunez[1], J. Orange[4], J. Majewski[1,3], D.S. Rosenblatt[1].* 1) Dept Human Genetics, McGill University, Montreal, QC, Canada; 2) Section of Metabolic Disease, The Children's Hospital of Philadelphia, Philadelphia, PA, USA; 3) McGill University and Genome Quebec Innovation Centre, Montreal, QC, Canada; 4) Division of Immunology, Children's Hospital of Philadelphia, Philadelphia, PA ,USA.

A 3-month-old infant presented with failure to thrive, megaloblastic anemia, severe neutropenia and thrombocytopenia, and increased plasma levels of homocysteine and methylmalonic acid. Studies in cultured fibroblasts showed a selective decreased ability to synthesize methylcobalamin from exogenous cyanocobalamin. Exome capture was performed on patient DNA using the Agilent SureSelect 50 Mb exome capture kit and sequencing with the Illumina Hiseq sequencer. Two sequence changes in the *MTHFD1* gene, which encodes a trifunctional folate metabolic enzyme containing methylenetetrahydrofolate (methyleneTHF) dehydrogenase, methenylTHF cyclohydrolase and formylTHF synthetase activities, were identified: c.727+1G>A, affecting the splice acceptor site of intron 8; and c.517C>T (p.Arg173Cys), which affects a conserved residue in the NADP-binding site of the methyleneTHF dehydrogenase domain of the trifunctional enzyme. Previous site-directed mutagenesis experiments have demonstrated that mutations affecting arginine 173 have a deleterious effect on enzyme function. Analysis of DNA from family members showed that the father was heterozygous for the c.727+1G>A mutation while the mother was heterozygous for c.517C>T; an unaffected sibling carried neither mutation. MTHFD1 catalyzes the reaction of formate, generated in the mitochondria, with THF to form formylTHF (required for purine biosynthesis) and its conversion to methyleneTHF (required for thymidylate synthesis, and the source of methylTHF required for homocysteine remethylation). This patient represents the first identified case of MTHFD1 deficiency. Our study demonstrates the capacity of exome capture and sequencing to identify novel causative genes even in cases where only a single patient is available for study.

1080F

Compound heterozygous mutations in the ERCC6 gene cause a severe form of Cockayne syndrome in an Amish isolate. *B. Xin, H. Wang.* DDC Clinic for Special Needs Children, Middlefield, OH.

Cockayne syndrome (CS) is a rare autosomal recessive multisystem disorder characterized by growth failure, mental retardation, progressive neurological dysfunction, premature aging, and photosensitivity. The clinical spectrum of CS encompasses a wide range of severity from severe prenatal forms to mild and late-onset presentations. The underlying cause of the disease is a defect in transcription-coupled DNA repair, specifically the nucleotide excision repair pathway. To date, most CS patients reported have been associated primarily with mutations in two genes, ERCC6 and ERCC8. Here, we report two patients with a severe form of CS from an Amish pedigree and the identification of causative mutations in ERCC6 gene. The patients presented with severe phenotype that includes failure to thrive, severe developmental delay, characteristic facial features, and congenital cataracts. DNA analysis revealed compound heterozygosity for 2 novel mutations in the ERCC6 gene in both patients: a 28-bp deletion in exon 5 (c.1293_1320del) and a splice-donor site mutation in intron 14 (c.2709+1G>T). The c.1293_1320del mutation leads to a frameshift and creation of a premature termination (p.Glu432LysfsX24) in translation; the splice site mutation c.2709+1G>T results in aberrantly spliced ERCC6 mRNA which leads to frameshift and production of truncated protein. Further targeted mutation analysis in the family showed that the 28-bp deletion was inherited by the paternal parent while the splice site mutation was maternally inherited. None of the unaffected siblings carried both mutations. Our findings indicate that loss of function of ERCC6 is responsible for this severe form of Cockayne syndrome and the possibility of compound heterozygosity in Amish which is extremely uncommon for a rare condition at an isolated founder population.

1081F

Molecular characterization of the disease genes in patients with Cornelia de Lange syndrome. *J.Y. Yang[1], J.Y. Wang[1], K.Y. Koo[1], C.H. Lee[1], S.J. Kim[2], J.E. Lee[2], J.S. Lee[1].* 1) Department of Clinical Genetics, Yonsei University, College of Medicine, Seoul 120-752, Korea; 2) DNA link, Seoul, Korea.

Cornelia de Lange syndrome (CdLS; OMIM #122470) is an autosomal dominant disorder that is classically characterized by typical facial features, severe growth and mental retardation, limb defects, hirsutism and other visceral system involvement. Currently, Heterozygous mutations in the cohesin regulator, NIPBL, or the cohesin structural components SMC1A (formerly SMC1L1) and SMC3 have been identified as causes of CdLS. The mutations in NIPBL and SMC1A genes correspond to 20% to 50 % or 5% of CdLS patients, respectively. This finding suggests genetic heterogeneity and other disease causing genes in CdLS. In this study, mutations in NIPBL and SMC1A genes were screened in 11 Korean patients with CdLS by PCR direct sequencing. Three patients showed mutations in NIPBL gene (27%) and one patient in SMC1A gene (9%), and overall detection rate of mutation was 36%. Array-based comparative genomic hybridization (array-CGH) was performed in seven patients who did not show mutation in NIPBL or SMC1A gene for whole genomic copy number variation screening with the resolution of 4X180K. Two patients showed DNA copy number alterations including a deletion on chromosome 5q12.1 and duplication on chromosome 16p11.2. This study suggests that genetic backgrounds of Korean patients with CdLS are heterogeneous, too. Moreover, whole exome sequencing among this group is going on in order to identify disease gene(s) for CdLS.

1082F

Exome sequencing identifies a *PLCG2* mutation in a dominantly inherited systemic inflammatory disease. *Q. Zhou[1], G. Lee[1], J. Brady[1], A. Sheikh[1], J. Khan[2], D. Kastner[1], I. Aksentijevich[1].* 1) National Human Genome Research Institute, Bethesda, MD, USA; 2) National Cancer Institute, Gaithersburg, MD, USA.

We have identified a family presenting with a chronic inflammatory disease characterized by recurrent blistering skin lesions, arthralgia, inflammatory eye and bowel disease. The affected father and daughter also have a history of recurrent sinopulmonary infections, and a mild immunodeficiency with a paucity of circulating antibodies (IgG or IgA). We performed exome sequencing using Agilent SureSelect Human All Exon 50 Mb Kit on three samples including the affected father and daughter, and the unaffected mother. A total of 15 Gb of mapped sequence was generated from paired-end 76 bp reads on the SOLiD™ System platform. The average coverage of each base in the targeted regions was 80 fold, and 80%; of these bases were covered sufficiently deeply for variant calling (>10x coverage). About 50,000 variants and 5,700 indels were called per sample with a quality score q>20. Exonic variants (non-synonymous and splice-site variants and coding indels) were filtered to exclude common variants identified in the dbSNP130 or 1000 Genomes databases along with benign variants as predicted based on the evolutionary conservation (GERP score). A total of 33 transcripts were identified as possible candidate genes. We focused our attention to six genes known to have a role in inflammation including the *PLCG2* gene that encodes phospholipase C. 2 (PLC. 2), an enzyme responsible for ligand-mediated signaling in B, NK, and mast cells and with a critical regulatory role in various inflammatory pathways. Recent work from our lab identified *PLCG2* exon skipping mutations in patients presenting with a distinct inflammatory disease manifesting with cold urticaria and immune dysregulation. The candidate missense variant, S707Y, is a de novo mutation in the father and was passed only to his affected daughter but not to an unaffected son. Furthermore, the S707Y mutation was not detected in 376 Ashkenazi Jewish healthy controls, 368 North American rheumatoid arthritis controls and 450 exome sequencing controls. The S707Y mutation affects a highly conserved residue in an autoinhibitory SH2 domain and is predicted to be probably damaging, however the effect of this mutation on the PLC. 2 activity is unknown. Preliminary study evaluating the effect of the S707Y variant in an overexpression system showed an increase in IP3 production relative to wild type protein suggesting a gain-of function mutation in the PLC. 2-mediated signal pathway.

1083F

Epistatic interaction of mutations in DOCK8 and CLEC7A as a cause of intractable diarrhea, eczema and immunodeficiency in four brothers. *D.L. Dinwiddie[1], S.F. Kingsmore[1], S. Caracciolo[2], F. Colombo[2], A. Prandini[2], G. Tabellini[4], M. Giacomelli[2], M.E. Cantarini[3], A. Pession[3], C.J. Bell[5], N.A. Miller[1], S.L. Hateley[5], C.J. Saunders[1], L. Zhang[6], G.P. Schroth[6], S. Parolini[4], R. Badolato[2].* 1) Children's Mercy Hospital, Center for Pediatric Genomic Medicine, Kansas City, MO 64108; 2) Clinica Pediatrica dell'Università di Brescia, Brescia 25123, Italy; 3) Divisione di Onco-ematologia Pediatrica e Terapia Cellulare, Università di Bologna, Bologna 40138, Italy; 4) Dipartimento di Scienze Biomediche e Biotecnologie,Università di Brescia, Brescia 25123, Italy; 5) National Center for Genome Resources, Santa Fe, New Mexico 87505; 6) Illumina Inc., Hayward, California 94545.

We report four brothers from a consanguineous Italian family with an immunodeficiency syndrome characterized by severe eczema, milk and egg allergies, recurrent infections, intractable diarrhea, failure to thrive and, in two of the four, lymphoma. One died at age 12 months of cardiovascular failure due to intractable diarrhea and another of T cell lymphoma. The three siblings surviving infancy were studied. All had severe T cell lymphopenia (from 160 to 1140 cells/μl), but normal levels of B lymphocytes and NK cells. An immunodeficiency syndrome, IPEX (Immunedysregulation, Polyendocrinopathy and Enteropathy, X-linked; OMIM #304790) was suspected but ruled out with negative FOXP3 mutation analysis and normal protein expression in CD25+ and CD127+ lymphocytes. 37.7 million nucleotides of all the exons in the genome were enriched 44-fold from genomic DNA of the proband. Next generation sequencing of the enriched exons yielded 171 million sequences, each 130 nucleotides long. ~95% of sequences aligned to the reference human genome uniquely, covering each exonic nucleotide ~135 times. A bioinformatic decision tree was used to identify and genotype nucleotide variants in the aligned sequences. Two homozygous variants were detected in the patient: The first was a nonsense mutation in the C-type lectin receptor gene (CLEC7A, p.Tyr238X, rs16910526), a pattern recognition receptor that signals via CARD9. This mutation has been reported to cause familial chronic mucocutaneous candidiasis (CANDF4, OMIM#613108). The second variant was a novel homozygous frameshift in exon 27 of the DOCK8 gene, c.3193delA. DOCK8 mutations cause hyper-IgE recurrent infection syndrome (OMIM# 243700), characterized by T lymphopenia, eosinophilia, elevated IgE, eczema, recurrent infections and severe food allergies. Sanger sequencing confirmed homozygosity for the DOCK8 and CLEC7A mutations in the three siblings and heterozygosity in their parents. The DOCK8 mutation is predicted to result in a premature stop codon 17 residues downstream of the deletion (Ser1065AlafsX17). The phenotypic severity and enteropathy despite a hypomorphic DOCK8 mutation appeared to reflect bridging defects (one null, the other hypomorphic) in two genes acting in parallel pathways. Previously reported cases of DOCK8 mutations were often characterized by viral cutaneous infections and not associated with enteropathy. Interestingly, this unusual phenotype resembles IPEX syndrome, despite normal FOXP3 sequence.

1084F

THE SPECTRUM OF MEFV MUTATIONS IN AN ARABIC COHORT. *A.A. Kohilan[1], R.Z. Taha[1], D.F. Ahram[1], S. Ayesh[2], J. Alami[1], H.I. El-Shanti[1].* 1) Shafallah Medical Genetics Center, Doha, Qatar; 2) Makassed Hostpital, Jerusalem.

Autoinflammatory diseases are a group of disorders characterized by seemingly unprovoked inflammation in the absence of high-titer autoantibodies or antigen-specific T cells. Familial Mediterranean fever (FMF) is an autosomal recessive disorder and is the archetypal autoinflammatory disease. It is characterized by recurrent self-limiting episodes of fever and painful polyserositis. FMF is prevalent in specific ethnic groups—namely, non-Ashkenazi Jews, Armenians, Turks, and Arabs. The gene responsible for FMF—MEFV—has been identified in 1997. There seems to be a distinctive clinical picture in Arab patients with FMF, and the range and distribution of MEFV mutations is different from that noted in other commonly affected ethnic groups. The aim of this study is to delineate the spectrum and distribution of MEFV mutations amongst an Arabic FMF patient cohort and to assist the genotype-phenotype correlation in these patients. We have collected DNA samples from 188 FMF patients (from Qatar, Jordan and Palestine) who have been clinically diagnosed with FMF, according to international and validated diagnostic criteria. We have designed primers to cover the entire genomic region of MEFV. As a first tier, mutation detection is done by resequencing the entire coding sequence and splice sites; as a second tier the rest of the genomic region including the promoter are resequenced. In the first tier, we have identified 191 out of 376 mutant alleles (50%) by resequencing the entire coding region and splice sites of MEFV. In addition, resequencing of the entire genomic region of 100 patients who had only one identifiable allele was carried resulting in the identification of specific haplotypes and we are currently investigating the phenotypic significance of these haplotypes. The spectrum of MEFV mutations in Arabs seems different from other ethnic groups commonly affected by FMF. The fraction of the identifiable disease causing alleles is the lowest amongst the commonly affected ethnic groups. The results of the genomic resequencing of MEFV may provide some insight into the role of non-coding sequences and may explain the molecular pathology of FMF. Thereby, we are currently working on the development of a low cost and high throughput technique to facilitate the resequencing of the entire genomic sequence of MEFV using the Next Generation sequencing technology.

1085F

Identifying causal mutations for Primary Immunodeficiencies (PIDs) in Southeast Asian region using whole exome sequencing. *J. Yang, W. Yang, PPW. Lee, S. Zeng, L. Zhang, PC. Sham, YL. Lau.* The University of Hong Kong, Pokfulam, Hong Kong.

PIDs are rare inborn errors of the immune system and vast majority of PIDs are monogenic diseases. Study of potential genetic defects of PID offers enormous opportunities to the understanding of host-pathogen interactions at the molecular and functional level. Like many other rare Mendelian disorders, disease genes of PIDs were mostly identified by linkage studies or candidate gene screening. Next generation sequencing, especially whole exome sequencing, provides an opportunity for identifying the causal mutations when linkage or candidate gene approach fail to identify the genetic cause. “Asian Primary Immunodeficiency Network (APID)” is a collaborative network with an on-line PID patient registry that contains a wealth of clinical and genetic data for researchers and clinicians. With this collection, we have reported the largest Chinese cohorts of common PIDs including chronic granulomatous disease, Wiskott-Aldrich syndrome and X-linked agammaglobulinemia, as well as rare genetic mutations which have not been described in the Chinese population. However, among the 259 patients tested, only 164 patients were confirmed to have molecular defects based on a candidate gene approach. We have selected about a dozen patients whose clinical and immunological phenotypes are definitive but no mutation in the currently known PID genes has been found through candidate gene screening for whole exome sequencing. These include agammaglobulinemia with absence of B cells and with BTK mutations excluded; severe combined immunodeficiency disorders; mendelian susceptibility to mycobacterial diseases; and autoimmune lymphoproliferative disorder. On average, we have around 70X coverage of the targeted exome, with about 94% of the regions covered 5X or above. Since for each patient, thousands of novel variants are identified, and pinpointing the causal mutations is a daunting challenge. The following approaches are adopted to help with the data analysis. Since recessive mutations and X-linked mutations in male patients are a strong possibility for some patients, homozygous mutations or compound heterozygous mutations, and mutations on the X-chromosome are preferentially considered. Several patients are from consanguineous marriage families, so homozygous mutations in long homozygous stretches in the genome (likely autozygous regions) are considered for causal mutation identification. Mutations with support from other affected members are also preferentially considered.

1086F

A rare case of trans-heterozygous autosomal dominant polycystic kidney disease with bilineal inheritance of PKD1 and PKD2 mutations. *M. Roifman[1], D. Chitayat[2], R. Teitelbaum[2], J.E. Murphy[3], K. Wang[4], N. He[4], C.F. Morel[3], Y. Pei[4].* 1) Division of Clinical and Metabolic Genetics, Department of Paediatrics, Hospital for Sick Children, University of Toronto, Toronto, ON, Canada; 2) The Prenatal Diagnosis and Medical Genetics Program, Department of Obstetrics and Gynaecology, Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada; 3) Fred A. Litwin Family Centre in Genetic Medicine, University Health Network and Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada; 4) Divisions of Nephrology and Genomic Medicine, University Health Network and University of Toronto, Toronto, ON, Canada.

Autosomal dominant polycystic kidney disease (ADPKD) is a phenotypically variable disorder, characterized by progressive renal and extra-renal cyst formation, and typically leads to end stage renal disease (ESRD) by late middle age. The vast majority of cases are caused by mutations in either the PKD1 or PKD2 gene, with the former conferring more severe disease and earlier age of onset of ESRD by approximately 20 years. Trans-heterozygous mutations involving both genes have been shown in transgenic mouse models to be viable, but cause more severe disease than does either mutation alone. Bilineal disease and trans-heterozygosity in two human siblings has been previously documented based on demonstration of a protein-truncating PKD2 mutation and a putative disease PKD1 haplotype associated with a high lod score (Am J Hum Genet 68: 355-363, 2001). Human trans-heterozygotes with a PKD1 and PKD2 mutation confirmed by direct gene sequencing have not been previously described. We report a case of ADPKD in a patient with trans-heterozygosity for a protein-truncating PKD2 mutation (c.2160InsA; p.Asn720FS4X) inherited from the paternal lineage and a missense PKD1 mutation (c.8510C>T; p.2767R>C) of unknown significance that affects a highly conserved amino acid inherited from the maternal lineage. The PKD1 mutation R2767C is predicted to be probably damaging by Polyphen (PSIC score difference of 2.65) and is associated with very mild renal disease in the proband's affected mother and grandmother, suggesting that it may function as a hypomorphic allele. MRI-based total kidney volume measurement is currently being performed to further delineate the genotype-phenotype correlation of all the affected members of this family. Our findings of bilineal ADPKD with a trans-heterozygous individual affected by mutations in both PKD1 and PKD2 have important implications for genetic counseling of the at-risk subjects (i.e. the risk of disease in the proband's siblings and her children is ¾ at birth). Moreover, our documentation of mild renal disease associated with a putative hypomrophic allele suggests that not all PKD1 needs to be associated with severe renal disease.

1087F

Autosomal dominant adult Neuronal Ceroid Lipofuscinosis (Kufs disease) maps to chromosome 20q13.33. *M. Cadieux-Dion[1], P. Lachance-Touchette[1], C. Meloche[1], S. Leonberg[2], F. Andermann[3], E. Andermann[3], S.F. Berkovic[4], G.A. Rouleau[1], P. Cossette[1].* 1) CHUM Research Centre, Notre-Dame Hospital, Excellence Centre For Neurosciences of the University of Montreal, Montreal, Quebec Canada; 2) Clinical Neurophysiology, Medford, New Jersey, USA; 3) Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada; 4) Epilepsy Research Centre, Austin Health, Victoria, Australia.

The neuronal ceroid lipofuscinosis (NCL) are a group of devastating neurodegenerative disorders with infantile (INCL, Santavuori-Haltia), late-infantile (LINCL, Jansky-Bielschowsky), juvenile (JNCL, Batten disease, Spielmeyer-Vogt) and adult (Kufs disease) onset. The clinical manifestations of NCLs include recurrent seizures, myoclonus, intellectual deterioration, vision impairments and early death. Pathology of NCLs is characterized by abnormal lysosomal accumulation of autofluorescent lipopigment in the neurons, and sometimes in the eye. Mutations in 8 genes have been so far associated with the various forms of NCLs in Humans. It has been recently shown that mutations in CLN6 gene, causing late-infantile onset NCL, are also associated with recessive forms of adult-onset NCL (Kufs disease). Although Kufs disease with autosomal inheritance has been described earlier (CLN4), the causative gene remains to be identified.

Microsatellite genome scan allowed us to localize the CLN4 locus on chromosome 20q13.33 with a maximal LOD score of 5.3 at SNP rs11204451 in a large North American family. This family contains 18 affected individuals over 4 generations and shows an autosomal dominant mode of inheritance. Analysis of haplotypes revealed key recombinants and allowed us to determine a 4 MB candidate region containing at least 80 genes. Based on a priorisation list, multiple candidate genes have been sequenced but, to date, no causative mutations have been found.

We now aim to identify the CLN4 gene by using Next Generation Sequencing. We captured the exome of 3 affected individuals from this CLN4 family with *Agilent SureSelect All Exon 50 MB kit* and underwent whole-exome sequencing using *Illumina Hiseq* platform. These later analysis are underway.

1088F

In situ detection of Globotriaosylceramide by MALDI MS-imaging in mouse model of Fabry disease. *R. Dobrovolny[1, 2], M. Volny[3], H. Faltyskova[3], P. Novak[3], V. Havlicek[3], J. Ledvinova[2], B. Asfaw[2], H. Hulkova[2], M. Elleder[2], L. Kuchar[2], R.J. Desnick[1].* 1) Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) First Faculty of Medicine of Charles University, Institute of Inherited Metabolic Disorders, Prague, Czech Republic; 3) Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic.

Fabry disease is an X-linked inborn error of glycosphingolipid metabolism caused by the deficient activity of lysosomal (-galactosidase A ((-Gal A) leading to the progressive accumulation of glycosphingolipids with terminal (-galactosyl residues. In patients with the classical phenotype, who have little or no enzymatic activity, the disease presents in the childhood and progresses to renal failure, cardiac involvement, cerebrovascular disease and early demise. A murine (-Gal A knock-out model has been generated which has no enzymatic activity and accumulates major glycosphingolipid, globotriaosyceramide (GL-3). To investigate the nature of GL-3 accumulation in the mouse model, MALDI MS-imaging (MSI) was used to investigate the spatial distribution of GL-3 in kidney tissue sections from wild-type and Fabry mice. In MALDI MSI, compounds are ionized directly from the tissue slice, which is attached on a conductive surface moving in set raster steps under a laser beam with resolution up to 50 μm. Sampling of the ions directly from the tissue into the mass spectrometer provides very rich mass spectra with respect to the number of ions, enabling simultaneous detection of different analytes during a single measurement. In the mice, MSI identified different GL-3 isotypes accumulated preferentially in the outer cortex of the kidney. The results of the MSI correlated well with the immunochemical detection with GL-3 specific antibodies in adjacent kidney sections. The murine results also were compared with available results from human normal and Fabry specimens. The results confirm the feasibility of the MSI method for the study of various lipidoses and glycolipidoses helping to elucidate the mechanisms underlying the accumulation of the substrates, even in cases where good quality antibodies are not available and when subcellular resolution is not needed.

1089F

Mucopolysaccharidosis type II: thirteen novel mutations in the IDS gene of the Czech and Slovak patients. *L. Dvorakova[1], G. Storkanova[1], M. Hrebicek[1], L. Stolnaya[1], H. Vlaskova[1], H. Poupetova[1], E. Hruba[1], M. Magner[2], J. Zeman[2].* 1) Inst Inherit Metabol Disorders, Charles Univ, First F Med, Prague 2, Czech Republic; 2) Dept of Paediatrics and Adolescent Medicine, Charles Univ, First F Med, Prague 2, Czech Republic.

Mucopolysaccharidosis type II (MPS II, Hunter syndrome) is a rare X-linked recessive lysosomal storage disorder caused by the deficiency of the enzyme iduronate sulfatase (IDS). The clinical diagnosis of MPS II was confirmed biochemically both by the demonstration of an increased excretion of urinary dermatan and heparan sulfates and by the deficiency of iduronate sulfatase activity in 30 patients from 25 Czech and Slovak families. In 20 probands the material for DNA analysis was available and a causative mutation was identified in all cases. In two patients the IDS-IDS2 recombination leading to altered transcript lacking exons 8 and 9 (Rec B) was found. The other mutations were family specific and included only five missense mutations, while the rest were clearly pathogenic mutations (3 nonsense mutations, 4 small and 2 gross deletions, 3 small insertions, and 1 splicing error). Thirteen pathogenic changes including two missense mutations located at amino acid positions 333 and 335, respectively, were not described previously. The genotype/phenotype correlation was not straightforward as an insertion leading to frameshift caused an attenuated as well as severe phenotype in two patients from the same family. Three patients with genotypes c.[1181-1G>A], c.[1038delC] and [RecB], respectively, receive the ERT. Samples from twelve mothers of probands were available, one de novo mutation was observed. A total of fourteen heterozygotes were identified in the families, all of them were asymptomatic. Peripheral blood cells were inspected for X-inactivation by HUMARA method in 11 heterozygotes. X-inactivation was skewed in one clinically asymptomatic heterozygote (80/20), in who the non-mutated allele was preferentially inactivated. X-inactivation of another clinically asymptomatic carrier with low IDS activity (11% of controls) was random. Thus, X-inactivation status did not correlate neither with the clinical nor the biochemical phenotypes in our cohort of carriers. Grant support: VZ MSMCR0021620806, MZ0VFN2005.

1090F

Fabry disease: identification and characterization of 12 novel alpha-galactosidase A mutations causing variable phenotypes. *D.P. Germain[1, 2], C. Boucly[3], E. Caudron[4], F. Jabbour[3], X. Jeunemaitre[5], N. Miri[3], M. Piraud[6], P. Prognon[4], G. Reboul[2], K. Benistan[2].* 1) Division of Medical Genetics, University of Versailles, Garches, France; 2) Expert Centre for Fabry disease, CHU Raymond Poincare, Garches, France; 3) Laboratory of Biochemistry, CHU Raymond Poincare, Garches, France; 4) Pharmacy, HEGP, Paris, France; 5) Department of Genetics, HEGP, Paris, France; 6) Laboratory of Biochemistry, HCL, Lyon, France.

Background: Fabry disease (FD, OMIM 301500) is an X-linked lysosomal storage disease resulting from mutations in the alpha-galactosidase A (GLA) gene. The disease is phenotypically highly heterogeneous with classic and variant phenotypes. Identification of the GLA mutation in families enables confirmation of carrier status in females, accurate genetic counselling, precise prenatal genetic testing, and study of genotype/phenotype correlations (1). **Patients and Methods**: The nature of the molecular lesions in the GLA gene was determined in 22 unrelated families with FD from France (n=18), Algeria (n=1), Bulgaria (n=1), Greece (n=1) and Morocco (n=1). Genomic DNA was isolated from leukocytes or dried blood spot (DBS) obtained from hemizgous males or heterozygous females and all coding regions and flanking sequences were PCR-amplified and analyzed by direct automated sequencing. Extensive clinical work-up was done prior to initiation of enzyme replacement therapy (ERT) or enzyme enhancement therapy (EET) together with measurement of alpha-gal A residual activity and determination of baseline urinary Gb3. **Results**: Twelve novel mutations were identified : p.M42R (c.125T>G), p.G43S (c.127G>A), p.G132E (c.395G>A), p.K168N (c.504A>C), p.I198T, (c.593T>C), p.Q212X (c.634C>T), IVS4 C(-3)>G, p.F295C (c.884T>G), p.L300P (c.899T>C), p.G328E (c.983G>A), p.G411D (c.1232G>C), and c.1086-1098 del13bp. Sequences obtained from DNA eluted from DBS in the PCR mix were of good quality. All novel mutations were private to one given family. For some mutations, enzyme activity was enhanceable in response to incubation of mutant cells with active site specific chaperone (1-deoxygalactonojirymicin). Clinical severity scoring, using the DS3 instrument (2), revealed variable phenotypes within the study population. **Discussion**: Our results further expand the spectrum of GLA mutations causing FD, allow precise heterozygote detection in families, help defining genotype/phenotype correlations and facilitate identification of candidate patients for clinical trials of enzyme enhancement therapy. **References**: 1) Germain DP. Fabry disease. Orph J Rare Dis 2010 ; 5 : 30. 2) Gianini et al. A validated disease severity scoring system for Fabry disease. Mol Genet Metab 2010; 99: 283-290.

1091F

Identification of 83 novel alpha-mannosidosis-associated sequence variants: Functional analysis of *MAN2B1* missense mutations. *H.M.F. Riise Stensland[1], H.B. Klenow[1], L.V. Nguyen[1], G.M. Hansen[2], D. Malm[3], O. Nilssen[1,4].* 1) 1Department of Medical Genetics, Division of Child and Adolescent Health, University Hospital of North-Norway, N-9038 Tromsø, Norway; 2) Department of Medical Biochemistry, Faculty of Medicine, University of Tromsø, N-9037 Tromsø, Norway; 3) Department of Internal Medicine, Division of Medicine, University Hospital of North-Norway, N-9038 Tromsø, Norway; 4) Department of Clinical Medicine-Medical Genetics, University of Tromsø, N-9037 Tromsø, Norway.

The lysosomal storage disorder alpha-mannosidosis is caused by deficiency of the enzyme lysosomal alpha-mannosidase (MAN2B1), a lysosomal enzyme involved in the ordered degradation of N-linked oligosaccharides. In this study, 130 unrelated alpha-mannosidosis patients from 30 countries, mainly of European origin, were subjected to mutation analysis. A total of 96 putative disease-causing mutations were identified, of these 13 had been reported previously. Hence, this study provides a new delineation of the mutational spectrum in alpha-mannosidosis, increasing the number of known disease causing mutations from 42 to 125. The 83 novel variants included 29 missense mutations, 24 smaller frameshifting mutations (duplications/insertions/deletions), 16 nonsense mutations, 10 splice site mutations, three larger deletions and one small in-frame insertion. In total, 256 of the 260 mutant alleles (98.5 %) were identified. Most of the mutations (81%) were unique to each family, further demonstrating the allelic heterogeneity of alpha-mannosidosis. However, one missense mutation, c.2248C>T (p.Arg750Trp), was detected in 50 patients from 16 countries, and accounted for 27.3 % of the disease alleles. Haplotype analysis of all reported patients with c.2248T-alleles using 3 intragenic *MAN2B1* cSNPs revealed that the c.2248T variant was present on four *MAN2B1* haplotype backgrounds, of which one major haplotype accounted for 95 % of the alleles. The distribution of the c.2248T associated haplotypes differed remarkably from those of the control populations, suggesting that the c.2248C>T has occurred on a few ancestral haplotype backgrounds where the major haplotype subsequently has spread by founder effects. In order to investigate their effect on the MAN2B1 protein, the putative disease-associated missense mutations and other selected variants were introduced into the human *MAN2B1* cDNA, expressed in cell-culture and assayed for MAN2B1 activity. The majority of the variants were inactive, however, ten of the putative disease-associated missense mutants showed MAN2B1 activity above background, and more detailed studies are necessary to further evaluate the pathogenicity of these variants.

1092F

Exome sequencing of trios identifies *de novo* mutations in novel candidate genes for permanent neonatal diabetes. *H. Lango Allen, R.C. Caswell, S.E. Flanagan, A.T. Hattersley, S. Ellard.* Molecular Genetics, Institute if Biomedical and Clinical Science, Peninsula College of Medicine and Dentistry, University of Exeter, Exeter, United Kingdom.

INTRODUCTION: Permanent neonatal diabetes mellitus (PNDM), defined as diabetes diagnosed before the age of 6 months, is most commonly caused by mutations in the K_{ATP} channel genes *KCNJ11* and *ABCC8* (40%), and the insulin gene *INS* (12%), with about 40% of PNDM patients still awaiting genetic diagnosis. We sequenced the exomes of eight PNDM patients negative for *KCNJ11*, *ABCC8* and *INS* mutations, and their unaffected, unrelated parents, to search for novel causes of isolated PNDM. By sequencing trios we expected to identify *de novo* pathogenic mutations, although we also looked for homozygous and compound heterozygous mutations. **METHODS AND RESULTS:** We performed 76bp paired-end single-lane sequencing of 8 trios on the Illumina GAII platform, using SureSelect Human All Exon Kit to capture 1.22% of human genomic regions corresponding to the CCDS exons. We aligned an average of 60 million reads to the whole human genome using BWA tool, and obtained 4-5 billion aligned bases per sample, of which ~70% were on-target. The mean target coverage was 55-70X; 83-90% of targeted bases were covered by at least 10 reads, 73-83% by at least 20 reads, and 62-70% by at least 30 reads. We used GATK pipeline, with local realignment around indels, recommended quality filters, and minimum coverage of 10 reads, to call 20-25,000 variants per individual, of which between 600 and 1000 were not present in dbSNP131. Using in-house MySQL queries we discovered up to 20 putative novel *de novo* missense mutations per patient. After manual inspection of parents' sequence alignment files for presence of poorly captured variants, and Sanger sequencing of those not detected in parents, each patient had zero or 1 (total 5) missense substitutions, and one had an 8bp deletion within a protein-coding region. Follow-up studies of these candidate genes are in progress within a larger PNDM cohort. We also identified several novel homozygous variants, though most of these are now present, at <1% frequency, in the latest 1000Genomes calls. Interestingly, in two patients, candidate gene sequencing identified causal mutations that were missed by exome sequencing - one in an exon with no or very little coverage, the other in an untargeted regulatory region. **CONCLUSIONS:** Exome sequencing of trios is a successful approach for identifying novel candidate genes for a monogenic disease, by looking for *de novo* mutations of major impact in patients with other common causes of the disease excluded.

1093F

Frequency of SPG48 in autosomal recessive and sporadic spastic paraplegia patients. *N.A. Schlipf[1], P. Bauer[1], S. Klimpe[3], K.N. Karle[2], M. Synofzik[2], J. Schicks[2], O. Riess[1], L. Schöls[2], R. Schüle[2].* 1) Department of Medical Genetics, Institute of Human Genetics, Tübingen, Baden-Württemberg, Germany; 2) Clinical Neurogenetics, Department of Neurology and Hertie-Institute for Clinical Brain Research and German Center of Neurodegenerative Diseases, University of Tübingen, Tübingen, Germany; 3) Department of Neurology, University of Mainz, Germany.

Hereditary spastic paraplegias (HSPs) are a rare and highly heterogeneous group of neurodegenerative disorders. The main neuropathological feature is the progressive degeneration of the corticospinal tracts, which leads clinically to a progressive lower limb spasticity and pyramidal weakness. The most frequent causes of autosomal recessive HSP (ARHSP) are mutations in the SPG11-gene. However, ARHSP families as well as sporadic cases negative for such mutations present a significant diagnostic challenge. Mutations in a gene implicated in DNA repair (*KIAA0415*) have been recently reported to be associated with spastic paraplegia type SPG48. Our objective was to determine the relative frequency of *SPG48/KIAA0415* mutations in a large cohort of 133 HSP patients, including sporadic and familial cases with evidence of recessive inheritance. We applied a targeted next-generation amplicon sequencing (NGS) approach to analyze all coding exons of the *SPG48/KIAA0415*-gene. The *SPG48* target regions were covered with an overall average coverage of 112-fold by this comprehensive molecular screening. Sequencing fragments which failed completely (5%, 133 out of 2261) or covered insufficiently (<10-fold) (12%, 279 out of 2261 fragments) were sequenced by Sanger sequencing. All sequence variants detected by NGS were validated by Sanger sequencing, too. Currently, after excluding known polymorphisms or silent mutations, 5 single nucleotide variants with uncertain or unlikely pathogenicity remained. Subject to future investigation will be a multiplex ligation dependent probe amplification assay (MLPA) targeting SPG48 to investigate copy number variations.

1094F

Cluster headache: exome capture of the elusive causal variants. *L. Southgate[1], S. Scollen[2], M.A. Simpson[1], R. McEwen[3], B. Zhang[4], W. He[4], M.E. Weale[1], T. Schlitt[1], L. Xi[4], C.L. Hyde[4], B. Dougherty[5], J.C. Stephens[4], M. Leone[6], C. Sjöstrand[7], M.B. Russell[8], S.L. John[4], R.C. Trembath[1].* 1) Department of Medical and Molecular Genetics, King's College London, Guy's Hospital, London, United Kingdom; 2) Pfizer, Neusentis, PTx Precision Medicine, Granta Park, Cambridge, United Kingdom; 3) Pfizer, Molecular Medicine, Ramsgate Road, Sandwich, Kent, United Kingdom; 4) Pfizer, PTx Precision Medicine, Eastern Point Road, Groton, CT; 5) Pfizer, Molecular Medicine, Eastern Point Road, Groton, CT; 6) Carlo Besta National Neurological Institute, Milan, Italy; 7) Department of Neurology, Karolinska University Hospital Huddinge, Stockholm, Sweden; 8) Department of Neurology, Research Centre, Akershus University Hospital, Oslo, Norway.

Cluster headache (CH) is regarded as the most severe of the primary headache syndromes, characterised by recurrent, relatively short-lived attacks of excruciating pain, often with regular periodicity. It is widely considered to be a neurovascular disorder with activation of hypothalamic, trigeminovascular and cranial parasympathetic systems. Recent estimates suggest a lifetime prevalence of about 0.1% and, whilst CH typically presents as a sporadic disorder, familial clustering supports autosomal dominant inheritance with reduced penetrance. We have previously reported a genome-wide linkage scan in a large cohort of Northern European families, which identified a number of putative linkage loci and provided substantial evidence for genetic heterogeneity. To advance our search for causative genes, we have undertaken exome sequencing in ten autosomal dominant CH pedigrees. Exome capture was performed for two affected subjects from each family using the Agilent SureSelect Target Enrichment System and sequenced on an Illumina Genome Analyzer IIx. Paired-end sequence reads were aligned to the reference genome and variants identified and annotated using an in-house data analysis pipeline. Under the assumption that novel rare variants underlie CH predisposition, variants were filtered against dbSNP, 1000 Genomes and >200 in-house controls to exclude known or common variation. By isolating identical novel heterozygous variants, shared between the two related affected subjects in each family, we identified 196 genes. Our previous linkage data enabled further prioritisation of putative candidates on a family-specific basis and we are currently completing the cosegregation analysis of these variants in the extended pedigrees. The power of exome sequencing to identify mutations in rare Mendelian disorders is now well-established; however, few data exist on the utility of this approach to unravel the genetic architecture in the context of locus heterogeneity or complexities such as incomplete penetrance and phenocopies. Our initial analyses are confounded by such complexities, as well as by the assumption that causative variation will be novel. Given the reduced penetrance and population prevalence of CH, alternative analysis models may be more appropriate. This study points to the need to develop larger data sets together with pathway based analysis, work presently ongoing, in order to further our understanding of the genetic basis of this debilitating disorder.

1095F

Comparison of functional prediction methods for nonsynonymous SNPs in exome sequencing-based study of Mendelian diseases. *P. Wei[1,3], X. Jian[2], E. Boerwinkle[1,2], X. Liu[1,2].* 1) Human Genetics Center, University of Texas School of Public Health, Houston, TX; 2) Division of Epidemiology, Human Genetics and Environmental Sciences, UTSPH, Houston, TX; 3) Division of Biostatistics, UTSPH, Houston, TX.

Next-generation sequencing (NGS) technologies have allowed for sequencing of the whole-exome of a large number of individuals, but it remains a considerable challenge to identify disease-causing variants from thousands of background polymorphisms. Functional prediction algorithms of nonsynonymous SNPs (nsSNPs) have shown to be promising in filtering for pathogenic mutations. While many prediction methods have been proposed, it is unclear which to use in practical applications. We performed a comparative study of nine functional prediction algorithms (LRT, MutationTaster, PANTHER, PhD-SNP, PMut, PolyPhen-2, SIFT, SNAP, and SNPs3D), three conservation scores (GREP, phastCons, and PhyloP), and an ensemble prediction method (weighted average of the normalized scores (WAS)). With a primary focus on exome sequencing-based studies of Mendelian diseases, we evaluated the predictive performance of different methods on two manually curated test sets of non-functional nsSNPs and deleterious ones that cause Mendelian disorders, taking advantage of the Uniprot database and data from the 1000 Genomes Project. MutationTaster's prediction score had the highest discriminative power with an area under the ROC curve (AUC) of 0.86, whereas PolyPhen-2's qualitative prediction had the highest sensitivity, ranging from 83% to 90% across test sets. We suggest, as a practical guideline, using PolyPhen-2 to filter for nsSNPs causing Mendelian diseases followed by MutationTaster to quantitatively prioritize functional nsSNPs. Our study also sheds light on the use of functional predictions in NGS-based association studies of complex traits, and provides novel insights into the value of conservation scores and ensemble-based prediction methods.

1096F

The complex function of FMR2P and its paralogs: insights into the molecular pathology of FRAXE intellectual disability. *B. Bardoni[1], M. Bensaid[1], D. Douguet[1], J. Gecz[2], M. Melko[1].* 1) Dept Human Genetics, CNRS UMR 6097, Valbonne, PACA, France; 2) SA Pathology (Women's and Children's Hospital), 72 King William Road, North Adelaide, SA 5006 Adelaide, AUSTRALIA.

FRAXE intellectual disability (ID) is a mild form of mental retardation. This disease is caused by the expansion of a CCG trinucleotide repeat in the 5' untranslated region of FMR2 (Fragile X Mental Retardation 2), resulting in its silencing. FMR2-null mice display impairment in conditioned fear and enhanced long-term potentiation, suggesting a role for FMR2 in regulating synaptic plasticity and memory formation. FMR2 (also called AFF2) belongs to the AFF (AF4/FMR2) family of genes including other 3 members AFF1, AFF3 and AFF4. While the role in transcription of AFF proteins was described shortly after their identification, more recently we have shown that FMR2P has properties of an RNA-binding protein, modulating alternative splicing via the binding to a G-quadruplex RNA, a structure that is known to behave as an exonic splicing enhancer. In particular, FMR2P modulates the alternative splicing of the exon 14 (expressing the Nuclear Export Signal) of the mRNA encoded by FMR1, the gene silenced in the Fragile X syndrome, another form of ID. When splicing is blocked, FMR2P is localized in the nucleolus, suggesting a role of this protein also in intranuclear transport of mRNA and/or mRNA maturation. We explored some functional properties of the AFF family (functional domains, intracellular localization, RNA-binding ability) triggered by the observation that AFF3 and AFF4, similarly to the AFF2/FMR2 protein, are localized in nuclear speckles and that their overexpression affects the dynamics and/or biogenesis of these structures. We showed that these proteins specifically bind the G-quadruplex RNA and modulate the splicing efficiency of a minigene reporter containing a G-quadruplex RNA in one alternatively spliced exon. We also obtained findings showing that AFF family members are involved in biogenesis/dynamics of nuclear speckles. In conclusion, we provided new evidence concerning the function of members of AFF family and new insight into the molecular pathology of FRAXE ID since our results suggest that a functional redundancy among AFF proteins can explain the mild nature of the cognitive impairment observed in FRAXE patients. In support of this hypothesis, increased expression of the AFF4 gene was identified in fibroblasts from FRAXE patients. By a two-hybrid screening in yeast we identified 3 new interacting proteins of FMR2P. We will present new data showing the ability of the partners of FMR2P to modulate its function.

1097F

Modeling Mental Retardation in Cornelia de Lange Syndrome (CdLS) with *Drosophila melanogaster*. *D. Xu[1], J. Li[2], W. Chen[3], A. Bell[4], N. Ferrick[4], T. Jongens[4], I. Krantz[5].* 1) Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA; 3) Dept of Neuronscience, University of Pennsylvania School of Medicine, Philadelphia, PA; 4) Dept of Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA; 5) Dept of Pediatrics/Human Gen, Children's Hosp Philadelphia, Philadelphia, PA.

Cornelia de Lange Syndrome (CdLS) is a dominant genetically heterogeneous diagnosis characterized by a striking constellation of cognitive impairment, growth delay and birth defects. Approximately 65% of CdLS probands have an identifiable mutation in cohesin structural (*SMC1A*, *SMC3*) or regulatory (*NIPBL* and *HDAC8*) genes. We, and others, have described a non-canonical role of cohesin in regulating gene expression, that is most likely the mechanism by which it manifests its phenotypic effect in CdLS. Homozygous mutation of cohesin genes in *Drosophila* (and other organisms) is embryologically lethal, while heterozygous loss has minimal, if any, reported phenotypic effect. Homozygous mutation of cohesin genes or disruption of cohesin structure has been shown to cause failure of . -neuron pruning, a post-mitotic event, during *Drosophila* brain development. It remains unclear if loss of one copy of these cohesin genes, more closely representing the gene dosage in CdLS probands, also causes any structural or functional brain differences in *Drosophila*. We investigated brain development in heterozygous fly mutants of *SMC1*, *Rad21*, *Rpd3* (*Drosophila HDAC8* homologue) and *Nipped-B*. . -neuron pruning defects have been observed in ~30% of the heterozygous mutant pupae. This indicates that heterozygous cohesin mutations indeed affect normal fly brain development, although the effect is not fully penetrant. Although showing normal locomotor activity, these heterozygous flies do display irregular sleep pattern, especially during dark periods. These mutants present decreased total sleep time consisting of a larger number of short sleep episodes compared with wild type flies. Preliminary courtship assay demonstrates a deficit in learning and memory function in the heterozygous mutant flies. Ongoing genome-wide expression analysis of the brain of these mutant flies will provide molecular insight into the effects on gene regulation of haploinsufficiency for cohesin genes. These experiments will be very important in understanding the alterations of the transcriptome in the *Drosophila* brain and how that translates into the cognitive status resulting from haploinsufficiency. These studies will help to validate if the heterozygous cohesin mutant flies echo the cognitive phenotype seen in CdLS probands and can serve as a valuable model to study the neurocognitive aspects of CdLS.

1098F

Identification of novel mutations in Xanthina dehydragenase gene in an Azorean family affected with hypouricemia. *I. Foroni[1,2], A.R. Couto[1,2], J. Bruges-Armas[1,2].* 1) Serviço Especializado de Epidemiologia e Biologia Molecular, Hospital de Santo Espírito de Angra do Heroísmo, Canada do Barreiro 9700-856 Angra do Heroísmo, Azores, Portugal; 2) Institute for Molecular and Cell Biology, Universidade do Porto, Portugal.

Background. Uric acid is the end-product of purine metabolism. It is converted in xanthine by the activity of the Xanthine oxidase enzyme. A deficiency of the enzyme causes low level of uric acid and the consequent accumulation of xanthine in blood serum. The disease, called Xanthinuria, is a rare autosomal recessive disorder that leads to renal lithiasis and in some cases renal failure. It is caused by mutations in the Xanthina deydrogenase gene located on chromosome 2p22. Methods. We examined an entire family composed of ten persons aged between 32 and 73 years. The proband and three siblings were affected with renal lithiasis and abdominal pain. The serum level of creatine clearance, urate clearance, and uric acid were analyzed. Mutational analysis on XDH gene was performed in order to find single-nucleotide polymorphism which can be associated to the desease. Results. The proband and the siblings showed low uric acid and high urate clearance levels. Within the analyzed coding region of XDH gene, a novel G to C base substitution at position 859 bp in exon 10 was identified in the proband and in the three siblings. The point mutation caused a CTG (Leu) to GTG (Val) nonsense mutation at codon 287. The healthy individuals of the family showed normal biochemical values and no base change. Conclusion. In the present study a novel point mutation was identified at the exon 10 of the XDH gene. This is the first report which associates a nonsynonimous mutation with a possible case of Xanthinuria in an Azorean family.

1099F

Mutation analysis in OTCD patients with emphasis on detection of large deletions. *G. Storkanova[1], H. Vlaskova[1], L. Stolnaya[1], H. Treslova[1], V. Stranecky[1], M. Hrebicek[1], R. Mihalova[2], L. Dvorakova[1].* 1) Inst Inherit Metabol Disorders, Charles Univ, First F Med, Prague 2, Czech Republic; 2) Inst Biol Med Genetics, First F Med, Charles Univ and General Univ Hospital, Prague 2, Czech Republic.

Ornithine transcarbamylase deficiency (OTCD, OMIM 311250) is the most common inherited defect of the urea cycle. Working diagnosis of OTCD is usually based on clinical signs, biochemical markers (elevated ammonia and glutamine in plasma, elevated urinary excretion of orotic acid) and results of the allopurinol loading test in females. As enzymological proof of OTC deficiency requires tissue sample from invasive liver biopsy, the diagnosis is commonly confirmed by mutation analysis. To detect the causative mutations we used sequencing of the X-linked OTC gene and its transcript, MLPA and SNP array technology. We have detected 26 hemizygotes and 44 carriers from 26 families. The found mutations included three large and two small deletions, two splicing defects, three nonsense mutations, 11 missense mutations, and one mutation abolishing the natural stop codon. The largest identified deletion was 444 kb deletion. This deletion included the whole OTC gene, TSPAN7 gene (Xp11.4) and larger part of RPGR gene (Xp21.1). The heterozygosity for the deletion was found in the proband's mother by MLPA. We have also revealed 9937 bp deletion including part of intron 4, whole exon 5, intron 5, exon 6 and part of intron 6 and 1 bp insertion in OTC gene. We have identified 5 heterozygotes in proband's family and the knowledge of this mutation was used for a prenatal diagnosis. Although OTCD is considered X-linked recessive disease, significant number of heterozygous females is clinically affected. In our cohort 9 heterozygotes manifest clinical symptoms. One of them carries a 24536 bp deletion/3 bp insertion. The deletion boundaries were detected by MLPA assay, DNA array analysis and direct sequencing of long range PCR product. The data shown here in accordance with results published by others show that large deletions are common in the OTC gene and sequencing of coding region of the gene alone is often not sufficient. Support: VZ MSMCR0021620806, MZ0VFN2005.

1100F

GENE EXPRESSION PROFILE IN AUTOSOMAL RECESSIVE CUTIS LAXA, TYPE 2. *D.Z. Scherrer, C.V. Maurer-Morelli, J.F. Vasconcellos, C.S. Rocha, A.H.B. Matos, C.E. Steiner.* Medical Genetics, Unicamp , Campinas, São Paulo, Brazil.

Autosomal recessive cutis laxa, type 2 (ARCL2), is a rare disorder of connective tissue in which the skin sags excessively, giving to the individual an aged aspect. It has been suggested that ARCL2 is the same entity described under the denominations of wrinkly skin syndrome and gerodermia osteodysplastica. Such confusion is caused, in part, by overlapping of the histological and clinical findings, thus nosology remains uncertain until the definitive elucidation of the molecular basis of these conditions. So far, there are only three genes implicated in several forms of cutis laxa; ATP6V0A2, SCYL1BP1, and PYCR1, however, they cannot explain the complete phenotype of this patients. The main aims of this study were to determinate the gene expression profile in ARCL2 by using microarray investigation and identify genes and pathways, which are possible altered in such condition in order to shed some light into the molecular mechanisms underlying this type of cutis laxa. Because cutis laxa is a rare condition, we performed gene expression profiling in skin tissue from two patients with ARCL2. This study was performed using the Human Genome U133 Plus 2.0 array (Affymetrix™), and analyzed using Affy and RankProd packages from Bioconductor. Genes were considered differentially expressed when the statistical significance was $p<0.01$. The analyses of overrepresented gene ontology categories were performed with the DAVID software, and the gene interactions and correlation networks were identified with the Ingenuity Pathways Analysis software. Transcriptional profiling by microarray analysis from ARCL2 patients detected 750 differentially expressed genes compared to healthy controls. The top enriched gene ontology categories analyzed by DAVID included the differentiation and development of keratinocytes, epidermis and ectoderms. Among the most activated signaling pathways analyzed by Ingenuity software were cancer, connective tissue development and function, skeletal and muscular systems as well as lipid metabolism, which include genes as GPX3, CCL27, TIMP3, and ADIPOQ. This is the first study using microarray investigation in the ARCL2 disease. Our results provide a complete view of cellular pathways differentially expressed in such condition and look forward to contribute to a better understanding of molecular mechanisms of ARCL2.

1101F

Apoptosome independent caspase 9 activation underlies Microphthalmia with linear skin defects (MLS) syndrome. *A. Indrieri[1], I. Conte[1,2], G. Chesi[1], D. Ghezzi[3], J. Quartararo[4], I. Ferrero[4], R. Tate[2], P. Goffrini[4], M. Zeviani[3], P. Bovolenta[5], B. Franco[1,6].* 1) TIGEM, Telethon Institute of Genetics and Medicine, Naples, Italy; 2) Institute of Genetics and Biophysics (IGB) Adriano Buzzati Traverso CNR, Naples, Italy; 3) Unit of Molecular Neurogenetics, Fondazione Istituto Neurologico Carlo Besta, Milan, Italy; 4) Department of Genetics, Microbiology, Anthropology and Evolution, University of Parma, Parma Italy; 5) Centro de Biología Molecular Severo Ochoa. CSIC-UAM, Universidad Autónoma de Madrid, Madrid. Spain; 6) Medical Genetics Services, Department of Pediatrics, Federico II University, Naples Italy.

Microphthalmia with linear skin defects (MLS) is an X-linked dominant male-lethal neuro-developmental disorder associated to mutations in the holocytochrome c-type synthetase (HCCS) transcript. Female patients display microphthalmia and linear skin defects, additional features include CNS malformation, mental retardation and cardiac defects. HCCS codifies a mitochondrial protein that catalyzes the attachment of heme to apo-Cytochrome (Cytc) c and c1. Defects in the yeast homologous heme lyases result in loss of respiratory growth. Cytc is released from mitochondria in response to a variety of intrinsic death-promoting stimuli leading to caspase-dependent cell death. We generated an animal model to study the molecular mechanisms underlying this rare genetic disease in the medaka fish (Oryzia latipes, ol). Consistent with a respiratory role we find Medaka olhccs mRNA to be highly expressed in the muscles, heart, CNS and in the eye where high mitochondrial activity is required. To analyze the olhccs function during development, three different morpholinos have been designed and injected resulting in a pathological phenotype, which resembles the human condition. As expected, morphants displayed a severe ocular phenotype with microphthalmia and coloboma associated with microcephaly and cardiac defects. Characterization of morphants revealed impairment of mitochondrial functions, overproduction of reactive oxygen species (ROS) and a strong increase of apoptosis in the CNS and in the eyes. This data confirm the role of HCCS in mitochondria and suggest that MLS should be considered a mitochondrial disease. Interestingly, our data showed an activation of mitochondrial dependent cell death triggered by activation of Caspase9 (Casp9) in a Bcl-dependent but apoptosome-independent manner suggesting that at least in some tissues apoptosis can occur in a non-canonical way. Casp9 activation relies on the formation of apoptosomes, composed of Cytc, Apaf1, and Casp9. Our data provide evidences for an Apaf1- and Cytc-independent Casp9 activation in vivo and suggest a possible tissue specificity for this event. Our model provides strong evidences that mitochondrial mediated apoptotic events may underlie microphtalmia providing new insights into the mechanisms of this developmental defect.

1102F

Successful strategy for rapid molecular diagnosis in complex I deficiency with custom target capture associated to next generation sequencing. *A.S. LEBRE[1], M. JAMBOU[1], M. RIO[1], P. DE LONLAY[1], N. BODDAERT[1], V. PROCACCIO[2], J.P. BONNEFONT[1], A. MUNNICH[1], A. RÖTIG[1], Z. ASSOULINE[1].* 1) Université Paris Descartes, AP-HP Hôpital Necker-Enfants Malades et Inserm U781 et U797, Départements de Génétique, Radiologie pédiatrique, Neurologie pédiatrique et des Maladies du développement, Paris F-75015 France; 2) Department of Biochemistry and Genetics and UMR INSERM, U771-CNRS6214, Angers University Hospital, Angers, France.

Leigh Syndrome (LS) is a rare disorder characterized by degeneration of the central nervous system. LS has been associated with mutations in the mitochondrial DNA (mtDNA) as well as with numerous nuclear genes, highlighting the extreme genetic heterogeneity of the disease. A molecular diagnosis is still missing for a large majority of LS patients. The need of a good patient characterization and the large number of candidate genes in LS remains a great challenge for molecular diagnosis. Our goal was i) to better characterize the LS patients; ii) to use a high throughput approach for sequencing and identification of causative mutations. This study was focused on LS patients with complex I deficiency. The patient selection was based on criteria including i) a common pattern of brain MRI imaging; ii) isolated complex I deficiency in muscle; iii) mtDNA involvement excluded; iv) complex I assembly defect identified on patient skin fibroblasts with BN-PAGE analysis. A targeted resequencing assay including complex I genes was designed relying on custom target capture (SureSelect, Agilent) coupled to next-generation sequencing. Specifically, we captured the entire sequence of 87 known as causative or candidate genes in LS and re-sequenced them on Illumina GaII platform. A total of 8 LS patients with complex I deficiency were selected including a positive control. Over 99.5% of the targeted regions were captured and sequenced with appropriate coverage and quality. The depth of sequencing coverage was extremely high (mean x103). Filtering of novel variants was performed by comparison to dbSNP130, in-house database (composed of 32 exomes) and 8 HapMap exomes. Of the variants recognized, 1 to 9 % represented new alterations and only one candidate gene was identified in 7/8 patients. All pathogenic mutations identified were confirmed by Sanger sequencing. Of the seven new patients analyzed, one known mutation in *NDUFS4* gene and nine new mutations in *NDUFV1, NDUFA11, C8ORF38* and *NUBPL* genes were identified. This report demonstrates the power of our strategy for rapid molecular screening in complex I deficiency. This study indicates that the use of next generation sequencing technology holds great promise as a tool for molecular diagnosis of LS. Compared to the full exome sequencing, this approach has the potential to generate fewer variants and to rapidly focus on mutations in strong candidate genes in well characterized patients.

1103F

Exome sequencing for unraveling the molecular basis of complex I deficiency. *H. Prokisch[1,2], T. Haack[1,2], K. Danhauser[2], B. Haberberger[1,2], P. Freisinger[3], S. Eck[1], M. Zeviani[4], TM. Strom[1,2], T. Meitinger[1,2].* 1) HelmholtzZentrum Munchen, Inst fur Humangenetik, Neuherberg, Germany; 2) Technical University Munich, Institute of Human Genetics, Munich, Germany; 3) Klinikum am Steinenberg, Reutlingen, Germany; 4) The Foundation `Carlo Besta' Institute of Neurology, Milan, Italy.

Faulty energy supply due to defective oxidative phosphorylation is the biochemical signature of the genetically heterogeneous group of mitochondrial disorders. The molecular basis of the disorder remains unknown for a majority of patients and there is an urgent need for new diagnostic and therapeutic strategies. We applied exome sequencing (50 Mb capture, 100x coverage) in a group (n=15) of unrelated patients with isolated mitochondrial respiratory chain complex I deficiency to screen for pathogenic mutations. In a first step we analyzed known structural subunits and assembly factors of complex I and identified pathogenic mutations in five patients. In a second step we searched for two mutated alleles in genes coding for mitochondrial proteins and identified the potential pathogenic mutation in two patients. Next we screened for two loss of function alleles in any gene and identified one novel gene involved in complex I activity. In the remaining 6 patients, none of the above filters led to the identification of a pathogenic mutation. We established a lentiviral based complementation assay to validate the pathogenic role of novel variants in fibroblasts of patients. Complementation testing and mutation screening for the novel genes in 150 additional index patients is ongoing. In conclusion, exome sequencing in combination with functional assaying provides both an effective tool for molecular diagnosis and for discovery of new genes mutated in patients with complex I deficiency.

1104F

Defective NDUFA9 as a novel cause of neonatally fatal complex I disease. *B.J.C. van den Bosch[1,2], M. Gerards[1,2], W. Sluiter[3], A.P.A. Stegmann[1], E.L.C. Jongen[1], D.M.E.I. Hellebrekers[1], E.H. Lambrichs[2], H. Prokisch[4,5], K. Danhauser[4,5], K. Schoonderwoerd[6], I.F.M. de Coo[7], H.J.M. Smeets[1,2].* 1) Unit Clinical Genomics, Department of Clinical Genetics, Maastricht University, Maastricht, Netherlands; 2) School for Oncology and Developmental Biology, Maastricht University Medical Centre, Maastricht, The Netherlands; 3) Centre for Lysosomal and Metabolic Diseases, Erasmus MC, Rotterdam, The Netherlands; 4) Institute of Human Genetics, Technische Universität München, Munich, Germany; 5) Institute of Human Genetics, Helmholtz Zentrum München, German Research Centre for Environmental Health, Neuherberg, Germany; 6) Department of Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands; 7) Department of Neurology, Erasmus MC, Rotterdam, The Netherlands.

Mitochondrial disorders are associated with abnormalities of the oxidative phosphorylation (OXPHOS) system and cause significant morbidity and mortality in the population. The extensive clinical and genetic heterogeneity of these disorders due to a broad variety of mutations in either the mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) impedes a straightforward genetic diagnosis. Several approaches exist to identify the underlying gene defect, such as (large scale) screening of candidate genes, linkage analysis, homozygosity mapping and/or whole exome/genome sequencing. Here we present a single Kurdisch patient from consanguineous parents with neonatally fatal Leigh syndrome and complex I deficiency in which the gene defect was identified by homozygosity mapping and subsequent positional candidate gene analysis of complex I genes. A pathogenic mutation was identified in the complex I subunit encoding NDUFA9 gene, changing a highly conserved arginine at position 321 by proline, which is the first mutation reported for NDUFA9. Complex I activity was restored in fibroblasts of the patient by lentiviral transduction with wild type but not mutant NDUFA9, confirming that the mutation causes the complex I deficiency and related disease. Our data shows that homozygosity mapping and candidate gene analysis is still an efficient way to detect mutations in (single) consanguineous patients with OXPHOS deficiency, especially when the enzyme deficiency in fibroblasts allows appropriate candidate gene selection and functional complementation.

1105F

Zebrafish model of Poikiloderma with Neutropenia Syndrome: towards the function of the causative *C16orf57* gene. *E.A. Colombo[1], S. Carra[2], E. Bresciani[2], L. Volpi[3], F. Cotelli[2], L. Larizza[1].* 1) Dip. Medicina, Chirurgia e Odontoiatria, Università degli Studi di Milano, via A. di Rudinì 8, Milano, Italy; 2) Dip. di Biologia, Università degli Studi di Milano, via Celoria 26, Milano, Italy; 3) Dip. Biologia e Genetica per le Scienze Mediche , Università degli Studi di Milano, via Viotti 3/5, Milano, Italy.

Poikiloderma with Neutropenia (PN; OMIM #604173) is a rare autosomic recessive genodermatosis characterized by poikiloderma, telangiectasia, hyperkeratosis and pachyonychia, and by the hematological sign of neutropenia, which accounts for recurrent infections in the pediatric age and susceptibility to myelodysplastic syndrome at later stages. Other symptoms include short stature, osteoporosis, osteopetrosis and fragile carious teeth. A few PN patients display craniofacial dysmorphisms, mainly midface hypoplasia, depressed nasal bridge and mild prognathism. *C16orf57* has been shown to be the causative gene of PN, and eighteen inactivating mutations have been detected so far in forty PN patients. However, little is known about this gene and the function of its encoded protein. As *C16orf57* is highly conserved from plants to humans, we decided to employ zebrafish (*Danio rerio*), an ideal vertebrate model for the study of human developmental genetic diseases and haematological malignancies, to gain insights into the C16orf57 function and the consequences of its disruption. The human *C16orf57* gene has a unique ortholog in zebrafish (zc16orf57), with a high degree of conservation both in the intron-exon structure and in aminoacid sequence (73,4% similarity and 46% identity). Time course RT-PCR analysis on cDNA from 2-4 cell cleavage stage up to the swimming larva (3 dpf) showed zc16orf57 transcripts in all analyzed stages, consistent with the parallel widespread expression of the gene tested by whole-mount in situ hybridization assay. Moreover zc16orf57 was detected by RT-PCR in various tissues of adult zebrafish (brain, eye, gills, heart, liver, muscle, ovary and testis) in agreement with its ubiquitous expression in humans. To directly assess the function of zc16orf57 during development we performed loss-of-function experiments injecting specific antisense, morpholino-modified oligomers. Injection of the splice-morpholino in embryos at one cell stage induces an abnormal severe phenotype that partially recapitulate the PN defects. The morphants show: a decreased skin pigmentation, reduction or lack of blood circulation, a small head with defects in early cartilages of pharyngeal arches, as highlighted by Alcian blue staining, and edema in the pericardial area that expands to the whole embryo.

1106F

Selenoprotein N deficiency in mice is associated with lung emphysema. *B. Moghadaszadeh[1], B.E. Rider[1], M.W. Lawlor[1], C.A. Owen[2], A.H. Beggs[1].* 1) Division of Genetics and Program in Genomics, The Manton Center for Orphan Disease Research, Children's Hospital Boston, Harvard Medical School, Boston, MA, USA; 2) Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.

Mutations of the selenoprotein N gene, SEPN1, cause multiminicore myopathy, a rare congenital myopathy characterized by muscle weakness, spinal rigidity and respiratory insufficiency. Patients' muscle biopsies are characterized by the presence of minicores that are indicative of areas of mitochondria depletion and myofibrillar disruption. As with other members of the selenoprotein family, selenoprotein N incorporates selenium in the form of selenocysteine (Sec). Most selenoproteins that have been functionally characterized are involved in redox reactions, with the Sec residue being located at the catalytic site of the protein. To model multimincore myopathy and learn more about selenoprotein N function, we generated a Sepn1-knock-out (KO) mouse line in which exon 9, which carries the Sec codon, has been deleted. Homozygous Sepn1-KO mice are fertile and their weight and lifespan are comparable to wildtype (WT) animals. Furthermore, muscle histology of these mice under baseline conditions appears normal. Since the Sepn1-KO mice presented no apparent phenotype under normal conditions, and we anticipated SEPN1 to be involved in redox reactions, we fostered oxidative stress in these animals by feeding them a vitamin-E deficient diet for 8 months and subjecting them to an endurance running program for 5 days prior to sacrifice. Under these conditions both WT and Sepn1-KO mice developed minicores in their muscles, and while not statistically significant, the number of minicores was slightly higher in KO animals. We also investigated the spontaneous activity of these mice by quantifying the number of rearings and distance traveled in 10 minutes and found that Sepn1-KO mice were less active than the WT. This difference was more pronounced when animals were subjected to oxidative stress. Selenoprotein N deficiency in humans is associated with a respiratory insufficiency syndrome, which so far has been thought to be caused primarily by diaphragm weakness. Remarkably, Sepn1-KO mice presented enlarged alveoli, compatible with development of emphysema beginning as early as 4 weeks of life and progressing with age. Interestingly this pulmonary phenotype is present in KO mice under normal conditions in the absence of oxidative stress induction. Our findings in mouse lungs suggest that selenoprotein N deficiency directly affects lung function, which may have a potential impact on management of respiratory insufficiency in patients with multiminicore myopathy.

1107F

Update on the roles and functions of OPA1 in mitochondria: a never ending story? *P. Amati-Bonneau[1,2,7], V. Procaccio[1,2,7], G. Lenaers[3,7], C. Hamel[3,7], V. Carelli[4,7], A. Martinuzzi[4,7], B. Wissinger[5,7], D. Milea[1,2,6,7], M. Ferré[1,2,7], A. Chevrollier[1,2,7], V. Desquiret-Dumas[1,2,7], N. Gueguen[1,2,7], P. Reynier[1,2,7], D. Bonneau[1,2,7].* 1) Departement of Biochemistry & Genetics, CHU Angers, Angers, France; 2) UMR INSERM U771-CNRS6214, Angers, France; 3) INSERM U583, Institute for Neurosciences of Montpellier, Montpellier,France; 4) Departement of Neurological Sciences, University of Bologna, Bologna, Italy; 5) Molecular Genetics Laboratory, Institutefor Ophthalmic Research, University Clinics Tuebingen, Tuebingen, Germany; 6) Departement of Ophthalmology, CHU Angers, Angers France; 7) ERMIO-N:European Research Project on Mendelian Inherited Optic Neuropathies.

Inherited optic neuropathies are one of the most common causes of vision loss in childhood and early adulthood. Mutations in the mitochondrial fusion protein OPA1 cause autosomal dominant optic atrophy (ADOA). The OPA1 gene encodes a mitochondrial dynamin-related GTPase, located to the mitochondrial inner membrane. Originally, OPA1 has been shown to play a key role in mitochondrial fusion and mitochondrial network organization. As of today, our laboratory in Angers has identified more than 230 OPA1 mutations (refer to our website: http://lbbma.univ-angers.fr/eOPA1) in a large cohort of 1500 patients. The large majority of OPA1 mutations result in protein truncation suggesting that haploinsufficiency could be the prime pathomechanism. However, a dominant-negative effect of mutated OPA1 could also play a role as seen with the R445H mutation consistently resulting in severe optic atrophy associated with hearing loss. The pathophysiology of OPA1-related diseases is still largely unknown. A primary degeneration of retinal ganglion cells (RGC) has been hypothesized but the mechanisms leading to this degeneration require further investigations. Other OPA1 functions have been recently identified, related to oxidative phosphorylation and maintenance of membrane potential, maintenance of mtDNA, and cristae organization and control of mitochondrial apoptosis through the compartmentalization of cytochrome c. Biochemical investigations of patient fibroblasts carrying OPA1 mutations have revealed a common OXPHOS coupling defect together with a reduction of the mitochondrial membrane potential. Alterations of the mitochondrial network have been observed in patient fibroblasts bearing OPA1 mutations. The severity of the mitochondrial network fragmentation seems to be well correlated with the most severely affected patients suggesting a link between OXPHOS impairment and mitochondrial alterations. Finally, significant mtDNA alterations have been reported in OPA1 patients as a strong indicator of the role of OPA1 in mtDNA maintenance. Recently, mouse models carrying OPA1 mutations have been generated exhibiting pathological features similar to ADOA including a progressive RGCs and optic nerve axon degeneration. This model should substantially help us to clarify the genetics and pathophysiology of OPA1 related disorders and useful to evaluate candidate therapeutic strategies.

1108F

Identification and functional analysis of SOX10 missense mutations in different types of Waardenburg syndrome. *A. Chaoui[1,2], Y. Watanabe[1,2], R. Touraine[3], V. Baral[1,2], M. Goossens[1,2,4], V. Pingault[1,2,4], N. Bondurand[1,2].* 1) INSERM U955, Creteil, France; 2) Université Paris-Est, Faculté de Médecine, Créteil, France; 3) CHU-Hôpital Nord, Service de Génétique, Saint Etienne, France; 4) AP-HP, Groupe Hospitalier Henri-Mondor, Service de Biochimie et Génétique, Créteil, France.

Waardenburg syndrome (WS) is an auditory-pigmentary disorder that exhibits varying combinations of sensorineural hearing loss and pigmentation defects. Four subtypes are clinically defined based on the presence or absence of additional symptoms. In particular, the absence of additional features characterizes WS2, whereas the association with Hirschsprung disease defines WS4. WS is genetically heterogeneous with six genes already involved, including SOX10. Since 1998, about 50 heterozygous mutations or deletions of this gene have been described in patients presenting with WS2 or WS4, with or without myelination defects of the peripheral and central nervous system (PCWH). The mutations characterized so far are mostly truncating mutations, removing part of the protein. Only 3 missense mutations were described. Here, we report the identification of new SOX10 missense mutations in 11 patients, associated with a variety of phenotypes ranging from WS2 to PCWH, and describe the functional consequences of each of these mutations on the main SOX10 characteristics and functions. Altogether, our functional assays highlight deleterious effects of all the mutations tested. Some mutants present with partial cytoplasmic and/or subnuclear redistribution, some lose their DNA binding and/or their transactivation capacities on various tissue specific target genes. Intriguingly, several mutants were redistributed in nuclear foci. To our knowledge, such redistribution was not described so far during functional studies of patients' mutations identified in other SOX genes. Whether it is a cause or a consequence of the mutant pathogenicity remains to determine, but it could help identify new SOX10 function and mode of action.

1109F

Characteristics of hepatic proteome expressions in Wilson disease. *B.H. Lee[1,2,3], J.M. Kim[2,3], S.H. Heo[2], J. Kim[4], J.H. Kim[2], G.H. Kim[1,2], J.H. Choi[1], H.W. Yoo[1,2,3].* 1) Pediatrics, Asan Med Ctr, Seoul, Korea; 2) Genome Research Center for Birth Defects and Genetic Disorders, Asan Med Ctr, Seoul, Korea; 3) Medical Genetics Center, Asan Med Ctr, Seoul, Korea; 4) Department of Pathology, Asan Med Ctr, Seoul, Korea.

Wilson's disease (WD) is an autosomal recessive disorder caused by a deficiency of the P-type copper-transporting ATPase (ATP7B). The most pathognomic feature of WD is copper accumulation, starting in hepatic tissue and progressing to extrahepatic tissues including the brain, which induce oxidative injury and pro-apoptotic conditions. Using Long-Evans Cinnamon (LEC) rats, an animal model of WD, the study was undertaken to identify proteins involved in the process of WD and to investigate their functional roles in copper-induced hepatotoxicity. LEC rats and LEA (Long-Evans Agouti) rats, a non-diseased LEC rats, aged 6, 12 and 24 weeks (3 each per group) were used. Their clinical and histopathological features were compared. Their protein profiles were comparatively analyzed using 2-DE and MALDI-TOF-MS, and validated by immunoblotting. In early stages, mitochondrial matrix proteins including agmatinase, isovaleryl coenzyme A dehydrogenase, and cytochrome b5 were down-regulated. As mitochondrial injuries progressed, along with subsequent apoptotic processes, expressions of malate dehydrogenase 1, annexin A5, transferrin, S-adenosylhomocysteine hydrolase, and sulfite oxidase 1 were differentially regulated. Notably, the expression of malate dehydrogenase 1 was down regulated while the annexin A5 was over-expressed in an age-dependent manner, indicating that these proteins may be involved in the WD process. In addition, pronounced under-expression of S-adenosylhomocysteine hydrolase in elderly LEC rats, also involved in monoamine neurotransmitter metabolism, indicates that this protein might be related to the development of neurological manifestations in WD. The results of our study help to understand the pathogenic process of WD in hepatic tissues, identifying the important proteins associated with the disease process of WD, and to investigate the molecular pathogenic process underlying the development of neurological manifestations in WD. Further investigations are needed whether the similar differential expression patterns of these proteins are also noted in other easily-accessible tissues, blood or urine, in WD patients with the demonstration of their disease-specific expressions, which will ultimately help to utilize them as potential biomarkers for the prediction of WD progression.

1110F

HRAS mutants identified in Costello syndrome patients have the ability to induce cellular senescence: possible association with the pathogenesis of Costello syndrome. *T. NIIHORI[1], Y. AOKI[1], N. OKAMOTO[2], K. Kurosawa[3], H. OHASHI[4], S. MIZUNO[5], H. KAWAME[6], J. INAZAWA[7], T. OHURA[8], H. ARAI[9], S. NABATAME[10], K. KIKUCHI[11], Y. KUROKI[12], M. MIURA[13], T. TANAKA[14], A. OHTAKE[15], I. OMORI[16], K. IHRARA[17], H. MABE[18], K. WATANABE[19], S. NIIJIMA[20], E. OKANO[21], H. NUMABE[22], Y. MATSUBARA[1].* 1) Dept Med Genet, Tohoku Univ Sch Med, Sendai, Japan; 2) Osaka Med Ctr & Res Inst for Maternal & Child Health, Osaka, Japan; 3) Kanagawa Children's Med Ctr, Yokohama, Japan; 4) Saitama Children's Med Ctr, Saitama, Japan; 5) Aichi Prefectural Colony Central Hosp, Aichi, Japan; 6) Dept Genet Couns, Ochanomizu Univ, Tokyo, Japan; 7) Dept Mol Cytogenet, Med Res Inst Sch Biomed Sci, Tokyo Med & Dent Univ, Tokyo, Japan; 8) Sendai City Hosp, Sendai, Japan; 9) Morinomiya Hosp, Osaka, Japan; 10) NCH, National Ctr of Neurol & Psychiatry, Tokyo, Japan; 11) Shimane Pref Central Hosp, Izumo, Japan; 12) Kurashiki Central Hosp, Kurashiki, Japan; 13) Tokyo Metropolitan Children's Med Ctr, Tokyo, Japan; 14) National Res Inst for Child Health & Development, Tokyo, Japan; 15) Dept Ped, Saitama Med Univ, Saitama, Japan; 16) Ctr Maternal, Fetal & Neonatal Med, Tokyo Metropolitan Bokutoh Hosp, Tokyo, Japan; 17) Dept Ped, Grad Sch Med Sci, Kyushu Univ, Fukuoka, Japan; 18) Dept Child Dev, Faculty of Life Sci, Kumamoto Univ, Kumamoto, Japan; 19) National Hosp Org Kokura Med Ctr, Kitakyushu, Japan; 20) Dept Ped, Juntendo Univ, Nerima Hosp, Tokyo, Japan; 21) Dept Ped, Jikei Univ Sch Med, Tokyo, Japan; 22) Dept Clin Genet, Kyoto Univ Hosp, Kyoto, Japan.

Costello syndrome (CS) is characterized by a distinctive facial appearance, failure to thrive, mental retardation and cardiomyopathy. In 2005, we discovered that heterozygous germline mutations in HRAS caused CS. Several studies have shown that mutations causing CS are clustered in codons 12 and 13, and mutations in other codons have also been identified. The functional profiling of HRAS mutations causing CS remains to be elucidated. In the current study, we identified G12S, G12A, G12C and G12D mutations in 21 patients and analyzed the clinical manifestations in these patients. To examine the functional difference among the mutations identified in CS, we characterized a total of nine HRAS mutants, including seven mutants in codon 12 or 13, K117R and A146T. The A146T mutant demonstrated the weakest Raf-binding activity, and the K117R and A146T mutants had weaker effects on downstream JNK signaling than mutants in codon 12 or 13. We demonstrated that HRAS mutants had the ability to induce senescence when overexpressed in human fibroblasts. Oncogene induced senescence is a cellular reaction that controls cell proliferation and is driven by oncogenic mutation, and it has been considered one of the tumor suppression mechanisms in vivo. Our findings suggest that the HRAS mutations identified in CS are sufficient to cause oncogene induced senescence and cellular senescence might contribute to pathogenesis of CS.

1111F

BMP2-Smad-Runx2 overexpression provides evidence for the involvement of a pro-osteogenic signalling pathway in pseudoxanthoma elasticum. *O. Vanakker[1], M. Hosen[1], P. Coucke[1], O. Le Saux[2], A. De Paepe[1].* 1) Center for Medical Genetics, Ghent Univiversity Hospital, Ghent, Belgium; 2) Department of Cell and Molecular Biology, John A. Burns School of Medicine, University of Hawai'i.

Aims. Pseudoxanthoma elasticum (PXE) is characterized by oculocutaneous and cardiovascular manifestations, due to mineralization and fragmentation of elastic fibers. The causal ABCC6 gene encodes a transmembrane transporter; however, the pathogenetic link with the elastic fiber abnormalities remains unknown. Current pathophysiological hypotheses include a role for oxidative stress and an unidentified PXE serum factor. We have previously shown that PXE patients have a diminished vitamin K (VK) serum concentration, leading to inefficient .-carboxylation or activation of VK-dependent inhibitors of mineralization, such as matrix gla protein (MGP). MGP is known to inhibit Bone Morphogenetic Protein 2 (BMP2), a master regulator of osteogenesis. Several pathways are associated with BMP2, one of which is the BMP2-Smad-RUNX2 pathway in which RUNX2 acts as a transcriptional regulator of proteins involved in mineralization or osteogenesis. We aimed to study the role of this signalling pathway in the PXE pathogenesis. **Methods & Results.** Alizarin red calcium stains were performed to detect mineralization around the whiskers of ABCC6 knockout mice and human PXE dermis. Immunohistochemical stains on calcium-positive adjacent slides for BMP2, Smad 1-4-5-8 and RUNX2 showed positive labeling, co-localizing with mineralization, in all tissues compared to controls. These qualitative results were quantitatively confirmed via qPCR on human PXE fibroblasts. Comparable qPCR results were obtained on control fibroblasts inoculated with human PXE serum. **Discussion & Conclusion.** Our results indicate the involvement of the pro-osteogenic cellular BMP2-Smad-RUNX2 pathway in PXE. Calcification-specific upregulation of RUNX2 provides a pathophysiological mechanism for the up- or downregulation of proteins previously implicated in PXE (such as osteocalcin, alkaline phosphatase, osteopontin, bone sialoprotein and VEGF-A), leading to ectopic mineralization or neovascularisation. In addition, our results for the first time merge three principal pathophysiological observations in PXE. Indeed, besides VK-deficiency also oxidative stress has been shown to contribute to RUNX2 overexpression and we show a similar effect of PXE serum on RUNX2 expression. Thus, our results provide evidence that vitamin K deficiency, oxidative stress as well as the PXE serum factor co-operate in an integrated pathophysiological process leading to ectopic mineralization in PXE.

1112F

Cohesin complex regulator ESCO2 and Replisome Progression complex Timeless and TIPIN constitute a complex that links establishment of sister chromatid cohesion and the replication stress checkpoint. *H. Vega[1,2], M. Gordillo[2,3], N. Sakai[4], HS. Rho[5,6], J. Boeke[5,7], H. Zhu[5,6,7,8], K. Inui[4], K. Ozono[4], EW. Jabs[2].* 1) Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI; 2) Department of Genetic and Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 3) Department of Surgery, Cornell University, New York, NY; 4) Department of Pediatrics, Osaka University, Osaka, Japan; 5) The Center for High-Throughput Biology, Johns Hopkins University, Baltimore, MD; 6) Department of Molecular Biology and Genetics, Johns Hopkins University, Baltimore, MD; 7) Department of Pharmacology and Molecular Sciences, Johns Hopkins University, Baltimore, MD; 8) The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD.

ESCO2 mutations cause Roberts syndrome (RBS). ESCO2 is member of the Eco1 family of acetyltransferases that is central to the establishment of sister chromatid cohesion, a process intimately linked to the passage of the replisome. Eco1 is aided during cohesion establishment by a number of non-essential factors associated to the replisome. However, little is known about how the replisome and the cohesion factors affect each other. Using a human protein chip we found that ESCO2 and ESCO1 physically interact with the replication stress checkpoint proteins Timeless and TIPIN members of the replisome progression complex. ESCO2 exist in two different complexes in the replisome, one with Timeless and other with PCNA. Timeless and PCNA siRNA depletion indicates that the Timeless-ESCO2 complex plays a more important role in sister chromatid cohesion than the PCNA-ESCO2 complex. RBS cells, deficient in ESCO2 activity showed increased spontaneous chromosome fragilities, reduced proportion of cells in the S phase without reduction in BrdU incorporation and faster progression through S phase. Under replication stress conditions RBS cells showed hypersensitivity to hydroxyurea, reduced levels of Timeless, abnormal electrophoretic mobility of TIPIN, deficient phosphorylation of Chk1 and histone H2AX, impair .H2AX, MDC1 and MRE11 foci formation while ATR foci are efficiently formed. Our results demonstrate a new role for ESCO2 in signal transduction and unravel a link between sister chromatid cohesion and the replication stress checkpoint.

1113F

Severe myopathy due to cofilin-2 deficiency in a knockout mouse model. *P.B. Agrawal[1,2], T. Savic[1], Z. Chen[1], M. Joshi[1], A.H. Beggs[1,2].* 1) Dept. of Medicine, The Manton Center for Orphan Disease Research, Program in Genomics, Children's Hospital Boston, Boston, MA; 2) Harvard Medical School, Boston, MA.

Background: Congenital myopathies (CMs) are rare, genetic skeletal muscle disorders that present with muscle weakness, delayed motor milestones, feeding/breathing difficulties. We have identified cofilin-2 (CFL2) to be the sixth gene mutated in nemaline myopathy, the most common CM. Cofilin-2 belongs to the AC family of actin-binding proteins that include cofilin-1 and destrin. The role of cofilin-2 in muscle development and thin filament dynamics is unknown. Objective: To create a cofilin-2 conditional knockout (KO) mouse model (COFI). To breed the COFI mice with various cre-recombinase expressing mice to create constitutive and striated muscle-specific Cfl2-KOs and to evaluate the KO phenotype. Design/Methods: COFI mice were created and bred with various cre-mice including those expressing it in zona pellucida (Zp3-cre) for a constitutive KO, cardiac and skeletal muscles-specific (Acta1-cre, Ckmm-cre with postnatal cre expression) and skeletal muscle-specific (Mef2c-cre). Results: Loss of cofilin-2 was associated with severe myopathy in the KO mice. The Cfl2-KO mice died by day 8 (constitutive KO, Cofi/Cofi:Acta1-cre/+, Cofi/Cofi:Mef2c-cre/+) and day 31 (Cofi/Cofi:Ckmm-cre/+) after birth. KO animals were significantly smaller in both length and weight compared to their wild type (WT) siblings. Their muscles demonstrated extensive areas of degeneration on H&E and NADH staining. On ATPase staining, slow fibers were significantly increased in the KO mice. On qRT-PCR, slow-fiber specific gene transcripts were significantly higher and fast-fiber specific gene transcripts lower in the KO mice compared to WT. Electron microscopy showed myofibers with minicores, nemaline bodies, absent Z lines, disrupted sarcomeres and actin accumulations. Immunofluorescence using phalloidin and anti-actin antibody showed actin accumulations in several fibers. Increased amounts of actin and alpha-actinin-2 were present in the KO mice compared to control on the Western blot analysis. Further, the skeletal muscle did not show any defects in the KO fetuses (E18.5) suggesting that cofilin-2 plays a critical role in muscle maintenance, but not in myofibrillogenesis. Conclusions: Cofilin-2 deficiency in mice causes severe myopathy and markedly reduced life span, and is associated with myofiber degeneration, nemaline bodies, cores and actin accumulations. Cofilin-2 is critical for the muscle maintenance.

1114F

Anoctamin 5 (ANO5) skeletal muscle subcellular localization is preserved in a LGMD2L patient carrying a splicing mutation. *V. Bolduc[1], T.C. Conte[1], R. Larivière[1], K.M. Boycott[2], H. Inoue[3], M. Itakura[3], Y. Robitaille[4], N. Leblanc[5], E. O'Ferrall[6], B. Brais[1,6].* 1) Laboratoire de neurogénétique, Centre de Recherche du CHUM, Montreal, Que, Canada; 2) Department of Genetics, Children's Hospital of Eastern Ontario, Ottawa, Ont, Canada; 3) Institute for Genome Research, The University of Tokushima, Tokushima, Japan; 4) CHU Ste-Justine, Montreal, Que, Canada; 5) Department of Pharmacology, University of Nevada, School of Medicine, Reno, NV, USA; 6) Department of Neurology and Neurosurgery, McGill University, Montreal Neurological Institute, Montreal, Que, Canada.

Recessive mutations in Anoctamin 5 (*ANO5*) were recently found to cause a limb-girdle muscular dystrophy type 2L (LGMD2L; MIM 611307). LGMD2L is characterized by late-onset progressive weakness of proximal limb muscles with prominent quadriceps atrophy and high serum creatine kinase levels. A total of 16 published and unpublished ANO5 mutations were uncovered to date, consisting of 8 missense, 2 nonsense, 2 splice site mutations, 1 single base pair duplication, and 3 indels. ANO5's function and localization in muscle is still unknown, but Ano1 and Ano2 function as calcium-activated chloride channel (CaCC) in other tissues. Our immunofluorescence data support that ANO5 localizes to intracellular membranes in a striated pattern in close proximity to Z-disks, with a partial co-localization with SERCA1 (a sarcoplasmic reticulum marker) and OPA1 (a mitochondrial marker). On a LGMD2L muscle section from a patient homozygous for a splice site mutation, we could observe preservation of the striated ANO5 signal, despite that this mutation was previously demonstrated to trigger the degradation of transcripts via the nonsense-mediated mRNA decay pathway. *ANO5* transcripts analyses from this LGMD2L patient using TA-cloning revealed that alternative splicing events occur, and that the coding frame is restored in a proportion of the transcripts. This result suggests that myocytes may use alternative splicing to produce truncated ANO5 isoforms in LGMD2L which may be partially functional, and may explain the preserved ANO5 staining observed in additionnal LGMD2L patients as well. A better understanding of ANO5 isoforms in normal and pathological states will help elucidate the pathophysiology of the anoctaminopathies.

1115F

A molecular genetic approach to the diagnosis of limb-girdle muscular dystrophies. *D. Monies[1], S. Majid[1], M. Al Muhaizea[2], H. Al Hindi[3], I. Al Homoud[2], A. Al Azami[1], D. Jaroudi[1], B. Baz[1], E. Naim[1], S. Al Haibey[1], BF. Meyer[1], S. Bohlega[2].* 1) Genetics, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 2) Neurosciences, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; 3) Department of Pathology & Laboratory Medicine, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia.

Autosomal recessive limb-girdle muscular dystrophy (LGMD2) is a genetically heterogeneous disorder. So far 15 genes have been identified which are responsible for LGMD2. Due to such a large number of genes and their size (e.g. TTN has 312 exons) the genetic diagnosis can be very costly and laborious. We have studied eighteen consanguineous LGMD2 families. The fifteen candidate genes were screened for homozygosity using the Affymetrix Cyto v.2 array and Chromosome Analysis Suite (ChAS) software. We excluded all but one candidate gene in ten families and 2 or 3 loci were identified in four and three families respectively. In one family none of the LGMD2 loci were homoallelic. This family was reclassified as having facioscapulohumeral muscular dystrophy (FSHD) and confirmed by a microdeletion of DUX4. To date the underlying mutations were identified for eight LGMD2 families. These consisted of six novel mutations in four genes with two of these being shared by more than 1 family. Our results demonstrate that in consanguineous populations, homozygosity screening is a much faster and less expensive molecular approach to the diagnosis of genetically heterogeneous disorders like LGMD2.

1116F

Molecular diagnosis of neuromuscular diseases by next generation sequencing. *N. VASLI[1], J. BOEHM[1], S. LEGRAS[2], J. MULLER[1], F. PLEWNIAK[2], C. KEIME[2], S. VICAIRE[3], B. JOST[3], JL. MANDEL[1,4], V. BIANCALANA[1,4], J. LAPORTE[1].* 1) Department of Translational Medicine and Neurogenetics, IGBMC, Illkirch-Graffenstaden, Alsace, France; 2) Bioinformatics platform , IGBMC, Illkirch-Graffenstaden, France; 3) Microarrays and sequencing platform, IGBMC, Illkirch-Graffenstaden, France; 4) Laboratoire de diagnostic gÉnÉtique, FacultÉ de MÉdecine, Strasbourg, France.

Introduction : Neuromuscular diseases (NMD) are debilitating disorder with a strong impact on the individuals and society. Despite tremendous research and clinical efforts, the molecular causes of NMD are still unknown for about 40% of patients and additional genes remain to be found. In order to provide a faster and cheaper molecular diagnosis for NMD patients and to detect different types of mutations, we have validated sequence capture and next generation sequencing using Agilent Sureselect liquid capture kit following by Illumina GAIIx sequencing. Results: Using targeted re-sequencing of 270 genes implicated in most of neuromuscular disorders in 7 patients with known mutations, we successfully retrieved the deleterious mutations in all of them (covering point mutations, intronic mutation 10nt from the exon, a small indel and a large hemizygous deletion). The coverage for one of the mutations was low and led to improvement of our protocol. In the second part of the project, after improving the capture step for uncovered regions in first trial, we pooled 4 tagged DNAs in each lane of Illumina GAIIx. In total we sequenced 16 patients (4 pools of 4 patients) with different types of mutations where we knew the mutations in half of them. We could successfully detect all the disease-causing variants in the 8 patients with known mutations. For patients with unknown mutations we prepared a list of candidate genes and are checking them by direct sequencing. More experiments are ongoing to prove these changes as disease-causing mutations. Conclusion: We conclude that next generation sequencing is a powerful approach to identify potential disease-causing variants, a prerequisite for genetic counseling and better healthcare. Although this technique is still in its infancy, it should allow reducing the time to genetic diagnosis and its cost. It might represent a first screening without the need for very detailed clinical criteria for inclusion that may be absent in atypical forms of the diseases or when the disease begins. In addition, the analysis might be proposed before the need of more invasive investigations such as biopsy. This emerging strategy is very likely to become a standard tool for routine genetic diagnosis.

1117F

Application of massive parallel sequencing to the detection of somatic *NLRP3* mutations in cryopyrinopathies. *E. Gonzalez[1], M. Ibañez[2], R. Merino[3], F. Rius[1], S. Plaza[1], J. Yague[1].* 1) Immunology Service (CDB), Hospital Clinic de Barcelona, Barcelona, Spain; 2) Pediatric Reumathology Dpt., Hospital del Niño Jesús, Madrid, Spain; 3) Pediatric Reumathology Dpt., Hospital de la Paz, Madrid, Spain.

Chronic infantile neurologic, cutaneous and articular syndrome (CINCA), also known as neonatal-onset multisystem inflammatory disease (NOMID), represents the severest phenotype among the cryopyrynopathies, which also includes Familial Cold Autoinflammatory Syndrome (FCAS) and Muckle-Wells syndrome. CINCA/NOMID is a severe, early-onset inherited autoinflammatory disease characterized by an urticaria-like rash, arthritis/arthropathy, neurologic involvement, and dysmorphic features. CINCA/NOMID has been associated with dominantly inherited *NLRP3* mutations, most of them located at exon 3. During the past years, *NLRP3* mutational analysis has been the only test to achieve a definitive diagnosis of cryopyrinopathies. However, Sanger-based sequencing analyses detect germline, disease-causing mutations in only 55-60% of CINCA/NOMID patients, suggesting for the presence of genetic heterogeneity. In a previous study, we found the novel and de novo p.D303H *NLRP3* variant in a Spanish CINCA/NOMID patient as the responsible of his disease. Interestingly, it was detected as a somatic mutation in hematopoietic and nonhematopoietic cell lineages. This work included subcloning and Sanger-sequencing of exon 3 of *NLRP3* as well pyrosequencing to quantify the amount of cells presenting the mutation. However, this strategy is expensive and time-consuming to apply as diagnostic tool in patients with a moderate clinical suspicious of cryopyrinopathies. The recent incorporation of massive parallel sequencers to research and clinic opens a new perspective to study the presence of somatic mutations in an easier way. We used a 454 GS Junior sequencer to test if this system could identify known germline and somatic mutations in exon 3 of *NLRP3* gene that were previously identified and quantified by traditional methods. For this proposal, we run three samples: -Sample 1: p.G307V in ~12% of cells -Sample 2: p.D303H in ~20% of cells -Sample 3: p.D303N in germline The test gave us the expected results finding ~10.5% of cells with the somatic mutation p.G307V in sample 1, ~16.7% of cells with the somatic mutation p.D303H in sample 2 and 46,7% of cells in sample 3 with the germline p.D303N mutation. Regarding the good results obtained in this first test, we are currently performing massive parallel sequencing of exon 3 of *NLRP3* in patients who have a clinical suspicious of cryopyrinopathies . We expect that we will be able to explain the molecular basis underlying their disease.

1118F

Molecular analysis in four Greek patients with retinal degeneration establishes a spectrum of clinical diagnosis: identification of four novel mutations in CHM, RS1, SPATA7 and RDS genes. *S. Kamakari[1,2], G. Koutsodontis[1,3], C. Tsika[3], I. Datseris[1], M. Tsilimbaris[3].* 1) Ophthalmic Genetics Unit, OMMA Institute of Ophthalmology, Halandri Athens, Greece; 2) School of Medicine, University of Athens, Athens, Greece; 3) Department of Ophthalmology, School of Medicine University of Crete, Iraklion, Greece.

Purpose: To identify the molecular cause in 4 Greek retinal dystrophy patients with uncertain clinical diagnosis. Methods: PCR amplification and sequencing of the full coding sequence and splice junctions of CHM,OAT,RS1,SPATA7 and RDS genes was selectively performed on 4 patients. LCA chip analysis was used in 1 case. Results: Case 1 was a 57 year old male patient diagnosed with either gyrate atrophy or chorioderemia. Pedigree analysis did not confirm the pattern of inheritance. No mutations were detected in the gyrate atrophy OAT gene, whereas a novel frameshift mutation c.223_224dupTG (p.Trp75Cysfsx52) in exon 4 was detected in the chorioderemia gene, CHM. Case 2 was a 10 year old male patient with possible diagnosis of Stargardt's disease. Thorough pedigree investigation revealed 8 male patients on the maternal side indicating a possible X-linked inheritance. A decision was made to analyse the proband for the X-linked retinoschisis gene, RS1, and a novel missense mutation, c.175T>G (p.Cys59Gly) was detected in exon 3. Case 3 was a 34 year old simplex patient diagnosed with retinitis pigmentosa. She had light perception at the time of seeking genetic testing and suffered from vision loss since birth. LCA chip analysis was selected rather than ARRP and led to the detection of the known heterozygous mutation c.322C>T (p.R108X) in exon 5 of the SPATA7 gene. Subsequent sequencing of the entire coding sequence of this gene revealed a second novel heterozygous splice mutation c.373-1G>C in intron 5. Case 4 was a 30 year old male patient who was diagnosed with Best disease, had choroidal neovascularisation and his pedigree analysis showed an autosomal dominant pattern of inheritance. Due to late age of onset, the peripherin/RDS gene was selected for analysis since mutations in this gene are the major cause of multifocal pattern dystrophy. A heterozygous 1bp deletion c.163delT (p.Ser55LeufsX10) was detected in exon 1 of the gene. The 4 mutations were not found in 92 control subjects. Conclusion: We established the molecular diagnosis in 4 Greek patients with retinal degeneration and uncertain clinical diagnosis by analysing the best candidate gene for each patient. All 4 identified potentially pathogenic mutations in the analysed genes CHM, RS1, SPATA7 and RDS gene were novel. An accurate molecular diagnosis predicts the patients' visual prognosis, allows carrier identification and genetic counseling and may be important for future gene-specific therapies.

1119F

A novel mutation at F9 gene Iranian hemophilia B patient. *L. Kokabee, M. Kokabee, S. Zeinali, M. Karimipoor.* Molecular Medicine Group, Biotechnology Department, Pasteur Institute of Iran, Tehran, Tehran, Iran.

Hemophilia B is an X-linked bleeding disorder caused by the defect or functional deficiency of coagulation factor IX. The disease is due to heterogeneous mutations in the factor IX gene (F9), located at Xq27.1. It spans about 34 kb of genomic DNA. In this study, mutation in F9 gene of an Iranian patient with severe hemophilia B was analyzed. For this purpose, after obtaining informed consent, genomic DNA was extracted from blood (referred from Esfahan Hemophilia Center) by standard methods. PCR amplification and single strand conformation polymorphism (SSCP) technique were performed for scanning of the all functional-important regions of the F9 gene. Abnormal SSCP profile was identified in exon 8 of this patient. Then, direct sequencing was done according to Sanger method. DNA sequencing of the fragment with definite band shift in SSCP showed a single base insertion at nucleotide 30845insT. This frame shift mutation causes premature stop codon at residue 242 (242X). This mutation has not been reported previously in the hemophilia B mutation database and due to producing premature termination codon causes severe form of the disease, as observed in the patient.

1120F

A family of oculofaciocardiodental (OFCD) syndrome with a novel *BCOR* mutation and genomic rearrangements involving *NHS*. *Y. Kondo[1], H. Saitsu[1], K. Nishiyama[1], Y. Tsurusaki[1], H. Doi[1], N. Miyake[1], T. Miyamoto[2], N.K. Ryoo[3], J.H. Kim[3], Y.S. Yu[3], N. Matsumoto[1].* 1) Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan; 2) Department of Obstetrics and Gynecology, Asahikawa Medical College, Asahikawa, Japan; 3) Department of Ophthalmology, Seoul National University College of Medicine, Seoul, Korea.

Oculofaciocardiodental syndrome (OFCD) is an X-linked dominant disorder, presenting congenital cataract, microphthalmia, dysmorphic face, dental radiculomegaly, and septal heart defects in affected female. Mutations in *BCOR* (encoding BCL-6-interacting corepressor) cause OFCD syndrome. Here, we report on a Korean family with common features of OFCD syndrome including congenital cataract, hypodontia, bilateral 2nd-3rd syndactyly, and septal heart defects in three affected females (mother and two daughters). Through the mutation screening and copy number analysis using genomic microarray, we identified a novel heterozygous mutation, c.888delG, in the *BCOR* gene and two duplications at Xp22.2-22.13 and Xp21.3 in all the three affected females in this family. The *BCOR* mutation may yield a premature stop codon (p.N297IfsX80) likely to be associated with nonsense mediated mRNA decay. The duplication at Xp22.2-22.13 partially involved the *NHS* gene causative for Nance-Horan syndrome, which is an X-linked disorder and shows similar clinical features with OFCD syndrome such as bilateral congenital cataracts, dental anomalies, facial dysmorphism and mental retardation in affected males, and in carrier females with milder presentation. X-inactivation study using peripheral blood leukocyte DNA indicated that normal allele, which was inherited from the healthy father, was mostly expressed. Considering the presence of bilateral 2nd-3rd syndactyly and septal heart defects, which is unique to OFCD syndrome, the mutation in *BCOR* is likely to be the major determinant of the phenotypes in this family.

1121F

Exome sequencing reveals a homozygous mutation in *DPH1/OVCA1* associated with syndromic craniosynostosis with ectodermal and developmental abnormalities. *C.M. Loucks[1], A.M. Innes[1], F.P. Bernier[1], D.R. McLeod[1], E.G. Puffenberger[2], K.M. Boycott[3], S.J. Childs[4], J.S. Parboosingh[1].* 1) Dept of Medical Genetics, University of Calgary, Calgary, AB, Canada; 2) Clinic for Special Children, Strasburg, PA, USA; 3) Dept of Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada; 4) Dept of Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, Canada.

Cranioectodermal dysplasia (CED) is a rare autosomal recessive disorder characterized by short stature, sagittal craniosynostosis and ectodermal abnormalities. In addition, the majority of patients have developed renal and retinal disease clinically consistent with CED being a ciliopathy, a classification supported by the identification of three genes involved in intraflagellar transport as causative for the disorder: *IFT122*, *IFT43* and *WDR35*. We ascertained three Hutterite children with a CED-like phenotype without significant renal or retinal disease but with intellectual disability. Given the known consanguinity in this population, a single nucleotide polymorphism array was performed on the patients to identify a homozygous region on chromosome 17 of approximately 10 Mb, containing 310 genes. Coding and flanking intronic regions throughout the genome were captured in one of these patients, followed by next generation sequencing to reveal over 50 000 variants. Looking at only variants present in the homozygous region on chromosome 17 reduced the number of variants to just over 500. Common variants were then excluded leaving 15 variants, most of which were intronic, intergenic or changes at poorly conserved positions. Subsequently, an additional Hutterite patient was identified with the same phenotype. Upon sequencing of coding or highly conserved variants, this patient was homozygous for only one variant surrounded by a small haplotype shared by all four patients. This variant results in the loss of the likely start codon of *DPH1/OVCA1*, a gene responsible for a post translational modification on a protein involved in translation elongation, characterized as a tumour suppressor in ovarian/breast cancer and associated with developmental abnormalities in the knockout mouse. Currently, work is being done to prove pathogenicity of this variant using zebrafish with initial studies suggesting evidence of perturbed sonic hedgehog signaling. Identifying the causative gene for CED in the Hutterites will allow for diagnostic testing within the population and will further our understanding of the complexities of ciliopathies in both the Hutterites and the general population.

1122F

MLL2 haploinsufficiency and nonsense-mediated mRNA decay as pathological mechanisms for the Kabuki syndrome. *G. Merla, C. Fusco, B. Augello, M.N. Loviglio, E.V. D'Addetta, A. Calcagni, L. Micale.* Medical Genetics Unit, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy.

Kabuki syndrome (KS), also known as Niikawa-Kuroki syndrome, is a rare, multiple congenital anomalies/mental retardation syndrome, mainly characterized by a very distinctive facial appearance and other clinical signs such as developmental delay, short stature, persistent fingerpads, and urogenital tract anomalies. We and others demonstrated that a large number of Kabuki patients carry mutations in MLL2 gene, the majority of which results in premature stop codon termination. MLL2 encodes an H3K4 histone methyl transferase which acts as an epigenetic transcriptional activator during growth and development. In our study we assessed whether the MLL2 protein haploinsufficiency may be a molecular mechanisms that causes Kabuki syndrome. We assayed whether MLL2 truncating mutations lead to non-functional MLL2 proteins or can result in mutant mRNA degradation through the nonsense-mediated mRNA decay (NMD), leading to MLL2 protein haploinsufficiency. Using Kabuki patients lymphoblastoid cell lines, expressing detectable levels of both MLL2 mRNA and protein, we tested whether selected nonsense mutations were substrate of NMD. By QPCR analysis we highlighted that the treatment of patient cell lines with the well-known NMD inhibitors puromycin and emetine significantly increased the steady-state levels of mutant MLL2 transcripts. Consistently, western blot analysis on patients total lysate detected a decrease MLL2 protein level. To date no functional studies have been carried out to elucidate the molecular events causing this syndrome. Our findings provide the first evidence that MLL2 transcripts carrying PTC mutations undergo NMD, which in turn reduces MLL2 expression, and further strengthen the model of haploiunsufficiency as a pathomechanism of the disease, at least in a subset of Kabuki patients.

1123F

Molecular Investigation into Cystic Fibrosis in Omani Patients. *A. Ouhtit[1], H. Al-Kindy[1], Q. Al-Salmi[2], S. Al-Yahyaee[1], M. Al-Bimani[1], M. Al-Nabhani[1].* 1) Genetics, College of Medicine & Health Sciences, Sultan Qaboos University, Al-Khod (Muscat), Al-Seeb, Oman; 2) Royal Hospital, Ministry of Health, Oman.

Introduction. Cystic fibrosis (CF) is the most common lethal autosomal recessive disorder among Caucasians (1: 3,000), and the CFTR gene is the most common mutated gene for CF, with ΔF508 as the most common mutation in Caucasians. Our preliminary pilot study in Omani population revealed a CF prevalence of 1:2,738. Objective. The objective of the present study was to determine the most common CFTR mutations in the Omani patients to establish a proper molecular genetics diagnosis of CF in Oman. Methods. Blood Genomic DNA samples from Omani patients were examined by PCR and sequencing analyses of the entire coding sequence of the CFTR gene. Results. While ΔF508 is the most common mutation in the west, S549R was the most common mutation in Oman (79%; for S549R and 16%; for ΔF508), but the rest of the patients (5%) showed 2 novel mutations in the CFTR gene. Conclusion. The common mutations identified in Omani patients will be further validated for the establishment of molecular genetic testing of CF in Oman.

1124F

A germline mutation in a family with Gaucher disease. *N. Tayebi[1], H. Saranjam[1], S. Chopra[2], H. Levy[2], E. Sidransky[1].* 1) Medical Genetics Branch, NHGRI, NIH Bethesda MD; 2) Children's Hospital Boston, Harvard Medical School, Boston, MA.

Gaucher disease (GD), the most common lysosomal storage disorder, results from the inherited deficiency of the enzyme glucocerebrosidase (GCase). Over 300 unique mutations have been identified in GBA, the gene encoding glucocerebrosidase. GD is autosomal recessively inherited, so affected individuals inherit mutations from both parents. We investigated a family with a baby diagnosed with type 2 GD. Direct sequencing of GBA demonstrated that the child's genotype was T323I /L444P. We also sequenced parental DNA. Mutation T323I was inherited from the father, but no mutation was detected in a DNA sample from the mother. This was confirmed by sequencing GBA in DNA from different maternal cells, including blood, skin fibroblasts, and buccal cells. Further analysis demonstrated that the mother's somatic cells had normal levels of GCase expression and enzymatic activity. Studies of 32 additional markers evaluated in the child and parents confirmed maternity. Based on the experiments performed, we propose that the L444P mutation is likely present only in the mother's germ line cells, and was passed on to the proband. This is the first report of the presence of a germ line mutation in Gaucher disease, and can have significant implications for genetic counseling in recessive disorders.

1125F

Complete scanning of the HFE gene in Dutch hereditary hemochromatosis patients. *M.J. van Belzen, S. Smith, E. Bakker.* Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands.

Hereditary hemochromatosis (HH) type 1 is an autosomal recessive disorder caused by mutations in the HFE gene. The disease is characterized by accumulation of iron, which mainly occurs in the liver and in a number of other tissues including the pancreas, joints, skin and heart. Clinical symptoms include fatigue, liver cirrhosis, diabetes mellitus due to pancreatic islet cell failure, arthritis, pigmentation of the skin and cardiomyopathy. Three mutations are frequent in Caucasian populations: p.Cys282Tyr (3%), p.His63Asp (15%) and p.Ser65Cys (1.4%). Our general testing strategy for patients suspected of having HH is to perform sequence analysis of exons 2 and 4 of the HFE gene for detection of these three mutations. In this study we performed sequence analysis of the remaining 4 exons of the HFE gene in 74 Dutch patients in whom only one of these mutations was identified in heterozygous form. All patients had increased ferritin levels and increased transferrin saturation. No other mutations were present in exons 2 and 4. To test for the presence of heterozygous deletions, MLPA for all six exons was performed with the commercial P347-A1 kit (MRC-Holland). Sequence analysis revealed the presence of one other mutation, the previously reported p.Leu183Pro in exon 3. This mutation was detected in heterozygous form in only one patient. Iron indices of this patient were highly increased, liver enzymes were elevated and clinical symptoms included arthritis and tanned skin. MLPA analysis did not detect any deletion or duplication of one or more exons of the HFE gene. The frequency of other HFE mutations proved to be low in the Dutch population. In only one patient in this cohort a second mutation could be identified (0.14%), and this patient showed several characteristics of manifest HH. Targeted mutation detection for the three most frequent mutations seems to be an adequate strategy in our population. Complete mutation scanning of the HFE gene should only be considered in those patients with a strong clinical suspicion of HH but without homozygosity or compound heterozygosity for the mutations in exons 2 and 4.

1126F

Candidate gene functional analysis in nonsyndromic sagittal craniosynostosis. *G. Yagnik[1], J. Liu[1], C. Stevens[1], A. Ghuman[1], S. Kim[1], J. Kim[1], E. Cherkez[1], D. Soung[2], H. Drissi[2], M. Buckley[3], T. Roscioli[3], S.A. Boyadjiev[1].* 1) Section of Genetics, Department of Pediatrics, University of California Davis, Sacramento CA,95817; 2) New England Musculoskeletal Institute, Department of Orthopaedics, Farmington, CT; 3) Department of Human Genetics, Radboud University Medical Centre, Nijmegen, Netherlands.

Premature fusion of one or more cranial sutures is a common malformation (~4 per 10,000 live births) known as craniosynostosis (CS). Approximately 85% of cases present as nonsyndromic craniosynostosis of unknown etiology. The remaining 15% are syndromic (i.e. associated with other anomalies) and follow Mendelian inheritance patterns. Previous work has shown that loss-of-function mutations in ALX4 and RUNX2 result in hypoossification of the skull, manifest as parietal foramina or large fontanels in the context of cleidocranial dysplasia. Overexpression of NELL1 results in isolated craniosynostosis while loss of its function results in skeletal hypoossification in mice models. In addition, LRIT3 was identified as a candidate gene for nonsyndromic CS via exome sequencing. Based on these data, we hypothesize that gain-of-function mutations in ALX4, RUNX2, NELL1 and possibly LRIT3 will accelerate osteoblast differentiation and result in premature suture fusion (craniosynostosis). In order to test this hypothesis, we sequenced the DNA of more than 200 sagittal probands for each of the four candidate genes. The sequencing identified a total of 21 highly conserved, rare, familial nonsynonymous variants, consisting of 18 SNPs and 3 deletions. Some of these variants have already been tested via dual-luciferase assays and have demonstrated the expected gain-of-function effect. Further functional analyses are underway.

1127F

Exome sequencing reveals a novel homozygous mutation in adult-onset autosomal recessive spinocerebellar ataxia with psychomotor retardation. *H. Doi[1,2], K. Yoshida[3], Y. Tsurusaki[1], N. Miyake[1], H. Saitsu[1], H. Sakai[1], Y. Kuroiwa[2], N. Matsumoto[1].* 1) Department of Human Genetics, Yokohama City University, Yokohama, Kanagawa, Japan; 2) Department of Clinical Neurology and Stroke Medicine, Yokohama City University, Yokohama, Kanagawa, Japan; 3) Division of Neurogenetics, Department of Brain Disease Research, Shinshu University, Matsumoto, Nagano, Japan.

Autosomal recessive cerebellar ataxias (ARCAs) are clinically and genetically heterogeneous disorders associated with diverse neurological and non-neurological features that occur before the age of 20. Currently, more than 20 causative genes for ARCAs have been identified, but approximately half of the ARCA patients remain genetically unresolved. Here, we present a Japanese family with two siblings showing psychomotor retardation and the slowly progressive type of ARCA without involvement of pyramidal tracts or peripheral nerves. To find a causative mutation, whole exome sequencing combined with homozygosity mapping was performed on both cases. As a result, only one homozygous missense mutation remained as a candidate for both cases. Expression analysis of the gene using a TaqManTM assay confirmed that the transcripts were highly expressed in human fetal and adult brain tissues as well as in the mouse brain (especially in the cerebellum). An immunohistochemical analysis clearly showed that the gene product is specifically localized in Purkinje cells of the cerebellum in humans and mice. These results strongly suggest that the mutation contributes to the degeneration of the cerebellum seen in both patients.

1128F

The role of the JAK-Stat3 signaling pathway in inherited photoreceptor (PR) degenerations (IPDs). *K.C. Jiang[1], M.J. Szego[1], A.N. Bramall[1], K.L. Wright[1], L.R. Pacione[1], W.W. Hauswirth[2], S.E. Egan[1], R.R. McInnes[1,3].* 1) Program in Stem Cell and Developmental Biology, Hospital for Sick Children Research Institute, Toronto, M5G1L7; 2) Department of Ophthalmology, University of Florida, Gainesville, Florida 32610-0284, USA; 3) The Lady Davis Institute, Jewish General Hospital, McGill University, Montreal H3T1E2.

The biochemical pathways that promote or resist PR death in IPDs are largely unknown. We previously reported identification of 9 up-regulated mRNAs encoding proteins that constitute a putative JAK-Stat3 cytokine signaling pathway in the retinas of *Rds*$^{+/-}$, *TgRHO(P347S)* and *Rd1*$^{-/-}$ mouse IPD models. By immunoblotting, we found the active forms of STAT3, pSTAT3^{Tyr705} and pSTAT3^{Ser727}, to be elevated from 2-8 fold in the retinas of these three models. Immunostaining of retinas from all three models showed the increased STAT3 and pSTAT3 to be predominantly in Müller glia, but *Stat3* mRNA expression was also found to be increased in mutant PRs using laser capture microdissection of the PR layer and qPCR: 2.4 fold (n=3, p<0.05) in 7 week old *Rds*$^{+/-}$ PRs and 5.4 fold (n=3, p<0.05) in postnatal (PN) day 12 *Rd1*$^{-/-}$ PRs. To determine the significance of up-regulated *Stat3* in mutant PRs, we first used subretinal injections of an AAV2-CBA-Cre virus, whose expression is PR-specific, to delete *Stat3* from *Stat3*$^{flox/flox}$; *Rd1*$^{-/-}$ mutant PRs. AAV2-CBA-Cre injected retinas had an average increased PR survival of 30%±6.5% (n=22; p<0.05) at PN13. In addition, when we crossed mice with PR-specific Cre expression (*Opsin-iCre* mice) to *Stat3*$^{flox/flox}$ mice with the *Rd1*$^{-/-}$ mutation, we observed 42% (n=12; p<0.001) and 44% (n= 11; p<0.001) increases in PR survival at PN13 and PN15, respectively. These results suggest that the increased *Stat3* expression in *Rd1*$^{-/-}$ PRs is pathogenetic. In distinct contrast, however, when PR *Stat3* was deleted from *TgRHO(P347S)* mice by crossing *Opsin-iCre* mice to *Stat3*$^{flox/flox}$ mice with the *TgRHO(P347S)* mutation, 40% (n=6; p<0.01) and 67% (n=5; p<0.001) decreases in PR survival were seen at PN20 and PN30, respectively. These results suggest that the increased *Stat3* expression in *TgRHO(P347S)* PRs is protective, a finding confirmed by a 38% increased survival at PN30 (n=4, p<0.05) of *TgRHO(P347S)* PRs over-expressing *wt Stat3* from a *ROSA26* transgene. We conclude that i) comparable changes in gene expression in mutant retinas can have strikingly different mechanistic effects on PR survival in different IPD models, and ii) over-expression of PR *Stat3* may have potential as a general therapy for IPDs, assuming that the *TgRHO(P347S)* model is more representative of IPDs as a whole, vs. the aggressive degeneration seen with *Rd1* mutations.

1129F

Mechanism and clinical severity of FIG4 mutations in Charcot-Marie-Tooth Disease. *M.H. Meisler[1], G.M. Lenk[1], S. Reddel[2], A.E. Grant[1], C.J. Ferguson[1], C. Towne[3], J.M. Jones[1], L.G. Biesecker[4], S.D. Batish[3], G. Nicholson[2].* 1) Dept Human Gen, Univ Michigan, Ann Arbor, MI; 2) University of Sydney, ANZAC Institute, Concord Hospital, Sydney, Australia; 3) Athena Diagnostics, Worcester MA; 4) NIH Intramural Sequencing Center, NHGRI, NIH, Bethesda MD.

Charcot-Marie-Tooth type 4J (CMT4J) is a severe recessive form of CMT caused by mutations of the lipid phosphatase FIG4. Eight new cases were identified through screening with the CMT gene panel. Three additional cases were found in a small cohort characterized by early onset and proximal as well as distal muscle weakness. These 11 previously unreported patients exhibited variable onset and severity, assymetric proximal as well as distal muscle weakness, EMG findings indicative of denervation in proximal and distal muscles, and frequent progression to severe amyotrophy and wheelchair dependence. Among the 16 known cases, fifteen have the compound heterozygous genotype FIG4I41T/null. The I41T mutation impairs interaction between the FIG4 protein and the associated scaffold protein VAC14 that is required for in vivo stability of the FIG4 protein, as demonstrated by the loss of FIG4 protein in mice homozygous for a null mutation of VAC14. FIG4-I41T is thus a hypomorphic allele encoding a protein that is unstable in vivo. A transgenic mouse model of CMT4J was generated by expression of the I41T mutant cDNA as a transgene in Fig4 null mice. Expression of only 10% of the normal level of FIG4 protein was sufficient to rescue survival and prevent neurodegeneration in the null mouse. A mouse line with lower expression survives for 3 to 6 months and provides a model of CMT4J that can be used to test therapeutic intervention to increase the abundance of the I41T protein above the threshold for survival. The observations in the mouse model suggest that up-regulation of the I41T allele in CMT4J patients could be therapeutic.

1130F

Demyelinating sensory-motor neuropathy with secondary axonopathy: a novel mutation in Connexin32. *A. Patitucci[1], C. Tortorella[2], M. Liguori[1], A. Magariello[1], R. Mazzei[1], FL. Conforti[1], W. Sproviero[1,3], L. Citrigno[1], A. Morabito[1], C. Ungaro[1], A. Gambardella[1,3], M. Muglia[1].* 1) Institute of Neurological Sciences, CNR, Mangone (CS), Italy; 2) Institute of Neurology, Department of Neurological and Psychiatric Sciences, University of Bari, Italy; 3) Institute of Neurology, University Magna Graecia, Catanzaro, Italy.

Charcot-Marie-Tooth (CMT) disease is a heterogeneous disorder of hereditary motor and sensory neuropathies characterized by progressive muscle wasting, distal muscle weakness, sensory loss, and hypo/areflexia. According to the inheritance pattern, CMT is classified as autosomal dominant, autosomal recessive, or X-linked CMT (CMTX). CMTX is the second most common form of neuropathy after CMT1A, with a frequency of approximately 20%; it is due to mutations in connexin 32 (Cx32). Cx32 protein is expressed in myelinating Schwann cells where it forms the functional channels that allow the rapid transport of ions and small nutrients between coupled cells. We report about a 55-year-old man who complained for progressive gait abnormalities started when he was 11 years old. In the following years he developed a moderate muscular hypothrophy in both his thenar and ipothenar eminences, bilateral steppage gait, and recently a subjective dysestesia in his upper and lower limbs. Electrophysiological exam revealed a generalized sensory-motor demyelinating neuropathy with secondary axonopathy. His mother and grandmother also have showed similar neurological symptoms and signs. Genomic DNA was extracted after receiving informed consent from the patient. The CMT1A duplication was tested by Real Time PCR using the relative method. Point mutations in Peripheral Myelin Protein 22 (PMP22), Myelin Protein Zero (MPZ) and Cx32 were screened by Polimerase Chain Reaction followed by direct sequencing. Duplications of 1.5 Mb on 17p11.12 locus responsible for CMT1A, PMP22 and MPZ mutations were excluded. The analysis of Cx32 gene revealed a c.171 G>T variation that leads to a glutammin-hystidin substitution at codon 57 (p.Gln57Hys); unfortunately the genetic diagnosis was not confirmed in the other relatives since they were not available for the DNA sampling. However the same nucleotide substitution was not found in 100 genotyped controls. In order to verify the pathogenic role of this Cx32 mutation, the PolyPhen program was used: the PSIC (Position-Specific Independent Counts) score difference for the two aminoacids Gln and Hys was 2.859 (>2.0), resulting in a probable damaging effect of the identified mutation. This is in line with previous evidence reporting the same substitution (p.Gln57Hys) but resulting from a different nucleotide change (c.171 G>T instead of c.171 G>C) as causative of a CMTX phenotype. Functional studies are needed in order to confirm this finding.

1131F

Identification of the genetic defect causing pontine autosomal dominant microangiopathy and leukoencephalopathy using exome sequencing. *S. Appenzeller, G. Kuhlenbäumer.* UKSH Campus Kiel, Exp. Medicine, Arnold Heller Str. 3, Building 41 24105 Kiel, Germany.

Statement of purpose: Purpose of this study is to identify the genetic defect causing pontine autosomal dominant microangiopathy and leukoencephalopathy (PADMAL) using exome sequencing. PADMAL is characterized by a leukencephalopathy with an inflammatory component, pontine lesions, slowly progredient dementia, tetraparesis and early death. The age of onset is between 12 and 60 years [1]. PADMAL is of great research interest because the pathology resembles that of the sporadic form of cerebral microangiopathy. Therefore the identification and functional analysis of the underlying genetic defect of PADMAL will help to further elucidate the pathomechanisms of PADMAL as well as contribute to a better understanding of more common sporadic forms of microangiopathies. Methods used: Linkage analysis, CNV analysis, exome sequencing, Sanger sequencing Summary: We ascertained a large multigeneration family with a new inflammatory form of microangiopathy called PADMAL. Variants in known genes causing microangiopathies (NOTCH3; TREX1, HTRA1) were excluded as causing PADMAL using linkage analysis and Sanger sequencing. We did not find Copy Number Variants >10 kb using Affymetrix Genome-Wide Human SNP Array 6.0 in two distantly related patients. Exome sequencing has previously been successfully applied in identifying the cause of monogenic diseases not amenable to linkage analysis (summarized in [2]). Exome sequencing of two distantly related individuals who share only a fraction of their genome is underway. Results will be available at the end of June. Conlusion: Recent studies have shown that exome sequencing can be successfully carried out to identify the cause of monogenic diseases. The results of the exome sequencing will be shown at the meeting. Literature: [1] MRI features of pontine autosomal dominant microangiopathy and leukoencephalopathy (PADMAL). Ding XQ, Hagel C, Ringelstein EB, Buchheit S, Zeumer H, Kuhlenbäumer G, Appenzeller S, Fiehler J. J Neuroimaging. 2010 Apr;20(2):134-40. [2] Novel genomic techniques open new avenues in the analysis of monogenic disorders. Kuhlenbäumer G, Hullmann J, Appenzeller S. Hum Mutat. 2011 Feb;32(2):144-51.

1132F

Whole-exome sequencing to tackle genetic heterogeneity in cortical malformations associated with eye and muscle defects. *M.C. Manzini[1], R.S. Hill[1], J. Rodriguez[1], J.N. Partlow[1], B. Barry[1], W.B. Dobyns[2], M.A. Salih[3], C.A. Walsh[1].* 1) Division of Genetics, Children's Hospital Boston, Boston, MA; 2) Genetic Medicine, Pediatrics, University of Washington, Seattle, WA; 3) Pediatric Neurology, King Kahlid University Hospital, King Saud University, Riyadh, Saudi Arabia.

Cobblestone lissencephaly and polymicrogyria (PMG) are cortical malformations sometimes associated with ocular defects and muscular dystrophy and appear to be caused by a disruption of normal cellular interactions with the extracellular matrix (ECM) in the basal lamina. The best-studied example are alpha-dystroglycanopathies, a group of disorders with a wide spectrum of heterogeneous brain, eye and muscle symptoms, where the transmembrane glycoprotein dystroglycan is not appropriately glycosylated perturbing its interactions with ligands in the ECM. Eight different genes, mostly involved in glycosylation, have been identified to date in alpha-dystroglycanopathies, explaining only 30-40% of cases. Several additional genes remain to be identified in the remaining 60-70% of cases and a different group of genes may be involved in patients with similar presentation where dystroglycan glycosylation is normal. In addition to extreme genetic heterogeneity, these disorders are also clinically heterogenous and the severity of the symptoms in the affected children often limits the ability to collect DNA from multiple cases in the same family to narrow the size of the linkage regions. Next-generation sequencing approaches allow us to bypass heterogeneity by analyzing each family individually. We have collected a large cohort of patients affected by cobblestone lissencephaly and/or PMG with ocular malformations and muscle defects and generated genome-wide SNP genotyping data on all affected individuals and family member. Since several of our families were consanguineous and showed likely autosomal recessive inheritance, we used homozygosity mapping to identify candidate regions. Because a large percentage of the genome resulted homozygous (3-10%) we then employed whole-exome sequencing to sequence all coding sequences in the genome and focused on the genes in the candidate regions. Multiple candidates were identified and as expected they displayed little overlap between different families with similar phenotypes underlining the genetic heterogeneity in these disorders.

1133F

Linkage and single exome analyses identify *ADCY5* as the gene for Familial Dyskinesia with Facial Myokymia. *W.H. Raskind[1,2], Y-Z. Chen[1], M.M. Matsushita[1], P.D. Robertson[3], M. Rieder[3], S. Girirajan[3], H. Lipe[4], E.E. Eichler[3,5], D.A. Nickerson[3], T.D. Bird[1,4,6].* 1) Dept Med, Univ Washington, Seattle, WA; 2) Dept Psychiatry and Behavioral Sciences, Univ Washington, Seattle, WA; 3) Dept Genome Sciences, Univ Washington, Seattle, WA; 4) Dept Neurology, Univ Washington, Seattle, WA; 5) Howard Hughes Medical Institute; 6) VISN-20 Mental Illness Research, Education, and Clinical Center, Department of Veteran Affairs, Seattle, WA.

Objective: Familial dyskinesia with facial myokymia (FDFM) is an autosomal dominant disorder that progresses from paroxysmal episodes to constant and is exacerbated by anxiety. In a five-generation family we previously mapped FDFM to chromosome 3p21-3q21. Although the 72.5 Mbp minimal linkage region was too large for traditional positional gene-by-gene mutation identification, we reasoned that the exome sequence of a single affected individual would be sufficient for this purpose. **Methods:** We performed whole exome sequencing in one affected individual and used a series of bioinformatic filters, including functional significance and presence in dbSNP or 1000Genomes databases, to reduce the number of candidate variants. Nine affected and three unaffected at-risk individuals in three generations of the family were used for co-segregation analyses. **Results:** The exome contained 23428 single nucleotide variants, of which 9391 were missense, nonsense or splice site alterations. The critical region contained 323 single nucleotide variants, five of which were not present in either of the sequence-databases. Adenylate cyclase 5 (*ADCY5*) was the only gene in which the variant (A726T) cosegregated perfectly with disease status and was not present in 1200 control exomes. This residue is highly conserved evolutionarily and the change is predicted to be damaging by both PolyPhen and Sift. **Interpretation:** ADCY5 is highly expressed in striatum, a major brain coordinator of movement. Striatal dysfunction is common in many movement disorders, including Huntington disease. Mice deficient in Adcy5 develop a movement disorder that is worsened by stress. We conclude that FDFM in this family results from a missense mutation in ADCY5. This study demonstrates the power of a single exome sequence in combination with linkage information to identify causative genes for rare autosomal dominant diseases.

1134F

Mutation in the Neurofilament Light Chain Gene (NEFL) associated with facial diplegia and marked elevation of creatine kinase. *B. Rautenstrauss[1, 2], N. Garcia-Angarita[1], B. Schlotter-Weigel[1].* 1) Friedrich-Baur-Institute, Ludwig-Maximilians-University, Munich, Bavaria, Germany; 2) Medizinisch Genetisches Zentrum, Munich, Bavaria, Germany.

Objective: to present a family with an unusual phenotype of CMT2E, caused by a known pathogenic heterozygote mutation Gln332Pro in the Neurofilament Light Chain gene (NEFL). Background: NEFL gene mutations gene are found in about 2% of the cases in a large cohort of CMT patients and cause autosomal dominant neuropathies classified either as axonal Charcot-Marie-Tooth (CMT) type 2E or demyelinating CMT type 1 (CMT1F). Methods: 3 affected members of a family with CMT2E neuropathy underwent clinical and electrophysiogical examination. Results: Age of onset was reported at age 17-25. In one case first symptoms started in the upper limbs. At presentation distal weakness and atrophy was found in variable degree dependent on disease duration. The oldest patient (> 60 years) was wheelchair bound and showed a severe phenotype with disabling proximal weakness in the lower limbs and marked atrophy distal in lower and upper limbs. As additional symptom he presented a striking facial diplegia unable to blow his cheeks or to whistle. Eyeclosure was still possible. Dysarthria and swallowing difficulties were absent, the tongue motility preserved. Sporadic fasciculations were noticed in the facial muscles. The youngest patient (28 years) was only mildly affected with slightly reduced foot extension (MRC 4/5+) but complained about myalgia and fasciculations in the lower limbs after physical activity. A blood test revealed a markedly elevated creatin kinase (CK) up to 1414 U/l whereas in the other patients CK was moderately elevated (500 U/l). There was no evident ataxia and only mild sensory deficits were documented. Tendon reflexes were decreased or absent. Electrophysiological studies of the mildly affected patient confirmed a purely axonal neuropathy with normal motor nerve conduction velocity (NCV) in upper and lower limbs, normal distal motor latencies (dmL) and low amplitudes. In the more affected patients demyelinating motor NCV ranging from 32-38 m/s were recorded pronouncly in the lower limbs. Conclusion: CMT neuropathies are genetically very heterogeneous. Up to date 13 different NEFL gene mutations have been identified and no definite phenotype-genotype correlation exists. Prominent ataxia, plantopalmar hyperkeratosis and hypakusis are described as particular features. To our knowledge this is the first report of facial diplegia in a family with CMT2E neuropathy. Furthermore the elevated CK seems a point of interest regarding the function of NEFL.

1135F

Identification and functional studies of MLC1 mutations in Chinese patients with megalencephalic leukoencephalopathy with subcortical cysts (MLC). *J. Wang[1], H. Xie[1], A. Dhauchak[2], J. Shang[1,3], H. Wei[1,3], Y. Wu[1], H. Xiong[1], Q. Gu[1], C. Ding[4], L. Wang[5], Z. Gao[5], D. Colman[2], X. Wu[1], Y. Jiang[1].* 1) Peking University First Hospital, Beijing, China; 2) Montreal Neurological Institute, Montreal,Quebec,Canada; 3) Shanxi Medical University, Taiyuan, Shanxi, P. R. China; 4) Department of Neurology, Beijing Children's Hospital Affiliated to Capital Medical University, Beijing, China; 5) Department of Neurology, Children's Hospital, Capital Children's Research Institute, Beijing, China.

Megalencephalic leukoencephalopathy with subcortical cysts (MLC, MIM# 604004) is an autosomal recessively inherited disease resulting from a deficiency of MLC1 protein. This study was to identify MLC1 mutations and perform their functional studies in Chinese patients with megalencephalic leukoencephalopathy with subcortical cysts (MLC). Methods The analysis of clinical features and MLC1 mutation screening were performed in 13 Chinese patients. The functional study of MLC1 variants was carried out by western blot, real-time PCR and confocal microscope. U373MG cells transfected by p.cDNA3.1-HA-MLC1 wildtype and mutants were immunoassayed with antibodies of HA and calnexin, an ER marker. Results A total of 10 MLC1 mutations were identified in these patients, including five novel missense mutations (c.65G>A, p.R22Q; c.95C>T, p.A32V; c.218G>A, p.G73E; c.823G>A, p.A275T; c.832T>C, p.Y278H), one novel splicing mutation (c.772-1G>C in IVS9-1), one novel small deletion (c.907_930del, p.V303_L310del), one known nonsense mutation (c.593delCTCA, p.Y198X) and two known missense mutations (c.206C>T, p.S69L; c.353C>T, p.T118M). Compared with the protein expression in WT, the protein expression in mutants A32V, G73E, S69L, T118M, Y198X, A275T and C278R were reduced in different level (all $p<0.05$), but no statistic differences was found for protein expression in mutant R22Q.Compared with mRNA expression in WT, that in mutants G73E, T118M, Y198X and A275T were at a lower level ($p<0.05$). WT MLC1 was at arstrocyte borders, whereas MLC1 mutants R22Q and A32V are mainly intracellularly localized, and S69L, G73E, T118M are mildly retained at ER. Besides, other mutations are mainly at cell borders. Conclusion Ten DNA variations of MLC1 gene were identified including seven novel mutations. MLC1 mutants associated with MLC likely disrupt MLC1 protein and mRNA expression and locolization. This would be the first report about MLC1 mutations in patients with megalencephalic leukoencephalopathy with subcortical cysts in China.(We thank the patients and their parents for their support and cooperation. This work was supported by the National Key Research Project `11-5' (2006BAI05A07), `973' Project of the Science and Technology Ministry of China (2007CB5119004), the National Natural Science Foundation of China (30973227), the International Collaborative Genetic Research Training Grant (NIH/FIC, No. D43 TW06176)) .

1136F

Genetic analysis and exome sequencing in Amyotrophic Lateral Sclerosis. *K.L. Williams[1,2], J.A. Solski[1], J.C. Durnall[1], A.D. Thoeng[1,3], S. Waraich[1,2], G. Rouleau[4], G.A. Nicholson[1,5], I.P. Blair[1,2].* 1) Northcott Neuroscience, ANZAC Research Institute, Concord, NSW, Australia; 2) Sydney Medical School, University of Sydney, NSW, Australia; 3) Department of Physiology, University of Sydney, NSW, Australia; 4) Ste-Justine Hospital Research Centre, Montreal, Canada; 5) Molecular Medicine Laboratory, Concord Hospital, NSW, Australia.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that causes the progressive degeneration of motor neurons. Familial ALS (FALS) accounts for approximately 10% of ALS cases with the remainder being sporadic (SALS). We aim to investigate known ALS genes and identify new ALS genes in a large cohort of Australian ALS families (n=165) using a combination of traditional genetic linkage approaches together with next-generation sequencing strategies. We also aim to develop a user-friendly pipeline for analysis of the raw exome sequencing data. We analysed 165 ALS families for mutations in known ALS genes and determined that mutations in these genes account for 26% of Australian ALS families, and comprise *SOD1* (17.0%), *FUS* (4.8%), *ANG* (1.8%), and *TARDBP* (2.4%) mutations. None of the identified mutations were present in a screen of 492 control chromosomes. To identify new loci for familial ALS, genome-wide linkage scans were performed in a subset of families that were negative for all known genes. An 8cM genome-wide microsatellite scan was carried out by deCODE-Iceland using 166 individuals (affected, unaffected and obligate carriers). Subsequent analyses have yielded significant and suggestive linkage to several chromosomal regions. Exome capture-sequencing (Agilent capture, SOLiD4 sequencing) has been performed among 39 individuals across 11 families. Bioinformatic analysis has required the development of a user-friendly pipeline, resulting in several new candidate ALS genes currently being screened through control cohorts. The genetic defects are yet to be identified among 74% of ALS families (122/165 families) within our cohort. The chromosomal regions implicated from our genome-wide linkage scans do not overlap previously identified loci, implicating substantial genetic heterogeneity. Linkage analysis in combination with exome capture and sequencing allows us a greater opportunity to identify novel ALS genes. The identification of these novel ALS genes will give insights into the biological basis of both familial and sporadic motor neuron degeneration, allow development of new disease models and provide new targets for therapeutic development.

1137F

Role of IL-1) in NLRP12-associated autoinflammatory disorders and resistance to anti-IL-1 therapy. *I. Jéru[1, 2, 3], V. Hentgen[4], S. Normand[5], P. Duquesnoy[1], E. Cochet[3], A. Delwail[5], G. Grateau[6], S. Marlin[7], S. Amselem[1, 2, 3], JC. Lecron[5].* 1) U.933, INSERM, Paris, France; 2) UMR S933, Université Pierre et Marie Curie-Paris6, Paris, France; 3) Service de Génétique et d'Embryologie médicales, Hôpital Trousseau, Assistance Publique Hôpitaux de Paris, Paris, France; 4) Service de Pédiatrie, Centre Hospitalier de Versailles, Le Chesnay, France; 5) EA 4331, Université de Poitiers, France; 6) Centre de référence Amyloses d'origine inflammatoire et Fièvre méditerranéenne familiale, Hôpital Tenon, Assistance Publique Hôpitaux de Paris, Paris, France; 7) Centre de référence des surdités congénitales et héréditaires, Hôpital Trousseau, Assistance Publique Hôpitaux de Paris, Paris, France.

Background. A new class of autoinflammatory syndromes called NLRP12-associated disorders (NLRP12AD) has been associated with mutations in the NLRP12 gene. Conflicting data on the putative role of NLRP12 in IL-1) signaling have been generated in vitro. Aim. This prospective study was undertaken to assess the secretion of IL-1) and three IL-1) -induced cytokines (IL-1Ra, IL-6 and TNF-( ) in patients' PBMC cultured ex vivo and to evaluate the patients' response to recombinant IL-1 receptor antagonist (IL-1Ra, anakinra), a major drug in the treatment of autoinflammatory disorders. Methods. Patients' disease manifestations and cytokine measurements were recorded before anakinra treatment was started, during 14 months of therapy, and after discontinuation of anakinra treatment. Results. Spontaneous secretion of IL-1) by patients' PBMC was found to be dramatically increased (80 to 175-fold) compared to controls. Consistently, anakinra initially led to a marked clinical improvement and to a rapid near-normalization of IL-1) secretion. However, a progressive clinical relapse occurred secondarily, associated with an increase in TNF-(secretion, persistent elevated levels of IL-1Ra and IL-6 and a reactivation of IL-1) secretion. Anakinra was discontinued after 14 months of therapy. Conclusion. Our findings provide in vivo evidence of the crucial role of IL-1) in the pathophysiology of NLRP12AD. This is the first time anakinra has been used to treat this disorder. This study provides new insights into the mechanisms underlying resistance to anti-IL-1 therapy observed in few patients with autoinflammatory syndromes. Our data also point to the potential interest of cytokine ex vivo measurements as predictors of response to treatment.

1138F

Arteriosclerosis, emphysema and abnormal elastogenesis in Schimke immuno-osseous dysplasia. *M. Morimoto[1,2], Z. Yu[3], J.M. Clewing[4], K. Choi[1,2], B. Najafian[5], C. Mayfield[6], G. Hendson[7], J.G. Weinkauf[8], A.K. Gormley[9], D.M. Parham[3], T. Lücke[10], C.F. Boerkoel[1,2].* 1) Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Rare Disease Foundation, Vancouver, BC, Canada; 3) Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 4) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 5) Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN; 6) Warren Clinic, Tulsa, OK; 7) Department of Anatomic Pathology, University of British Columbia and Children's and Women's Health Centre of British Columbia, Vancouver, BC, Canada; 8) Department of Medicine, University of Alberta, Edmonton, AB, Canada; 9) Department of Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 10) Department of Neuropediatrics, Children's Hospital, Ruhr-University Bochum, Germany.

Background SWI/SNF-related matrix-associated actin-dependent regulator of chromatin,
subfamily A-like protein 1 (SMARCAL1) deficiency diffusely alters gene expression yet
causes pathognomonic features characteristic of Schimke immuno-osseous dysplasia (SIOD),
an autosomal recessive multisystem disorder. We have hypothesized that SMARCAL1
deficiency causes disease by pathologically altering the expression of specific genes in affected
tissues.

Methods To study the arteriosclerosis and emphysema observed in SIOD, we analyzed the gene expression
and tissue morphology of SMARCAL1-deficient patient and mouse aorta and lung by
quantitative PCR, gene expression arrays, histopathology and immunohistochemistry.

Results SMARCAL1-deficient patient and mouse aorta and lung have markedly reduced
elastin mRNA and protein expression and abnormal organization of elastin fibers in the aorta.
Although the decreased expression of elastin could be arising from a direct effect of
SMARCAL1 deficiency on the elastin gene, we also find markedly reduced expression of
known transcriptional regulators of elastin expression.

Conclusion These observations provide one explanation for the arteriosclerosis and
emphysema of SIOD and a model for understanding other features of SIOD.

1139F

Clinical, Molecular, and Diagnostic Features of Hermansky-Pudlak Syndrome Type 3. *T. Gahl[1,2], R. Hess[2], T. Markello[2], E. Doherty[1], K. O'Brien[2], H. Doward[2], B. Gochuico[2], N. Cardillo[2], A. Cullinane[2], W. Gahl[2,3], M. Huizing[2].* 1) Virginia Tech Carilion School of Medicine, Roanoke, VA; 2) Medical Genetics Branch, NIH/NHGRI, Bethesda, MD; 3) Intramural Clinical Director NHGRI, OCD, NIH/NHGRI, Bethesda, MD.

Hermansky-Pudlak syndrome (HPS) is a heterogeneous disorder of albinism, bleeding and sporadic other features including recurrent infections, granulomatous colitis and pulmonary fibrosis. There are 8 human HPS subtypes. All subtypes are caused by mutations in genes coding for proteins that are organized into four complexes, designated adaptor complex-3 (AP3; HPS subtype 2), Biogenesis of Lysosome-related Organelles Complex (BLOC)-3 (HPS subtypes 1 and 4), BLOC-2 (subtypes 3, 5, and 6), and BLOC-1 (subtypes 7, 8, and 9).

After analyzing our NIH HPS cohort of 266 individuals with albinism and absent platelet dense bodies, HPS-3 was the most common subtype after HPS-1. More patients were affected with HPS-1 (195 patients; 148 with north-west Puerto-Rican 16-bp founder duplication, 47 with other mutations) than HPS-3 (34 patients; 9 with central Puerto-Rican 3,9-kb deletion, 25 with other mutations). And there were more unique mutations in *HPS1* (26) than in *HPS3* (23), but both these genes harbored significantly more mutations than other HPS genes: *HPS2* (4), *HPS4* (15), *HPS5* (14), *HPS6* (11), *HPS8* (1), *HPS9* (1). These findings classify HPS-3 as a major HPS subtype.

We report 16 novel *HPS3* mutations, including missense, nonsense, frameshift, and splice site mutations, located throughout the *HPS3* gene. We show that BLOC-2 deficiency can be diagnosed by Western blotting of patients' cell extracts using HPS5 antibodies, assisting in subtyping HPS-3 patients. Clinical analysis of the 12 unreported HPS-3 patients carrying these mutations confirmed the relatively milder phenotype of HPS-3. In contrast to AP3 and BLOC-3 deficient patients, there are no cases of molecularly subtyped HPS-3 patients (or other BLOC-2 patients) with abnormal lung findings consistent with pulmonary fibrosis, death from respiratory failure, or need for lung transplant. However, HPS-3 patients are at risk, like all other HPS subtypes (15-20%), for an inflammatory bowel disease which includes granulomatous colitis distinctive from but as severe as Crohn's disease.

1140F

Oligogenic Disease Models and `Clan Genomics'. *J. Lupski[1, 2, 3], J. Belmont[1,2], E. Boerwinkle[4,5], M. Bainbridge[1,5], C. Gonzaga-Jauregui[1], R. Gibbs[1,5].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, 77030; 2) Department of Pediatrics, Baylor College of Medicine, Houston, Texas, 77030; 3) Texas Children's Hospital, Houston, Texas, 77030; 4) Genome Sequencing Center, Baylor College of Medicine, Houston, Texas, 77030; 5) University of Texas Health Sciences Center, Houston Tx 77030.

With increasing DNA data and consideration of phenotypic heterogeneity, the dichotomous view of genetic disease as Mendelian versus common/chronic can be replaced by general oligogenic models. Using whole genome sequencing and whole exome DNA capture reagents, we have characterized more than 30 families with Mendelian disease and ~3,000 samples with common disease. The allele discovery supports oligogenic models as we have found genetic effects that add or modify the impact of primary disease mutations. In an individual with Charcot-Marie-Tooth disease we identified a pair of causative, recessive alleles in SH3TC2, and were able to correlate the single alleles with additional phenotypes: one subclinical axonal neuropathy; and the other with predisposition to carpal tunnel syndrome. Next, we identified the genetic cause of a DOPA Responsive Dystonia by discovering compound heterozygous, rare, deleterious mutations in SPR. The discovery altered patient management by the addition of a serotonin precursor that subsequently has alleviated symptoms which were not addressed by L-DOPA therapy alone. Interestingly, co-segregation with an apparent heterozygous null allele for SPR suggests a possible role for a haploinsufficiency in SPR activity in fibromyalgia; a condition that responds to SSRI. In studies of left ventricular non compaction (LVNC) we have found mutations in multiple genes - some with early onset and some that do not present before the age of 30. Both extremes may be present in a single family-in one such `solved' case we find a rare mutation is primarily responsible for the LVNC, but is modified by a common, protein changing mutation to yield a more severe phenotype. We have articulated similar models in multiple syndromes (Noonan syndrome, ciliopathies) and in restricted data sets from complex disorders e.g. autism. With the current pace of systematic collection and characterization of Mendelian families, it will be possible to complete and generalize these models, and to potentially associate overlapping phenotypes between the models by utilizing variant data from genome-wide studies. The impact of rare variants in these models means that the most important thing for individuals regarding inherited alleles is what their near relatives gave to them - the population from which you come is not that relevant. We term this `clan genomics', and postulate the general emergence of family data that govern clusters of disease predispositions.

1141F

Carrier screening in Romanian population for GJB2-35delG gene mutation causing non-syndromic deafness. *E. Severin[1], C. Dragomir[2], A. Stan[2], N. Scribann[3], L. Savu[2].* 1) Dept Human Genetics, Carol Davila Univ Med Pharm, Bucharest, Romania; 2) Genetic Lab SRL - Bucharest, Romania; 3) Georgetown Univ.Medical Ctr, Washington, DC, U.S.A.

Objective. The aim of carrier screening was to estimate the carrier frequency of the GJB2-35delG gene mutation in Romanian population. The 35delG mutation is known to be the leading one involved in hereditary hearing loss in European populations. Design. We performed a carrier screening to identify carriers of a hearing loss gene mutation among fetuses with normal hearing parents using prenatal molecular testing procedures. Samples of amniotic fluid (N=366) and chorionic villi (N=11) were taken from an unselected group of 377 unrelated pregnant women. Genomic fetal DNA was extracted and analyzed by PCR multiplex assays. Results. The rate of carriers for 35delG GJB2 mutation was 3.45% (13/377 or 1 in 29)) comparable to that of the most South-Eastern European populations. The genetic tests were considered for carrier detection and early diagnosis and not for termination of pregnancy. Conclusions. The study suggests a need for detecting the carriers. This is the first step for the construction of a national database, and provides information for health planners and policy makers to help them in planning programs and allocation resources. The impact of carrier screening results was good. No women regretted having had the test even their unborn child was identified as carrier.

1142F

5'UTR region mutations in *ENG* gene cause hereditary hemorrhagic telangiectasia. *K. Damjanovich[1], C. Langa[2], C. Bernabeu[2], F.J. Blanco[2], J. McDonald[1,3], D.A. Stevenson[4], P. Bayrak-Toydemir[1,5].* 1) ARUP Laboratories, Salt Lake City, UT, USA; 2) Centro de Investigaciones Biologicas, Madrid, Spain; 3) University of Utah, Department of Radiology, Salt Lake City, UT, USA; 4) University of Utah, Department of Pediatrics, Salt Lake City, UT, USA; 5) University of Utah, Deptartment of Pathology, Salt Lake City, UT, USA.

Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant vascular disorder characterized by arteriovenous malformations, epistaxis, and telangiectasias, caused by mutations in the coding regions of activin A receptor type II-like (*ACVRL1*) and endoglin (*ENG*) genes. However, in approximately 15% of cases mutations have not been found in the coding regions of these genes, by sequencing and deletion/duplication testing. Non-coding regions, particularly the 5'UTR region, are important in gene regulation. Knowing this importance, we reviewed our HHT clinical database and found an *ENG* variant, c.1-9G>A in two cases; homozygous in one of the cases. This variant is predicted to affect translation, yet not alter the reading frame. Expression analyses indicated the c.1-9G>A variant is a hypomorphic mutation. This prompted us to screen 154 cases for variants in the 5'UTR region that could affect transcription. These cases, with 2 or more HHT clinical criteria, had been found to be negative for mutations in the coding regions of *ACVRL1* and *ENG*. We found a c.1-127C>T mutation, which is predicted to affect translation initiation, and alters the reading frame. This mutation was found to be in a family linked to the *ENG* gene, which previous sequencing and deletion/duplication analysis revealed no mutations in the coding regions of *ACVRL1* and *ENG*. We performed a segregation study for this family, and found the mutation only in affected individuals. The mutation was found in two other cases, in one of which the mutation was found in an affected sibling. In vitro expression studies with mutant constructs indicated downregulation of the endoglin protein, which emphasizes the importance of the 5'UTR region of the *ENG* gene in the disease pathogenesis of HHT. We sequenced 134 normal cases in which our mutations were not found, further supporting the role of these mutations in the disease, and underscoring the necesary inclusion of the 5'UTR region of the *ENG* gene in clinical testing for hereditary hemorrhagic telangiectasia.

1143F

Minor splicing defect in Microcephalic Osteodysplastic Primordial Dwarfism type 1 (MOPD1, Taybi-Linder syndrome). *P. Edery[1], C. Marcaillou[2], M. Sahbatou[3], A. Labalme[1], J. Chastang[1], R. Touraine[4], E. Tubacher[3], F. Senni[1], M.-B. Bober[5], S. Nampoothiri[6], P.-S. Jouk[7], E. Steichen[8], S. Berland[9], A. Toutain[10], C.-A. Wise[11], D. Sanlaville[1], F. Rousseau[2], F. Clerget-Darpoux[12], A.-L. Leutenegger[13].* 1) Dept Gen, Biol Est Ctr, Lyon-Bron, France; Inserm U1028, CNRS UMR5292, University Lyon 1, CRNL, TIGER, Lyon, France; 2) Integragen, Evry, France; 3) Fondation Jean Dausset, CEPH, Paris, France; 4) Genetics Service, CHU Saint-Etienne, France; 5) Dept Pediatrics, Division Med Genetics, Wilmington, USA; 6) Dept Ped Genetics, Amrita Institute Med Science, Cochin, India; 7) Genetics and Procreation Dept, CHU Grenoble, France; CNRS UMR5525, University Joseph Fourier, Grenoble, France; 8) Dept Pediatrics, University Hosp Innsbruck, Austria; 9) Section Clin Genetics, Dept Pathology, St Olavs Hospital, Trondheim, Norway; Center Med Genetics Mol Medecine, Haukeland University Hospital, Bergen, Norway; 10) Genetics Service, Bretonneau Hospital, CHRU Tours, France; Inserm U930, François Rabelais University, Tours, France; 11) Center Musculoskeletal Research, Texas Scottish Rite Hospital Children, Dallas, USA; 12) Inserm U669, Villejuif, France; Paris Sud University, UMR-S669, Villejuif, France; 13) Inserm U946, Paris, France; Hematology Institute, Paris Diderot University, UMR-S946, Paris, France.

The spliceosome, a ribonucleoprotein complex that includes proteins and small nuclear RNAs (snRNAs), catalyses RNA splicing through intron excision and exon ligation to produce mature messenger RNAs, which, in turn serve as templates for protein translation. Using targeted massively parallel sequencing, we identified mutations in the *U4atac* snRNA component of the minor spliceosome in patients with brain and bone malformations and unexplained postnatal death (MOPD1, Taybi-Linder syndrome, Leutenegger A.-L. et al, Am J Hum Genet 2006;79:62-6; Edery P. et al, Science 2011;332:240-3). We also showed that minor splicing efficiency is affected in fibroblasts derived from Taybi-Linder patients and that expression of several genes containing minor introns, but not all of them, is decreased in patients' cells. We are currently performing RNA-seq (whole transcriptome) analysis in patients fibroblasts to identify signalling pathways linked to the disease phenotype. We shall present our preliminary results on new candidate pathways for abnormal neuronal migration defects and unexplained early death, which are two important features of the disease.

1144F

Mutations in SERPINF1 cause Osteogenesis Imperfecta Type VI. *E. Homan[1], F. Rauch[2], I. Grafe[1], C. Lietman[1], J. Doll[3], B. Dawson[1], T. Bertin[1], D. Napierala[1], R. Morello[4], R. Gibbs[1], L. White[1], R. Miki[5,6], D. Cohn[5,6], S. Crawford[3], R. Travers[2], F. Glorieux[2], B. Lee[1,7].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Genetics Unit, Shriners Hospital for Children and McGill University, 1529 Cedar Avenue, Montreal, Quebec, Canada H3G 1A6; 3) Department of Surgery, NorthShore University Health System Research Institute, Evanston, IL 60201, USA; 4) Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA; 5) Departments of Molecular, Cell and Developmental Biology and Orthopaedic Surgery, University of California, Los Angeles, CA 90095, USA; 6) International Skeletal Dysplasia Registry, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA; 7) Howard Hughes Medical Institute, Houston, TX 77030, USA.

Osteogenesis Imperfecta (OI) is a spectrum of genetic disorders characterized by low bone mass and fragility. It is caused by dominant mutations affecting the synthesis and/or structure of type I procollagen or by recessively inherited mutations in genes responsible for the post-translational processing/trafficking of type I procollagen. In all but one class of OI, the histopathological and clinical features cannot predict the causative gene or functional defect among affected individuals. However, recessive OI type VI is characterized by a pathognomonic increase in the amount of unmineralized osteoid, thereby suggesting a distinct disease mechanism. OI type VI is of unknown etiology and identifying the disease gene will add to our understanding of matrix mineralization and identify potential therapies. To identify the gene region, homozygosity mapping was carried out using the genomic DNA of 3 members of a consanguineous French Canadian family. A single region of homozygosity was shared by all three of these related OI VI patients defined by the markers rs8074026 and rs1362761. Next generation sequencing of one patient from this family identified homozygosity for a stop mutation in exon 4 (g.4130C>T, p.R99X) of serpin peptidase inhibitor, clade F, member 1(SERPINF1) which was confirmed by Sanger sequencing in all affect family members. The stop codon mutation was predicted to result in nonsense mediated decay (NMD) of the SERPINF1 transcript and realtime RT-PCR of patient fibroblasts confirmed that this is the case. Sanger sequencing the 8 exons of SERPINF1 in 10 unrelated patients identified additional mutations. Interestingly, the g.4130C>T mutation was identified in additional patients of French Canadian origin, suggesting the presence of a founder allele in the population. SERPINF1 encodes the 50kDa secreted protein, Pigment Epithelium-derived Factor (PEDF). Serum PEDF levels were undetectable in OI type VI patients in contrast to classical OI patients (caused by mutations affecting type I collagen) in whom PEDF serum levels were comparable to normal controls. These data suggest that mutations in SERPINF1 resulting in loss of PEDF cause OI type VI. This provides an avenue for proper OI diagnosis of patients, whereas PEDF could be measured in serum for confirmation. In addition, PEDF is a secreted protein, suggesting that therapy with recombinant PEDF could be used as a potential therapy for OI type VI patients.

1145F

Spondylothoracic dysostosis, spondylocostal dysostosis type 2, and the mutational spectrum of MESP2: protein modelling helps explain phenotypic differences from the genotype. *P.D. Turnpenny[1], M. Owens[2], K. Stals[2], E. Young[2], S. Phadke[3], I. Verma[4], O. Braaten[5], M. Nielsen[6], L. Bonafé[7], A. Cornier[8], R. Caswell[9], S. Ellard[2,9].* 1) Clinical Genetics Department, Royal Devon & Exeter Hospital, Exeter, United Kingdom; 2) Molecular Genetics Laboratory, Royal Devon & Exeter Hospital, Exeter, United Kingdom; 3) Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India; 4) Center of Medical Genetics, Sir Ganga Ram Hospital, Rajinder Nagar, New Delhi, India; 5) Department of Paediatrics, Ullevål University Hospital, Oslo, Norway; 6) Department of Clinical Genetics, Leiden University Medical Center, Leiden, Belgium; 7) Laboratory of Molecular Paediatrics, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; 8) Department for Molecular Medicine, La Concepción Hospital, San Germán, Puerto Rico; 9) Institute if Biomedical and Clinical Science, Peninsula College of Medicine & Dentistry, University of Exeter, Exeter, United Kingdom.

The mesoderm posterior 2 (MESP2) gene (MIM 605195) is a key component of the Notch signalling pathway and is integral to the determination of segmental boundary formation in axial skeletal development. It is a small gene of 2 exons with a bHLH transcription domain located within exon 1. There are two very different phenotypes associated with recessive mutations in the MESP2 gene, though both affect all vertebral segments: 1. A mild form of spondylocostal dysostosis—type 2 (SCD02, MIM 608681, the closest phenotype to `Jarcho-Levin syndrome')—with limited truncal shortening and good lung capacity; 2. The severe phenotype—spondylothoracic dysostosis (STD)—giving rise to the classical `crab-like' appearance on a chest radiograph, which is sometimes lethal in early childhood due to respiratory insufficiency. Both give rise to generalised segmentation defects of the vertebrae and an early radiological `tramline' effect of ossified vertebral pedicles that is not seen in SCD cases due to the more commonly mutated DLL3 gene (SCD01). We report a wide range of 11 published and unpublished mutations alongside the radiological phenotypes. Severe, STD cases generally had frameshift or nonsense mutations in exon 1 that result in a truncated protein that is susceptible to degradation by the nonsense-mediated decay (NMD) pathway. Two SCD02 cases had the same 4-bp duplication mutation (c.500_503dup, p.Gly169fs) in exon 1, creating an open reading frame and premature termination codon in exon 2; another SCD02 case is a compound heterozygote for substitution mutations within the bHLH domain, predicted to reduce DNA binding and transcriptional MESP2 activity. One non-lethal STD phenotype is homozygous for a deletion (c.776delC, p.Pro259fs) resulting in an open reading frame and extended termination codon at 480 in exon 2. Various mechanisms may explain the severe phenotype, including the possibility that the additional sequence interacts functionally with transcriptional coactivators, resulting in the sequestration of such factors away from functional sites of transcription. In general the genotype-phenotype correlation depends on mutational site, effect on BHLH binding, and NMD. This new MESP2 developmental gene data aids our understanding and recognition of the SCD/STD axial skeletal phenotypes.

1146F

Biochemical and Molecular data of Patients with Osteogenesis Imperfecta type II-IV in the Netherlands 1991-2011. *F.S van Dijk[1], D. Micha[1], N. Ameziane[1], M. Huizer[1], E.D. Setijowati[1,2], T. Uithuisje[1], A. Maugeri[1], G. Pals[1].* 1) Clinical Gen, VU Med Ctr, Amsterdam, Netherlands; 2) Center of Biomolecular Research, Faculty of Medicine, Diponegoro University, Indonesia.

Objectives: OI (Osteogenesis Imperfecta) is currently known to be genetically heterogeneous with an estimated 90% due to heterozygous causative variants in the COL1A1 or COL1A2 gene and approximately 10% due to causative variants in recessive OI-related genes and other unknown genetic causes. Methods: In order to determine the proportion dominant versus recessive causes of OI types II-IV in our diagnostic laboratory, we studied the molecular and biochemical data from all index patients referred to us with from 1991 until 2011, with a clinical diagnosis of OI type II, II/III, IV, I/IV. Results : More than 500 causative variants were found in COL1A1 or COL1A2. In 9 index patients bi-allelic recessive variants in CRTAP, LEPRE1, PPIB and FKBP10 were found. Not all patients were analyzed for all currently known OI-related genes. Currently, we are performing targeted exome sequencing for known and candidate genes of OI to complete the molecular data on all patients. Conclusions: We present an overview of molecular and biochemical data of patients referred to our DNA laboratory with a clinical diagnosis of OI type II-IV. Several patients with a clear clinical/radiological diagnosis of OI type II/III/IV without causative variants in the known genes for OI will be discussed more extensively.

1147F

Targeted re-sequencing of the remaining 22q11.2 region in atypical DiGeorge patients. *B.A. Nowakowska[1,2,7], J.K.J. Van Houdt[1], A. Swillen[1], K. Devriendt[1], S. Bouquillon[3], F. Novara[4], C. Le Caignec[5], M. Piotrowicz[6], W. Hawula[6], A. Kutkowska-Kazmierczak[7], E. Obersztyn[7], J.R. Vermeesch[1].* 1) Center for Human Genetics, K.U. Leuven, Belgium; 2) Foundation for Polish Science (FNP), Poland; 3) Hôpital Jeanne de Flandre, CHRU de Lille, France; 4) Genetica Medica, Universita` di Pavia, Italy; 5) CHU Nantes, Service de Génétique Médicale, Nantes, France; 6) Department of Genetics, Polish Mother's Memorial Hospital - Research Institute, Lodz, Poland; 7) Institute of Mother and Child, Warsaw, Poland.

The 22q11.2 deletion syndrome, also called the DiGeorge syndrome (OMIM 188400) is the most common chromosomal deletion syndrome in humans with an incidence of 1 in 2-4000 live births. Although the clinical presentation of 22q11.2 syndrome is variable, the major clinical characteristics of the syndrome are intellectual disability, congenital heart anomalies, velopharyngeal abnormalities and characteristic facial appearance. In addition, to the variability amongst patients with the “typical” phenotypic features, occasionally (about 1/100) 22q11.2 deletion carriers present atypical malformations. Despite intensive studies it remains unclear what causes this phenotypic variability of patients with the same deletion. We hypothesize that the distinct rare features are caused by unmasking a recessive allele which occurs at low frequency in the general population. In our study we focused on patients with 22q11.2 deletion and one of the phenotypic features outside the traditional 22q11.2 spectrum, like anorectal malformation, arthrogryposis, polymicrogyria, eye anomalies, inner ear malformations and laryngeal web. For identification of the functional role of genes within the common deletion we captured coding parts of the remaining 22q11.2 region using custom designed Nimblegen capture arrays, and re-sequenced the enriched samples with 454 GS FLX Titanium chemistry. SNP analysis of the remaining 3 Mb region, in 26 individuals with deletion and rare, atypical phenotypic feature, showed surprising high diversity of genomic variants in re-sequenced genes. The recurrent deleted region harbors approximately 50 genes. Each patient carried five to eighteen non-synonymous mutations. Overall, mutations were identified in an astonishing 29 genes and several patients carried stop mutations (which are thus nullisomic). For some of those, the nullisomic mice were shown to be embryonic lethal. Further analysis performed based on comparison of variants present in patients with the same phenotypic feature against variants found in patients without that characteristic and candidate genes responsible for particular feature, will be presented.

1148F

Phage display predicts regions on MMACHC that bind its partner MMADHC in human intracellular cobalamin metabolism. *I.R. Miousse[1], M. Plesa[2], D.S. Rosenblatt[1], J.W. Coulton[2].* 1) Department of Human Genetics, McGill University, Montreal, Canada; 2) Department of Microbiology and Immunology, McGill University, Montreal, Canada.

Protein-protein interactions are essential in human vitamin B_{12} (cobalamin) intracellular metabolism. Two key proteins involved in the early part of this pathway, MMACHC and MMADHC, have recently been shown to interact *in vitro* and *in vivo*. Mutations in *MMACHC* and *MMADHC* are the cause of rare inborn disorders characterized by a defect in the synthesis of methylcobalamin and adenosylcobalamin, cofactors for methionine synthase and methylmalonyl-CoA mutase, respectively. The human *MMADHC* gene, cloned as a fusion to maltose binding protein plus a hexahistidine tag, was expressed in *Escherichia coli*. The protein was purified on FPLC with Ni^{2+}-NTA and the tag removed with TEV protease. After immobilizing homogeneous MMADHC protein, its surface was probed with a library of bacteriophages displaying random sequences on their tail proteins (phage display). Unbound phages were washed off and bound phages were eluted and amplified for a second round of affinity-selection. From two commercially available phage libraries, 181 unique sequences with affinity for MMADHC were identified. By aligning affinity-selected sequences with the amino acid sequence of MMACHC, we assembled four regions that are predicted to interact with MMADHC. Three of these four regions are located in the C-terminal domain of MMACHC, corresponding to amino acids 221 to 238, 232 to 250 and 261 to 280. The C-terminus of MMACHC shares similarity with the C-terminus of TonB, a protein that mediates import of cobalamin in gram-negative bacteria. Interestingly, this region of TonB also mediates protein-protein interactions in bacteria, highlighting a potential similarity in function between the two proteins.

1149F

DFNB59 gene mutation screening using PCR-SSCP/HA technique in non-syndromic genetic hearing loss in province of Iran. *F. Azadegan-Dehkordi[1], E. Farrokhi[2], M. Saedi-Marghmaleki[3], M. Abolhasani[1], Gh. Banetalebi[3], M. Montazer-Zohouri[4], M. Hashmzadeh-Chaleshtori[5].* 1) Human Genetics, Cellular and Molecular Reserch Center, Shahrekord, Chaharmahal va Bakhtiyari, Iran; 2) MSc of Biochemistery, Cellular and Molecular Reserch Center, Shahrekord University of Medical Sciences, Iran; 3) BSc of Laboratory Sciences, Cellular and Molecular Reserch Center, Shahrekord University of Medical Sciences, Iran; 4) PhD Student of Medical Genetics, school of Medical Sciences-Tarbiat Modares University, Iran; 5) Professor of Human Genetics, Cellular and Molecular Reserch Center, Shahrekord university of Medical Sciences,Iran.

Background and aim: Hearing Impairment (HI) is the most prevalent Neurosensory disorder which is heterogenous and can also occur due to environmental causes. The majority of hearing deficiencies are of genetic origin affecting about 60% of the HI cases. A novel gene DFNB59 encods pejvakin has been recently shown to cause deafness. This study aims to determine the frequency of DFNB59 gene mutations in coding region the gene in Booshehr province. Method: In this descriptive experimental study, we investigated the presence of DFNB59 gene mutations in Exons (2-7) of the gene in 80 deaf subjects. DNA was extracted using standard phenol -chloroform method. The screening of gene mutations was performed by PCR-SSCP/HA procedure. Finally, the possible mutations were confirmed by direct sequencing. Results: In all, 9 polymorphisms 793C>G were found in 80 non-syndromic, genetic hearing loss subjects studied. However no DFNB59 gene mutation was identified. Conclusion: We conclude that the association of DFNB59 gene mutations with hearing loss is very low in samples studies.

1150F

Ubiquitous Expression of Glucosylceramide-Synthesizing Enzyme Accelerates Disease Phenotype in a Gaucher Disease Mouse Model. *S. Barnes[1], Y-H. Xu[1,3], Y. Sun[1,3], W. Zhang[2], K. Setchell[2,3], G. Grabowski[1,3].* 1) Dept Human Gen, Children's Hosp Med Ctr, Cincinnati, OH; 2) Div Path Lab Med, Children's Hosp Med Ctr, Cincinnati, OH; 3) Dept Pediatrics Univ of Cincinnati Coll Med, Cincinnati, OH.

Several viable mouse models of GD have been generated by introducing point mutations into the mouse GCase (gba1) locus that are common in Gaucher patients, including N370S, V394L or D409H, or D409V. Disease phenotypes in these mouse models have somewhat milder phenotypes than their human counterparts. We hypothesized that increasing substrate synthesis in these mice should produce phenotypes that more closely resemble those in humans. We created transgenic mice overexpressing the mouse glucosylceramide-synthesizing enzyme, UDP-glucose:ceramide glucosyltransferase (GCS) driven by a ubiquitous ROSA promoter and cross bred these mice into the D409V/null (9V/null) line. RT-PCR and Northern blot showed high levels of transgenic GCS expressed in all tissues tested compared with endogenous GCS. Western blot analyses detected substantial increases in GCS protein above WT levels, particularly in the lung and liver. This correlated with 2-fold increases in GCS activity compared to non-transgenic controls by in vitro assays using NBD-ceramide as a substrate. Glycosphingolipids analyses by LC/MS revealed ~2 fold increases in tissue-specific glucosylceramide species in brain, liver, spleen and lung tissues of 9 week old 9V/null mice expressing transgenic GCS (tgGCS). Increased CD68 immunostaining (markers of activated macrophage) was observed in the liver and lung of 9V/null mice expressing transgenic GCS compared to their non-transgenic littermates, mild effects were observed in brain. Overall, the presence of transgenic GCS increased the substrate load to levels comparable to 20 week old mice and accelerated the disease phenotype at least 3 weeks earlier than expected for 9V/null mice. This mouse model may be useful in determining the threshold level of substrate flux that influences phenotypic development and to test the efficacy of newer therapeutic approaches including pharmacological chaperones and substrate reduction.

1151F

Transgenic mouse model for MADA disease displays severe pathology in skin organization and a specific transcriptional profile in cutaneous fibroblasts. *M.R. D'Apice[1], A. Vielle[2], M. Bertoli[3,4], S. Sambucini[5], S. Mariani[4], S.M. Lepore[4], D. Minella[4], B. Testa[4], G. Rossi[6], G. Chillemi[7], G. Prosperini[7], S. Bueno[7], M. Federici[8], A. Botta[4], F. Amati[4], G. Novelli[1,3,4].* 1) Laboratory Medicine , Policlinico Universitario Tor Vergata, Rome, Italy; 2) The Gurdon Institute, University of Cambridge, Cambridge, UK; 3) Ospedale San Pietro Fatebenefratelli UOSD di Genetica Medica, Rome, Italy; 4) Department of Biopathology, University of Rome Tor Vergata, Rome, Italy; 5) Istituto di Ricerche di Biologia Molecolare, Pomezia, Italy; 6) Department of Public Health and Cell Biology, Tor Vergata University, Rome, Italy; 7) CASPUR, Consortium for Supercomputing Applications, Rome, Italy; 8) Department of Internal Medicine, University of Rome Tor Vergata, Rome, Italy.

Mandibuloacral Dysplasia type A (MADA; OMIM#248370) disease is characterized by postnatal growth retardation, craniofacial dysmorphism, skeletal and cutaneous features, lipodystrophy, and metabolic complications. Affected patients have a homozygous p.R527H mutation in the LMNA gene encoding nuclear lamin A/C. Since the pathogenic mechanisms of this mutation are still unknown, we generated a MADA transgenic mouse model by the random insertion of the human p.R527H LMNA cDNA. We evaluated the effects of p.R527H over-expression by pathological, histological, cellular and molecular examination. The LMNA mutation determines some aging phenotypes in adult transgenic mice. These include decreased body weight and skin thinning and disorganization. A mild significant glucose intolerance and insulin resistance was revealed when the animals were undergone to a diet rich in fat. Investigations of growth rate, life span, wound healing, and ossification ability of transgenic mice not showed significant differences compared to wild-type littermate. Mutant MADA fibroblasts, isolated from dorsal skin, showed alteration of nuclear shape, delayed cellular proliferation and increase of senescence. Additionally, their transcriptional profile showed significant alterations in the expression of gene involved in different cellular pathways. Although these data suggest that our MADA mouse model summarizes only some characteristics of the complex human phenotype, transgenic fibroblasts show an altered nuclear and cellular phenotype and a specific gene expression confirming the role of lamin A in signalling pathways affecting transcription regulation.

1152F

Pathogenic orphan transduction created by a non-reference, rare LINE-1 retrotransposon. *S. Solyom[1], A.D. Ewing[1], D.C. Hancks[1], M. Matsuo[2], Y. Takeshima[2], H. Awano[2], H.H. Kazazian[1].* 1) Institute for Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Kobe University, Kobe, Japan.

Retrotransposons ("jumping genes") are highly abundant mobile genomic elements. In particular, the long interspersed element-1 (LINE-1) class comprises 17% of the human genome. These autonomous non-long terminal repeat retrotransposons move by a potentially mutagenic "copy and paste" mechanism via an RNA intermediate that is reverse transcribed and inserted into the genome. Recently, the retrotransposition-mediated insertion of a new transcript was described as a novel cause of genetic disease, Duchenne muscular dystrophy, in a Japanese male (Awano et al. 2010). In this work, it was presumed that the inserted sequence was a single-copy, non-coding RNA transcribed from chr. 11q22.3 that was reverse transcribed and integrated into exon 67 of the dystrophin gene on chr. X. Because LINE-1 insertions are often associated with 3' transduction of adjacent genomic sequences, we suspected that the insertion might result from a 3' transduction of a LINE-1. However, no LINE-1 was present in the DNA upstream of the single copy sequence from chr. 11q22.3 in the human reference genome. Here we demonstrate through PCR-based analysis that a polymorphic, non-reference full-length LINE-1 is situated in the maternal and proband genomes at chr. 11q22.3 directly upstream of the sequence that was inserted into the dystrophin exon. This full-length LINE-1 is rarely present in the Japanese population. Thus, the likely explanation for the mutagenic insertion in the proband is a LINE-1-induced 3' transduction event with a severe 5' truncation upon insertion, such that only the 3' transduced sequence was inserted. This is the first example of LINE-1-induced human disease caused by an "orphan" 3' transduction, without insertion of the LINE-1 sequence. Therefore, any insertion of a non-repetitive sequence bearing the hallmarks of retrotransposition should be further investigated for a LINE-1 mediated transduction event.

1153F

A Novel Gene Controls Macular Retina Development, Corpus Callosum and Hippocampus Morphogenesis as well as Immune Function. *P. Bitoun[1], E. Pipiras[2], B. Benzacken[2], A. Delahaye[2].* 1) Medical Genetics, CHU Paris-Nord, Jean Verdier Hospital, Bondy, France; 2) Embryo-Cytogenetics CHU Paris-Nord, Jean Verdier Hospital, Bondy, France.

Background: Childhood Macular dystrophy usually presents around age 5-15 as Stargardt disease associated with ABCA4 mutations. The authors report a consanguineous family with a congenital syndromic macular dystrophy. Materials and Methods: Patients were examined using ocular biomicroscopy, color vision, ERG, VEP, Multifocal ERG, retinal Optical Coherescence Tomography (OCT), brain MRI karyotyping, ABCA4 sequencing, screening for known AR retinitis pigmentosa gene mutations and 105k Agilent Array CGH platform. Results: This 5 year-old proband presented with congenital nystagmus and visible macular dystrophy without auto-fluorescence, 20/200 distance vision and absent macular formation on Optical Coherence Tomography. ERG was normal while 15' and 30' pattern VEP absent response pointed to macular bundle dysfunction. She had complete corpus callosum agenesis discovered prenatally and an MRI showed hippocampi hypoplasia, she had severe liver infection manifesting as fatigue and daily afternoon sleep attacks at school with normal EEG and cytolytic hepatitis with 20x N elevation of ASAT and ALAT associated with infectious mononucleosis combined with group A beta streptococcal infection lasting for 4 months. Her 10 year-old brother with reportedly normal vision was found to have absent foveal peak on multifocal ERG bilaterally and slight hypoplasia of the junction of the posterior third of the corpus callosum. The younger 4 year-old sister was also found to have macular dystrophy without nystagmus and 20/60 vision bilaterally. The Proband was found to carry a small 115kb homozygous deletion of the 3q25 region on array CGH also present in both siblings while parents carried the same heterozygous deletion. Proband was found to harbour a double variant ABCA4 allele in cis with missense S2255I and c.6282 + 7G>A (IVS45 + 7 G>A) the pathogenicity of which are widely debated in the literature and inherited from father but not present in the other siblings. Discussion: The contribution of this ABCA4 variant in explaining the more severe proband phenotype is discussed while the macular dystrophy of the sister and, to a lesser degree, the brother confirms the role of the gene deletion alone in the etiology of the macular dystrophy . This observation identifies a developmental gene involved in retinal macular specification and development as well as corpus callosum and hippocampus morphogenesis as well as immune function.

1154F

Novel mutations in CNGA3 and CNGB3 cause Achromatopsia in the Newfoundland Population. *L. Doucette, J. Green, C. Black, G. Johnson, TL. Young.* Discipline Gen, Memorial Univ Newfoundland, St. John's, NF, Canada.

Intro: Achromatopsia is an autosomal recessive (AR) congenital disease of the cone photoreceptor affecting 1-50,000 individuals worldwide. This disorder causes severely reduced visual acuity, loss of colour vision, photophobia, and nystagmus. The majority of cases are caused by mutation of one of three genes, CNGB3 (50% of cases), CNGA3 (20-30%), and GNAT2 (2%). This study aimed to identify the molecular etiology of AR achromatopsia in five families from Newfoundland. Methods: We obtained 13 samples (eight affected; five unaffected) from five families and performed direct sequencing of three previously associated achromatopsia genes, CNGB3, CNGA3, and GNAT2. Identified variants were checked in dbSNP, underwent segregation analysis where possible, and were compared to ethnically matched controls. Mutated amino acids were checked for conservation to assess pathogenicity. Results: This approach revealed 18 variants (14 were SNPs). Two variants (CNGA3 c.1580 T>G; and CNGB3 c.887_896del) were novel and were absent in ethnically matched population controls. The CNGA3 c.1580 T>G mutation causes the replacement of a leucine residue at position 527 with an arginine (L527R). This mutation was homozygous in family 1491 and conservation analysis of the L527 residue found that it is completely conserved across 15 species. This mutation was also identified as a compound heterozygote with the previously described c.1479 T>C: p.R427C allele in family 1723. The second novel mutation, c.887_896del, was found in CNGB3 and is predicted to cause a frameshift, creating a stop codon nine amino acids downstream (p.T296NfsX9). This 10 bp deletion in CNGB3 was found to be compound heterozygous in family 1713 with the commonly reported c.1148delC mutation. We also identified the c.1148delC in the homozygous state in two other families (1442, and 1492). This study has identified two novel mutations and two previously described mutations, solving a total of five families. This observed heterogeneity confirms the complexity of achromatopsia and illustrates that different genes and mutations can be responsible for this disorder even in a genetically isolated population such as in Newfoundland.

1155F

RD3 mutations are responsible for GUCY2D-like LCA. *I. PERRAULT[1,2], S. HANEIN[1,2], N. DELPHIN[2], S. GERBER[1,2], N. ABOUSSAIR[1,3], A. MEGARBANE[4], C. EDELSON[5], C. LEOWSKI[6], J.-L. DUFIER[7], O. ROCHE[7], A. MUNNICH[1,2], J. KAPLAN[1,2], J.-M. ROZET[1,2].* 1) Genetics and epigenetics of metabolic, sensorineural diseases and birth defetcs, INSERM U781-Paris Descartes University & Institute of genetic diseases IMAGINE, Necker - Enfants Malades University Hospital, Paris, France; 2) Genetics Department, Necker - Enfants-Malades University Hospital, Paris, France 3Institute for Medical Genetics, Rabat, Morroco; 4Department of Medical Genetics, Saint Joseph University Hospital, Beyrouth, Lebanon, 5Ophthalmo-Pediatric Department, Fondat; 3) Institute for Medical Genetics, Rabat, Morroco; 4) Department of Medical Genetics, Saint Joseph University Hospital, Beyrouth, Lebanon; 5) Ophthalmo-Pediatric Department, Fondation Ophtalmologique Adolphe de Rothschild, Paris, France; 6) Institut National des Jeunes Aveugles, Paris, France; 7) Ophthalmology Department-Paris Descartes University, Necker - Enfants Malades University Hospital, Paris, France.

Purpose: Leber congenital amaurosis (LCA) is the earliest and most severe retinal degeneration. It may present as a congenital stationary cone-rod dystrophy (LCA type I) or a progressive yet severe rod-cone dystrophy (LCA type II). Fifteen LCA genes have been identified which account for approximately 70% of cases. A unique splice-site mutation in the gene RD3 was reported to cause LCA. The purpose of this study was to assess the involvement of RD3 in a large cohort of unrelated patients affected with LCA and to look for possible genotype-phenotype correlations. Families and Methods: We selected 190 unrelated LCA patients originating worldwide with no mutation in known LCA genes. The two coding exons and intron-exon boundaries of the RD3 gene were screened using direct sequencing. The clinical files of patients harbouring RD3 were revisited to search for genotype-phenotype correlations. Results: Two original RD3 mutations were identified in 5 families originating from countries on the Shores of the Mediterranean (Algeria, Morocco n = 2, Lebanon and Turkey). The two mutations are expected to truncate severely the protein. They segregated with the disease in all five families and were not found in a control panel of 113 unrelated healthy individuals, 25/113 of who originated from Mediterranean countries. One of the 2 mutations segregated with the disease in 4/5 families. Segregation analysis of microsatellite markers flanking the mutation supported the hypothesis of a founder effect in 3/4 families by showing the transmission of a small common haplotype (1Mb) with the disease. Haplotype studies and Bayesian calculations suggested that the mutation occurred 100-150 generations ago. All Patients presented with LCA type I but with moderate or absent hyperopia. Discussion and conclusion: This study gives support to the implication of RD3 in LCA families. Yet, the identification of homozygous mutations in consanguineous families originating from closely-related populations suggests that rare population-specific mutations might exist. All patients presented with LCA type 1. Interestingly, this phenotype was originally described in patients with mutations in the gene encoding GUCY2D which trafficking in photoreceptors depends on RD3. Finally, none of the patients (2-31 yrs) had renal failure, neurological symptoms or mental retardation. Owing to the retina-specific pattern of expression of RD3, it is likely that the disease may remain restricted to the retina.

1156F

Transcriptional Regulation of B°AT1 (Slc6a19), the Gene Mutated in Hartnup Disorder. *E. Tumer, S. Broer, T. Juelich.* Research School of Biological Sciences, The Australian National University, Canberra, Australia.

Hartnup disorder is an autosomal recessive disorder caused by mutations in the B°AT1 (Slc6a19) gene encoding the major transport system for neutral amino acids across the intestinal brush-border membrane. B°AT1 protein is expressed at the apical membrane of intestinal villus enterocytes along the villus-crypt axis. Mechanisms regulating B°AT1 expression along crypt to villi tips are still unknown. In this study a combination of experimental approaches were used to identify potential factors of mouse B°AT1 transcriptional regulation. Potential binding sites of transcription factors within the promoter region of B°AT1 gene were predicted with Genomatix Matinspector (genomatix.de), and the compared with changes in global gene expression pattern between crypts and villus cells using Microarray. We co-transfected these transcription factor candidates into Hek293 with B°AT1 promoter constructs of different sizes. Co-transfection of Hnf4(-expression plasmid increased B°AT1 promoter activity by 20 times when compared to the basal luciferase activity. Also, Hnf1(co-transfection showed 3 to 5 fold higher luciferase activity to a promoter only construct. The binding sites for this transcription factors are confirmed by site-directed *in vitro* mutagenesis. Both transcription factors are highly expressed in the crypt and villus cells. Sox9 is another possible transcription factor candidate; Sox9 co-transfection was sufficient to abolish the promoter activation caused by Hnf1(and Hnf4(. We hypothesize that Sox9, a transcription factor known to be involved in the differentiation of stem cells into mature enterocytes, plays a crucial role in the repression of B°AT1 in the crypt, while Hnf1a and Hnf4a were shown to interact readily with the B°AT1 promoter *in vitro*..

1157F

Expression and characterization of the ALAS2 carboxy-terminal gain-of-function mutations causing X-linked protoporphyria. *D.F. Bishop, V. Tchaikovskii, I. Nazarenko, M. Balwani, D. Doheny, R.J. Desnick.* Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY.

X-Linked Protoporphyria (XLP) (MIM #300752) is a cutaneous porphyria resulting from deletions in the last exon of the erythroid-specific 5-aminolevulinate synthase gene (ALAS2). These exon 11 deletions result in either a carboxy-terminal truncation or a replacement of the carboxy-terminus with a different polypeptide sequence. They are expressed as “gain of function” mutations that increase the enzymatic activity of ALAS2, leading to the accumulation of nearly equal amounts of free- and Zn-protoporphyrin IX in erythrocytes. The subsequent release from erythrocytes results in protoporphyrin accumulation in other tissues, particularly in the vasculature of the skin and liver. Patients exposed to light experience a painful burning sensation, due to the photosensitivity of the porphyrin in the skin vasculature. To date, the reported studies of XLP-causing mutations have used assays of crude extracts of recombinantly-expressed mutant proteins fused to the maltose binding protein (MBP) (Whatley et al., Am. J. Hum. Genet. 83:408-414, 2008). To further characterize the functional consequences of these mutations, the two previously reported ALAS2 exon 11 mutations, c.1706_1709delAGTG and c.1699_1670delAT, and a third, new XLP mutation, c.1734delG, were expressed in *E. coli*, the MBP fusion protein removed, and the mutant recombinant enzymes purified to homogeneity. The novel c.1734delG mutation resulted in a carboxy-terminal replacement of the last 7 residues with 12 new residues; SMSPPMPEKPAA. In contrast to the previous report of up to a 200-fold increase of ALAS2 activity *in vitro*, the c.1706_1709delAGTG and c.1699_1670delAT enzymes had 2- to 3-fold increases in both specific activity and the V_{max} for glycine. Both had a 1.5-fold increase in the V_{max} for succinyl CoA. Surprisingly, the homogeneous c.1734delG mutant enzyme had normal specific activity, a 25% reduced V_{max} for glycine, and a 50% reduced V_{max} for succinyl CoA. The affinity constants for both substrates were normal, as were the thermostabilities at 45°C for all three enzymes. Wild-type ALAS2 had two bands on SDS PAGE, the expected band of ~54 kDa and a proteolytically clipped band of ~52 kDa that was enzymatically active *in vitro*, but predictively less active *in vivo*. The c.1734delG mutation led to reduced proteolysis, increasing the amount of the ~54 kDa form that is active *in vivo*, thereby providing a different gain-of-function mechanism for this XLP mutation.

1158F

Mutational screening of ARID/BRIGHT and JmJC domains in *JARID1C/KDM5C* gene in Brazilian men with intellectual disability of unknown etiology. *A.P. Gonçalves, N.F. Rodrigues, M. Campos Jr, J.M. Santos, M.M.G. Pimentel, C.B. Santos-Rebouças.* Department of Genetics, State University of Rio de Janeiro, Rio de Janeiro, Brazil.

X-linked intellectual disability (XLID) is a heterogeneous set of genetic conditions caused by mutations in more than 90 different genes located at X chromosome, which affects approximately 2-3 males per 1000. Among these genes, mutations in the *Jumonji AT-rich interactive domain 1C* (*JARID1C*) gene were recently identified as potential etiologic candidates for XLID. *JARID1C* gene encodes a transcriptional regulator that has histone demethylase activity specific for di- and trimethylated histone 3 lysine 4 (H3K4) and is indispensable for epigenetic regulation. JARID1C protein contains several conserved motifs, including ARID/BRIGHT and JmJC domains. ARID/BRIGHT displays a possible involvement in the modification of the chromatin structure, whereas JmJC domain catalyzes novel histone modifications, being responsible for the demethylase activity of JARID1C. In this study, we investigated mutations in the *JARID1C* gene by screening exons 3, 4 and 5 (ARID/BRIGHT domain) and exons 11, 12 and 13 (JmJC domain) in 115 patients with ID of unknown etiology (103 from families with possible XLID and 12 from ID affected sib pairs). Genomic DNA was extracted from peripheral blood and samples were amplified by PCR, followed by direct Sanger sequencing analysis. All patients had normal karyotypes and mutations in *FMR1*, *FMR2* and *MECP2* genes were ruled out. We identified two silent mutations: the variant c.564G>A (Lys188Lys), located at exon 5 and the variant c.1794C>G (Pro598Pro), situated at exon 13. While the former was previously described in both XLMR families and control samples, being considered benign, the latter has never been reported. Nonetheless, a silent mutation at the same 1794 position (c.1794C>T; Pro598Pro) was previously found among probable XLID families, but was absent in controls. *In silico* analyses were performed to verify if the c.1794C>G variant could affect possible Exonic Splicing Enhancers (ESEs) of splicing. Rescue-ESE software did not identify any ESE in the region flanking the c.1794C>G mutation, whereas ESE finder software predicted modifications of the ESE pattern from the wild type sequence to the mutated one. Evidence have demonstrated possible harmful role of silent variants, suggesting that they could interfere, in some situations, with mRNA folding, translation speed, besides altering ESEs pattern. In this sense, the c.1794C>G mutation should not be definitively classified as benign and more investigative studies are needed.

1159F

Disease-associated NEMO mutations as a tool to unravel the key molecular mechanisms in NF-2B activation. *A. Pescatore[1], E. Esposito[1], J. Gautheron[2], G. Courtois[2], M.V. Ursini[1].* 1) Human Molecular Genetics , IGB-ABT-CNR, Naples, Italy; 2) INSERM U781, Hôpital Necker, Paris, France.

NF-2B is a ubiquitous transcription factor implicated in a wide array of physiological and pathological processes such as development, immunity tissue homeostasis and inflammation. The genetic disease that mainly contributed to elucidate of the role of NF-2B alteration in human disorder is Incontinentia Pigmenti (IP, OMIM #308300), an X-linked dominant genodermatosis caused by mutations in NEMO. IP presents with skin lesions appearing at birth and evolving in four typical stages. In addition, other neuroectodermal tissues are affected and often, a severe clinical presentation involving mental handicap was reported. NEMO is the essential subunit of the kinase complex IKK, the regulatory complex required for the canonical NF-2B activations. Further progress on the comprehension of the IP physiopathology depends on a deeper insight into the mechanisms of action of NEMO mutated protein in the context of IP pathology. Starting from the NEMO screening in a large cohort of IP patients from EU we identified few missense NEMO mutations. Among them we will present a complete analysis of two NEMO missense mutations and one short internal deletion found in IP patients. Studying the selected mutations in several cell lines: (1) we identified the lysine residues of NEMO, which are targeted by TRAF6-dependent non-degradative ubiquitination, a posttranslational regulatory modification that is essential for NEMO function; (2) we characterized the domains participating in NEMO/TRAF6 interaction and we provide genetic and molecular evidences supporting the role of this physical interaction in NF-2B activation; (3) we produced exciting new information concerning the identity of the NF-2B-dependent signaling pathways such as IL-1beta and TNF-alfa that play a critical role in the manifestations of the disease. More important, dissecting specific molecular interactions within the NEMO-mediated NF-2B activation could suggest new targets for the development of potentially effective therapeutic strategies, not only for this specific pathology but also for more common pathologies resulting from NF-2B dysfunction. Supported by Telethon GGP08125 and Association Incontinentia Pigmenti France (http://www.incontinentiapigmenti.fr).

1160F

Regulation of Dendritic Spine Characteristics in Mouse Brains by Members of the Fmr1 Gene Family. *R. Zong[1], Z. Fang[1], D. Parghi[2], D. Nelson[1].* 1) Molec & Human Gen, Baylor College Med, Houston, TX; 2) Intellectual and Developmental Disabilities Research Center (IDDRC), Baylor College of Medicine, Houston, TX.

Absence of the RNA-binding protein FMRP (Fragile X Metal Retardation Protein) causes Fragile X syndrome, the most common form of inherited mental retardation. Abnormal dendritic spine development was observed in both human patients and in mice lacking FMRP. There is increasing evidence to support that FMRP modulates synaptic plasticity by negatively regulating translation of mRNAs, however specific targets and molecular mechanism in vivo for this action remain largely unknown. Qualitative examination of human brain autopsy material has shown that fragile-X patients exhibit abnormal dendritic spine lengths and shapes on parieto-occipital neocortical pyramidal cells. In animals, similar quantitative results have been obtained in Fmr1 knockout mice as dendritic spines were examined by using the Golgi-Cox method. We have examined dendritic spines in mice with mutations in Fmr1, as well its paralogs Fxr1, and Fxr2 using the Golgi method, and carried out quantitative analysis of dendritic spine length, morphology, and density to demonstrate the regulation of dendritic spine development by FMRP, FXR1P, and FXR2P. Behavioral studies have also suggested that Fxr1 and Fxr2 share functional overlap with Fmr1 in mice. In this study, we demonstrate that mice lacking any of these proteins (FMRP, FXR2P, or FXR1P) individually exhibit abnormal dendritic spines in the visual cortex. Phenotypes include increased spine length, elevated spine density, and an immature morphology. To further demonstrate the functional overlap of these proteins, abnormal dendritic spine phenotypes were found to be exaggerated in Fmr1/Fxr2 double knockout mice. We also found elevated spine densities in Purkinje neurons of mutant mice. Our data are consistent with the concept that this class of RNA binding proteins together plays an important role in normal spine structural maturation and possibly in pruning. Finally, we demonstrate the ability to establish and/or rescue dendtitic spine abnormalities in adult mice that have alleles which allow condition expression or ablation of FMRP. This finding suggests that this aspect of the Fragile X phenotype may be amenable to treatment beyond the juvenile period.

1161F

The Role of Methylation in Reversible Gene Expression in The Airways of Former Smokers. *GL. Stewart[1], EA. Vucic[1], R. Chari[2], S. Lam[1], WL. Lam[1].* 1) Dept. of Integrative Oncology, BC Cancer Research Centre, Vancouver, BC, Canada; 2) Dept. of Genetics, Harvard Medical School, Boston, MA.

Background: Smoking related disease including lung cancer (LC) is a leading cause of morbidity and mortality, and by 2030, it is predicted to kill one in every 6 people in the world. Surprisingly, half of newly diagnosed LC patients are former smokers (FS), who remain at an elevated risk of LC years after quitting. Cigarette smoke induces DNA damage in airway and lung tissues at the genomic and epigenomic levels, where it is associated with changes to gene expression. Upon smoking cessation, both reversible and irreversible changes in gene expression have been described in studies on current (CS), FS, and never smokers. As DNA methylation is both a heritable and reversible epigenetic gene regulatory mechanism that is highly altered in cells lining the airways of smokers, we hypothesize that the reversible nature of genes differentially expressed in bronchial epithelial cells in the airways of CS and FS, may be due to changes in DNA methylation Methods: Bronchial epithelial cells were obtained from brushings of small airways (< 2mm diameter) during bronchoscopy from CS (n=12) and FS (n=6). Global gene methylation profiles were generated using the Illumina Infinium Human Methylation27 platform. Methylation results were aligned to the set of reversibly expressed genes described in previous studies, and genes that were differentially expressed and methylated upon smoking cessation were selected for further study. Results: Methylation and expression analysis identified 9 genes to be overexpressed and hypomethylated, and 3 genes to be underexpressed and hypermethylated in CS relative to FS. The former 9 included GPX2 and GSTA2, genes involved in central metabolic pathways activated in response to halogenated and polycyclic aromatic hydrocarbons, and detoxification of reactive oxygen intermediates. These pathways have been previously shown to be upregulated in CS. The latter 3 included SYF2 and CXCL6, involved in cell cycle regulation and inflammatory disease, respectively. Conclusion: The identification of differentially methylated and expressed genes between CS and FS may provide insight into the mechanism of smoking related disease. Significantly, as DNA methylation is a reversible gene regulatory mark, this knowledge may lead to the application of preventative epigenetic therapeutics for CS and FS and help reduce the burden of smoking related disease.

1162F

Genome-wide association analysis of genetic determinants for lethal prostate cancer. *R. Szulkin[1], S. Benlloch[2], A. Amin Al Olama[2], D. Easton[2], R. Eeles[3], Z. Kote-Jarai[3], M. Guy[3], K. Govindasami[3], H. Grönberg[1], F. Wiklund[1], PRACTICAL.* 1) Department of Medical Epidemiology and Biostatistics,Karolinska Institutet, Stockholm, Sweden; 2) Centre for Cancer Genetic Epidemiology, University of Cambridge, Strangeways Laboratory, Cambridge, UK; 3) The Institute of Cancer Research, Sutton, Surrey UK.

Given a prostate cancer diagnosis today, it is hard to predict how the disease develops. Prognostic tools, such as clinical stage (T), pathological grade (Gleason) and prostate-specific antigen (PSA) levels perform suboptimally in distinguishing those who will develop a lethal prostate cancer from those with a less aggressive disease. Thus, there is an urgent need for improved tools to guide clinicians in treatment decisions. There is a strong genetic component in prostate cancer development and several risk susceptibility regions have been identified. However, none of these have been proven to discriminate between lethal and non-lethal prostate cancer. We have performed a genome-wide association study (GWAS) to identify inherited genetic variants associated with prostate cancer mortality. In the first stage, we examined the association between SNPs (Single-nucleotide Polymorphisms) and mortality in a combined analysis of a Swedish study, CAPS (Cancer of the Prostate in Sweden) and a British study, UK 1. The CAPS study included 1,932 prostate cancer cases of whom 422 had died from the disease at the end of follow-up (June 2009), genotyped using the Affymetrix 500k array. UK1 included 1820 patients, of whom 206 patients were deceased, genotyped using the Illumina 550k array. Imputation using Hapmap CEU as reference was used to estimate genotypes for a common set of 1.6 million SNPs for analysis. In the combined analysis 10 SNPs were associated at p<10-5 and 78 SNPs at p<10-4. The strongest associations were found in chromosomal regions 7q11 (SNP rs17139313, p=4.3*10-8) and 7q31 (SNP rs2299556, p=9.1*10-7). These were genotyped in a further 18,194 prostate cancer patients, of which 712 had died from the disease, within the PRACTICAL (PRostate cancer AssoCiation group To Investigate Cancer Associated aLterations in the genome) consortium. Neither association replicated in PRACTICAL. Genotyping of the 5,000 top survival associated SNPs, from the first stage is currently ongoing. These results emphasise that large sample sizes are required to reliably identify genetic determinants of cancer survival.

1163F

The Role of Notch Signaling in the Pathogenesis of Osteosarcoma. *J. Tao[1], M. Jiang[1,4], T. Bertin[1], F. Gannon[2], L. Donehower[3], B. Lee[1,4].* 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Pathology and Immunology, Baylor College of Medicine, Houston, TX; 3) Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX; 4) Howard Hughes Medical Institute, Houston, TX.

Human osteosarcoma (OS) is the most common primary bone cancer, comprising approximately 20% of all bone tumors and about 5% of pediatric tumors overall. Most OS occurs sporadically and our understanding of its molecular basis is still limited. Recently, we found that human and mouse p53 mutant osteosarcoma tumor samples show evidence of upregulated Notch signaling. Moreover, chemical or genetic inhibition of Notch signaling decreased tumor growth in a xenograft mouse model of human osteosarcoma. Notch signaling plays an important role in the developmental processes and tissue homeostasis. Altered Notch signaling has been associated with several cancers in which Notch can act both as an oncogene and tumor suppressor depending on its spatiotemporal expression. To generate a mouse osteosarcoma model and examine the role of Notch signaling in bone tumorigenesis, we utilized a bitransgenic mouse model that constitutively expresses a single copy of Notch NICD in osteoblasts. The resulting bitransgenic Notch gain-of-function (GOF) mice developed osteosclerosis at early age. Histomorphometric and molecular analysis of cavarial and long bones indicated that Notch could stimulate proliferation of immature osteoblasts while inhibiting their differentiation into mature osteoblasts. This gain of function phenotype was reminiscent of osteoblastic tumors. Indeed, aging studies of these GOF mice showed that they spontaneously developed bone tumors with complete penetrance and had a survival mean time of 9.5 months. To explore a genetic interaction of Notch and p53 pathways in the pathogenesis of OS, the GOF mice were bred with p53-conditional mice. We found that p53-null GOF mice showed significantly shortened latency prior to tumorigenesis, as mice developed OS at a mean time of 5 months. This result suggests that the genetic interaction of Notch and p53 plays a critical role in OS development. Together, our data support that p53-Notch interaction may be a dominant mechanism in OS pathogenesis.

1164F

Molecular Study of PCA3 gene variations in Iranian patients affected with prostate cancer. *A. TAVAKOLI TAMEH, S. MATOO, A. YASARI MAZANDARANI, B. SEDAGHATI KHAYAT, N. HATAMNEJADIAN, SH. ABADPOUR, M. MAHDAVI, A. RASHID FARROKHI, A. EBRAHIMI.* Dr. Ahmad Ebrahimi Molecular Genetics , PhD Parseh Medical Genetics Counselling Center, Floor 7, No.75, Royan Alley,Keshavarz Bolv. Tehran, Iran Tel-Fax: +98 21 88966579 ,88996889 E-mail: ae35m@yahoo.com.

Introduction: Prostate Cancer is the most common cancer in men over 35 years old. Using biomarkers for early diagnosis in this cancer could be useful. Early researches show that increasing PCA3 expression in patients with prostate cancer could be a remarkable diagnostic biomarker in decision-making for biopsy. So studying the molecular changes of PCA3 gene in prostate cancer can be helpful. Materials and methods: Fifty patients and 100 healthy people were chosen according to the clinical examinations and preclinical work ups. After genetic counseling and preparing consent information, the proband blood samples were collected and DNA samples were extracted. The PCR product using intronic primers were amplified and then analyzed by direct sequencing. Results: The analysis showed various sequence, including some new SNPs and mutations. Studies also suggest that patients with prostate cancer have a rational pattern of STR population in exon 3 of PCA3 gene. This study same as previous molecular studies in Iranian population, revealed that there is a high level of heterogeneity in Iranian multi-ethnic population. Key words: Prostate cancer, PCA3, STR, PCR.

1165F

Exploring a *PALB2* c.3113G>A breast cancer multiple-case family for other genetic risk factors via exome sequencing. *Z.L. Teo[1], D.J. Park[1], F. Odefrey[1], F. Hammet[1], T. Nguyen-Dumont[1], H. Tsimiklis[1], B.J. Pope[2], A. Lonie[2], G.G. Giles[3], J.L. Hopper[4], M.C. Southey[1], ABCFS.* 1) Genetic Epidemiology Laboratory, Department of Pathology, The University of Melbourne, Victoria, Australia; 2) Victorian Life Sciences Computation Initiative , Victoria, Australia; 3) Cancer Epidemiology Centre, The Cancer Council Victoria, Victoria, Australia; 4) Centre for Molecular Environmental Genetic and Analytical Epidemiology, School of Population Health, The University of Melbourne, Carlton, Victoria, Australia.

PALB2, the binding partner of BRCA1 and BRCA2, is involved in homologous recombination repair. Studies of multiple-case breast cancer (BC) families have reported that mono-allelic *PALB2* mutations, on average, increase BC risk two to six fold. Proven genetic modifiers of BC risk for *BRCA1* and *BRCA2* mutation carriers are now numerous. Despite being associated with small changes in risk individually, collectively they may account for considerable risk modification for some women. In a population-based study of BC we used five population-based families and estimated the cumulative BC risk of carrying the *PALB2* c.3113G>A mutation to be 91% (95%CI=41-100) by age 70, similar to 65% (95%CI=44-78) and 45% (95%CI=31-56) average risk estimates for *BRCA1* and *BRCA2* mutation carriers respectively. One of these pedigrees, negative for *BRCA1*and *BRCA2* mutations, has ten diagnoses of BC (5 under the age of 50 years). The pedigree structure does not allow for all cases to be explained by a single genetic factor and two affected women do not carry the *PALB2* mutation (both maternal side). In view of such a high risk estimate and observations of non-*PALB2* mutation carrier cases in this pedigree, we hypothesise that there may be other genetic risk factors segregating through this family. Presence of other genetic risk factors, in addition to *PALB2* c.3113G>A, could alter the risk for mutation carriers and/or convey BC risk to family members who are not *PALB2* mutation carriers. These additional factors could further explain the BC cases in non-*PALB2* mutation carriers, the multiple cases of early onset BC and the high estimated risk of c.3113G>A in this family. We performed whole exome capture followed by massively parallel sequencing on four strategically selected affected females of this family: the proband, her sister and their paternal and maternal cousin. In this family, we have identified 15 predicted protein damaging variants in genes with plausible relevance to cancer aetiology. Eight such variants are protein truncating or are mutations predicted to have strong effects on splicing efficiency, two of which have been observed in key DNA repair genes. In summary, six of the genes have roles in DNA repair, three in cell cycle checkpoints regulation, one controls telomerase activity and another has been reported to predispose to prostate cancer. Further analyses of these candidate genes to determine their relevance to BC predisposition will be presented.

1166F

The chromosome 2p21 region harbors a complex genetic architecture for association with risk for renal cell carcinoma. *J. Toro[1,2], S.S Han[1], M. Yeager[1,3], L.E. Moore[1], M.H. Wei[1], R. Pfeiffer[1], M.P. Purdue[1], M. Johansson[4], G. Scelo[4], C.C. Chung[1,3], V. Gaborieau[4], D. Zaridze[5], K. Schwartz[6], N. Szeszenia-Dabrowska[7], F. Davis[8], V. Bencko[9], V. Janout[10], D. Albanes[1], L. Foretova[11], L. Burdett[1, 3], C.D. Berg[12], W.R. Diver[13], J. Virtamo[14], J.D. McKay[4], N. Chatterjee[1], P.S. Rosenberg[1], N. Rothman[1], P. Brennan[4], W.H. Chow[1], S.J. Chanock[1].* 1) Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA; 2) Medical Service, Veterans affairs Medical Center, Washington, DC.,USA; 3) Core Genotyping Facility at the Advanced Technology Center of the National Cancer Institute, NIH, SAIC-Frederick Inc., National Cancer Institute-Frederick, Frederick, Maryland, USA; 4) International Agency for Research on Cancer (IARC), Lyon, France; 5) N.N. Blokhin Cancer Research Centre, Moscow, Russia; 6) Karmanos Cancer Institute and Department of Family Medicine, Wayne State University, Detroit, Michigan, USA; 7) Department of Epidemiology, Institute of Occupational Medicine, Lodz, Poland; 8) Division of Epidemiology and Biostatistics, School of Public Health, University of Illinois at Chicago, Chicago, USA; 9) Charles University in Prague, First Faculty of Medicine, Institute of Hygiene and Epidemiology, Prague, Czech Republic; 10) Palacky University, Olomouc, Czech Republic; 11) Department of Cancer Epidemiology and Genetics, Masaryk Memorial Cancer Institute, Brno, Czech Republic; 12) Division of Cancer Prevention, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA; 13) Epidemiology Research Program, American Cancer Society, Atlanta, Georgia, USA; 14) Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland.

In follow-up of a recent genome-wide association study (GWAS) that identified a locus in chromosome 2p21 associated with risk for renal cell carcinoma (RCC), we conducted a fine mapping analysis of the 120kb region flanking the EPAS1 gene. We genotyped 59 tagged common single nucleotide polymorphisms (SNPs) across a 120kb region flanking the EPAS1 gene in 2,278 RCC cases and 3,719 controls of European background. Genotypes surrounding EPAS1 were imputed using two publicly available reference data sets: 1000 Genomes Project March 2010 release and Phase III HapMap. To validate the association signals, top hits were genotyped in five studies (2,480 RCC cases and 4,203 controls). A conditioned analysis was performed to determine if the effect for new SNPs was independent of the previously reported markers in the GWAS. In an analysis of controls, SequenceLDhot was used to identify recombination hotspots between two signals. We observed a novel signal for rs9679290 (P= 5.75x10-8; OR= 1.27, 95% CI: 1.17-1.39 per allele). Imputation of common SNPs surrounding rs9679290 using HapMap III and 1000 Genomes data yielded two additional signals, rs4953346 (P= 8.66 x 10-10; OR=1.31, 95% CI: 1.20-1.43 and rs12617313 (P = 9.82 x 10-9; OR=1.33, 95% CI: 1.31-1.46, both highly correlated with rs9679290 (r2 greater than 0.95), but interestingly did not correlate with the two SNPs reported in the GWAS: rs11894252 and rs7579899 (r2 =0.05 and 0.08 with rs9679290). Genotype analysis of rs12617313 confirmed an association with RCC risk (P=1.72 x 10-9; OR= 1.28, 95% CI: 1.18-1.39 per allele), which could be independent of the previously reported GWAS association signal based on a conditional analysis and the presence of an intervening recombination hotspot detected using SequenceLDhot software. Cases with germline EPAS1 variants in the new region were more likely to have tumor VHL alterations, with the strongest association observed for rs4953346 (P= 0.01, OR= 2.41, 95% CI, 1.24-4.67). Our investigation suggests a second locus separated by a recombination hotspot in the chromosome 2p21 and a more complex genetic architecture for common variants in this region associated with RCC.

1167F

Prevalence of *BRCA* mutations among black South African women with breast cancer. *E.J. van Rensburg, M.D. Sluiter, C.M. Dorfling, N. Roelofse.* Dept Human Genetics, University of Pretoria, Pretoria, South Africa.

Cancer of the breast is the most common cancer in South African women. When arranged by population group, breast cancer ranks first in white and Asian women and second in black females. Overall the lifetime risk for developing cancer of the breast is 1 in 27 for all South African women, ranging from 1 in 12 for white women to 1 in 51 for black women (Mqoqi et al., 2004: Incidence of histologically diagnosed cancer in South Africa, 1998-1999, National Cancer Registry). It has been estimated that 15 - 20% of female breast cancers are due to inheritance of highly penetrant mutations in either of two breast cancer susceptibility genes, *BRCA1* and *BRCA2*. Germ-line mutations within the *BRCA*-genes are responsible for different proportions of inherited susceptibility to breast cancer in different populations. To date there is little information regarding the prevalence of *BRCA* mutations in black South Africans. In order to determine the proportion of black South African women who carry *BRCA* gene mutations we screened a hospital-based cohort of 287 black women (unselected for family history of cancer), using PTT and PCR-SSCP/Hetero-duplex analysis. MLPA analysis was carried out to detect large rearrangements in BRCA1. The ages at diagnosis of breast cancer ranged from 18 - 85 years, with 202 of the women diagnosed at or before age 55. Three frame-shift mutations (with one recurring 4 times), one novel large genomic rearrangement in *BRCA1* and, four frame-shift and one nonsense mutation in *BRCA2* were identified in 13/287 (4,5 %) of the women - all of whom were diagnosed before age 55 (13/202; 6,4%). In this group, 13,6% of women diagnosed with breast cancer at 18 - 35 years of age carried a *BRCA* mutation. Only one of the 13 women with a *BRCA* mutation reported a family member with breast cancer (an affected sister), thus family history of cancer is not an indicator of *BRCA* mutation status in this cohort. Using this data one can calculate the incidence of *BRCA*-associated breast cancer among black women in South Africa. With the lifetime risk of breast cancer for black women at 1 in 51, this would translate to approximately 1 in 1150 persons of the general population.

1168F

Novel Tools to Study of the EGFR/HER2 Pathway Utilizing Targeted Integration via Zinc Finger Nucleases and an SH-2 Domain Based Biosensor. *D. Vassar, H. Zhang, N. Zenser, F. Zhang, A. Samsonov, J. Fetter, D. Malkov.* R&D, Cell Based Assays, Sigma-Aldrich, St Louis, MO.

HER2 and EGFR are both members of the ErbB family of receptor tyrosine kinases.The study of this receptor family,especially HER2,is of high therapeutic interest as their overexpression has been associated with numerous types of cancer. We have developed novel genetically engineered cell lines as unique research tools to study these receptors. Zinc finger nucleases(ZFNs) are synthetic proteins engineered to bind DNA at a sequence-specific location and create a double strand break. The cell's natural machinery repairs the break in one of two ways: non-homologous end joining or homologous recombination. We utilized the homologous recombination pathway to insert a transgene into a defined location of the specific genes.In this case, the desired location was the near the C-terminus of HER2 and/or EGFR and the transgene chosen was a fluorescent reporter protein.The fluorescent protein was stably integrated into the cells preserving endogenous regulation of gene expression and protein function. The specificity of the target integration process allows for the integration of multiple reporter genes into a single cell line. We therefore tagged both EGFR and HER2 in the human ovarian adenoma cell line, SKOV-3,to permit study of their interactions in cellular processes and develop cell based assays for efficient screening of compound libraries. In addition to endogenous tagging of specific genes, we also designed a natural domain-based, genetically encoded biosensor for EGFR by transduction with lentivirus. The biosensor is composed of a protein-binding domain, derived from natural domain-binding proteins,fused to a fluorescent protein that serves as a reporter.Specifically, a biosensor consisting of the SH2 domain of the adaptor protein,Grb2,fused to GFP was developed and tested in human cell lines having endogenous EGFR expression such as the A549. The biosensor was either transiently transfected or stably expressed in the cells using an exogenous promoter.In each case the EGFR biosensor showed robust redistribution towards the cell membrane indicative of receptor activation. Unlike biochemical assays and immunohistochemical staining, both approaches described here allow for live cell imaging studies of endogenous EGFR and HER2 activity making them ideal for high content analysis assays.Our findings suggest that both strategies can be applied to the study of other receptor tyrosine kinases and possibly additional cellular signaling pathways.

1169F

Interleukin-10 Promoter Polymorphism (-1082 G/A) and Gastric Cancer. *A. Venkateshwari[1], B. Amar Chand[1], D. Krishnaveni[1], B. Prabhakar[2], K. Pandu Ranga Rao[2], P. Anitha[2], N. Pratibha[3], A. Jyothy[1].* 1) Cell Biology, Institute of Genetics and Hospital for Genetic Dis, Hydrabad, Andhra Pradesh, India; 2) Department of Gastroenterology, Osmania General Hospital, Hyderabad - 500 001, India; 3) Department of Genetics, Osmania University, Hyderabad, India.

Gastric cancer (GC) is one of the most commonly diagnosed malignancies and remains a considerable public health problem worldwide. Gastric cancer is a genetic disease developing from a multifactorial, multigenetic and multistage process with the involvement of both genetic and environmental risk factors. Genetic variation in genes encoding cytokines and their receptors, determine the intensity of the inflammatory response, which may contribute to individual differences in the outcome and severity of the disease. Interleukin (IL)-10, an important anti-inflammatory and immuno-suppressive cytokine produced by T-lymphocytes, down-regulate the expression of Th1 cytokines and acts as co-stimulatory molecule and regulate angiogenesis in various cancers. In the present study, we investigated the association between the IL-10 -1082 G/A polymorphism and the susceptibility to gastric cancer in south Indian population. A total of 164 endoscopically and histopathologically confirmed gastric cancer patients and 160 healthy control subjects were included in the study. The genotyping of IL-10 -1082 G/A polymorphism was carried out by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). Statistical analysis was applied to test for the significance of the results. The distribution of IL-10 genotypes at -1082 G/A were GG 19.51 %, GA 36.59% and AA 43.9 % in gastric cancer patients and GG 31.25 %, GA32.5 % and AA36.25 % in control subjects. We observed increased frequency of low expresser allele A in GC patients in comparison to controls [OR = 1.49 (CI = 1.089-2.036), p value = 0.0156]. Thus, the allele A of IL-10 gene promoter polymorphism may be one of the important genetic risk factor in the development of gastric cancer.

1170F

TP53 Codon 72 Polymorphism and risk of Acute leukemia. *S. Vure[1], N. Dunna[1], K. Sailaja[1], D. Surekha[1], S. Rajappa[2], D. Raghunadharao[2], S. Vishnupriya[1].* 1) Department of Genetics, Osmania UNiversity, Hyderabad, AP,; 2) Department of Medical Oncology, Nizams Institute of Medical sciences, Hyderabad, India.

TP53 is the mostly commonly mutated gene in many cancers. The TP53 tumor suppressor protein is involved in multiple cellular processes, including transcription, DNA repair, genomic stability, senescence, cell cycle control and apoptosis. A common single nucleotide polymorphism located within the proline rich region of TP53 gene at codon 72 in exon 4 encodes either proline or arginine. TP53 Arg 72 is more active than TP53 Pro 72 in inducing apoptosis. The frequency of TP53 Arg 72 allele is higher than TP53 Pro72 allele. This might indicate that the hyper mutability observed for TP53 Arg allele might lead to unstable protein and lack of tumor suppressor ability leading to myeloid malignancies. The aim of the study was to understand the association of 72 codon polymorphism with acute leukemia development and prognosis of the disease. The present study includes 288 acute leukemia cases comprising of 147 acute lymphocytic leukemia (ALL), 141 acute myeloid leukemia (AML) and 245 control samples for analysis of TP53 Codon72 polymorphism using PCR-RFLP method. Significant association of homozygous arginine genotype with acute leukemia was observed (χ2-133.53; df-2, p - < 0.000). When data was analyzed with respect to clinical variables, the elevation in Mean WBC, Blast %, LDH levels and slight reduction in DFS in ALL cases with arginine genotype was observed. AML patients with Pro/Pro had elevated WBC, Blast%, LDH levels with slightly reduced DFS. Unlike other polymorphisms, the genotype association with respect to clinical variables was found to be different in ALL and AML. Hence our study indicates that Arg/Arg genotype might confer increased risk to development of acute myeloid leukemia.

1171F

Genome-wide analysis of genetic alterations in a mouse mutagenesis model of acute lymphoblastic leukemia. *L. Wei[1], J. Dang[1], J. Zhang[2], L. Janke[1], L. Weyden[3], A. Rust[3], D. Adams[3], J. Downing[1], C. Mullighan[1].* 1) Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN; 2) Department of Computational Biology and Bioinformatics, St. Jude Children's Research Hospital, Memphis, TN; 3) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1HH, U.K.

Genome-wide profiling studies of acute lymphoblastic leukemia (ALL) have identified multiple novel recurring genetic alterations of unknown pathogenic significance. Alterations of PAX5 (encoding the lymphoid transcription factor PAX5) by heterozygous mutations in over 30% of B-ALL cases, suggesting that PAX5 is a haploinsufficient tumor suppressor. To test this hypothesis, we mutagenized Pax5+/+ and Pax5+/-) mice with the alkylating agent ENU or Moloney murine leukemia virus (MLV), and performed genome-wide analysis of genetic alteration in the resulting tumors. Pax5 haploinsufficiency significantly increased the penetrance and reduced the latency of B-ALL in both models including Array-CGH, Sanger sequencing, exome sequencing (39 tumors), and retroviral integration site (RIS) sequencing (128 tumors) identified additional genetic alteration in B lymphoid transcriptional regulators in the tumors, notably Pax5 sequence mutations identical or similar to those in human ALL in 60% of ENU tumors of 128 tumors. We also identified recurring (>2 samples) non-silent mutations in 86 genes in addition to Pax5, 11 of which are known to be mutated in cancer. For these known cancer genes, most of the mutations were identical to mutations previously observed in human cancer and leukemia, including (known/total) Jak1 (4/5), Jak3 (9/11), Ptpn11 (7/7), Kras (4/4), Nras (3/3). Indel mutations were identified in Cdkn2a (n=1) and Notch1 (n=1) in T-lineage ALL. Sh2b3 (Lnk) encodes a negative regulator of JAK2 signaling that is mutated in myeloproliferative disease, and harbored non-silent mutations in 4 cases (1 frameshift, 2 nonsense, and 1 missense). As expected, tumors from ENU-treated mice had a higher burden of sequence mutations (23.1±12.5 mutated genes/case) than the MLV-induced tumors (7.7±3.8 mutated genes/case, $P<0.01$). Grouped by tumor type, myeloid tumors harbor the fewest mutations (8.8±9.5 mutated genes/case), compared with B-cell tumors (20.2±12.5 mutated genes/case) or the single T-cell tumor studied (37 mutated genes). These results confirm the role of PAX5 in leukemogenesis and show that leukemogenesis commonly requires the acquisition of multiple mutations. Our data also suggest that screening of human leukemia samples for the novel mutations identified in these studies may identify new genes and pathways contributing to the development of ALL.

1172F

Genetic Variation in the APC gene associates with moderate APC allele-specific expression and is correlated with increased susceptibility to common forms of colorectal cancer. *M.J. White[1], M. Curia[2,3], S. De Iure[2], L. De Lellis[2,3], S. Veschi[3,4], S. Mammarella[2], J. Bartlett[1], A. Di Iorio[5], C. Amatetti[6], M. Lombardo[6], P. Di Gregorio[7], P. Battista[4], R. Mariani-Costantini[2,3], S.M. Williams[1], A. Cama[2,3].* 1) Department of Molecular Physiology and Biophysics, Vanderbilt Univ, Nashville, TN; 2) Department of Oncology and Experimental Medicine, “G.d'Annunzio” University, Chieti-Pescara, Chieti, Italy; 3) Aging Research Center (Ce.S.I) University, “G.d'Annunzio” Foundation, Chieti, Italy; 4) Department of Human Movement Sciences, “G.d'Annunzio” University, Chieti-Pescara, Chieti, Italy; 5) Department of Medicine and Sciences of Aging, University “G.d'Annunzio” Foundation, Chieti, Italy; 6) Division of Oncology, “S.Spirito” Hospital, Pescara, Italy; 7) Division of Immunotransfusion, “SS. Annunziata” Hospital, Chieti, Italy.

Although germline variation in allele-specific expression (ASE) has been shown to associate with highly penetrant familial cancers, its role in sporadic common cancers is less well-defined. ASE of the adenomatous polyposis coli (APC) gene plays a role in familial adenomatous polyposis (FAP), a familial colon cancer. We hypothesized that moderate ASE variation in the APC gene contributes to susceptibility to common forms of colorectal cancer (CRC). There were two main aims of this study: First, we investigated whether APC ASE was associated with sporadic cancer by performing germline analysis in CRC cases (n=53) and controls (n=68) using denaturing high performance liquid chromatography (DHPLC). We compared the means, medians, and variances of ASE between cases and controls. Our results showed that while there was no significant difference in average ASE values between cases and controls (mean p=0.57, median p=0.45), there were significant differences in the distribution of ASE values between cases and controls; cases had a significantly larger variance (p=0.0004). Further analyses confirmed that cases had a significantly higher proportion of individuals more than 1.0 (p=0.001, OR=3.97) or 1.645 (p=0.005, OR=13.46) standard deviations from the mean than controls, confirming that deviations of ASE values from the overall mean were associated with increased risk of CRC. Second, we investigated whether genetic variants in the APC gene were associated with its ASE. Twenty-three tag SNPs were genotyped in the APC gene, and eight passed quality control and were polymorphic. Four markers were significantly associated with APC ASE in the case/control analyses, rs41115 (additive model p=0.0091), rs41115 (dominant model p=0.0030), rs467033 (dominant model p=0.0427), rs971517 (additive model p=0.0010), and rs2431507 (p=0.0247). Three markers were significantly associated with APC ASE in case-only analyses, rs41115 (p=0.0058), rs41115 (p=0.0013), and rs971517 (p=0.0103). None of the markers showed significant association with APC ASE in controls. In conclusion, our study detected a wider distribution in APC ASE among CRC cases compared to CRC controls, found that CRC risk seemed to increase with the degree of ASE, and discovered genetic variants in APC that were associated with ASE. Our combined results support the conclusion that APC, a gene that predisposes to FAP, also plays an important role in sporadic cancer via ASE.

1173F

Role of NEU1 deficiency in cancer development and progression. *S.J. White-Gilbertson, E. Machado, D. van de Vlekkert, S. Moshiach, E. Bonten, A. d'Azzo.* Genetics, St. Jude Children's Research Hospital, Memphis, TN.

Deficiency of the lysosomal sialidase NEU1 results in the lysosomal storage disease sialidosis. Type I sialidosis is a catastrophic pediatric disease while Type II, or adult onset sialidosis, is a relatively mild condition caused by gene mutations which preserve residual activity of NEU1. A recent paper reported on a family with three siblings with Type II sialidosis, who all developed a different type of cancer. This genetic study suggests a link between NEU1 deficiency and cancer susceptibility, prompting us to dissect possible links between NEU1 and cancer. Our own research into NEU1 deficiency, performed in the mouse model of sialidosis, has revealed a novel function of NEU1 as an inhibitor of lysosomal exocytosis. In the absence of NEU1, its substrate LAMP-1 accumulates, increasing the number of lysosomes docked at the PM and ready to engage in lysosomal exocytosis. As a result, lysosomal contents, including active proteases such as cathepsins, are aberrantly released extracellularly, most likely impacting the extracellular matrix structure and composition. We hypothesized that this phenotype could be advantageous for cancer cells, which are known to extensively modify their extracellular matrix. We have therefore examined the expression of NEU1 in a variety of cancer cell lines from four cancer types: breast carcinoma, colon carcinoma, Ewing's sarcoma, and alveolar rhabdomyosarcoma. For each type of cancer examined, lower levels of NEU1 activity correlated with increased expression of over-sialylated LAMP-1, as our model would predict. Here we report on a correlation between a low-NEU1, highly exocytic phenotype and the invasive capacity of cells. In some cases, the invasiveness of tested cell lines was known. For instance, the syngeneic system of SW480 and SW620 colon cancer lines is composed of cells derived from a primary or metastatic tumor, respectively, from the same patient. In other cases, such as for Ewings sarcoma and rhabdomyosarcoma, invasive potential of the tested cell lines was determined in our hands using an ex vivo model of peritoneal invasion. These studies establish a new paradigm for understanding the spread of cancer: invasive potential is enhanced by degradation of extracellular matrix via lysosomal exocytosis of active proteases. (Supported in part by NIH grant RO1 GM060950 and ALSAC).

1174F

Patient satisfaction survey of a comprehensive *BRCA* information resource. *J.L. Wiggins, K. Kohut, E.K. Bancroft, R.A. Eeles, K. Myhill, A. Stormorken, S. Thomas, S. Shanley.* Cancer Genetics, The Royal Marsden NHS Foundation Trust, Sutton, United Kingdom.

Families with *BRCA* gene mutations have expressed a need for reliable and evidence-based information. We developed a comprehensive *BRCA* resource pack as a pilot project aiming to empower *BRCA* mutation carriers to make informed decisions and help them discuss genetic issues with at-risk relatives. Method: Charity funding was obtained for production of a resource pack. The format was a small, plain binder including information on the *BRCA* genes; screening options, reducing risk, sharing information with at-risk relatives and information sources. The information pack was distributed to 150 consecutive *BRCA* mutation carriers attending either their annual genetics follow-up appointment or the department-run *BRCA* support group over a six month period. A feedback form was included in the pack, to be returned by post after reading the resource. Results: Thirty-three individuals returned the feedback form (response rate 22%). Ninety-one per cent of respondents were female, 84% were over 40 years of age, 79% had known about their *BRCA* mutation status for more than a year and 46% had a personal history of cancer. Overall satisfaction with the resource pack was very high. Comments from patients included `Wonderful resource to reduce sense of fear and isolation' and `Clear, easy to understand and informative. Will keep referring to it time and again'. Action was taken by 85% of patients as a result of receiving the information resource including identifying and sharing information with at-risk family members and investigating options for risk-reducing surgeries. Patients almost exclusively thought the pack should be presented when receiving their genetic test result. Conclusion: The patients who received this pack have attended the genetics clinic for follow up on an annual basis since their diagnosis. The feedback suggests that there is a role for a detailed written resource and ongoing genetic support even among a well educated group of *BRCA* patients. Additional funding has been obtained have the resource pack available in our clinic for all eligible patients.

1175F

Somatic mutations in GRM1 alter receptor activity and promote tumor progression. *M. Willard[1], J. Esseltine[2], M. Lajiness[1], I. Wulur[1], S. Ferguson[2], T. Barber[1].* 1) Translational Science, Eli Lilly and Company, Indianapolis, IN; 2) Robarts Research Institute and Department of Physiology and Pharmacology, University of Western Ontario London, Ontario, Canada.

Emerging data indicates that GPCRs have a critical role in cancer. Specifically, accumulating evidence suggests that modulation of GPCR function may promote cancer progression and metastasis. Metabotropic glutamate receptor type 1 (GRM1) and its role in physiological processes in the nervous system have been well-documented. Recently, the role of GRM1 in cancer progression has emerged. Somatic mutations altering the coding region of GRM1 have been detected in colorectal cancer, glioblastomas, and lung cancer but the functional significance of these mutations is unknown. Here we demonstrate that cancer-associated mutations of GRM1 lead to altered receptor localization, interactions, and activity, and consequently, abnormal cellular behavior. These findings suggest that somatic mutations in GRM1 promote tumor progression through deregulated receptor activity, and highlight the importance of evaluating GRM1 antagonists to block both the normal and mutant forms of the receptor.

1176F

A replication study and genome-wide scan of single nucleotide polymorphisms associated with pancreatic cancer risk and overall survival. *J.A. Willis[1], S.H. Olson[2], S. Mukherjee[1], M. Gönen[2], R.C. Kurtz[3], R.J. Klein[1].* 1) Program in Cancer Biology and Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY; 2) Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY; 3) Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY.

Background: Pancreatic adenocarcinoma is a rapidly fatal disease with a poor long-term prognosis. To better understand the role of inherited genetic variations in this disease, we performed a combined replication and discovery study of single nucleotide polymorphisms (SNPs) associated with pancreatic cancer risk and overall survival (OS). **Experimental Design:** Germline DNA from 495 pancreatic cancer cases and 285 healthy controls was genotyped at SNPs previously reported to be associated with pancreatic cancer risk or clinical outcome. We analyzed putative risk SNPs under a logistic regression model (adjusted for age and gender) for replication of their reported effects on risk, as well as under a Cox Proportional Hazards model for the discovery of novel effects on OS. Similarly, we analyzed putative “clinical outcome” SNPs for replication of their reported effects on OS as well as for novel effects on risk. Further, we performed a two-stage genome-wide association study (GWAS) of OS using a subset of 252 cases genotyped by whole-genome SNP arrays. **Results:** Seven putative risk SNPs were analyzed in our study. At a nominal significant threshold of $p < 0.05$, we replicated the known risk effects of SNPs rs505922 (*ABO*) ($p = 0.002$, per-allele OR = 1.67, 95% CI 1.20-2.31) and rs9543325 ($p = 0.03$, per-allele OR = 1.39, 95% CI 1.03-1.88). Of the 24 putative “clinical outcome” SNPs analyzed in our study, we replicated the effects of SNPs rs9350 (*EXO1*) ($p = 0.005$, per-allele HR = 1.29, 95% CI 1.08-1.53) and rs2953993 (*POLB*) ($p = 0.05$, per-allele HR 1.39, 95% CI 1.01-1.92). However, no putative risk SNPs or putative “clinical outcome” SNPs were found to be associated with OS or risk, respectively. To discover novel SNPs associated with OS, we conducted a GWAS of ~300k SNPs in 252 cases (stage 1), followed by validation of 27 candidate SNPs in 226 additional cases (stage 2). We identified a novel SNP rs16867625 located in a non-genic region of chromosome 8q22 to be putatively associated with OS (combined stage 1 and 2 $p = 5.66 \times 10\text{-}6$, per-allele HR = 2.04, 95% CI 1.50-2.78). **Conclusions:** The effects of SNPs on pancreatic cancer risk and overall survival were replicated in our study, though further work is necessary to understand the functional mechanisms underlying these effects. More importantly, the putative association with OS identified by GWAS suggests that GWAS may be useful in identifying SNPs associated with clinical outcome in pancreatic cancer.

1177F

Colorectal and other cancer risks for carriers and non-carriers of a DNA mismatch repair gene mutation from mutation carrying families: a prospective cohort study. *A.K. Win[1], D.D. Buchanan[2], J.P. Young[2,3], N.M. Lindor[4], R.W. Haile[5], G. Casey[5], D.J. Ahnen[6], G.G. Giles[7], I. Winship[8,9], F.A. Macrae[10], K. Tucker[11], J. Goldblatt[12], M. Clendenning[2], J.A. Baron[13], S.N. Thibodeau[14], S.R. Gunawardena[14], B. Bapat[15,16], S. Gallinger[15,17], L. Le Marchand[18], P.A. Newcomb[19], J.L. Hopper[1], M.A. Jenkins[1], The Colon Cancer Family Registry.* 1) Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, The University of Melbourne, Parkville, Victoria, Australia; 2) Familial Cancer Laboratory, Queensland Institute of Medical Research, Australia; 3) School of Medicine, University of Queensland, Herston, Queensland, Australia; 4) Department of Medical Genetics, Mayo Clinic, Rochester, Minnesota, USA; 5) Department of Preventive Medicine, University of Southern California, Los Angeles, California, USA; 6) Denver VA Medical Center, School of Medicine, University of Colorado, Denver, Colorado, USA; 7) Cancer Epidemiology Centre, Cancer Council Victoria, Carlton, Victoria, Australia; 8) Department of Medicine, The University of Melbourne, Parkville, Victoria, Australia; 9) Genetic Medicine, The Royal Melbourne Hospital, Parkville, Victoria, Australia; 10) Colorectal Medicine and Genetics, The Royal Melbourne Hospital, Parkville, Victoria, Australia; 11) Hereditary Cancer Clinic, Prince of Wales Hospital, Randwick, New South Wales, Australia; 12) Genetic Services of Western Australia and School of Paediatrics and Child Health, University of Western Australia, Perth, Australia; 13) Department of Medicine, University of North Carolina, Chapel Hill, North Carolina, USA; 14) Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA; 15) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada; 16) Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada; 17) Cancer Care Ontario, Toronto, Ontario, Canada; 18) University of Hawaii Cancer Center, Honolulu, Hawaii, USA; 19) Cancer Prevention Program, Fred Hutchinson Cancer Research Center, Seattle, WA, USA.

Background: Carriers of germline mutations in DNA mismatch repair (MMR) genes are known to have substantially increased risk of colorectal, endometrial and some other cancers. However, cancer risk for their non-carrier relatives is not established. The aim of this study was to determine whether cancer risks for carriers and non-carriers from families segregating an MMR gene mutation are increased above that of the general population. Methods: We prospectively followed a cohort of 446 unaffected carriers of an MMR gene mutation (161 in *MLH1*, 222 in *MSH2*, 47 in *MSH6* and 16 in *PMS2*) and 1029 unaffected relatives without mutations every five years at recruitment centers of the Colon Cancer Family Registry. For comparison of cancer risk with the general population, we estimated country-, age- and sex-specific standardized incidence ratios (SIRs) of different cancers separately for carriers and non-carriers. Results: Over a median follow-up of 5 years, mutation carriers had an increased risk of colorectal cancer (SIR 20.48, 95% confidence interval, CI 13.08 - 33.42, P <0.001), endometrial cancer (SIR 30.62, 95% CI 14.37 - 76.57, P <0.001), pancreatic cancer (SIR 10.68, 95% CI 2.25 - 106.11, P = 0.02), gastric cancer (SIR 9.78, 95% CI 2.07 - 95.85, P = 0.02), renal cancer (SIR 11.22, 95% CI 3.92 - 48.23, P <0.001), bladder cancer (SIR 9.51, 95% CI 2.38 - 38.04, P = 0.001) , ovarian cancer (SIR 18.81, 95%; CI 6.26 - 85.19, P <0.001) and breast cancer (SIR 3.95, 95% CI 1.95 - 9.17, P = 0.001). Their non-carrier relatives had no increased risk of any cancer, including colorectal cancer (SIR 1.02, 95% CI 0.43 - 3.06, P = 0.97). Conclusions: Despite their family history of cancer and the presence of an MMR gene mutation in their families, we found no evidence that the relatives who did not harbor their family specific mutation were at increased risk of colorectal or other cancers and, therefore, may be considered at similar risk as the general population.

1178F

Further support for *RAD51C* gene's contribution to hereditary breast and ovarian cancer susceptibility. *R. Winqvist[1], M. Vuorela[1], J.M. Hartikainen[2], K. Sundfeldt[3], A. Lindblom[4], A. von Wachenfeldt Wäppling[5], M. Haanpää[1], U. Puistola[6], M. Anttila[7], V.-M. Kosma[2], A. Mannermaa[2], K. Pylkäs[1].* 1) Lab of Cancer Genetics, Dept of Clinical Genetics and Biocenter Oulu, Univ of Oulu, Oulu Univ Hospital, Oulu, Finland; 2) School of Medicine, Inst of Clinical Medicine, Pathology and Forensic Medicine, Univ of Eastern Finland, and Dept of Clinical Pathology, Kuopio Univ Hospital, Kuopio, Finland; 3) Inst of Clinical Sciences, Dept of Obstetrics and Gynecology, Sahlgrenska Academy at Gothenburg Univ, Sweden; 4) Dept of Molecular Medicine and Surgery, Karolinska Inst, Stockholm, Sweden; 5) Dept of Oncology-Pathology, Karolinska Univ Hospital, Karolinska Inst, Stockholm, Sweden; 6) Dept of Obstetrics and Gynecology, Univ of Oulu, Oulu Univ Hospital, Oulu, Finland; 7) Dept of Obstetrics and Gynecology, Univ of Eastern Finland, Kuopio Univ Hospital, Kuopio, Finland.

Currently known susceptibility genes such as *BRCA1* and *BRCA2* explain less than 25% of familial aggregation of breast cancer, which suggests the involvement of additional susceptibility genes. RAD51C, a RAD51 paralogue involved in homologous recombination, was recently identified as a predisposing factor both in Fanconi anemia and breast cancer. In the initial report, *RAD51C* (alias *FANCO*) mutations were shown to confer a high risk for both breast and ovarian tumors, but most of the replication studies published so far have failed to identify any additional susceptibility alleles. Here, we report a full mutation screening of the *RAD51C* gene in 147 Finnish familial breast cancer cases and in 232 unselected ovarian cancer cases originating from Finland and Sweden. Additionally, in order to resolve whether common *RAD51C* SNPs are risk factors for breast cancer, we genotyped five tagging single nucleotide polymorphisms, all located within the gene, from 993 Finnish breast cancer cases and 871 controls for cancer associated variants. Whereas none of the studied common SNPs associated with breast cancer susceptibility, mutation analysis revealed two clearly pathogenic alterations. *RAD51C* c.-13_14del27 was observed in one familial breast cancer case and c.774delT in one unselected ovarian cancer case, subsequently shown to have a background of cancer in the family, thus confirming that *RAD51C* mutations are implicated in breast and ovarian cancer predisposition, although their overall frequency seems to be low. Independent identification of the very recently reported *RAD51C* c.774delT mutation in yet another patient originating from Sweden suggests that it might be a recurrent mutation in that population and should be studied further. The reliable estimation of the clinical implications of carrying a defective *RAD51C* allele still requires the identification of additional mutation positive families.

1179F

Chromosome 17 anomalies inferred by high-density genotyping of benign, borderline and malignant serous ovarian cancer specimens. *P.M. Wojnarowicz[1], S.L. Arcand[2], K. Klein Oros[3], D. Provencher[4,5], A.-M. Mes-Masson[4,6], C. Greenwood[3,7], P.N. Tonin[1,2,8].* 1) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 2) The Research Institute of the McGill University Health Centre; 3) Division of Clinical Epidemiology, Lady Davis Research Institute, Jewish General Hospital, Montreal; 4) Centre de recherche du Centre hospitalier de l'Université de Montréal/ Institut du cancer de Montréal; 5) Department of Obstetrics and Gynecology, Université de Montréal; 6) Department of Medicine, Université de Montréal; 7) Department of Epidemiology, Biostatistics and Occupational Health, McGill University; 8) Department of Medicine, McGill University.

Epithelial ovarian cancer (EOC) of the serous histopathological subtype is the most lethal gynecological malignancy, and current knowledge of EOC pathogenesis is limited. Tumours are classified based on pathology as benign (BOV), borderline or low malignant potential (LMP), low-grade (LG) or high-grade (HG) tumours, which may represent distinct disease states or progressive phases of a continuum. Chromosome 17 (Chr17) aberrations occur frequently in EOC, with partial loss of specific genomic regions or complete loss of heterozygosity (LOH) occurring in 50-70% of HG tumours. Molecular genetic, functional and transcriptome analyses also have implicated a number of Chr17 genes in EOC, including *TP53* (17p13.1), which is the most frequently mutated gene in HG tumours. We have applied the Illumina 610-Quad BeadChip SNP array to further investigate Chr17 abnormalities across these disease states, namely 33 BOV, 59 LMP, 12 LG, and 87 HG serous EOC tumour samples. We found that 100% of the BOV samples were heterozygous for Chr17, whereas allelic imbalance (AI), LOH and/or interstitial breaks were found in LMP, LG, and HG tumour samples at frequencies of 5.1%, 50%, and 94.3%, respectively. We found 100% of the BOV samples as well as 100% of the heterozygous LMP, LG, and HG tumours to be *TP53* mutation negative, while 83.33 % of LG and 96.3% HG tumour samples that exhibited AI, LOH and/or interstitial breaks anywhere on Chr17 were mutation positive. Other loci of interest such as the *BRCA1*, *ERBB2*, and *NF1* regions were also surveyed for aberrations as these regions are implicated in EOC. Low frequency of *ERBB2* amplification and homozygous deletion affecting *NF1* were also observed in two and four HG cases, respectively. Interestingly, one LMP sample also showed deletion of the *NF1* region, this sample was also the only LMP to be *TP53* mutation positive. Thus Chr17 genomic content may be a good indicator of disease state and also aid in identifying those LMP samples that may progress to more aggressive disease.

1180F

MicroRNA-93 inhibits colorectal tumorigenesis and decreases early relapse of human colorectal cancer. *I. Yang[1,2], H. Tsai[3,4,6], K. Chen[1], P. Tsai[1], S. Huang[1,6], W. Chou[1,6], J. Wang*[1,3,4,6], S. Juo*[1,5].* 1) Department of Medical Genetics, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 2) Department of Nursing, Shu Zen College of Medicine and Management, Kaohsiung, Taiwan; 3) Department of Surgery, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 4) Cancer Center and Division of Gastroenterology and General Surgery, Department of Surgery, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan; 5) Cancer Center and Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 6) Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.

MicroRNAs are short noncoding RNAs that serve an important role in post-transcriptional regulation. Accumulating evidence has demonstrated that deregulation of miRs participates in the cancer development and outcomes. Using microRNA arrays, we identified that microRNA-93 has substantially different expression levels between early (recurrence within 12 months after surgery) and non-early relapse of colorectal cancer (CRC). The replication study including 35 early relapsed and 42 non-early relapsed CRC further confirmed over-expressed microRNA-93 in non-early relapsed samples. Cellular studies showed that over-expression of microRNA-93 inhibited colon cancer cell proliferation and migration but not invasion. The cell cycle studies revealed that microRNA-93 caused accumulation of the G2 population. However, microRNA-93 could not induce cell apoptosis or necrosis. Functional studies showed that microRNA-93 could suppress CCNB1 protein expression leading to cell cycle arrest in the G2 phase. Moreover, microRNA-93 could repress expression of ERBB2, p21 and VEGF, all of which are involved in cell proliferation. We also demonstrated that microRNA-93 suppressed tumor growth in null mice. Taken together, the present study including human, cell and mouse data consistently demonstrated that microRNA-93 can inhibit tumorigenesis and reduce CRC early recurrence, which may have potential clinical applications for the prediction and intervention of CRC patients.

1181F

DNA methylation in lung cancer. *H. Zamakhshari, M. Abdalla, Y. Haj-Ahmad.* Norgen Biotek Corp, Thorold, Ontario, Canada.

Lung cancer has the highest rate of mortality in both genders due to the late diagnosis. The current method of diagnosis is mainly based on biopsy which is Invasive and can be done only after visualizing the tumor by x-ray. Early detection of the lung cancer can make a big difference in the life expectancy of the patient therefore a new approach is needed. Cancer biomarker has been the main focus over a last decade. A biomarker can be any molecule (DNA, RNA or protein) that can indicates an alteration in physiology from normal. These alterations can be generated from genetic and epigenetic abnormalities of certain genes. The objective of this study is to identify the epigenetic changes including the DNA methylation in lung cancer cell lines which can be used as potential biomarker for the early diagnosis of Lung Cancer. This was based on the hypothesis that a difference may be found between the cancerous and normal cell lines and such a difference may lead to the identification of a biomarker(s) for the early detection of lung cancer. DNA was isolated from two Small cell lung cancer (SCLC) and two Non-small cell lung cancer (NSCLC) cells lines as well as from a normal lung cells line. Four tumour suppressor genes (RASSF1-A,RAR) , APC and P53) were studied for their aberrant methylation using Methylation Sensitive Restriction Enzymes PCR. The isolated DNA was digested with DpnI that will cleave DNA only when its recognition site is methylated and a set of primers were designed to amplify the region flanking the DpnI - recognition site. The housekeeping gene GAPDH was used as a control where the designed GAPDH primer will amplify a region that doesn't have a DpnI - recognition site. Digesting the DNA did not affect the amplification of the tumour suppressor genes as it was amplified by house keeping gene GAPDH. The tumour suppressor gene RASSF1, RAR-) , APC and p53 was not methylated in the lung cancer cell line when digesting with the enzyme DpnI. The same approach was also tested by digesting DNA with two different restriction enzymes HapII and HhaI. It was found that p53 was methylated in H164 and A549 cell line when digesting with the enzymes HapII and HhaI.

1182F

Spontaneous metastasis in a mouse model of testicular germ cell tumours. *J. Zechel[1], G. MacLennan[2], J. Heaney[1], J. Nadeau[1,3].* 1) Department of Genetics, Case Western Reserve University School of Medicine, Cleveland, OH; 2) University Hospitals Case Medical Center, Institute of Pathology, Cleveland OH; 3) Institute for Systems Biology, Seattle, WA.

Testicular germ-tumours (TGCTs) are the most common cancer in men between the ages of 15 and 35 and worldwide incidence has doubled in the last 40 years. TGCTs have a high rate of metastasis, which increases mortality rates and treatment-associated morbidity. Despite the importance of metastasis in human TGCTs and in part because of the lack of a suitable animal model, little is known about acquisition of metastatic potential or modes of dissemination. Here we describe a new model of spontaneous TGCT metastasis the 129 family of mice and demonstrate that these are true metastases from primary testicular cancers, rather than independent extragonadal germ cell tumours. Among males from six genetically distinct strains that had a primary TGCT, similar rates of metastases were found. Additionally, metastases were found in anatomical sites that are consistent with TGCT metastasis in humans. Various lines of evidence support their germ cell origin and the pluripotent nature of these metastases, including the existence of several tissues of endodermal, mesodermal and ectodermal origin, and presence of OCT4 and SSEA-1 pluripotency markers. Moreover, metastases were never observed in males in the absence of a primary testicular cancer. Genetic analysis of pairs of primary TGCTs and metastases from individual mice revealed shared DNA copy number variants, implying that metastases originated from a primary TGCT. Together these results provide the first evidence for spontaneous TGCT metastasis in mice and show that these metastases originate from primary TGCTs rather than independently from ectopic stem cells.

1183F

Next-generation sequencing of multiple samples derived from the same BRCA1-positive patient with ovarian carcinoma. *J. Zhang[1], E. Lalonde[1], L. Li[2], W. Gotlieb[3], A. Ferenczy[4], W. Foulkes[2], J. Majewski[1].* 1) The Department of Human Genetics, McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, Canada; 2) Program in Cancer Genetics, Departments of Oncology and Human Genetics, McGill University, Montreal, Quebec,Canada; 3) Departments of Obstetrics & Gynecology and Oncology, McGill University, Montreal, Quebec,Canada; 4) Departments of Pathology and Obstetrics and Gynecology, McGill University and Jewish General Hospital, Montreal, Quebec, Canada.

In contrast to the whole human genome sequencing, exome sequencing targets only protein-coding sequences of the genome, and provides a more efficient strategy to search for genetic variants underlying rare monogenic diseases. Metastasis to distant tissues is the leading cause of cancer mortality, but the mechanism underlying this process remains unknown. We are interested in investigating the genetic causes of metastatic tumors using high-throughput sequencing. For the purpose of this project, we have collected three separate tumour samples from one patient: a primary ovarian tumour, a pre-treatment metastatic tumour and a post-chemotherapy residual metastatic tumour in the stomach region (in the omentum and peritoneum). The samples have been subjected to whole exome capture and sequencing to detect mutations, such as SNVs and small indels at the DNA level. We hope to identify how mutations appear and disappear during the progression of the disease. This work could lead to improved understanding of the key events leading to recurrence and could potentially affect treatment for the affected patients.

1184F

Neural teneurins are expressed in human tumors and tumor-derived cell lines. *A. Ziegler[1], G. Di Capua[1], J.E. Oyarzún[1], I.E. Roa[2], J.A. Brañes[3], P. Casanello[3], G. Repetto[1].* 1) Center for Human Genetics, Faculty of Medicine, Clínica Alemana-Universidad del Desarrollo; 2) Division of Pathology, Clínica Alemana de Santiago; 3) Perinatology Research Laboratory (PRL) & Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynaecology, School of Medicine, Pontificia Universidad Católica de Chile.

Teneurins are a family of transmembrane proteins involved in embryonal development of the central nervous system, where they promote axonal guiding and neural networking. In a previous proteomics-based study, we had identified differential expression of teneurin-2 in malignant pleural mesothelioma versus lung adenocarcinoma, and had proposed that teneurin-2 could be a potential biomarker for tumor discrimination. Purpose: We evaluated 12 human tumor-derived cell lines to identify additional tumor types that could express teneurin-2 or teneurin-4. Based on the evidence obtained, we analyzed human ovarian tumors for the presence of teneurin-2 and teneurin-4 mRNA and protein to assess the patterns and frequency of expression. Methods: In cell lines, expression of teneurin mRNAs and their transcript variants was assessed by RT-PCR. Teneurin protein expression was detected by immunohistochemistry. Expression of teneurins in frozen tumors and control biopsies was determined by comparative real-time RT-PCR and immunohistochemistry. The study was approved by the participating institutions' Ethical Committees, and tissues were obtained with informed consent of patients. Results: In cell lines, teneurin-2 and teneurin-4 mRNA were expressed in ovarian and breast cancer cells, but infrequently in gastric cancer cells. Characterization of teneurin transcripts in Skov3 ovarian cells revealed multiple alternative splicing sites, and that expression of exons does not always coincide with the structure postulated in public sequence sources. The presence of teneurin mRNAs correlated with expression of teneurin proteins, as evidenced by immunohistochemical detection. In contrast to cell lines, we found differential expression of teneurins in a preliminary set of 8 ovarian tumors. While expression of teneurin-2 was seldom, teneurin-4 was detected in 6/8 tumors. Immunohistochemistry revealed a membrane localization pattern in tumors but not in surrounding stroma. Experiments are ongoing to analyze additional ovarian tumors and non-malignant controls for presence of teneurin-4. Conclusions: We describe for the first time the expression of teneurins in ovarian and breast cancer cell lines. Seventy five percent of human ovarian tumors expressed teneurin-4. Our data suggest that some teneurins might harbor potential as tumor biomarkers. Studies into functional contribution of teneurins to the tumor phenotype are underway. Supported by Fondecyt 1100605.

1185F

Identification of a novel *NBN* truncating mutation from targeted next-generation sequencing of men with familial prostate cancer. *K.A. Zuhlke[1], A.M. Ray[1], C.M. Robbins[3], W.D. Tembe[3], C.A. Salinas[1], J.D. Carpten[3], E.M. Lange[4], W.B. Isaacs[5], K.A. Cooney[1,2].* 1) Department of Internal Med, Hem/Onc, University of Michigan Medical School, Ann Arbor, MI; 2) Department of Urology, University of Michigan, Ann Arbor, MI; 3) Translational Genomics Research Institute, Phoenix, AZ; 4) Department of Genetics, University of North Carolina School of Medicine, Chapel Hill, NC; 5) Department of Urology, The Johns Hopkins University School of Medicine, Baltimore, MD.

Nibrin (*NBN*), located on chromosome 8q21 is a gene involved in DNA double-strand break repair that has been implicated in the rare autosomal recessive chromosomal instability syndrome known as Nijmegan Breakage Syndrome. Nijmegan Breakage Syndrome is characterized by microcephaly, growth retardation, immunodeficiency, and cancer predisposition. Individuals who are heterozygous for *NBN* mutations are clinically asymptomatic, but display an elevated risk for certain cancers including, but not limited to, ovarian and prostate cancer as well as various lymphoid malignancies. In this study, 94 unrelated familial prostate cancer cases from the University of Michigan Prostate Cancer Genetics Project (n= 54) and Johns Hopkins University (n=40) were subjected to targeted next-generation sequencing of the exons and UTRs of *NBN*. One individual diagnosed with prostate cancer at age 52 was identified to have a heterozygous 2117 C>G mutation in exon 14 of the gene, which results in a premature stop at codon 706 (S706X). Sanger sequencing of three additional male relatives revealed that the father, who has prostate and bladder cancer, also carries the S706X allele as does the proband's brother who has no cancer at this time. A paternal uncle, who was diagnosed with prostate cancer at age 70, does not carry the S706X allele. NBN is involved in double-strand break repair as a component the MRE11/RAD50 genomic stability complex. The S706X mutation truncates the protein in a highly conserved region of NBN near the MRE11 binding site, thus suggesting a role for *NBN* mutations in prostate cancer susceptibility.

1186F

Cytogenetic and molecular 11q23/MLL findings in infants with acute leukemia. *M.S. Gallego[1], M.C. Coccé[1], C. Alonso[2], J. Rossi[3], A. Bernasconi[3], E. Alfaro[2], M.S. Felice[2].* 1) Genetics Department, Hospital de Pediatría Prof. Dr. Juan P. Garrahan. Buenos Aires, Argentina; 2) Hemato-Oncology Department,Buenos Aires, Argentina. Hospital de Pediatría Prof. Dr. Juan P. Garrahan. Buenos Aires, Argentina; 3) Immunology Department, Hospital de Pediatría Prof. Dr. Juan P. Garrahan. Buenos Aires, Argentina.

Acute leukemia in infants (<1 year of age) is an infrequent disease with unique epidemiological, biological and clinical features, and the frequency of 11q23/MLL rearrangements has been described in about 50% and 80% of these rare cases with “de novo” acute myeloid leukemia (AML) or acute lymphoblastic leukemias (ALL) respectively. On the other hand, chromosomal rearrangements involving band 11q23 occur in 5-6% of all AML as well as in 5-10% of all ALL cases and in 85% of the secondary therapy-related myeloid We present the 11q23/MLL rearrangements detected in 167 infants diagnosed and treated in our Hospital from 1990 to 2011. Cytogenetic studies were performed by G-banding and in situ fluorescence hybridization (FISH) and characterized by RT-PCR for MLL/AF4, MLL/AF9 and MLL/ENL fusion genes. Patients were treated according to the Interfant-99/06 (n=76) and AML-99 (n=78) protocols. Chromosomal 11q23 abnormalities and/or MLL rearrangements were identified in 52(64)% of ALL and in 24 (28)% of AML patients, being t(4;11)/MLL-AF4, t(9;11)/MLL-AF9 and t(11;19)/MLL-ENL the most frequent translocations. We also found complex structural rearrangements, atypical t(4;11) translocations, inversion 11q, insertions and uncommon translocations involving 11q23. Some of these rearrangements were clarified by FISH using painting probes and split FISH. Since the incorporation of molecular techniques, the comparison between cytogenetic and molecular studies has shown high reciprocal agreement. In 14% and 6%, the alterations were detected either only by cytogenetic or by molecular studies, respectively. Response to induction of infants with 11q23/MLL abnormalities was: for ALL: 44 complete remission (CR) (90%), 3 deaths and 2 null responses, and for AML: 14 CR (68%), 7 deaths and 1 null response. From 44 ALL who achieved CR, 17 relapsed and 13 died in CR, and from 14 AML who achieved CR, 9 relapsed and 1 died in CR. The 5 years pEFS(SE) was 28 (6)% for ALL and 15 (8)% for AML (p= 0.122). Our findings emphasize the value of performing both, molecular and cytogenetic studies, including split FISH, to improve the detection of MLL rearrangements, taking into account the importance of its prognosis impact.

1187F

Genomic imbalances detected by oligoarray CGH in clear cell renal cell carcinoma (CCRCC). *F. Gong[1,2], C. Wang[2], X. Wang[1], S. Li[1], J. Lee[1].* 1) OUHSC, Oklahoma City, OK., USA; 2) The First Hospital of Jilin University, Changchun 130021, P.R. China.

Renal cell carcinoma (RCC) accounts for about 3% of all new cancer cases and the incidence rates for all stages have been steadily rising over the last 3 decades. Despite the introduction of new treatment regimens, surgical resection remains the only curative therapy for CCRCC. Unfortunately, up to 50% of patients undergoing nephrectomy for clinically localized CCRCC will develop local recurrence or distant metastasis. These statistics highlight the need for detecting CCRCC at early stages and identifying markers that can predict prognosis and response to therapy. Application of array CGH to identify chromosomal imbalances of CCRCC tumor specimens may help to differentiate relevant subsets of tumors, which would allow better prognostic and therapeutic decision. After obtaining the patients' signed consent forms, total genomic DNA was isolated from 50 fresh CCRCC tumor tissues by using commercial kits. Eleven out of 50 samples were Fuhrman stage I, 24 were stage II and the rests were stage III. Oligoarray CGH was performed on the 720k oligonucelotide chip (Roche/NimbleGen System Inc.) and experimental procedures were performed according to the manufacturer's protocols with minor modifications. Our data showed that all the samples had genomic imbalances, either losses or gains. The common regions with losses were 3p (45/50), 8p (14/50), 4q (8/50), 6q (7/50), 10q (6/50), 13q (6/50), plus monosomy of chromosomes 9 (10/50) and 14 (15/50). The regions with gains were 5q (35/50), 8q (9/50), 1q (8/50), plus gain of extra chromosomes 12 (9/50), 16(8/50), 20(8/50), and 22(6/50). Interestingly, no single case with stage I had a loss or gain of chromosome 1, but 17 out of 39 cases with stage II and III had either loss or gain involving chromosome 1. The result shows the 3p lose and 5q gain were the most significant imbalances detected in our 50 CCRCC samples, which were likely due to the fact that the unbalanced chromosome rearrangements involving the chromosomes 3 and 5. It also showed that cases with early stage did not have chromosome 1 rearrangement, but high frequency of rearrangement of chromosome 1 in the late stage of tumor. The findings warrant further investigation of whether stability of chromosome 1 could serve as a maker for tumor subtype classification of the CCRCC or not.

1188F

Definition of chromosome 8q copy number changes in paediatric solid tumors. *N.Y. Martin[1], D. Belluoccio[2], G.B. Peters[3], J.A. Byrne[1].* 1) Children's Cancer Research, KRI at Children's Hospital at Westmead, Sydney, NSW 2145, Australia; 2) 347 Burwood Hwy, Forest Hill, VIC 3131, Australia; 3) Department of Cytogenetics, The Children's Hospital at Westmead, Sydney, NSW 2145, Australia.

Chromosome 8q gain is one of the most common cytogenetic events in cancer. As either the entire chromosomal arm or different focal regions can be gained, chromosome 8q likely harbors multiple oncogenes. Proposed amplification targets include *MYC*, *MAL2*, *WWP1*, and *TPD52*, but others remain to be identified. It is not known whether the same or different genes are targeted in different tumour types. Chromosome 8q gain has also been poorly characterized in paediatric tumours, relative to more common adult cancers.

Methods: Genomic DNA was extracted from 19 paediatric solid tumours (3 osteosarcomas, 7 Ewing sarcomas, 3 rhabdomyosarcomas and 6 neuroblastomas) and 6 breast cell lines as controls. The SALSA Multiplex ligation-dependent probe amplification **(MLPA)** P014-A1 kit (MRC-Holland) was used to detect possible exonic amplifications or deletions. This includes probes with target sequences in 22 chromosome 8q genes, 8 chromosome 8p genes, and 10 reference genes. Agilent **array CGH** analyses will also be performed to refine and extend the results obtained using **MLPA** analyses.

Results: All 19 tumour samples showed increased copy number at one or more chromosome 8q loci. Increased copy number was most frequently noted at *SLA* (at 134.1 MB) and *RECQL4* (at 145.7 MB) which were gained in 12/19 (63%) and 14/19 (74%) tumours, respectively, and 4/6 breast cell lines. The *SLA* gene was predominantly gained in Ewing sarcoma (5/7 cases) and osteosarcoma (3/3 cases), whereas *RECQL4* was predominantly gained in Ewing sarcoma (6/7 cases), neuroblastoma (5/6 cases) and rhabdomyosarcoma (2/3 cases). *TPD52* (at 81.1 MB) was gained in 3/3 osteosarcoma cases and 4/6 breast cell lines, which is consistent with previous reports. Although *MYC* (at 128.8 MB) showed increased copy number in 6/6 breast cell lines analysed, only 2/19 (11%) tumours (2 neuroblastoma cases) showed *MYC* gain.

Conclusion: Increased copy number at chromosome 8q loci is a common finding in pediatric solid tumours, and of the chromosome 8q loci examined, *SLA* and *RECQL4* were most frequently gained. Analyses of these tumour samples using **array CGH** may provide further information about chromosome 8q genes which are specifically or commonly targeted in different paediatric solid tumours.

1189F

Does transient abnormal myelopoiesis occur in the absence of Down Syndrome, or is it really AML? *E.M. Tegg[1,2,4], C.E. Wren[2], A.G. De Paoli[3], A.M. Johnston[4], C. Williams[3].* 1) Haematology, Menzies Research Institute, Hobart, Australia; 2) Cytogenetics Department, Royal Hobart Hospital, Hobart, Australia; 3) NPICU, Royal Hobart Hospital, Hobart, Australia; 4) Haematology Department, Royal Hobart Hospital, Hobart, Australia.

Case history A well 1 day old preterm male neonate, born at 34 weeks gestation with a birth weight of 2740 g, was noted to have a petechial rash. Full blood examination revealed anaemia (Hb 109 g/L), thrombocytopenia (22 x 109/L), and a large proportion of pleomorphic blasts (40%). Immunophenotyping confirmed these as myeloblasts (CD13, CD33, CD56 and some expressing CD117). The differential diagnosis included transient abnormal myelopoiesis (TAM) associated with Down syndrome (DS) or acute myeloid leukaemia (AML). The infant had no compelling clinical features of DS. Mild hypotonia was consistent with his stated gestation and epicanthic folds were in keeping with the South-East Asian background of his mother. Cytogenetic analysis on unstimulated cells in the peripheral blood confirmed the sole abnormality as trisomy 21 in the blast cell line. A phytohaemagglutinin (PHA) stimulated culture of T lymphocytes and analysis of 100 cells from a buccal smear both revealed a normal male karyotype in all cells (46,XY). A provisional diagnosis of non-DS TAM was made and supportive treatment included red cell transfusion and multiple platelet transfusions. Two weeks after diagnosis, the platelet count had not improved with the blast count remaining at approximately 10%. At four weeks post diagnosis, there were no circulating blasts, the platelet count had recovered (106 x 109/L) and the child was discharged home. Discussion: Although the current World Health Organisation classification of haematological malignancies describes TAM as a unique disorder of DS, it is clear from this case and other cases in the literature that TAM can occur in infants in the absence of DS. In this case trisomy 21 was identified only in the peripheral blast line. The most appropriate long-term follow-up for infants with TAM remains unclear. Whilst there is a well recognized increased risk for subsequent development of leukaemia in these infants there is currently no treatment to lower this risk. The longer-term follow up of this case and a systematic review of the literature will be presented.

1190F

The role of pregnancy on hemangioblastomas in von Hippel-Lindau disease: a retrospective French study. *C. Abadie[1, 4], I. Coupier[1, 2, 4], S. Bringuier-Branchereau[3], S. Deveaux[4], S. Richard[4, 5], French National Cancer Institute network "VHL disease and inherited predisposition to kidney cancer.* 1) Service de Génétique Médicale, Unité d'Oncogénétique, CHU Montpellier, Montpellier, France; 2) Service d'Oncogénétique, CRLCC Val d'Aurelle, Montpellier, France; 3) Département d'Information Médicale, CHU Montpellier, France; 4) Réseau National et Centre Expert National Cancers Rares PREDIR, Service d'Urologie, AP-HP, Hôpital de Bicêtre, Le Kremlin-Bicêtre, France; 5) Laboratoire de Génétique Oncologique EPHE, CNRS FRE 2939, Faculté de Médecine Paris-Sud, Le Kremlin-Bicêtre et Institut de cancérologie Gustave Roussy, Villejuif, France.

Von Hippel-Lindau disease (VHL) is a dominantly inherited disorder predisposing to highly vascularized tumors including hemangioblastomas of the central nervous system (CNS) and the retina. The disease results from germ-line mutations in the VHL tumour-suppressor gene that plays a key role in the cellular response to tissue hypoxia and angiogenesis. CNS and retinal hemangioblastomas occur in about 75% and 60% patients, respectively. Only a few case reports and one study on a small population of VHL patients (Grimbert et al., Am. J. Gyn. Obst. 1999,180:110-1) were previously interested in a potential deleterious role of pregnancy on hemangioblastomas which express progesterone receptors. We present a retrospective and comparative French study in 269 women from 172 families coming from the national VHL clinical database. The aim was to analyse the onset of new hemangioblastomas and potential tumoral complications in patients according to their gestational status. Available data of imaging follow-up of CNS and retina were collected in 176 women with at least one pregnancy and 93 women with none. When taking account of the relative frequency regarding the period of follow-up, more significant complications of previous hemangioblastomas were seen in women during pregnancy (P=0.022) and it was also significant during a period of 3 months postpartum (P<0.001). To our knowledge, this work represents the first study analysing the effect of pregnancy in a very large series of women with VHL. This study underlies the necessity of follow-up patients closely during pregnancy. Thus, a magnetic resonance imaging without injection is systematically required during the fourth month of pregnancy in each patient with previously known CNS lesions.

1191F

Global gene expression profiles in ovarian cancer linked to Lynch syndrome. *K. Bartuma[1], K. Harbst[1, 2], S. Malander[1], M. Jönsson[1], Z. Ketabi[3], A. Måsbäck[4], G. Jönsson[1, 2], M. Nilbert[1, 3].* 1) Department of Oncology, Institute of clinical sciences, Lund University, Lund, Sweden; 2) CREATE health strategic center for translational cancer research, Lund University, Lund, Sweden; 3) Clinical Research Center, Hvidovre Hospital, Copenhagen University, Hvidovre, Denmark; 4) Department of Pathology, Institute of Laboratory Sciences, Lund University, Lund, Sweden.

Lynch syndrome is caused by defective DNA mismatch repair and has been associated with an increased risk of ovarian cancer with a particular overrepresentation of endometrioid and clear cell tumors. The more common colorectal cancers in Lynch syndrome show distinct genetic profiles related to mismatch repair, which led us to investigate gene expression profiles in hereditary ovarian cancer. Ovarian cancers from 25 Lynch syndrome mutation carriers and 42 sporadic cases were included in a case-control design. The Illumina Whole Genome DASL assay was used for expression profiling and significance analysis of microarrays was performed to identify differentially expressed genes. Differential expression between Lynch syndrome-associated and sporadic ovarian cancers involved 335 genes with up-regulation of the mTOR and MAPK/ERK pathways. Clear cell cancers are overrepresented in Lynch syndrome and this subset showed a distinct expression profile with 668 differentially expressed genes with up-regulation of the HER2 signaling and apoptosis signaling pathways. Global gene expression profiles differ between hereditary and sporadic ovarian cancer and demonstrate distinct profiles linked to clear cell histologies. These data identify key signaling pathways as potential therapeutic targets in ovarian cancer linked to Lynch syndrome.

1192F

Endometrial cancer as a red flag for Lynch syndrome identification. *P.O. Chappuis[1,2,3], M. Genevay[4], P. Petignat[5], S. Myit[6], P. Benusiglio[1], S. Arcioni[3], L. Fonteneau[1], R. Suchy[3], L. Rubbia-Brandt[4], P. Hutter[3,7].* 1) Division of Oncology; University Hospitals of Geneva; Switzerland; 2) Division of Genetic Medicine; University Hospitals of Geneva; Switzerland; 3) Unit of Medical Genetics; Institut Central des Hôpitaux Valaisans, Sion; Switzerland; 4) Division of Clinical Pathology; University Hospitals of Geneva; Switzerland; 5) Division of Gynaecology; University Hospitals of Geneva; Switzerland; 6) Division of Histocytopathology; Institut Central des Hôpitaux Valaisans, Sion; Switzerland; 7) Laboratory of Molecular Oncology; University Hospitals of Geneva; Switzerland.

BACKGROUND: Women carrying germline mutations in DNA mismatch repair (MMR) genes responsible for Lynch syndrome (LS) have a 40-60% lifetime risk of developing endometrial cancer. According to the Amsterdam criteria and Bethesda guidelines, identification of LS is mostly determined by personal/family history of colorectal cancer. Tumor pre-screening with immunohistochemistry (IHC) for detection of the main MMR proteins and search for microsatellite instability (MSI) in incident cases of endometrial cancer is presented. **METHODOLOGY:** A population-based study designed for patients with colorectal and endometrial cancer diagnosed < age 75 in the Geneva and Valais cantons, Switzerland has been set up in 05.2009. After written consent, a phone interview collected detailed personal/family history and a complete pedigree is drawn. Tumor tissue pre-screening is based on IHC for MLH1, MSH2, MSH6, PMS2, the main MMR proteins, and search for MSI using 3 monomorphic microsatellites (BAT-25, BAT-26, CAT-25). *BRAF* c.1799T>A/p.V600E is searched when MLH1 protein is not expressed. In case of positive pre-screening, a genetic counselling is proposed and selective testing for germline mutations in MMR genes is discussed. **RESULTS:** Between 05.2009 and 05.2011, 26 endometrial cancers have been pre-screened for LS. Mean age at diagnosis was 58.3 (31-73.4) years and 3 (11.5%) patients were < age 50. Endometrioid carcinoma is the most frequent (96%) subtype of endometrial cancer and 22 patients had FIGO stage I/II disease, 4 stage III/IV. Three (11.5%) cases fulfilled Bethesda guidelines and 1 (3.8%) the Amsterdam criteria. Five (19.2%) tumors demonstrated loss of expression of ⁄ 1 MMR proteins and/or MSI. All patients with positive pre-screening have been seen in genetic counselling and one patient declined testing. Three genetic tests for germline MMR mutations have been completed. One *MSH2* pathogenic mutation (c.367-?_645+?del) has been identified in a 36 years old patient who did not meet any clinical criteria for LS. Moreover, one *MLH1* unclassified variant (c.545+61T>C) has been characterised is another index case. **CONCLUSION:** Endometrial cancer can be the single familial presentation of LS. Pre-screening of all endometrial cancer using IHC for MMR proteins and/or search for MSI should be considered given the limitations of the Amsterdam criteria and Bethesda guidelines in gynaecologic population. *Supported by a Swiss Cancer League grant and the Dumont-Moerlen fund.*

1193F

Determining a role for germline mutations in intermediate risk breast and ovarian cancer susceptibility genes in the Belgian population. *K. De Leeneer, A. De Jaegher, B. Crombez, I. Coene, A. De Paepe, B. Poppe, K. Claes.* Center for Medical Genetics, Ghent University Hosp, Ghent, Belgium.

Germline mutations in the coding and splice regions of BRCA1&2 are identified in approximately 15% of patients selected for genetic testing in the Belgian population. It is estimated that germline mutations in intermediate risk genes like PALB2, ATM, CHEK2, BRIP1 and BARD1 account for an elevated breast and ovarian cancer risk in approximately 5% of familial breast cancer. In this study we evaluated the mutation prevalence of PALB2 mutations in 285 Belgian patients from 256 independent BRCA1/2 mutation negative families with a young age at onset and/or family history of breast and/or ovarian cancer. The complete UTR, coding and splice site regions of PALB2 were analyzed with High resolution melting followed by Sanger sequencing of the aberrant melting curves. In silico predictions of variants with an unclear clinical significance was performed with the Alamut software. In total we identified 20 unique sequence variants in PALB2 of which 6 are previously unreported. Three novel unequivocal mono allelic mutations (PALB2 c.2834+1G>T c.2888delC and c.3423del4) were detected and shown to segregate with the disease in the families. PALB2 c.2834+1G>T is a splice site mutation which undoubtedly will lead to aberrant splicing; c.2888delC and c.3423del4 are both frame shift mutations in the WD40 protein domain, responsible for the interaction with BRCA2. Furthermore, we detected in nine patients 4 novel sequence variants (PALB2 c.-158G>C, c.498T>C, c.995T>A and c.1520C>G) of which the clinical significance is currently unknown. Several in silico analyses predict that PALB2 c.995T>A (p.Leu332His) could affect protein function. However, segregation analysis revealed that the mother of the patient, diagnosed with breast cancer at the age of 45 years did not carry the sequence variant. Deleterious PALB2 mutations were only identified in familial breast cancer patients (3/208= 1.4%) and not in 48 sporadic patients with early onset or bilateral breast cancer. Interestingly, the average age at diagnosis for the mutation carriers (62y; range 48-71) was significantly higher compared to the average age at diagnosis (39y, range: 25-78) of the non carriers. This limited role for PALB2 germline mutations in the Belgian population, suggests locus heterogeneity and further investigations in other intermediate risk breast and ovarian cancer susceptibility genes (ATM, CHEK2, BRIP1 and BARD1) will be undertaken.

1194F

Characterization of unclassified variants in DNA mismatch repair genes in Lynch syndrome. *M. Dominguez Valentin[1], LJ. Rasmussen[2], C. Therkildsen[3], T. Van Overeem Hansen[4], I. Bernstein[5], M. Nilbert[1,3].* 1) Department of Oncology, Institute of Clinical Sciences, Lund University, 22100 Lund, Sweden; 2) Center for Healthy Aging, Copenhagen University, Copenhagen, Denmark; 3) Clinical Research Centre, Hvidovre University Hospital, Copenhagen University, 2650 Hvidovre, Denmark; 4) Department of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; 5) HNPCC Register, Department of Gastroenterology, Hvidovre University Hospital, Copenhagen University, 26550 Hvidovre, Denmark.

Lynch syndrome (LS) is the most common form of hereditary colorectal cancer (CRC) and accounts for 2-5% of all CRC (Lynch et al. 2005). LS is caused by germline mutations in mismatch repair (MMR) genes. Patients suffering from this syndrome inherit mutations in one allele of any of four MMR genes, most frequently MSH2, MLH1 or MSH6 and more rarely in PMS2 (Couch et al. 2008). The majority of the disease-predisposing MMR gene alterations cause truncations and thus loss of function of the affected protein (Lützen et al. 2008). A significant proportion of DNA variants found in patients suspected of having LS are unclassified variants (UV) (32%, 18% and 38% for MLH1, MSH2 and MSH6, respectively), whose pathogenicity remains unclear (Castillejo et al. 2011). Our previous study on suspected Danish LS population, found 36% of UV, being 26% of MLH1, 30% of MSH2 and 44% of MSH6 (Nilbert et al. 2009). This study aims to evaluate the pathogenicity of 15 UV found in 20 suspected Danish LS families (3 UV of MLH1 in 6 families, 5 UV of MSH2 in 5 families and 7 UV of MSH6 in 9 families) by applying co-segregation analysis, co-occurrence analysis (other MMR gene mutation in the family), biochemical diagnosis (IHC and MSI analysis), in silico predictions (SIFT, Polyphen, MAPP-MMR and P-mut programs) and functional assays. We analyzed the pedigrees and performed IHC, MSI and in silico analysis of all the families. In order to characterize the subcellular localization of the mutant proteins, we evaluated the co-localization of the mutant proteins by confocal laser scanning microscopy. Our results suggest normal subcellular localization of the 4-tested MSH2 and all tested MSH6 proteins. Based on the characterization of protein-protein interactions in MMR complexes containing MSH2 and MSH6 mutant proteins, we performed yeast-two hybrid and GST fusion protein (pull-down) assays for all 5-tested MSH2. We observed that all tested MSH2 UV were able to interact with MSH6. Currently, we are developing specific functional assays for MLH1 and MSH6 UV. Using these approaches, evidence for pathogenicity will be collected, which has clinical implications for recommendations for surveillance.

1195F

Substantial unexplained variation in cancer risks for MLH1 and MLH2 mutation carriers. *J.G. Dowty[1], A.K. Win[1], D. Buchanan[2], R.J. Macinnis[1], N. Lindor[3], S.N. Thibodeau[4], G. Casey[5], S. Gallinger[6], L. LeMarchand[7], P. Newcomb[8], R. Haile[5], J. Goldblatt[9], S. Parry[10], F.A. Macrae[11], J.L. Hopper[1], M.A. Jenkins[1], the Colon Cancer Family Registry.* 1) School of Population Health, University of Melbourne, Parkville, Victoria, Australia; 2) Familial Cancer Laboratory, Queensland Institute of Medical Research, Brisbane, Queensland, Australia; 3) Departments of Medical Genetics, Mayo Clinic, Rochester, Minnesota; 4) Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota; 5) Department of Preventive Medicine, University of Southern California, Los Angeles, California; 6) Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada; 7) University of Hawaii Cancer Research Center, Honolulu, Hawaii; 8) Cancer Prevention Program, Fred Hutchinson Cancer Research Center, Seattle, Washington; 9) Genetic Services and Familial Cancer Program of Western Australia, Perth, Western Australia, Australia; 10) New Zealand Familial Gastrointestinal Cancer Registry, Auckland City Hospital, Auckland, New Zealand; 11) Department of Colorectal Medicine and Genetics, The Royal Melbourne Hospital, Parkville, Victoria, Australia.

Background: Germline mutations in the DNA mismatch repair genes MLH1 and MSH2 are associated with substantially increased risks of colorectal cancer (CRC), endometrial cancer (EC) and certain other cancers. Due to the rarity of these mutations, previous studies have been underpowered to provide precise estimates of risks. **Methods:** We studied 167 MLH1 and 225 MSH2 mutation-carrying families comprising 17,352 members from the Colon Cancer Family Registry. Probands were recruited either because they had a family history of cancer (n=274) or from cancer registries independently of family history (n=118). Hazard ratios (HRs) of cancer risks for carriers compared with the general population and age-specific cumulative risks for carriers (penetrance) were estimated using modified segregation analysis conditioned on ascertainment. Heterogeneity in risks for carriers was modeled with a polygenic risk modifier (as in the BOADICEA model). **Results:** The age-specific incidence of CRC for male MLH1 mutation carriers was estimated to be 222 times (95% CI: 152-324) that for the population at ages 40 years and younger but only 6.1 times (95% CI: 2.4-15.5) after age 60 years. This decline ($p<0.0001$) in the CRC HR with age was also evident for female MLH1 mutation carriers ($p=0.002$) but was less marked. Male MLH1 mutation carriers on average had higher CRC HRs than female carriers ($p=0.004$). The estimated CRC HRs for MSH2 mutation carriers were similar to those for MLH1, with any differences consistent with chance ($p>0.3$). The estimated EC HRs also decreased sharply with age ($p=0.0004$ and 0.001 for MLH1 and MSH2, respectively) and no difference by gene was observed ($p=0.7$). There was strong evidence for an unmeasured polygenic modifier of risk ($p<0.0001$). The estimated average cumulative risks (95% CI) of CRC to age 70 years were 44% (35-54) for male carriers and 38% (30-48) for female carriers, and corresponding EC risks were 22% (14-31). However for carriers in the lowest vs. highest quartiles of polygenic risk (respectively) these were 4.9% vs. 93% for male CRC, 3.5% vs. 88% for female CRC and 1.5% vs. 59% for EC. **Conclusions:** This international study shows that, although the average cancer risks for MLH1 and MSH2 mutation carriers are similar, there is substantial unexplained variation in risks due to differences by mutation or by genetic or environmental modifiers. This finding has implications for the counseling and clinical management of mutation carriers.

1196F

From 2000-2010 - A Review of the First Pediatric Cancer Genetics Program in North America. *H. Druker[1,2], S. Meyn[1,2], N. Parkinson[1,2], D. Malkin[1,2].* 1) Hospital Sick Children, Toronto, ON, Canada; 2) University of Toronto, Toronto, ON, Canada.

The Cancer Genetics Program at The Hospital for Sick Children was created in July of 1999. It is the first of its kind in North America. It is housed in the second largest pediatric oncology department in North America with an average of 295 new cancer diagnoses made every year. Individuals are seen if the primary risk is NOT for breast/ovarian cancers, familial GI cancers in adults, or retinoblastoma. Referrals are accepted if at least one member of the extended family has/had a childhood cancer or may be at risk for developing cancer. From July 1, 1999 to December 31, 2010, the Cancer Genetics Program registered 1842 patient-visits. Of those 69.5% were new referrals and 29.5% were follow-up appointments. Of the new referrals, 68.0% underwent genetic testing that yielded an interpretable result. Variants of unknown significance were excluded. 78.6% received negative results, while 21.4% received positive results. Individuals with a positive result or with a suggestive family history of cancer were provided with screening guidelines in accordance with published literature or expert judgment. The most commonly tested condition was Li-Fraumeni Syndrome (41.3%), followed by VHL (9.8%), FAP (7.6%), Hereditary Wilms (5.1%), MEN2 (4.7%), Rhabdoid Tumour Syndrome (4.5%), PGL (3.4%), Gorlin (3.2%), Fanconi Anemia (2.5%), NF2 (2.5%), CCHS Syndrome (2.3%), MEN1 (2.3%) and other (10.8%). The highest number of positive results was identified amongst those tested for MEN1 (54.6%), followed by Gorlin (53.3%), Fanconi Anemia (50%), VHL (45.7%), CCHS (45.5%), PGL (25.0%), Rhabdoid Tumour Syndrome (23.8%), FAP (22.2%), NF2 (16.7%), HLRCC (16.7%), Hereditary Wilms (12.5%), and LFS (11.8%). No mutations were identified amongst those tested for Cowden Syndrome or Familial Neuroblastoma. In summary, 68.0%; of new referrals underwent genetic testing that yielded an informative result. This number is expected to rise as more hereditary cancer predisposition genes are identified.

1197F

Characterisation of Genetics Aspects of Paragangliomas and Pheochromocytomas in the Quebec French-Canadian Population. *N. Dumas[1], M. Edmont[1], S. Grunenwald[2], I. Lévesque[2], M. Binet[1], N. Burnichon[3], A. Gimenez-Roqueplo[3], I. Bourdeau[2,1].* 1) Service de Médecine Génique, Centre hospitalier de l'Université de Montréal (CHUM), Montreal, QC, Canada; 2) Service d'endocrinologie, Département de médecine et Centre de recherche, Centre hospitalier de l'Université de Montréal (CHUM), Montréal, Québec; 3) Service de génétique, Assistance Publique - Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Paris, France.

Background: Paragangliomas (PGL) and pheochromocytomas (PCC) are rare tumors that may affect children and adults. To date, mutations in various genes have been identified as a risk factor for developing PGL and PCC including *VHL* (Von Hippel Lindau), *RET* (Multiple Endocrine Neoplasia type 2) and *NF1* (neurofibromatosis) and the genes encoding 3 of the 4 subunits of the mitochondrial enzyme succinate dehydrogenase (*SDHB*, *SDHC*, and *SDHD*). A mutation in one of the *SDHx* genes is present in 40% of individuals affected by head and neck PGL, and 10% of individuals with sympathetic PGL and PCC. Approximately 4% of hereditary PGL/PCC can be attributed to mutations in the *SDHC* gene. Methods: Following genetic counselling, 26 French Canadian patients (10 PCC, 16 PGL) consented to genetic testing of germline DNA. The 4 exons of the *SDHD* gene, the 8 exons of the *SDHB* gene, the 3 exons of the *VHL* gene, the 6 exons of the *SDHC* gene, and 7 exons (8, 10, 11, 13, 14, 15, 16) of the *RET* gene were amplified and directly sequenced. MLPA was performed for the *VHL* and *SDHx* genes. Results: One patient with PCC (10%) had a mutation in the *SDHB* gene (c.725G>A). Six patients with PGL (37%) were carriers of an *SDHx* mutation: two patients (33%) had an *SDHD* mutation (c.112C>T, c.119_125del), the same heterozygous non-sense mutation at codon 133 of exon 5 of the *SDHC* gene (c.397C>T, p.Arg133X) was found in 4 patients (67%). This genetic alteration leads to an amino acid change from arginine to a stop codon, causing premature truncation of the protein. These results are in contrast to the higher frequency of *SDHB* or *SDHD* mutations reported in other populations. Pedigrees were constructed and analysed for common ancestors for the patients with the *SDHC* mutation using a database containing information on residents of Québec since the 17th century (Balsac). Initial data from the genealogical research shows 10 possible common ancestors (5 couples) to the 4 index-cases with the *SDHC* mutation. Conclusion: Further genealogical studies including comparison to a control group, are needed to determine whether the *SDHC* mutation identified in our population truly represents a founder mutation. Haplotype analysis will also be used to further characterise this apparent *SDHC* founder mutation and to confirm the unique genetic distribution of *SDHC* mutations in French Canadians.

1198F

BRCA1 and BRCA2 mutational spectrum and evidence for a BRCA1 founder mutation in Luxembourg families with breast-ovarian cancer. *H. Grootkoerkamp[1], C. Bock[1], M. Sand[1], F. Lemoine[2], S. Meyer[2], S. Rauh[3], JL. Guarin[4], P. Hilbert[5], K. Dahan[5,6].* 1) ZithaKlinik, Luxembourg, L-2763 Luxembourg, Tumor Klinik; 2) Centre Hospitalier Emile Mayrisch , L-4240 Esch-sur-Alzette, Service d'Oncologie; 3) Centre Hospitalier Emile Mayrisch , L-4602 Niederkorn, Service d'Oncologie; 4) Centre de Génétique Humaine, Université Catholique de Louvain, B-1200 Brussels; 5) Centre de Génétique, Institut de Pathologie et de Génétique, B- 6041 Gosselies; 6) Laboratoire National de Santé, L-1911 Luxembourg Division d'Hématologie, Service de Conseil Génétique.

Genetic testing for inherited mutations in BRCA1 and BRCA2 has become integral to the care of women with a severe family history of breast or ovarian cancer, but in comparison with other European countries the prevalence of BRCA1/BRCA2 mutation in Luxembourg isn't well established. Here, we performed a multicenter study to determine the mutational spectrum and identify possible BRCA1 and BRCA2 founder mutations in the Luxembourg population. Between 2002-2010, 122 families were selected on the following criteria ; (i) two or more breast cancers under the age of 50 years, or (ii) three cases of breast cancer (BC) at any age or (iii) both breast and ovarian cancer (OC) cases or (iv) one BC diagnosed under the age of 35 years. Small mutations were screened by genomic sanger sequencing (/) or by amplicon sequencing with Multiplicom (www.multiplicom.com) using GS-FLX 454 standard chemistry (Roche) and large rearrangements using multiplex ligation dependent probes. A total of 15 mutations in the BRCA1/2 genes were identified in 122 families studied (15/122, 12%). All but four of the mutations were detected within the BRCA1 gene (11/15, 73,3%). Majority of mutations are unique nucleotide changes except for the large BRCA1 deletion comprising exons 13-15 which was identified in 4 unrelated families (4/15, 26%). No other rearrangement was detected. Mutational analysis of BRCA1/2 genes showed a relative low frequency of causing-disease changes in Luxembourg and illustrated the dominant role of the BRCA1 exon 13-15 deletion, a change previously observed in Asian population and found in 4 of 122 high-risk families referred for genetic counseling.

1199F

Differential effects of Pot1b and telomerase deficiency in adrenocortical dysplasia (*acd*) mice. *C.E. Keegan, H. Kocak, E.L. Macke, K.W. Wilson, G.A. Osawa.* Departments of Pediatrics and Human Genetics, Univ Michigan, Ann Arbor, MI.

Telomeres are nucleoprotein structures that solve both the end-replication problem and the end-protection problem. Dysfunctional telomeres can result from critically short telomeres or unprotected telomeres, leading to genomic instability. While telomerase prevents critical telomere shortening, the shelterin complex protects telomeres by suppressing DNA damage responses. In humans, Dyskeratosis Congenita (DC) is a rare genetic disorder characterized by extremely short telomeres, bone marrow failure, and mucocutaneous manifestations, caused by mutations in telomerase and its associated proteins and in the shelterin complex member *TINF2*. Mouse knockouts of *Terc*, the telomerase RNA component and *Pot1b*, a shelterin component, have been proposed as models for DC. Adrenocortical dysplasia (*acd*), a hypomorphic mutation encoding the shelterin component Tpp1, has overlapping features with *Terc* and *Pot1b* deficient mice, including growth retardation, infertility, and reduced survival. To better understand the *in vivo* interactions of Tpp1 with Pot1 and telomerase, we generated crosses of these three existing viable mouse models with known telomere dysfunction and features of DC. In our cross of *acd* mice to *Pot1b* null mice, *acd* mutant mice in the F_2 generation exhibited a slower growth rate than mice with other genotypes, as expected from prior studies. However, there were no surviving *acd/acd*, *Pot1b*$^{-/-}$ or *acd/acd*, *Pot1b*$^{+/-}$ mice, suggesting that deficiency of both Tpp1 and Pot1b is incompatible with survival. Furthermore, *acd/acd*,*Pot1b*$^{-/-}$ embryos were absent at E13.5, when homozygous *acd* mutant embryos are present in Mendelian ratios, suggesting a synergistic interaction. In addition, it has previously been shown that *Terc* haploinsufficiency significantly exacerbates the *Pot1b* null phenotype. Thus, to determine the effect of Terc deficiency on the *acd* mutant phenotype, we crossed early generation (G1) *Terc* deficient mice to *acd* mice, and we found that double mutant mice in the F_2 generation are viable and appear to have a phenotype resembling adult *acd* mutant mice. Future work will include isolation of MEFs from *acd* mutant mice with *Terc* deficiency and with *Pot1b* deficiency to investigate the cellular consequences of these mutations. Our data thus far indicate that *Pot1b* deficiency exacerbates the *acd* mutant phenotype, while *Terc* deficiency does not have a significant effect. This work was supported by American Cancer Society RSG GMC-115995 to CEK.

1200F

A large intragenic duplication in the Folliculin (*FLCN*) gene causing Birt-Hogg-Dubé syndrome with an interesting phenotype. *D.H.K. Lim[1,2], C. Morgan[2], P.K. Rehal[2], F. Bond[2], L. Walker[3], D. Halliday[3], F. Macdonald[2], E.R. Maher[1,2].* 1) Department of Medical & Molecular Genetics & Centre for Rare Diseases and Personalised Medicine, University of Birmingham, United Kingdom; 2) West Midlands Regional Genetics Service, Birmingham Women's Hospital, Edgbaston, Birmingham, United Kingdom; 3) Department of Clinical Genetics, Churchill Hospital, Oxford, United Kingdom.

Birt-Hogg-Dubé syndrome (BHD), caused by germline mutations in Folliculin (*FLCN*), is an autosomal dominant cancer predisposing genodermatosis characterised by fibrofolliculomas (benign tumours of the hair follicles), lung cysts +/- spontaneous pneumothorax and an increased lifetime risk of renal cancer. The function of Folliculin remains to be fully understood but it has been shown to be involved in the mammalian target of rapamycin (mTOR), AMP-activated protein kinase (AMPK) and transforming growth factor (TGF-)) signalling pathways.

In collaboration with the European Birt-Hogg-Dube Consortium, we previously established a locus specific database (LSDB) for *FLCN* variants available at www.lovd.nl/flcn. Most mutations reported to date have been point mutations and small insertions/deletions. Recently large genomic deletions and a single small duplication in *FLCN* have been reported.

We present a family with 5 affected individuals over 2 generations with a clinical diagnosis of BHD. The proband presented with typical histologically confirmed fibrofolliculomas and developed an unusual renal cell carcinoma at age 36 years. The phenotypic features of the other affected individuals of the family will be presented along with other interesting findings including a carcinoma of the caecum in the proband's father and chronic inflammatory conditions in the other affected individuals.

Sequencing of the *FLCN* gene identified a novel exon 11 missense variant (c.1198G>A, p.Val400Ile) that did not segregate with the disease in the family. Multiplex Ligation-dependant Probe Amplification (MLPA) revealed a large duplication of exons 2 to 11, which was subsequently confirmed to be in tandem by long range PCR (LR-PCR) on genomic DNA and RNA studies.

The identification of a large duplication of the *FLCN* gene further expands the mutation spectrum in BHD. We recommend MLPA analysis in patients who show clinical features of BHD, but in whom a disease causing mutation cannot be identified on routine sequencing. MLPA analysis of the *FLCN* gene will improve the diagnostic yield of mutation screening and allow more accurate genetic counselling.

1201F

Tumor suppression in *Apc*$^{Min/+}$ mice by prostaglandin D_2. *H. Lin[1], B. Tippin[1], A. Kwong[1], J. Park[1], A. Materi[1], V. Buslon[2], S. French[2], S. Narumiya[3], Y. Urade[4], E. Salido[5].* 1) Division of Medical Genetics, Harbor-UCLA Medical Center, Torrance, CA; 2) Department of Pathology, Harbor-UCLA Medical Center, Torrance, CA; 3) Department of Pharmacology, Faculty of Medicine, University of Kyoto, Japan; 4) Department of Molecular Behavioral Biology, Osaka Bioscience Institute, Japan; 5) CIBERER, Hospital Universitario Canarias, La Laguna, Tenerife, Spain.

Prostaglandin D_2 (PGD_2) appears to prevent intestinal tumors, as shown by more adenomas in *Apc*$^{Min/+}$ mice that lack hematopoietic prostaglandin D synthase (HPGDS) and fewer tumors in mice that over-express this enzyme (Park et al. Cancer Res 2007; 67: 881-9). PGD_2 binds to receptor PTGDR, and PGD_2 metabolites bind to peroxisome proliferator-activated receptor gamma (PPARG). Therefore, we hypothesized that knockouts of the genes for PPARG or PTGDR may reduce tumor suppression by PGD_2. Two sets of experiments were used to test these predictions. First, we produced *Apc*$^{Min/+}$ mice with transgenic lipocalin-type prostaglandin D synthase (PTGDS), with and without heterozygous *Pparg* knockouts (104 mice, including controls). Next, in separate experiments, we produced *Apc*$^{Min/+}$ mice with *Ptgdr* knockouts (40 mice, including controls). Mice were sacrificed at 14 weeks. Intestines were removed in one piece, cleaned, opened lengthwise, fixed in formalin, coiled into “Swiss rolls,” embedded, sectioned, and mounted on slides for histology. To score adenomas, we used 24 Swiss roll sections spaced 150 μm apart for experiments on *PTGDS* transgenic mice and *Pparg* knockout mice. We used 10 Swiss roll sections spaced 250 μm apart for experiments on *Ptgdr* knockout mice. We grouped tumors into 3 size categories: intravillar lesions; other small tumors (seen on only one section); and large tumors (seen on multiple sections). Compared to results from our earlier report, transgenic PTGDS was less tumor suppressive than transgenic HPGDS. Nonetheless, *PTGDS* transgenic mice had lower median numbers of large adenomas (50% of control; $P = 0.022$) and lower levels of MYC mRNA in the colon. Heterozygous *Pparg* knockouts alone did not affect numbers of tumors, but *Pparg* knockouts blunted the suppression of large tumors by transgenic PTGDS. More dramatically, *Ptgdr* gene knockouts (homozygous or heterozygous) raised the median number of tumors 2-fold, compared to the median number among *Apc*$^{Min/+}$ controls ($P = 0.0005$). The increase was 2.6-fold for homozygous *Ptgdr* knockouts (8 knockout mice and 9 controls; $P = 0.0015$). The data establish the concept of PGD_2 as a tumor suppressing molecule, whose effect is mediated through receptor PTGDR in *Apc*$^{Min/+}$ mice. Inflammatory mechanisms involving T cells and dendritic cells may be involved. The results may suggest ways to prevent tumors in familial adenomatous polyposis and sporadic colorectal neoplasia by use of PTGDR agonists.

1202F

Gynecologic phenotypes in women with PTEN Hamartoma Tumor Syndrome. *J. Mester, C. Eng.* Genomic Med Inst, Cleveland Clinic, Cleveland, OH.

PTEN Hamartoma Tumor Syndrome (PHTS) is a molecularly-defined umbrella term used to describe individuals with Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome, and other conditions who have germline mutations of the PTEN tumor suppressor gene, localized to 10q23. Genotype-phenotype analysis, not designed to look at gynecologic phenotypes, and case reports suggest increased, but unknown, prevalence of endometrial carcinoma of the uterus (EC) in CS patients. By expert opinion, EC was included as a major criterion towards the clinical diagnosis of CS. Other gynecologic (GYN) tumors and anomalies count towards minor diagnostic criteria. However, the prevalence and natural history of GYN findings have not been systematically studied in a prospectively accrued series of CS patients. In our series of 122 PTEN pathogenic mutation-positive females recruited by relaxed CS diagnostic criteria (CS or CS-like) or presence of a known germline PTEN mutation, 85 were adults >18 yrs. Of these 85, 15 (18%) were found to have EC. Median age at EC diagnosis was 47 yrs (range 20-64), compared to 61 yrs in the general population. Based on SEER data, 0.194 cases were expected, giving an age-adjusted Standardized Incidence Rate (SIR) of 77.4 (95% CI 44.95-124.7, p<0.001). All EC with documented histology were endometrioid lesions, FIGO grade I or II . While most patients were overweight or obese (average BMI 31.6 kg/m2), patients with EC trended towards having even higher BMI (p=0.055). Of the 85 mutation positive adult females, 40 had fibroid tumors, which was not significantly associated with obesity (p=0.14). Among all 122 mutation positive females 10 (8.2%) had 11 ovarian tumors, 2 of which were malignant, and 6 had GYN developmental anomalies (4 bicornuate uterus, 1 duplicated cervix, 1 hypoplastic ovary). Current National Comprehensive Cancer Network screening guidelines for CS, which are utilized for patients with PHTS, advise patient education and prompt response to symptoms of EC. Prophylactic hysterectomy or uterine surveillance is not mentioned but we would advise clinicians to have a low threshold to offer these options to PHTS patients given both high prevalence and high SIR of EC in this population. Based on these data, we would not add a recommendation for ovarian cancer screening or oophorectomy, but would advise physicians to closely follow adnexal masses and understand the potential for malignancy.

1203F

Genetic testing for BRCA1 and BRCA2 mutations in women with hereditary predisposition to breast and ovarian cancer in Croatia. *V. Musani[1], M. Levacic Cvok[2], I. Susac[3,4], P. Ozretic[1], M. Sabol[1], D. Car[1], D. Eljuga[4], Lj. Eljuga[4], S. Levanat[1].* 1) Rudjer Boskovic Institute, Zagreb, Croatia; 2) Rudjer-Medikol Diagnostics, Zagreb, Croatia; 3) Croatian League Against Cancer, Zagreb, Croatia; 4) Eljuga polyclinic, Zagreb, Croatia.

Among women, breast cancer is the most commonly diagnosed cancer after non-melanoma skin cancer, and it is the leading cause of cancer deaths in Croatia. Ovarian cancer is in the fifth place, both in incidence and mortality. Epidemiological data indicates that 5-15% of breast cancer and 10% of ovarian cancer cases are hereditary, and germline mutations in the tumor suppressor genes Breast Cancer Gene 1 (BRCA1) and Breast Cancer Gene 2 (BRCA2) account for the majority of hereditary breast and ovarian cancers. BRCA1 and BRCA2 are involved in a variety of cellular processes, including maintenance of genome stability, DNA repair via homologous recombination, transcriptional regulation, cell cycle control, chromatin remodeling and ubiquitinylation. Mutations are scattered throughout both genes and include disease-causing mutations as well as unclassified variants. The contribution of BRCA1 and BRCA2 mutations to hereditary breast and ovarian cancer in Croatia is unknown. The purpose of this study was to estimate the incidence and spectrum of pathogenic mutations in BRCA1 and BRCA2 genes in high risk women in Croatia. The screening was performed with high resolution melting analysis, direct sequencing and semi-quantitative multiplex PCR method (Cvok et al. Clin Chem Lab Med 2008;46(10):1376-83). Protocols were certified by EMQN (European Molecular Genetics Quality Network). The complete coding sequences and exon-intron boundaries analyses of both genes were carried out on 160 women with family history of breast and ovarian cancer. Up to now 13 pathogenic mutations were detected, eight in BRCA1, with one previously unpublished, and five in BRCA2 (three previously unpublished). 22 BRCA1 and 36 BRCA2 unclassified variants and polymorphisms were also identified, of which two in BRCA1 and eight in BRCA2 were not previously published. This is the first molecular investigation of the hereditary predisposition to breast and ovarian cancer in BRCA1 and BRCA2 genes in Croatia. Samples were collected from different regions of the country and the level of pathogenic mutations and distribution of polymorphic variants will contribute to population statistics for 4.5 million population of Croatia. This study was funded by The Terry Fox Run 2009 donation and supported by The Terry Fox Foundation and Croatian League Against Cancer.

1204F

Birt-Hogg-Dubé syndrome in a large Hutterite family. *R. Perrier[1], K. Dornan[2], J. Parboosingh[1], W. Davidson[3].* 1) Department of Medical Genetics, University of Calgary, Calgary, AB, Canada; 2) Division of Medical Genetics, Alberta Health Services, Calgary, AB, Canada; 3) Division of Respirology and Department of Community Health Sciences, University of Calgary, Calgary, AB, Canada.

Birt-Hogg-Dubé syndrome (BHD) is an autosomal dominant condition characterized by cutaneous manifestations (fibrofolliculomas, trichodiscomas, and achrocordon-like growths), cystic lung disease and spontaneous pneumothoraces, and predisposition to renal cell carcinoma. We describe a large Hutterite family, originally descended from a Mennonite couple, with 33 affected individuals. The proband in this family was diagnosed with BHD after presenting with a personal and family history of multiple spontaneous pneumothoraces. A heterozygous mutation in the *FLCN* gene (c.59delT) resulting in premature protein truncation was identified. Following the diagnosis of BHD, cascade testing was offered to at-risk relatives in the extended family with 63 people agreeing to formal testing. The familial *FLCN* mutation was identified in 33 people over 3 generations with an age range of 24-71 years at the time of testing. There was a history of spontaneous pneumothoraces in 21% (N=7). The average age at first diagnosis of pneumothorax was 35.9 years (range 20-47 years). In the 28 people who had a chest CT scan, the degree of lung parenchymal cystic changes varied from normal parenchyma to extensive, bilateral cystic disease. The prevalence of cystic lung disease was 89%. Fibrofolliculomas were present in 54%. One incident renal cancer (an unclassified renal cell carcinoma) was diagnosed by screening abdominal CT scan in a healthy 40 year old male. The only other malignancy was a lung adenocarcinoma in an obligate carrier diagnosed at age 37. As consanguineous marriages are common in the Hutterite population, one couple was identified in which both partners were found to carry the *FLCN* mutation, giving rise to the possibility of offspring with homozygous *FLCN* mutations. This couple has three healthy young children who have not been offered genetic testing given their age. Animal data from mice suggests that homozygous loss of *FLCN* would likely cause an embryonic lethal phenotype, so it is unlikely that there would be any reproductive consequences for this family with the exception of possible reduced fertility. This large Hutterite cohort with Birt-Hogg-Dubé syndrome provides a unique opportunity to describe the variable phenotype associated with the c.59delT *FLCN* mutation, and in the future will allow for study of the natural history of BHD in a prospective manner.

1205F

Use of Whole Exome Sequencing to Identify the Molecular Basis of Susceptibility to Lymphoid Malignancies in Childhood. *B.C. Powell[1,2], M. Delario[2], L. Jiang[3], L. Trevino[3], R. Zabriskie[2], M. Kimmel[4], L.C. Strong[5], D.A. Wheeler[1,3], R.A. Gibbs[1,3], S.E. Plon[1,2].* 1) Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept. of Pediatrics, Texas Children's Cancer Center, Baylor College of Medicine, Houston, TX; 3) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX; 4) Dept. of Statistics, Rice University, Houston, TX; 5) Dept. of Pediatrics, University of Texas M.D. Anderson Cancer Center, Houston TX.

Introduction: Knowledge of cancer susceptibility genes impacts prevention, surveillance and treatment for patients and their at-risk family members. These same genes and their signaling pathways are also important in sporadic cancers. However, the underlying genetic basis of many childhood cancers remains unknown. Aims and Methods: Our goal is to identify novel high risk cancer susceptibility genes by analysis of germline DNA from small cohorts of childhood cancer families enrolled in research protocols through three pediatric cancer genetics programs in Texas. Variants are discovered by sequencing of all gene coding regions (using coding exons from VEGA, CCCDS and RefSeq on a capture platform comprising 42 Mb of target followed by sequencing on Illumina HiSeq). We have used this whole exome sequencing of constitutional (non-tumor) DNA to study 7 kindreds in which multiple closely related individuals have developed lymphocytic leukemia or lymphoma. Relationships among affected individuals in these kindreds suggest inheritance that is autosomal dominant (AD, 4 kindreds), AD vs. X-linked (2 kindreds) or indeterminate (1 kindred). Variants are further characterized based on segregation with cancer in family, informatic prediction of pathogenicity of missense changes and presence in both control and tumor sequencing datasets. Results and Conclusions: Greater than 20X coverage was obtained for 91-95% of each targeted exome. One early finding is a nonsense variant of *TP53* (c.916C>T; p.R306X) in a kindred containing 5 cases of high-risk ALL and only one other solid tumor. This result is surprising in that prior studies have found that p53 does not underlie familial leukemia/lymphoma and leukemia is not a major feature of Li Fraumeni syndrome suggesting that a leukemia modifier may also segregate in the family. In another kindred, we analyzed variants shared among 3 individuals with cancer. 95% of these variants were previously reported in dbSNP. The remaining 54 novel SNVs were further reduced to 33 by subtraction of variants also found in the healthy non-transmitting parent, 7 of which are predicted by bioinformatic analysis to be functionally important. Integration of data across similarly-affected kindreds will provide a means for identification of novel susceptibility genes for rare and common cancer types, as well as identification of unexpected cancer phenotypes due to mutations in known genes. Supported by CPRIT RP101089 and 5R01CA138836 to SEP.

1206F

Linkage Analysis and Whole-Exome Sequencing on Families with Multiple Lipomatosis. *F. Probst[1], R. Corrigan[1], R. Zabriskie[2], D. Murdock[3], R. Hamid[4,5], G. Tiller[6], J. Phillips[4], N. Kramer[7], J. Graham, Jr.[7], M. Bainbridge[3], W. Jin[2], L. Wang[2], R. Gibbs[3], S. Plon[1,2].* 1) Molec & Human Gen, Baylor Col Med, Houston, TX; 2) Pediatrics - Hematology & Oncology, Baylor Col Med, Houston, TX; 3) Human Genome Sequencing Center, Baylor Col Med, Houston, TX; 4) Pediatrics, Vanderbilt University School of Medicine, Nashville, TN; 5) Cancer Biology, Vanderbilt University School of Medicine, Nashville, TN; 6) Genetics, Kaiser Permanente, Los Angeles, CA; 7) Medical Genetics Institute, Cedars-Sinai Medical Center, UCLA School of Med, Los Angeles, CA.

Multiple lipomatosis (OMIM %151900) is an autosomal dominant condition that leads to the development of multiple encapsulated lipomas, predominantly on the trunk and limbs. We have recently ascertained two large families that exhibit this genetic disorder in several living generations. Most affected family members did not develop tumors until later in life, typically in the late teens or early 20s in males, or in the late 20s or early 30s in females. One individual was affected in infancy and by age 4 years had undergone two major surgical procedures to remove large lipomas from his back. Two (out of eight) of the affected women in the larger pedigree developed breast cancer, one in her 40s and the other in her 50s, suggesting that multiple lipomatosis may confer an increased risk for breast cancer. Conversely, the gene that is responsible for multiple lipomatosis may also be involved in the pathogenesis of sporadic breast cancers. Linkage analysis on the larger family was performed on data from 700K SNP arrays using MERLIN software, which revealed a single linkage peak with a LOD score of greater than 3.0, located on the short arm of chromosome 1. However, an additional peak with a LOD score in the range of 2.5 to 3.0 was also noted, as were nine peaks with LOD scores between 2.0 and 2.5. Exome capture of four affected family members from the larger family was performed with the Roche NimbleGen SeqCap EZ Human Library v2.0 capture reagent, and sequencing of the output DNA was performed on the Applied Biosystems SOLiD platform. The data were aligned to the hg18 reference genome using BFAST v0.4.6d, and variants were called with SAMtools. This analysis yielded two potential candidate mutations that were seen in all four individuals, one of which was under the main linkage peak. Candidate gene sequencing and linkage analysis on the second family are currently in progress, as is candidate gene sequencing in several smaller families, in order to determine the underlying gene (or genes) responsible for this disorder.

1207F

Does age at cancer diagnosis depend on parent of origin of BRCA mutation? *I. Shapira, D. Budman, M. Akerman, L. Weiselberg, V. Vinciguerra, J. D'Olimpio, C. Devoe, C. Chavez, K. Cheng, L. Donahue, S. Cohen.* Hofstra North Shore LIJ School Of Medicine New York.

Background: Recently reported epidemiologic evidence shows that daughters who inherit Lynch syndrome mutations from their fathers get cancer at an earlier age than those inheriting mutations from their mothers suggesting that autosomal dominant DNA mismatch repair genes may be subject to imprinting effects. Biallelic expression is not the norm for all genes in humans. Imprinting effect evident in autosomal dominant hereditary paraganglioma leads to tumors only if inherited from paternal germline. Cancer penetrance in carriers of BRCA mutation may be determined by the parental origin of mutation Methods: From 2004-2010 we analyzed 1456 consecutive patients who presented for genetic counseling. In 168 patients with BRCA 1 or BRCA 2 the parent of origin for the mutation was known. Of 168 patients 2 had both BRCA1 and BRCA2 mutated paternally inherited. The mean age of BRCA mutation diagnosis was 44 years of age with a range of 21-67 years of age. All the patients in our cohort were born after 1940. 75 inherited the deleterious BRCA mutation from their fathers (53 affected) and 91 individuals inherited the deleterious mutation from their mother (61 affected). No ovarian cancers were identified in our cohort of BRCA 2 mutations of maternal inheritance. Two-sample t-test was used to compare the mean age at diagnosis in patients with BRCA 1 or 2 mutations of paternal or maternal inheritance. Results: Maternal Paternal 2 sample t-test p-value Br Ca BRCA1 46 + 11 39 + 7 p< 0.0281 Br Ca BRCA2 51 + 10 41 + 5 p< 0.0007 Ov Ca BRCA2 56 + 7 44 + 3 p< 0.0080 Significantly younger age at breast and ovarian cancer diagnosis was observed in paternal inheritance of BRCA mutations. Conclusion: Maternal and paternal inherited BRCA alleles may not be exchangeable. Women with paternally inherited mutations in BRCA gene mutations have cancers diagnosed at earlier age compared with women who inherit the gene mutations from their mothers. In this small sample clear differences at age of cancer diagnosis are apparent in paternal inheritance of BRCA gene mutation. If this observation duplicates in larger cohorts results will have important implications for recommendation of surgical risk reduction in BRCA mutation carriers.

1208F

A founder XPD mutation among Iraqi Jews: Estimation of the most recent common ancestor (MCRA). *H. Slor[1,2], S. Orgal[1], Z. Keren[3], S. Koka[3], O. Bittermann Deutch[4], E. Azizi[5], F. Pavlotzki[5], R. Yaniv[5], M. Korostishevsky[1], K.H. Kraemer[6], P.C. Hanawalt[7], G. Spivak[7], T.C. Falik-Zaccai[3,8].* 1) Dept Human Gen, Tel Aviv Univ Sch Med, Tel-Aviv, Israel; 2) Biotechnology, Tel Hai Academic College, Upper Galilee,Israel; 3) Institute of Human Genetics, Western Galilee Hospital, Naharia, Israel; 4) of Dermatology, Western Galilee Hospital, Naharia, Israel; 5) Department of Dermatology, Sheba Medical Center, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; 6) National Cancer Institute, NIH, Bethesda, MD, USA; 7) Department of Biology, Stanford University, Palo Alto, Ca, USA; 8) Rappaport Faculty of Medicine, Technion, Israeli Institute of Technology, Haifa Israel.

Xeroderma pigmentosum (XP) is a rare autosomal recessive disease characterized by hypersensitivity to UV irradiation due to a defect in nucleotide excision repair (NER). XP patients have a high frequency of squamous and basal cell carcinomas as well as malignant melanomas appearing at a very early age (Kraemer et al., 2007). The prevalence of XP in western European population was estimated to be 0.9 per million live births (Kleijer et al., 2008) . Certain geographically isolated populations have a higher prevalence, especially where consanguinity is common; in Japan the rate is 1 in 22,000 (Hirai et al., 2006). Among the 450,000 Iraqi Jews in Israel, we estimate the incidence of XPD to be 1 in 10,000. We describe a cohort of 13 unrelated XP patients representing 13 families with a total of more than 40 XP patients. All the patients are of Kurdistani and Iraqi Jewish origin. They exhibit mild to medium sun sensitivity, and a relatively low frequency of skin tumors (basal cell and squamous cell carcinomas) compared to other XP patients. There were no eye abnormalities, No hearing loss, and no XP type neurological abnormalities except for loss of tendon reflexes. All patients were found to belong to the XP-D complementation group by host cell reactivation and cell fusion assays. Sequence analyses revealed that all the patients were homozygous for a p.R683Q mutation in the XPD gene. These patients did not have the neurological abnormalities seen in XP patients with the common p.R683W mutation at the same amino acid site (Lehmann, 2001). All the Jewish Iraqi XP patients carry the same homozygous haplotype along 420 kb downstream and 503 kb upstream of the XPD gene as determined by SNP analysis. These data suggest a founder mutation. Further SNP analysis further away from the above distances identified several different variations indicating recombination events from which the most common recent ancestor (MRCA) was estimated to be about 60 generations ago, which correspond to about 1500 years. This large cohort might expand our understanding of the genotype-phenotype correlations for the p.R683Q mutation in the XPD gene. References 1)Hirai Y,et al, Mutat Res 601:171-178 (2006). 2)Kleijer WJ,et al, Cockayne syndrome and trichothiodystrophy. DNA Repair (Amst) 7:744-750 (2008). 3)Kraemer KH,et al,Neuroscience 145:1388-1396 (2007). 4)Lehmann AR: Genes Dev 15:15-23 (2001).

1209F

Further phenotypic characterization of segmental neurofibromatosis type 1 in a large patient cohort. *J.S. Solomons[1], J. Jamieson[1], S. Huson[2], D. Shears[1], U. Kini[1].* 1) Clinical Genetics, Oxford Radcliffe NHS trust, Oxford, United Kingdom; 2) Genetic Medicine, St. Mary's Hospital, Manchester, United Kingdom.

Introduction: Segmental Neurofibromatosis type 1 (SNF1) is a rare variant of NF1 characterised by limited, segmental distribution of cutaneous NF1 lesions. Somatic mosaicism caused by post zygotic mutation of the NF1 gene leads to an SNF1 phenotype. The phenotype in SNF1 can be variable with usually unilateral and rarely bilateral and either symmetric or asymmetric distribution. There is a paucity of reports in literature on phenotypic characterization, complications, malignancy and reproductive risks on large cohorts of patients with SNF1. **Aim**: To study phenotypic variation, complications, risk of malignancy and reproductive risks in a large cohort of patients with SNF1. **Methods**: Review of case notes of all patients with SNF1 referred to Oxford regional genetics NF1 centre from 1993 to date. **Results**: Eighty one patients with SNF1 were reviewed at the Oxford regional NF1 centre over a period of 17 years. Some of the complications diagnosed in our patient cohort included 22 patients with plexiform neurofibromas , 3 spinal Neurofibromas, 3 optic gliomas and 2 sphenoidal wing dysplasias. Six of our patients were diagnosed with other forms of benign and malignant tumours not commonly associated with NF1 such as teratomas, breast tumours, lymphomas, lipomas and hemangiomas. We present a detailed report of further phenotypic characterization, malignancy and reproductive risks based on observations in our patient cohort. **Conclusion**: We present here the phenotypic variability of SNF1 seen in the largest cohort of patients to our knowledge reported so far. A larger cohort of patients need to be studied in order to determine more accurate reproductive and malignancy risks.

1210F

Novel mutation in tp53 I195S in a patient with Li-Fraumeni. *T. Wegman-Ostrosky[1,2], L. Taja-Chayeb[3], J. Sanchez-Corona[1], C. Moran-Moguel[1], C. Migues-Muñoz[3], S. Vidal-Millan[4].* 1) Medicina Molecular, CIBO, IMSS, Guadalajara, Jalisco, Mexico; 2) CUCS, Universidad de Guadalajara; 3) Investigación Básica , Instituto Nacional de Cancerología , México; 4) Investigación Clínica, Instituto Nacional de Cancerología México.

Li Fraumeni Syndrome (LFS) is a dominantly inherited cancer syndrome characterized by a high risk of developing soft tissue sarcoma, osteosarcoma, pre-menopausal breast cancer, brain tumors, adrenocortical carcinoma, and a variety of other neoplasms. The main cause of LFS is a germline mutation in the p53 gene, and there are nearly 600 mutations reported word wild. Individuals with LFS have increased risks for developing cancer at younger ages. In individuals who have a TP53 mutation, the median age of diagnosis of cancer is 25 years. We present a case of 9 years old boy with multiple primary tumors and family background of several members who had cancer. A previously healthy boy of 9 years old, had history of rounding of her face, acne on her forehead and signs of virilization were noted. On admission he had acne on the forehead and increased oiliness of the facial skin. A mass of 4 x 4 cm was palpable on left torax. There was axillary hair and genitalia showed signs of virilization. Abdominal computed tomography confirmed the finding of a right adrenal mass and a second mass on the 5th rib. The biopsies of both tumors confirm adrenocortical carcinoma in the first mass and osteosarcoma in the biopsy from the rib. DNA was isolated from peripheral blood leukocytes. Five pairs of primers were used to amplify exons 2-9 of p53. After corroborating a correct amplification, samples were analyzed in a DHPLC (Transgenomics™) device. Heterozygote profiles were identified in the amplicon of the exon 5/6 by visual analysis of the chromatograms, comparing peak shapes with a wild-type sample. Samples were sequenced to identify sequence change in samples with an aberrant DHPLC profile in these fragments. Electropherograms were analyzed in both sense and antisense direction for presence of mutations and a point mutation in the codon 195 was detected. The sequences obtained were compared with the reference TP53 (GenBank X54156). Results were compared with the following three databases: International Agency for Research on Cancer (IARC) database; and the P53 Knowledgebase. In the nucleotide 13344 a trasvertion T -> G was detected. This mutation, I195S, is located in the DNA binding domain of P53 therefore destroys the ability of the protein to bind to its target DNA sequences, and thus prevents transcriptional activation of these genes. I195S has not been reported has a germline mutation, so is the first case of Li-Fraumeni Syndrome inflicted by the mutation I195S.

1211F

Mutation analysis of the *Rak* gene in Finnish breast cancer families. *T. Heikkinen[1], LM. Pelttari[1], K. Aittomaki[2], C. Blomqvist[3], H. Nevanlinna[1].* 1) Department of obstetrics and gynecology, Helsinki university central hospital, Helsinki, Finland; 2) Department of clinical genetics, Helsinki university central hospital, Helsinki, Finland; 3) Department of oncology, Helsinki university central hospital, Helsinki, Finland.

Inherited susceptibility to breast cancer consists of germline mutations in various genes, most notably in *BRCA1* and *BRCA2*. High breast cancer risk is also a part of rare cancer syndromes such as Cowden Syndrome caused by mutations in the *PTEN* gene. PTEN (phosphatase and tensin homolog) is a regulator of the PI3K/Akt oncogenic pathway that is often dysregulated in cancer. Rak (FRK, Fyn related kinase) is a kinase protein belonging to Src family which directly interacts with PTEN by phophorylating it on amino acid residue Tyr336 thus stabilizing the protein. The *Rak* gene is located on the chromosomal region 6q21 overlapping with the recently published Nordic breast cancer linkage region 6q14.2-21.3 (Arason et al. Breast Cancer Res 2010). We sequenced the protein coding regions, exon-intron boundaries, and 5'UTR of the Rak gene in 116 breast cancer patients belonging to 111 non-BRCA1/2 families. *CHECK2* 1100delC and *PALB2* 1592delT mutations were also excluded. We identified 10 variants on the *Rak* gene of which four have not been reported previously. Two variants changed the amino acid sequence of the protein product: a common polymorphism c.364GA (rs3756772), p.Gly122Arg, and in two families a novel variant c.1451GA, p.Arg484His. Neither of these missense variants however had predicted functional consequences on the protein in SIFT, Polyphen, or SNPs3D prediction tools and were not evolutionarily conserved. The two novel 5'UTR variants -239TC and -160CG, both found in single families, were evolutionary conserved among primates, but not among other mammals. The -160CG variant was not present in another breast cancer patient (cousin) from the same family. The fourth novel variant was an intronic variant IVS6+42AG found in three unrelated patients. Mutations in the *Rak* gene seem unlikely neither to contribute in a large scale to hereditary breast cancer nor to explain the 6q linkage.

1212F

Association of Variants in Estrogen-Related Pathway Genes with Prostate Cancer Risk and Prognosis. *S.K. Holt[1], E.W. Kwon[2,3], R. Fu[1,4], S. Kolb[1], Z. Feng[1,4], E.A. Ostrander[2], J.L. Stanford[1,5].* 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Cancer Genetics Branch, National Human Genome Research Institute, NIH, Bethesda, MD; 3) Program in Human Genetics and Molecular Biology, Johns Hopkins University School of Medicine, Baltimore, MD; 4) Department of Biostatistics, University of Washington, Seattle, WA; 5) Department of Epidemiology, University of Washington, Seattle, WA.

Estrogen signaling plays a key role in prostate cancer (PCa) development and progression. Through mediation by its receptors, estrogen has been shown to have both carcinogenic and anti-carcinogenic effects on the prostate, as well as defining distinct molecular subclasses of PCa cells. Thus, genetic variation within this pathway may contribute to both disease risk and outcomes. We report results from a hypothesis testing population-based genetic association study of single nucleotide polymorphisms (SNPs) in estrogen-related candidate genes ESR1, ESR2, CYP19A1 CYP1A1, and CYP1B1. We evaluated PCa risk conferred by 73 tagSNPs genotyped in 1,453 incident PCa cases and 1,351 age-matched controls using logistic and multinomial regression. Assessment of recurrence/progression and PCa-specific mortality after an average of 10 years of follow-up was carried out using adjusted Cox proportional hazards regression. PCa risk was associated with 12 SNPs found in ESR1, CYP1A1, and CYP1B1. Only the association with CYP1B1 rs1056836 (OR CC vs. CG or GG = 1.3, 95% CI 1.3 95% CI 1.1-1.5) remained significant after adjustment for multiple comparisons. As secondary analyses, we examined SNP genotypes by dietary phytoestrogen intake, body mass index, and family history of PCa. For outcomes analyses, there were 331 cases with recurrence/progression events and 88 cases who died of PCa. Analyses of outcomes are underway, with preliminary findings suggesting that genomic variation in ESR1, ESR2, CYP1A1, and CYP19A1 are associated with PCa-specific mortality. Complete results with respect to prognosis will be shown at conference presentation. These data provide strong evidence that germline genetic variation of CYP1B1 is associated with risk of PCa and that germline genetic variation may contribute to PCa outcomes. Future studies are needed to understand the functional consequences of these genetic variations. Larger studies are needed to confirm these associations with PCa risk and prognosis and to identify genetic predictors in the estrogen pathway for specific subsets of patients with distinct environmental exposures or genetic backgrounds.

1213F

Association of Genetic Polymorphisms in the Androgen Pathway and Prostate Cancer Risk. *E.M. Kwon[1,2], S.K. Holt[3], R. Fu[3,4], S. Kolb[3], Z. Feng[3], J.L. Stanford[3,4], E.A. Ostrander[2].* 1) Program in Human Genetics, Johns Hopkins University School of Medicine, Baltimore, MD, USA; 2) Cancer Genetics Branch, National Human Genome Research Institute, NIH, Bethesda, MD, USA; 3) Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; 4) Dept. of Epidemiology, School of Public Health, University of Washington, Seattle, WA, USA.

Prostate cancer (PC) is the most frequently diagnosed solid tumor and second leading cause of cancer deaths in U.S. men. Multiple genome-wide association studies (GWAS) have identified over 40 single nucleotide polymorphisms (SNPs) that are significantly associated with PC risk, including genes in the androgen pathway (e.g., *KLK3*). Androgens play a central role in mediating prostate carcinogenesis and genes involved in the androgen pathway are therefore important candidates for PC susceptibility. In this hypothesis-testing study, we evaluated risk of PC in association with variants in 22 genes that are either involved in androgen metabolism or have been shown to interact with AR. Using samples from a population-based case-control study of Caucasian and African American men, 187 SNPs were genotyped in 1,458 cases and 1,351 age-matched controls. PC risk was estimated using adjusted unconditional logistic regression and multinomial regression models. Our single SNP analyses showed suggestive evidence (p<0.05) for an association of PC risk with 13 SNPs in 8 genes: *NKX3.1, HSD17B3, AKR1C3, CYP17A1, KLK3, JAK2, NCOA4* and *STAT3*. The most significant effect on risk was seen with rs2253502 in *HSD17B3* (odds ratio, OR=0.57, 95% CI: 0.39-0.84). In addition, 60 SNPs in 15 genes were associated with clinical features of PC such as stage, Gleason score, and a composite aggressive score; of these, 5 SNPs in 4 genes (*CYP17A1, HSD17B4, NCOA4*, and *SULT2A1*) were associated with more aggressive disease with p<0.01. Pathway-based association analysis is currently underway to test cumulative effect from multiple SNPs within the androgen metabolism pathway. Our preliminary findings show significant gene-gene interactions (p<0.0001) between a SNP rs10883783 in *CYP17A1* and a SNP rs7039978 in *HSD17B3* conferring 1.44-fold increased risk of PC. These results confirm that variants in genes involved in the androgen metabolism pathway are associated with risk of PC. Our results replicate previously reported associations of SNPs in *NKX3.1, HSD17B3, ARK1C3, CYP17A1* and *KLK3*. In addition, novel associations were observed for SNPs in *JAK2, NCOA4, HSD17B4*, and *SULT2A1*. The latter results as well as those for SNPs associated with clinical features and gene-gene interactions need further replication in larger studies.

1214F

Frequency of *BRCA1* and *BRCA2* mutations in prostate cancer. *D. Leongamornlert[1], E. Saunders[1], M. Tymrakiewicz[1], N. Mahmud[1], E. Castro[1], C. Goh[1], T. Dadaev[1], M. Guy[1], L. O'Brien[1], E. Sawyer[1], A. Hall[1], R. Wilkinson[1], The. UKGPC Collaborators[1], D. Easton[2], D. Goldgar[3], R. Eeles[1,4], Z. Kote-Jarai[1].* 1) Oncogenetics, The Institute of Cancer Research, Sutton, Surrey, United Kingdom; 2) Centre for Cancer Genetic Epidemiology, University of Cambridge, Cambridge, UK; 3) Department of Dermatology, University of Utah, Salt Lake City, US; 4) The Royal Marsden NIH Foundation Trust, London, UK.

The aetiology of prostate cancer (PrCa) is poorly understood. A family history of prostate cancer, particularly at a young age, is a strong risk factor for the disease, indicating that inherited factors are important in PrCa. Relative risk of prostate cancer (PrCa) in *BRCA1* and *BRCA2* mutation carriers ≤ 65 years has been estimated to be up to 1.85 and 7.3-23, respectively. We previously estimated that 2.3% of PrCa cases diagnosed ≤ 55 years harbour a *BRCA2* mutation and PrCa among *BRCA2* carriers has been shown to be more aggressive, with poorer survival. To further evaluate the role of *BRAC1* and *BRCA2* genes in PrCa predisposition we screened 914 cases for *BRCA1* mutation and 1821 for BRCA2 mutation. Patients were recruited through the UKGPCS (UK Genetic Prostate Cancer Study Collaborators) aged 36 to 86 years but the study was enriched for cases with an early age of onset, (~88% men diagnosed ≤ 65 years). We analyzed the entire coding region of the *BRCA1* gene using Sanger sequencing and the *BRCA2* gene using a novel high-throughput multiplex fluorescent heteroduplex detection system developed for the ABI3130xl genetic analyser BRCA1 was also analysed for large re-arrangements by MLPA for 460 cases. We identified 4 deleterious mutations and 33 unclassified variants (UV) in *BRCA1* and 19 deleterious mutations and 69 UVs in *BRCA2*. Based on studies in breast/ovarian cancer families, most UVs in these genes are likely to be non-pathogenic. Three of the *BRCA1* mutation carriers were affected at age ≤ 65 years and one developed PrCa at 69 years, whilst all the carriers of frameshift mutations in *BRCA2* were affected at age ≤ 65 years. The frequency of deleterious *BRCA1* mutation in this study is 0.43% and the frequency of *BRCA2* mutation is 1.05 % for all ages and 1.20 % for cases diagnosed ≤ 65 years. Using previously estimated carrier frequencies germline *BRCA1* mutation confers an increased relative risk of PrCa approximately 3.6 fold (95% CI 1.0-9.3) and this translates to a ~7.6% cumulative risk by age 65. We estimate that germline *BRCA2* gene mutation confers an increased relative risk of PrCa by age 65 of approximately 7 fold (95% CI 4.5-11), corresponding to a cumulative risk of ~15% by age 65. This study indicates a rationale for routinely testing young onset PrCa cases for germline *BRCA2* mutations, to enable the delivery of targeted treatments.

1215F

Evidence for a *hTERT* repressor gene on human chromosome 3p21.3 by using chromosome engineering. *T. Ohhira[1], S. Abe[2], H. Tanaka[2], T. Notsu[3], S. Horike[4], D-L. Qi[1], C. Fujisaki[1], G. David[2], M. Oshimura[1], H. Kugoh[1].* 1) Biomedical Science, Regenerative Medicine, Yonago, Tottori, Japan; 2) Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, Indiana , USA; 3) Regenerative Medicine and Therapeutics, Graduate School of Medical Science, Tottori University, Tottori, Japan; 4) Frontier Science Organization, Institute for Gene Research, Kanazawa University, Kanazawa, Japan.

Telomeres are the repeated sequence located on both ends of chromosomes in eukaryotes, which protect the end of the chromosome from deterioration or from fusion with neighbouring chromosomes and their eventual loss is thought to result in cellular senescence. Unlike normal somatic cells, most tumor cells had an activation of telomerase, which stably maintains telomere length by adding DNA sequence repeats (”TTAGGG” in all vertebrates) to the 3' end of DNA strands in the telomere regions. The RCC23 cell line derived from human renal cell carcinoma had telomerase activity and loss of heterozygosity on the short arm of chromosome 3. We and others have previously demonstrated that the introduction of a human chromosome 3 into RCC23, via microcell-mediated chromosome transfer (MMCT), repressed transcription of the *hTERT* gene, that is a key component and controlling factor required for telomerase activity. In this study, we performed an analysis of hTERT expression level in RCC23 cells transferred truncated chromosome 3 fragments (RCC23 #3del), to identify chromosomal region that carry *hTERT* suppressor gene. By development of chromosome engineering, we found a telomerase repressor region located within a 7-Mb interval on chromosome 3p21.3. These results provide important information regarding *hTERT* regulation and a unique method to identify *hTERT* repressor elements. Ref; S. Abe et al., Genome Integrity., 1., 2010.

1216F

Frequency of haplotype (C667T/A1298C polymorphisms) of MTHFR in Mexican breast cancer patients. *AM. Puebla[1], A. Solorzano[2], A. Ramos[2], LE. Figuera[3], MP. Gallegos[1].* 1) Inmunofarmacologia, CUCEI, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico; 2) Laboratorio de Genetica Molecular, Division de Medicina Molecular, CIBO, IMSS; 3) Division de Genetica, CIBO, IMSS.

The methylenetetrahydrofolate reductase (MTHFR) gene is important for folate metabolism, and the polymorphisms, C677T and A1298C, have been observed to reduce the enzymatic activity; C677T is present at high penetrance in Mexican populations. These polymorphisms has been associated with breast cancer in other population but no included the Mexican population. In the present study we determinate the frequency of haplotypes of C667T/A1298C polymorphism of MTHFR gene in 110 patients with breast cancer and 249 healthy controls from Mexican population. The frequency observed of 10% (23/220) and 16.6 % (83/492) of CC haplotype; 36% (79/220) and 37% (182/492) of CA haplotype; 40%(88/220) and 29% (142/492) of TA haplotype; similar frequency was observed in the TC haplotype 11% (24/220; 54/492) in both study groups; 3% (6/220) and 8% (38/492) heterozygous haplotype in cases and controls respectively. The statistically different ($p<0.05$) was observed in the TA haplotype with OR 1.64(95%CI 1.16-2.32). These results suggest that the TA haplotype of C667T/A1298C polymorphism on the MTHFR is associated with Mexican breast cancer patients.

1217F

Rapid and Precise Inference of Copy Number Alterations using Graphics Processing Units. *G.K. Chen[1], C. Curtis[1], K. Wang[1,2].* 1) Dept of Preventive Medicine, University of Southern California, Los Angeles, CA 90089, USA; 2) Zilkha Neurogenetic Institute, Dept of Psychiatry, University of Southern California, Los Angeles, CA 90089, USA.

Heritable copy number variations (CNVs) in the human genome have been extensively characterized, and numerous computational approaches are available for robustly detecting such variants in germline DNA. Somatic alterations in cancer genomes present a landscape of variation that differs from germline CNVs in that these copy number alterations (CNAs) tend to be more abundant and are typically broader. In addition to the general challenges in assigning copy number states, methods for detecting CNAs must deal with issues that are unique to tumor samples. For example, tumor specimens frequently exhibit aneuploidy as a result of genomic instability and those of epithelial origin (e.g. breast, colon) often contain a significant fraction of normal cells, known as stromal contamination. While stromal contamination can be ameliorated by techniques such as laser capture microdissection, such an approach is laborious and seldom applied in large-scale genomic studies. A second issue is that there are many possible copy number states and stromal contamination levels, which presents computational challenges if investigators are interested in precise characterization of CNAs. As such, modeling the true allelic copy number states as an estimated mixture of normal and tumor copy numbers, with the estimate of the magnitude of stromal contamination in a locus-specific manner, can lead to more precise CAN inference. We propose a continuous time Hidden Markov Model for high-resolution SNP genotyping arrays that allows users to specify an arbitrary number (up to several thousand) of hidden states, dictated by the maximum number of states, possible genotypes, and the granularity of mixture estimates. Our algorithm exploits massively parallel computing cores on graphics processing units (GPUs), enabling HMM training to proceed in a matter of minutes in contrast to days or months using a serial implementation. Our software, written in OpenCL, allows for portability across a wide range of target platforms such as multi-core CPUs, and GPU adapters from vendors like ATI or nVidia. We present results that demonstrate that our method performs as accurately as the current state of the art methods (which also model stromal contamination), but achieves results using only a fraction of the memory and CPU time required by these methods, allowing investigators who may not have access to a large computing cluster to feasibly carry out analyses on a large number of tumor samples.

1218F

Copy number variation analysis in 134 unrelated patients with mutation negative adenomatous polyposis. *S. Horpaopan[1], S. Vogt[1], I. Spier[1], A.M. Zink[1], K. Wöllner[1], S. Herms[1,2], M. Draaken[1,2], A. Kaufmann[1], D. Stienen[1], S. Uhlhaas[1], E. Holinski-Feder[3,4], M.M. Nöthen[1,2], P. Hoffmann[1,2], S. Aretz[1].* 1) Institute of Human Genetics, University of Bonn, Germany; 2) Dept. of Genomics, Life & Brain Center, University of Bonn, Germany; 3) University Hospital of the Ludwig-Maximilians-University, Campus Innenstadt, Munich, Germany; 4) MGZ - Center of Medical Genetics, Munich, Germany.

Background: Adenomatous polyposis syndromes are characterised by multiple colorectal adenomas and a high lifetime risk of colorectal cancer. Germline mutations of the APC and MUTYH genes cause the autosomal dominant Familial Adenomatous Polyposis (FAP) and autosomal recessive MUTYH-associated Polyposis (MAP), respectively. However, in up to 50% of families no germline mutation could be identified although a genetic cause is likely. Copy number variants (CNVs), in particular, heterozygous microdeletions, contribute significantly to the mutation spectrum of known hereditary tumor syndromes and thus it could be hypothesised that those heterozygous deletion CNVs might also be the underlying cause in yet unidentified genes responsible for monogenic adenomatous polyposis syndromes. **Methods:** Genomic DNA from 134 unrelated mutation negative polyposis patients was used for genome-wide SNP genotyping with the HumanOmni1-Quad BeadArray (Illumina). Putative CNVs were identified by the QuantiSNP v2.2 algorithm, filtered according to various criteria by use of the Cartagenia Bench™ software, by in-silico-analysis, and by comparison with 531 healthy controls, and validated by qPCR. **Results:** 35 unique heterozygous deletion CNVs (size: 10-613 kb) containing 38 protein coding genes could be validated in 33 of 134 patients (25%) but not in healthy controls. 25 genes are partly or completely deleted, in 13 more the deletion affects intronic regions only. Additionally, 47 unique duplication CNVs (size: 25-856 kb) from 38 patients (28%) were validated by qPCR. 49 out of the 106 involved genes are partially duplicated which might point to potential loss-of-function effects. 9 of 25 partial/whole deleted genes and 30 of 49 partial duplicated genes are expressed in the intestine. All CNVs are present only once in the whole cohort; all except eight patients harbor just one CNV. Candidate adenoma genes include protein kinases, transcription factors, and potential tumor suppressors. **Conclusions:** By applying stringent filter criteria, we identified a group of rare deletion and duplication CNVs which might contain predisposing genes for adenoma formation. After prioritization of the included genes according to function and pathway, upcoming work is sequencing the coding regions of the most interesting candidates in all patients to look for pathogenic point mutations. The study was supported by the German Cancer Aid (Deutsche Krebshilfe).

1219F

Cutaneous Somatic Mosaicism in Hereditary Leiomyomatosis and Renal Cell Cancer. *B.G. Hammond[1], R. Madani[1], M. Pithukpakorn[2], G.M. Glenn[2], C. Cabala[2], O. Toure[2], W.M. Linehan[2], M.H. Wei[2], J.R. Toro[1,2].* 1) Dermatology, Washington, DC Veterans Affairs Medical Center, Washington, DC, USA; 2) National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA.

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is the autosomal dominant predisposition to uterine and cutaneous leiomyomas (CL) and renal cell cancer. Patients with HLRCC develop solitary, segmental and/or disseminated CLs. Our objective was to investigate the frequency of inactivation of the second FH allele in CL and compare the pattern of loss of heterozygosity (LOH) in segmental and non-segmental CL. Two or more skin biopsies were obtained from CLs in a segmental distribution and at least one skin biopsy from a non-segmental CL from each patient. DNA was extracted from microdissected CL and normal tissues, and genotyped with one intragenic FH marker and 12 polymorphic markers surrounding the FH locus covering a 17 kb region. DNA sequencing was conducted to screen for FH somatic and germline mutations. A total of 87 CLs were biopsied from 47 individuals with HLRCC. 87% (76/87) of CLs had a somatic mutation or LOH. LOH at the FH locus was detected in 84% (73/87) of CLs of which 74% (57/77) showed deletions > 8.3 kb. In every case of LOH, the germline mutation was retained, and the wild-type sequence was lost. Three CLs that did not show LOH revealed a frameshift FH mutation. Only a single alteration was found in each CL and no somatic mutations were found in matched normal fibroblasts and peripheral blood leukocytes DNA. To investigate the functional consequences of second allele inactivation, the FH enzyme activity in 10 CLs from three HLRCC patients was measured. All CLs showed almost absent FH enzyme activity compared to fibroblasts from unaffected skin from the same patient. DNA specimens (2-7 per lesions) obtained from a segmental CL showed the same LOH pattern (size and polymorphic markers) in each patient (N=11). In contrast, DNA from a non-segmental CL (N=12) showed a unique LOH pattern distinct from segmental or other non-segmental CLs on the same patient. This is the largest molecular and genetic investigation of CL from HLRCC patients. The high frequency of second allele genetic alterations and nearly absent FH enzyme activity in CLs strongly supports that FH is a tumor suppressor gene. Our findings suggest that segmental CL may arise from a second hit in an early progenitor cell. In contrast, second hits in independent lesions occur later and randomly in smooth muscle-like differentiated cells. Therefore, the timing of the loss of second FH allele may predict the pattern and distribution of CLs.

1220F

Description of BRCA mutations, UVs, SNPs and haplotypes in North-Eastern Romanian population. *L. NEGURA[1], D. AZOICAI[1], M. MATEI[1], G. POPOIU[2], A. CONEAC[3], N. UHRHAMMER[4], Y.-J. BIGNON[4], A. NEGURA[2].* 1) Immunology, University of Medicine and Pharmacy Gr. T. Popa, IASI, IASI, Romania; 2) Biochemistry and Molecular Biology, Alexandru Ioan Cuza University, IASI, Romania; 3) University of Medicine and Pharmacy Iuliu Hatieganu, Cluj-Napoca, Romania; 4) Centre Jean Perrin, Clermont-Ferrand, France.

Breast cancer is the most common cancer in women worldwide, including Romania, where its annual incidence has increased significantly to 14,000 during the last decade. In 30% of all cases there is a familial segregation, while in at least 10% a clear Mendelian inheritance is usually shown in different genetic backgrounds/populations. Germinal mutations in predisposition genes like BRCA1 or BRCA2 are responsible for up to 90% of cancer cases in hereditary breast and ovarian cancer (HBOC) families. Screening for BRCA mutations and polymorphisms is nowadays standard practice in the western world, allowing medical follow-up and genetic counseling of risk patients from HBOC families. Currently, most laboratories performing diagnostic analysis of the BRCA genes proceed to PCR amplification of exons and intron-exon boundaries, coupled to a pre-screening step to identify anomalous amplicons. The techniques employed for the detection of mutations and SNPs have evolved over time and vary in sensitivity, specificity and cost-effectiveness. We adapted several pre-screening techniques and imagined new ones in order to reduce the number of sequenced BRCA amplicons. MLPA screen was implemented for detecting large gene deletions or duplications. Multiplex-PCR and allele-specific PCR were used for rapid identification of known recurrent mutations in our region. In-house PCR-RFLP targeted novel BRCA mutations. Heteroduplex analysis by mismatch-specific endonuclease was used to pre-screen unknown mutations and to identify anomalous amplicons prior to sequencing. All detected variations were confirmed by forward and reverse dideoxy sequencing. We combined all these techniques in a pre-screening model. We present the first study regarding BRCA mutations and SNPs in Romanian population. We observed a variety of BRCA mutations, which may place the North-Eastern Romanian population somewhere between western populations (few recurrent, many rare or unique mutations) and eastern ones (recurrent mutations responsible for the majority of predisposed families, very few novel or unique mutations). Two missense variants of unknown significance (UVs) were observed. We used 14 prevalent SNPs to define BRCA1 alleles, and identified 7 different BRCA1 haplotypes in our population, with 5 canonical previously described and 2 novel haplotypes. This study was possible with partial financial support from the Romanian Ministry for Education and Research, by the CNCSIS grant PN-II-PD-557/2010.

1221F

Prostate Cancer predisposition loci and risk of metastatic Prostate Cancer in Asian Indians. *Y. TAN[1], J. AHN[2], T. REBBECK[3], R. MITTAL[4], A. MANDHANI[4], B. MITTAL[4], R. HAYES[2], H. RENNERT[1].* 1) Department of Pathology, Weill Cornell Medical College, New York, NY; 2) NYU School of Medicine, New York, NY; 3) University of Pennsylvania, Philadelphia, PA; 4) Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India.

Objectives: Prostate cancer varies widely by geographic location and ethnicity. American men have a high prostate cancer (PC) risk, but most have localized disease. In contrast, Asian Indians have a low PC risk, but most are diagnosed with metastatic disease. Genetic contribution to PC risk is well-established, but less is known about gene variation-associated PC in low-risk populations. The objective of this study was to investigate the role of selected susceptibility single nucleotide polymorphisms (SNPs) based on their genome-wide association with PC in the Cancer Genetic Markers of Susceptibility (CGEMS) in Asian-Indian men with advanced PC. Methods: DNA samples obtained from 121 cases and 216 age-matched controls from Asian Indians (Northern India) were genotyped for 15 SNPs at 11 different loci (JAZF1, 11q13, MSMB, 10q23, IL16, 8q24, HNF1B, CDH13, CPNE3, CTBP2, and 8p21). The association between SNPs and the risk for metastatic PC was analyzed by logistic regression. Age adjusted Odds Ratios (OR) were estimated under each possible genetic model, and the best fitted model was selected. Results: Six SNPs are significantly associated with risk for metastatic prostate cancer in Asian Indian. Among those six, four are located on 8q24: rs4242382 (OR=2.34, 95% CI=1.44-3.80); rs1447295 (OR=1.93, 95% CI=1.19-3.15); rs1016343 (OR=1.67, 95% CI= 1.09-2.57); rs6983561 (OR=1.89, 95% CI=1.13-3.14), one from MSMB rs10993994 (OR=1.82, 95% CI=1.14-2.88), and the other one from JAZF1 rs10486567 (OR=0.54, 95% CI=0.34-0.86) . The best fitted genetic model for the six significant SNPs is either Dominant or Log-additive. Conclusions: Our results support the role of selected susceptibility SNPs in 8q24, MSMB, and JaZF1 in metastatic PC in Asian-Indian men. This study warrants additional investigations of these genes in PC etiology to identify markers that can better predict individuals that are more likely to develop lethal PC.

1222F

Spatio-temporal dynamics of ATM protein at DNA damage sites define a distinct sub-pathway of the DNA double strand break response network. *M.S. Meyn[1,2,3], P. Bradshaw[1,2].* 1) Prg in Gen & Genomic Biol, Hosp Sick Children, Toronto, ON, Canada; 2) Dept of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 3) Dept of Paediatrics, University of Toronto, Toronto, ON, Canada.

Human cells use complex signaling networks to detect and respond to DNA damage in genomic DNA. ATM, the protein kinase inactivated in the cancer syndrome Ataxia-Telangiectasia, controls the major DNA Double Strand Break (DSB) response pathway. Current models assume that ATM is a critical component of . H2AX-associated megabase-sized chromatin complexes that develop around induced DSBs and serve as a platform for DSB repair. To better understand the behavior of ATM at DNA breaks we use UVA light + Hoechst dye to introduce spatially-defined DNA breaks within chromatin in human fibroblasts.

Photo-induction of DNA damage, equivalent to ~10 Gy . -radiation, causes rapid recruitment of ATM to damage sites. ATM recruitment occurs after MRE11/Rad50/NBS1 (MRN) and prior to DNA damage response factors . H2AX, 53BP1 and MDC1. Unlike . H2AX, ATM accumulation peaks 10-30 minutes post damage, then declines with kinetics paralleling those of DSB rejoining. While induced foci of . H2AX and many other DSB response proteins enlarge and diffuse with time, ATM forms discrete non-diffusible, MDC-1 independent foci. ATM foci formation requires MRN, but at 1 hr post damage, ATM foci are spatially separate from MDC1-independent MRN foci which themselves colocalize with homologous recombination (HR) proteins RPA, Rad51 and FANCD2. The non-diffusible nature of DNA damage-associated endogenous ATM foci is confirmed by FRAP analysis of GFP-tagged ATM. Auto-phosphorylation of serine 1981 is key for ATM foci formation, as mutating serine 1981 to alanine impairs GFP-ATM accumulation and over-expression of the telomeric protein TRF2, an ATM kinase inhibitor whose binding spans the S1981 residue, also attenuates GFP-ATM accumulation at DNA damage sites. Further, transient depletion of the chromatin regulator HMGN1 results in serine 1981 phosphorylation and activation of ATM signalling. Our results argue against current DSB response models as they indicate that at least 3 classes of complexes form at DSBs: . H2AX-associated megabase complexes; discrete foci of HR proteins; and discrete foci of ATM. ATM foci dynamics suggest ATM is not a major component of . H2AX-associated megabase complexes and does not directly facilitate HR-mediated repair. Rather, our findings suggest that ATM rapidly accumulates at localized sites of damage in an MRN-dependent manner, then is displaced as DNA breaks are either rejoined or processed for recombinational repair.

1223F

Single-cell RT-PCR cDNA subtraction and expression profiling of putative bipotential B-macrophage progenitors improves the identification of both normal and leukaemic haematopoietic development. *E. Sakhinia[1,2], Y. Heshmat[2], G. Brady[3].* 1) Molecular Medicine Division, Tabriz university of Medical Science, Tabriz, Iran; 2) 2- Tabriz Genetic Analysis Centre (TGAC), Sheikh Alraeis Clinic, Specialized & Subspecialized Outpatient Clinic of Tabriz Medical Sciences University, Tabriz, I.R.Iran; 3) Novel Therapies Division, EpiStem Ltd , Manchester,UK.

The molecular mechanisms underlying haematopoiesis are as yet unclear, but they will certainly be reflected and often driven by changes in gene expression. Identification of changes in gene expression accompanying haematopoietic differentiation is, therefore, likely to further our understanding of both normal and leukaemic haematopoietic development. In recent years, there has been increasing evidence for the existence of naturally occurring haematopoietic precursors, which are restricted to B cells/macrophages (B/M precursors) and are also a target for neoplastic transformation. Further understanding of B/M precursors would therefore provide potential insights into the regulation of both normal and leukaemic haematopoietic developments. The aim of this study was to examine early haematopoietic precursors in order to evaluate whether B cells and macrophages share a common restricted development precursor and if this is reflected at the molecular level. Our approachs were 1) Enrich for B/M specific genes by applying cDNA subtraction to foetal liver fractions enriched for B/M precursors. 2) Identify and characterise individual B/M precursors 3) Use cDNA subtractions, cloning and sequencing to analysis putative B/M specific genes. Analysis of the first set of cDNA subtractions indicated that B/M specific transcripts were rare in cDNA prepared from pools of enriched cells and an initial screen of cDNA clones failed to identify useful B/M markers. To further refine the approach single cell analysis was performed and resulted in the identification of three individual putative B/M precursors. The DNA sequencing showed that three of the subtracted clones have no similarities in the gene bank suggesting that subtraction is more reliable for discovering unknown stage specific and rare genes. But this does not negate the fact that the single cell approach alone is capable of identifying novel genes. Generally, the sequencing results confirm that the cDNA being used is still sound and that the whole approach is working, including sequencing. The approach taken is also enriching for rare unknown genes.

1224F

In Silico Estimation of Probabilities in Favor of Pathogenicity for Mismatch Repair Gene Missense Substitutions. *B.A. Thompson[1], M.P. Vallee[2], J.C. Herkert[3], C. Tessereau[4], E.L. Young[5], I.A. Adzhubei[6], B. Li[7], P. Gaildrat[9], R. Bell[5], S.D. Mooney[8], P. Radivojac[7], S.R. Sunyaev[6], T. Frebourg[9], R.M.W. Hofstra[3], R.H. Sijmons[3], K. Boucher[5], A. Thomas[10], D.E. Goldgar[11], M.S. Greenblatt[12], A.B. Spurdle[1], S.V. Tavtigian[5].* 1) Queensland Institute of Medical Research, Herston, Queensland, Australia; 2) International Agency for Research on Cancer, Lyon, France; 3) Department Genetics, University Medical Center Groningen, Groningen, The Netherlands; 4) Genetics of Breast Cancer Team, Cancer Research Center of Lyon, Centre Leon Berard, Lyon, France; 5) Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah School of Medicine, Salt Lake City, UT; 6) Division of Genetics, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 7) School of Informatics and Computing, Indiana University, Bloomington, IN; 8) Buck Institute, Novato, CA; 9) Department of Genetics, University Hospital and Inserm U614, Institute for Biomedical Research and Innovation, Rouen University, Rouen, France; 10) Department of Internal Medicine, University of Utah School of Medicine, Salt Lake City, UT; 11) Department of Dermatology, University of Utah School of Medicine, Salt Lake City, UT; 12) Department of Medicine, University of Vermont, Burlington, VT.

Clinically useful classification of rare missense substitutions observed during genetic testing is one of the outstanding problems in clinical genetics. Perhaps the most developed solution is the Bayesian “integrated evaluation” used to assess substitutions observed during testing of the breast cancer susceptibility genes BRCA1 and BRCA2. Here we take an important step towards an analogous system for the mismatch repair (MMR) genes (MLH1, MSH2, MSH6, PMS2) that confer colon cancer susceptibility in Lynch syndrome. We calibrated in silico methods to estimate probabilities of pathogenicity for MMR gene missense substitutions. We developed a qualitative MMR gene missense substitution classification system based on the IARC five-class system. After excluding uncertain variants, there were 81 missense substitutions that 1) could be classified as pathogenic, likely pathogenic, likely not pathogenic, or not pathogenic and 2) had no evidence of causing a splice defect. In parallel, we built and curated protein multiple sequence alignments for each of the four MMR genes, with all four alignments based on a fixed tree of metazoan sequences. We used six different missense substitution analysis tools (Align-GVGD, MAPP, MutPred, PolyPhen-2.1, SIFT, and Xvir) to score the missense substitutions. The tools' outputs were calibrated by regression against the classifications of the 81 missense substitutions. All six tools were reasonably effective at missense substitution analysis, generating correlation coefficients (R^2) of 0.39 - 0.54, regression P-values of 10E-10 and lower, and areas under the ROC curve of 0.85 - 0.91. MAPP was the most accurate stand-alone tool tested. Benefits of combining two tools into a single classification model were explored. Taking into account problems of colinearity and the requirement that secondary tools make a significant contribution to combined models, we found MAPP + MutPred to make the best combined model and, excluding those two tools, PolyPhen-2.1 + Align-GVGD to make the second best combined model. Because the integrated evaluation can accept prior probabilities as a continuous variable, our in silico models avoid binary classification in either their inputs or outputs. By design, their outputs are interpretable as probability in favor of pathogenicity. The best model has an area under the ROC curve of 0.93, and 54% of input substitutions receive probabilities of either >90% or <10% with only one apparent classification error.

1225F

The GWAS-identified prostate cancer associated SNP, rs10993994, transcriptionally regulates microseminoprotein-beta (MSMB) levels. *X. Xu[1], J.E. Hayes[1], H.G. Lilja[2,3,4], R.J. Klein[1,2].* 1) Cancer Biol & Gen, Sloan-Kettering Cancer Ctr, New York, NY; 2) Department of Laboratory Medicine, Division of Clinical Chemistry, Lund University, Malmö University Hospital, 205 02 Malmö, Sweden; 3) Reproductive Medicine Centre, Lund University, Malmö University Hospital, 205 02 Malmö, Sweden; 4) Department of Clinical Laboratories, Surgery (Urology) and Medicine (GU-Oncology), Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA.

Background: Recent genome wide association screens have identified a single nucleotide polymorphism (SNP), rs10993994, as a locus that confers genetic predisposition to prostate cancer. This SNP is located in the proximal promoter of micro-seminoprotein beta (*MSMB*). *MSMB* codes for one of major secretory products of the prostate,) -MSP/PSP94. Though the function of) -MSP is unknown, the protein and a peptide derivative have properties suggestive of tumor suppressors. We have previously shown that rs10993994 genotype is strongly correlated with physiological) -MSP levels in the serum and semen of young healthy men.

Objectives: We hypothesize that rs10993994 is the functional variant responsible for the prostate cancer association signal, and acts through reducing *MSMB* levels, a putative tumor suppressor. Consequently, our objective is to elucidate the regulatory mechanisms underlying rs10993994's influence on *MSMB*/) -MSP levels.

Results: Through promoter reporter activity analysis, we have determined that single nucleotide difference at rs10993994 causes a reduction in in vitro transcriptional activity. We also find evidence for allele-specific expression of *MSMB* in a cell line heterozygous for rs10993994. We have found several transcription factors with differential in silico predicted binding potential to the rs10993994 locus. We are currently testing each of these in turn through chromatin immunoprecipitation and siRNA-mediated knockdown of predicted transcription factors.

Conclusions: Our results support the hypothesis that rs10993994, a prostate cancer associated SNP identified from recent GWAS studies, is the functional variant responsible for the association signal. One criticism of genome wide association studies has been the lack of functional follow-up to association signals. Our preliminary findings suggest a compelling possible causal pathway, in the case of rs10993994's association with) -MSP levels and subsequent prostate cancer development. Our ongoing studies will resolutely determine the role of rs10993994 in *MSMB* regulation.

1226F

Genes with Novel SNPs and Indels in the Early-onset Female Breast Cancer Identified through Whole Exome Sequencing. *C. Lee[1], N. Leng[2], W. Kuo[3], H. Yang[4], K. Nobuta[2], Y. Lu[5], C. Chang[1], C. Lin[5], C. Chen[6], W. Yao[6], K. Chiu[1], A. Cheng[5], C. Shen[6], K. Chang[3,7], C. Haudenschild[2], C. Chen[1].* 1) Genomics Research Center, Academia Sinica, Taipei, Taiwan; 2) Genomic Services, Illumina, Inc., Hayward, CA, US; 3) Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan; 4) Molecular and Genomic Epidemiology Research Center, China Medical University Hospital, Taichung, Taiwan; 5) Department of Oncology, National Taiwan University Hospital, Taipei, Taiwan; 6) Institute of BioMedical Sciences, Academia Sinica, Taipei, Taiwan; 7) Department of Surgery, Cheng Ching General Hospital, Taichung, Taiwan.

The incidence rates of early-onset breast cancer has been increasing strikingly in Taiwan in last two decades. Clinical characteristics of early-onset breast cancer are very different between patients in Taiwan and Western countries. The proportion of estrogen receptor (ER)-expressed breast cancer with good prognosis is much higher in Taiwan than in Western countries. Whole exome sequencing was used in this study to explore novel single nucleotide polymorpgisms (SNPs), and insertions and deletions (indels) involved in the development of early-onset breast cancer in Taiwan. Paried blood and tumor tissue samples were collected from 68 patients with breast cancer diagnosed at ages 40 years old or younger. They were classified into five subtypes by the expression of ER, progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and Ki67 antigen. There were 37 luminal A [ER(+), PR(+/-), HER2(-), Ki67 low], 10 luminal B [ER(+), PR(+/-), HER2(-), Ki67 high], 12 luminal/HER2(+) [ER(+), PR(+/-), HER2(+)], 4 HER2(+) [ER(-), PR(-), HER2(+)] and 5 triple negative [ER(-), PR(-), HER2(-)] cases. Whole exome sequencing was performed by Illumina GAIIx system, with more than 8GB data in each sample. Using hg19 as a reference, SNPs and indels were identified by CASAVA 1.7 software; and genes with novel SNPs and indels were annotated by ANNOVAR software. Synonymous or non-frameshift mutations were excluded. Novel SNPs and indels were further ascertained by removing variations that were reported in 1000 Genome Project and dbSNP database. The remaining novel SNPs and indels in paired blood and tumor tissues of each patient were compared to find genes with novel somatic mutations. A total of 2881 genes with novel SNPs and indels were identified in 68 patients. One gene with novel mutation was found in 21 patients (31%), and another 10 genes with novel mutations were found in 14 (21%) to 19 (28%) patients. There were 13 genes with novel mutations observed in six or more patients affected with luminal-related subtypes only. There was one gene with novel mutations identified in all patients affected with HER2(+) subtype and another gene with novel mutations was also observed in all patients of triple(-) subtype. The findings suggest different genetic mutations are involved in the carcinogenesis of various subtypes of breast cancer. These common mutated genes in specific subtypes might provide new therapy targets.

1227F

The genomic landscape of childhood pre-B acute lymphoblastic leukemia. *J. Spinella[1], V. Saillour[1], J. Healy[1], M. Larivière[1], A. Alter[1], C. Richer[1], M. Bourgey[1], S. Busche[2], B. Ge[4], A. Montpetit[4], T. Pastinen[2,4], D. Sinnett[1,3].* 1) Sainte-Justine UHC Research Center, University of Montreal, Montreal, Qc, Canada; 2) Department of Human Genetics, McGill University, Montreal, QC, Canada; 3) Department of Pediatrics, Faculty of Medicine, University of Montreal, Montreal, Qc, Canada; 4) McGill University and Genome Quebec Innovation Centre, Montreal, QC, Canada.

Precursor B-cell acute lymphoblastic leukemia (pre-B ALL) is the most frequent pediatric cancer. Increased understanding of the pathobiology of B-cell ALL has led to risk-targeted treatment regimens and increased survival rates. However, the underlying causes of this pediatric cancer are still unclear. We are using next-generation sequencing technology to better understand the genomic landscape of pre-B ALL and to build a catalogue of variations involved in pediatric ALL onset and/or progression. Using a unique “quartet” design that involves matched tumor (at diagnosis) and normal (remission) samples, as well as DNA from both parents, we will be able to identify germline variations (which can modify the risk of disease and/or therapeutic responses) and somatic mutations. Here, we report the deep-sequencing of the whole exomes and the partial miRNomes of 30 childhood B-cell ALL quartets. Using the Agilent SureSelect Kit combined with multiplex paired-end sequencing (ABI SOLiD 4), we captured over 4.0 Gb of sequence on average per sample with a mean coverage of 40X. Genome-wide genotyping (Illumina's Omni 2.5 array) was also performed on all samples for quality control. For each individual, approximately 97% of the targeted bases were covered ⁄ 1X and 80% of the targeted bases passed our thresholds for variant calling (⁄ 8X coverage, MQV ⁄ 30). According to these criteria, about 30,000 SNPs were found per individual. Using this quartet design we were able to incorporate parental sequence information to reduce sequencing errors and facilitate the identification of overlapping candidate disease-causing variants. From the identified repertoire of germline variants and based on several criteria (PolyPhen annotation, known allele frequency etc.), we were able to highlight both rare (about 80 missense variants per individual) and common variants that are strong functional candidates for association testing. We also investigated the somatic mutation profiles and identified both recurrent and private leukemia-specific mutations; our goal now is to validate potential driver mutations that could play a direct role in leukemogenesis through functional assessment. Ultimately, this work will provide invaluable insights to understand the genetic mechanisms underlying pediatric ALL which could lead to the development of powerful clinical tools to improve detection, diagnosis and treatment of this childhood cancer.

1228F

A genome-wide association study to identify genetic markers associated with endometrial cancer grade. *T.A. O'Mara[1,2], D. Duffy[2], D.J. Thompson[3], S. Ahmed[4], K. Ferguson[2], C.S. Healey[4], G. Montgomery[2], M. Shah[4], J. Morrision[3], P.P. Pharoah[3,4], A.M. Dunning[4], P.M. Webb[2], D.F. Easton[3,4], A.B. Spurdle[2], ANECS.* 1) Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, QLD, Australia; 2) Division of Genetics and Population Health, Queensland Institute of Medical Research, Brisbane, QLD, Australia; 3) Department of Public Health and Primary Care, University of Cambridge, Strangeways Research Laboratory, Cambridge, UK; 4) Department of Oncology, University of Cambridge, Strangeways Research Laboratory, Cambridge, UK.

Endometrial cancer is the most malignancy of the female genital tract. Although endometrioid endometrial cancer (80% of cases) generally carries a good prognosis, some patients with this tumour subtype relapse within two years. Identification of genetic markers associated with prognosis could inform clinical decision-making for management at diagnosis, and inform development of chemotherapeutic agents targeting aggressive disease. Genome-wide association studies (GWAS) have been successful in identifying common genetic variation involved in cancer susceptibility. Presently there are limited published studies using GWAS data to identify single nucleotide polymorphisms (SNPs) associated with tumour prognostic indicators, such as grade. We used case data from an endometrial cancer case-control GWAS to assess association of SNPs with endometrial cancer grade. Genome-wide genotyping of 1285 Australian and British women with endometrioid endometrial cancer and reporting Caucasian ethnicity was performed using the Illumina 610K BeadChip. After applying quality control measures, data on 583,366 SNPs for 1220 cases with grade information were used in the analysis. Regression analyses assessing SNP association with grade (1, 2 or 3), adjusting for study group (Australian or British) were performed using PLINK software. Gene-based analysis was performed using the Versatile Gene-Based Association Study (VEGAS) program. Fifty-seven SNPs were found to be significant at <10 4. Two genes were identified by VEGAS as having a p-trend<10-6. Validation is underway of two variants with evidence of association with higher endometrial cancer grade (p-trend<10-6) in 10 independent datasets from Australia, UK, Belgium, Sweden, Norway and Germany. These SNPs are located in or near genes not previously reported to be involved in cancer aetiology or prognosis and, if confirmed, would represent novel gene targets. Neither of these SNPs fall into the top 1500 SNPs prioritised for validation of association with risk. Results to date suggest that genetic alleles associated with prognostic features, such as cancer grade, may be distinct from those associated with predisposition. GWAS analysis of tumour prognostic features is thus likely to improve understanding of biological pathways influencing outcome for endometrial cancer patients.

1229F

Precise Characterization of Stromal Transcriptional Response to Developing Tumors Using RNAseq. *R.O. Bainer[1], C. Frankenberger[2], J. Menon[2], C. Chavarria[1], K. Michelini[1], P. Melsted[1], Y. Gilad[1], M.R. Rosner[2].* 1) Human Genetics, The University of Chicago, Chicago, IL; 2) Ben May Department for Cancer Research, University of Chicago, Chicago, IL.

The ability of cancer cells to survive and grow at primary and metastatic sites is influenced to a large degree by the surrounding cells in the tumor environment. In this process, tumor cells secrete signals that recruit specific cell types to their immediate vicinity and induce these cells to produce factors critical to tumor development, while the surrounding normal tissue, or stroma, detects and responds to developing lesions. While the clinical importance of this complex dialogue between the tumor and the surrounding tissue has long been appreciated, its molecular basis is not well understood. In particular, studies attempting to characterize interactions between tumors and the surrounding tissue have been confounded, largely due to technical reasons relating to the difficulty of precisely identifying and separating the developing tumor cells from normal cells nearby. Recent developments in massively parallel sequencing technology offer an opportunity to overcome this difficulty and precisely identify factors associated with the stromal response to developing tumors on a genomic scale. By sequencing RNA from a mouse xenograft model whereby human tumor cells are introduced into a murine stromal background, we were able to identify changes in gene expression levels at the tumor-stromal interface. Using this strategy we were able to unambiguously assign reads to either the tumor or stromal transcriptomes, with an estimated misassignment rate approaching 0.01%. These methods have allowed us to directly compare transcript levels in stroma containing developing tumors to those levels observed in naive stroma, and subsequently identify a set of candidate factors likely involved in the stromal response to developing lesions. We then extended this method to contrast stromal responses to near-isogenic tumors with differing metastatic potential and invasive phenotype, thereby identifying candidate factors likely to be specifically relevant to metastatic colonization. In this experiment we utilize RNA sequencing technology in an innovative way to increase our understanding of the interplay between tumors and their local environment.

1230F

Somatic Mutation of *GNA11* in an Optic Nerve Sheath Dural Melanoma. *S.C. Benes[1,2], M.H. Abdel-Rahman[2], E. Dosunmu[3], E. Craig[2].* 1) Eye Center of Columbus, 262 Neil Ave. Suite 210, Columbus, OH 43215; 2) The Ohio State University, Department of Ophthalmology, 915 Olentangy River Rd. Columbus, OH 43212; 3) Mayo Clinic, Department of Ophthalmology, Rochester, MN.

In this study we investigated the molecular genetics of a case of optic nerve dural melanoma. We also report the biological behavior of two additional cases of optic nerve dural melanoma. Molecular genetic alterations in chromosomes 3, 6p, 8q were studied by genotyping. Somatic mutations in codons 183 and 209 of *GNAQ* and *GNA11* genes were investigated by restriction fragment length polymorphism. We identified a heterozygous somatic mutation in codon 209 of *GNA11*. No alterations of *GNAQ*, chromosome 3, 6p or 8q were identified in the patient whose tissue was studied with molecular genetics. Somatic mutations in *GNA11* are unique for uveal melanoma and have not been identified in other types of melanomas. This is the first report of this mutation in optic nerve sheath dural melanoma. It suggests that dural melanomas may have a similar cellular origin to uveal melanomas. The biologic behavior of two additional optic nerve dural melanomas with metastasis to the liver and death at 22 and 36 months supports the commonality between primary uveal and primary dural melanomas suggested by the newly described *GNA11* somatic mutation.

1231F

The clinical and genetic phenotypes of PMS2 HNPCC/Lynch syndrome families in Ireland. *A. Magee[1], V. McConnell[1], M. Loughrey[2], G. McCluggage[2], D. Allen[3], A. Green[4], C.M.J. Tops[5], R. Charlton[6].* 1) NI Regional Genetics Service, Belfast City Hospital Trust, Belfast BT9 7AB Northern Ireland, UK; 2) Institute of Pathology, Royal Victoria Hospital, Belfast Trust, BT12 6BA, Northern Ireland, UK; 3) Department of Pathology, Belfast City Hospital Trust, Belfast BT9 7AB Northern Ireland, UK; 4) National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin 12, Ireland; 5) Department of Clinical Genetics, LDGA, Leiden University Medical Centre, Leiden, Netherlands; 6) Yorkshire Regional DNA Laboratory, St James's University Hospital, Leeds LS9 7TS, UK.

Of the four mismatch repair (MMR) genes linked to HNPCC/Lynch syndrome, PMS2 has the lowest frequency, being reported in <5% of families. MMR immunohistochemistry (MMRIHC) helps identify cases where molecular testing is likely to identify a germline mutation. Eight probands with isolated loss of staining for PMS2 were identified. Six have confirmed germline PMS2 abnormalities; in one case no abnormality was identified. Molecular testing is underway in the eighth case. Five families were ascertained through a proband with colorectal cancer, the age at diagnosis ranging from 34 to 70. Two probands had endometrial cancer at ages 49 and 55. One had synchronous endometrial and ovarian cancers at age 38. Two families meet the Amsterdam criteria and five others fulfil the revised Bethesda criteria. The remaining pedigree has an isolated case of endometrial cancer at age 49 with no other HNPCC-associated cancers. Four probands have the same exon 2 mutation, c.137G>T(p.S46I), suggesting a possible founder mutation. One has an exon 9-10 deletion. The proband with synchronous endometrial and ovarian tumours has an exon 9 mutation, c.956C>T (p.P319L) plus a variant in exon 11, c.1211C>G (p.P404R). The tumour spectrum reported in these families includes:- melanoma; transitional carcinoma of the kidney and bladder; Merkel cell carcinoma; Hodgkins lymphoma; haemophagocytic lymphohistiocytosis (HLH); gastric, breast, bile duct and pancreatic carcinoma. Where possible, MMRIHC has been used to clarify which tumours were linked to PMS2. The Merkel cell tumour had originally been reported by the family as male breast cancer; MMRIHC showed normal staining. Carrier testing has excluded the case of HLH. Our data expands the genotype/phenotype correlation for PMS2 mutations.

1232F

Copy number and low frequency somatic mutation detection using multiplexed digital PCR. *D.R. Link[1], Q. Zhong[1], S. Bhattacharya[1], S. Kotsopoulos[1], J. Olson[1], V. Taly[2], A.D. Griffiths[2], J.W. Larson[1].* 1) RainDance Technologies, Lexington, MA; 2) Institut de Science et d'Ingenierie Supramoleculaires (ISIS), Strasbourg, France.

The ability to multiplex quantitative polymerase chain reactions (qPCR) is important for many clinically relevant research assays. However, current qPCR multiplexing capacity is typically limited to 4-fold by the spectral overlap of the fluorophores and the assay development process often requires lengthy testing to build standard calibration curves. Single molecule digital PCR (dPCR) in small, 5 picoliter volumes offers an attractive alternative to qPCR that is both more flexible and more precise. Based on a novel multiplexing approach for dPCR in picoliter droplets, we take advantage of the very high numbers of reactions possible within droplets (more than 10 million from 50 microliters of starting template), as well as the high probability that amplification of a single target DNA molecule will initiate within each droplet. By varying the concentration of different fluorogenic probes of the same color and having a narrow size distribution of droplets, it is possible to identify different probes on the basis of fluorescence intensity. Here we will report highly sensitive and absolute quantitative capabilities of this approach to analyzing copy number variation using multiplexed assays as high as ten-plex or more and demonstrate accurate detection of rare DNA mutations in a background of wild type DNA.

1233F

Next-gen sequencing of single human cells for detection of copy number variations and mutations. *J. Langmore[1], E. Kamberov[1], T. Tesmer[1], L. Patel[2], K. Pienta[2].* 1) Rubicon Genomics, Inc, Ann Arbor, MI 48108; 2) Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, MI 48109.

Next-Generation sequencing of single cells must be accurate, reproducible, rapid, and inexpensive for use in research and diagnostics. This is difficult because most methods of single-cell amplification produce stochastic and systematic bias in sequence representation and large amounts of extraneous DNA--making single-cell analysis problematic. Under specific conditions stochastic variation and background can be eliminated, leaving only reproducible bias. Using a modified PicoPlex NGS prep kit (Rubicon Genomics), individual flow-sorted cloned cancer and normal cells were amplified and prepared for NGS using indexed adaptors. Each cell was prepared in a four-step, single-tube process with no intermediate purifications followed by pooling cells before size separation. The elapsed time between cell collection and the start of cluster formation was <4 hours. Accuracy and reproducibility of the copy-number and mutation results were validated comparing replicate single cell samples to unamplified DNA using both NGS and PCR. Multiplexing was validated by pooling 4, 8, and 16 cells in single lanes.

The quality of single-cell sequencing on the Illumina GAIIx and HiSeq were evaluated. The GAII gave about 30M reads per lane of which 98-99% were high-quality in read 1 and 91-97% in read 2, with 80-98% mapping to the human genome. Only 0.5% of P-E reads mapped inconsistently due to inversion and 1.5% due to distance. There was very little evidence of chimeric, bacterial, or other spurious sequences. The HiSeq can be used to accurately call amplification or loss of 10kb regions of single cells, whereas variations of about 100kb were determined from 8 indexed single cells.

For patient cancer samples this strategy enables initial screening of many cells to indentify and characterize cell lineage. Individual specific cells can be characterized at higher resolution using additional NGS, microarray and PCR analysis. Multiplexed NGS of single-cells has relevance to characterization of tumor cells (e.g., circulating tumor cells and disseminated tumor cells for cancer research, blastomeres for pre-implantation genetic screening and diagnostics, and stem cell populations to test for heterogeneity).

1234F

Context-dependent estimates of substitution rates in human, chimpanzee and gorilla indicate acceleration in the human lineage. *N. Rustagi[1], I. Gorlov[2], SE. Plon[3,4], M. Kimmel[1], W. Amos[5].* 1) Statistics, Rice University , Houston , TX; 2) The University of Texas MD Anderson Cancer Center, Houston, Texas; 3) Genome Sequencing Center,Houston,Texas; 4) Texas Children's Cancer Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas; 5) Department of Zoology, University of Cambridge, Cambridge, UK.

Although it is often assumed that mutations accumulate randomly and in a strictly clock-like fashion increasing numbers of exceptions are coming to light. In terms of rate, a notable example is the hominid slowdown, which is widely reported but has yet to be quantified in the detail afforded by complete genome datasets. In terms of randomness, in addition to the well-known examples of transitions being commoner than transversions and the high mutability of CpG motifs, the DNA of many species deviates strongly from random in the frequencies of the 16 possible dinucleotide pairs. By implication, sequence context impacts on which mutations occur and how likely they are. To explore these issues in detail, we use a form of relative rate test using the exon and intron sequences of Human (H), Chimpanzee (C) and Gorilla (G), with Orangutan (O) as the out-group. Context was analyzed by considering each base in terms of its immediately flanking bases, giving 64 possible combinations (4 bases x 16 flanking dinucleotides). Relative mutation rates were determined using three way alignments of HCO, HGO, and CGO, generated using the Galaxy (http://galaxy.psu.edu/) program, using only alignments >100 bases in length. To reduce the influence of repeated sequences and poorer alignments, each candidate triplet is considered for analysis with probability 0.5 and only triplets where the flanking bases are identical in all three species are used to estimate context-sensitive substitution rates. A regression-based technique was developed to reconcile the substitution frequencies from the pairwise comparisons. Further, the estimated substitution rates were corrected to yield an ultrametric (balanced) species tree. We conclude that (1) the overall substitution rate in Humans significantly exceeds those in both Chimpanzee and Gorilla and (2) all mutation rates are strongly context-dependent, the extent varying between bases and between the three species. For example, the mutation rate of `A' in `CAT' is approximately fourfold greater than of `A' in `GAA'. We discuss the impact these findings have on our understanding of great ape evolution, including the evolution of disease-causing missense mutations. Supported by CPRIT grant RP101089 to NR, SEP and MK.

1235F

Differential expression profile of microRNA in medulloblastoma. *D.R. Lucon[1], C.S. Rocha[1], C.M. Morelli[1], D.P. Cavalcanti[1], S.S. Aguiar[2], S.R. Brandalise[2], J.A. Yunes[2].* 1) Departamento de Genética Médica - FCM, UNICAMP, Campinas, Brazil; 2) Centro Infantil Boldrini, Campinas, Brazil.

Background: Medulloblastoma is one of the most common pediatric cancers, likely originating from abnormal development of cerebellar progenitor neurons. The molecular mechanism contributing with medulloblastoma is poorly understood. MicroRNA has been shown to play an important role in development of the central nervous system. We report a series of microRNAs differentially expressed in medulloblastoma in comparison to normal fetal cerebellum and depict putative target genes. Methods: Five surgical specimens were obtained from patients with medulloblastoma and three normal cerebellum tissues were obtained from fetal autopsy. Total RNA was extracted by Trizol according to manufacturer's instructions, with an additional overnight precipitation step at -20°C with isopropanol. Five hundred micrograms of RNA were labeled with the 3DNA FlashTag Biotin HSR (Genisphere) and hybridized to GeneChip miRNA Arrays (Affymetrix). Data was acquired using a GeneChip Scanner 3000 7G (Affymetrix). The data processing was done in R environment (www.r-project.org) and the packages Affy and RankProd from Bioconductor were used for microRNA expression profile analysis. MicroRNAs were considered differentially expressed when the statistical significance was $p \leq 0.01$. The prediction of potential targets of selected microRNAs was done using the miRGen Targets and miR-Ontology Database softwares. Results: A total of 102 microRNAs were differentially expressed in medulloblastoma. Of these, 60 were upregulated and 42 downregulated. The miR-138, miR-184, miR-219-2-3p and miR-206 were among upregulated microRNAs while miR-199a-3p, miR-199b-3p, and miR-21 among downregulated ones. Target genes of these microRNAs include PTEN (miRs-138 and 21), OTX2 (miRs-138, 184 and 206), PAFAH1B1 (miRs-21, 138, 206 and 219-2-3p) and NOVA1 (miRs-199a-3p, 199b-3p, 21, 138 and 184) which are known to be involved in transcription regulation, cell differentiation, neuronal migration and nervous system development. Conclusion: This is the first time that miR-184, miR-219-2-3p, miR-199a-3p and miR-199b-3p were described in medulloblastoma. Interestingly, miR-21 was previously reported as upregulated in medulloblastoma in comparison to adult cerebellum, while we found it downregulated in comparison to fetal cerebellum. The result of our study hopes to shed some light into the molecular mechanisms of medulloblastoma.

1236F

Role of common genetic variants in pre-micro RNA in susceptibility and prognosis of Esophageal Cancer. *M. Umar[1], R. Upadhyay[1], S. Kumar[2], U.C. Ghoshal[3], B. Mittal[1].* 1) Department of Genetics, Sanjay Gandhi Post Grad Inst of Med Sc(SGPGIMS), Lucknow, India; 2) Department of Radiotherapy, Sanjay Gandhi Post Grad Inst of Med Sc(SGPGIMS), Lucknow, India; 3) Department of Gastroenterology, Sanjay Gandhi Post Grad Inst of Med Sc(SGPGIMS), Lucknow, India.

Introduction: MicroRNAs (miRNAs) are 21-23 nucleotide non-coding RNAs that regulate expression of about one third human genes at the posttranscriptional level through mRNA degradation or translation repression. miRNAs can act as oncogenes or tumor suppressor genes and known to be deregulated in several cancers. Differential expression of several miRNAs in Esophageal cancer (EC) and association of unique miRNA signatures with EC and its clinical phenotypes suggest critical role of miRNAs in cancer. Genetic variants in miRNAs have been also shown to affect progression, diagnosis, and therapeutics response of various malignancies; however their role in EC is controversial. Therefore, we aimed to evaluate role of common genetic variants in cancer related pre-miRNAs (affecting maturation/expression of respective miRNA) in susceptibility/survival outcome of EC patients in northern Indian population. Methods: In hospital based case control study, we genotyped four common polymorphisms in pre-miRNA: mir-196a-2 C>T (rs11614913), mir-146a G>C (rs2910164), mir-499 T>C (rs3746444) and mir-423 C>A (rs6505162) in 309 incident EC cases (including 153 followed-up cases) and 309 controls using PCR/PCR RFLP based methods. Results: Although, none of pre-miRNA genetic variants were associated with EC or its clinical phenotypes (histopathology, tumor location or lymph node metastasis) independently, combined effect of risk genotypes of four pre-miRNA polymorphisms associated with increased risk of EC in dose response manner: OR = 1.0, 1.5, 1.6 for 0-1, 2-3, 4 risk genotype (Ptrend = 0.008). Specifically, patients with⁄ 2 risk genotypes of pre-miRNA polymorphisms had 1.5 fold higher risk of EC compared to patients with 0-1 unfavorable genotype (OR = 1.56, 95% CI = 1.13-2.14, P = 0.007). Kaplan Meier and Cox Regression analysis showed no effect of pre-miRNA genetic variants on survival outcome of EC patients. Furthermore, no significant interaction of pre-miRNA polymorphisms with environmental risk factors was noticed. Conclusion: Combined risk genotypes of four common polymorphisms in pre-miRNAs seem to be associated with increased risk of EC, however no prognostic implication of these pre-miRNA genetic variants in EC was observed. Acknowledgment: Financial support from Council of Scientific and Industrial Research (CSIR), Department of Biotechnology (DBT), Department of Science and Technology (DST) and Indian Council of Medical Research (ICMR), Government of India.

1237F

The role of somatic mtDNA mutations in cancer. *E. Chen-Quin[1], M. Fowler[1], Y. Xie[2].* 1) Biology and Physics, Kennesaw State University, Kennesaw, GA; 2) Computer Sciences and Information Systems, Kennesaw State University, Kennesaw, GA.

Mutations in nuclear encoded mitochondrial genes Succinate Dehydrogenase (SDH-B, C, and D) and Fumarate Hydratase (FH) cause hereditary cancers of the head and neck and implicate a role for mitochondrial dysfunction in tumor formation. Likewise, somatic mutations from the mitochondrial chromosome (mtDNA) are found in 65% of tumors; but whether they play a carcinogenic role or are only a side effect remains unclear. Relative to the nuclear genome, the 10x faster mutation rate of mtDNA renders association of specific mutations at too low of power to link any one lesion with cancer. Current technology also does not allow transfection of individual mtDNA mutations for in vivo or in vitro testing of its role in cancer. It remains upon new computational methods to clarify the tumorigenic role of mtDNA mutations. BCI simulation shows CytB to have somatic cancer missense changes in positions of unusually high conservation (Stafford and Chen-Quin 2010), suggesting that tumors select for mutations in highly functional residues. It also shows CO1 to only mutate in positions of low conservation, suggesting tumor growth requires CO1 function. Using our BCI simulation method, new results from a meta-analysis of 350+ published mtDNA tumor sequences shows cancer mutations in ND6 to occur preferentially in amino acid positions of high conservation. These positions also coincide with observed cancer mutations from other species. We also present identification of COMS (combinations of two or more mutations found in two or more independent tumors; Zhidkov 2009) from a large dataset. Zhidkov 2009 identified recurring cancer mtDNA mutation combinations (COMS) in a limited data set, noting that somatic cancer mutations significantly coincide with ancient haplogroup-defining polymorphisms rather than with younger population polymorphisms. The overlap between somatic cancer mutations and ancient polymorphisms was posited as due to similar selective forces at the tumor and population level. To determine whether similar mutation COMS occur in tumor data from other laboratories, we analyze a mutation data set three times the size of the original analysis (411 tumors, 27 articles). From the additional data, we report COMS related, but not identical to, those in Zhidkov 2009. These other COMS also frequently identify somatic cancer mutations that coincide with ancient population polymorphisms.

1238F

MUTYH mutation spectrum in Brazil: first report of a large deletion in a MAP patient. *G.T. Torrezan[1], F.C.C. Silva[1], A.C. Krepischi[2], E.M.M. Santos[2], B.M. Rossi[2], D.M. Carraro[1].* 1) Genomic and Molecular Biology Laboratory, CIPE, A. C. Camargo Hospital, São Paulo, Brazil; 2) CIPE, A. C. Camargo Hospital, São Paulo, Brazil.

MUTYH-associated Polipose (MAP) is a hereditary colorectal cancer predisposing syndrome caused by biallelic mutations in MUTYH gene with a clinical phenotype that can occasionally resembles Familial Adenomatous Polyposis or Lynch syndrome (LS). We sequenced the entire coding region of MUTYH gene in 7 unrelated probands affected by polyposis and negative for mutations in APC gene. In addition, 51 LS patients non-carriers of germline mutations in the mismatch repair genes MLH1, MSH2, MSH6 and PMS2, were screened for the hotspot MUTYH mutations p.Tyr179Cys and p.Gly396Asp and for the presence of a 4.2 Kb MUTYH deletion identified in this study. Direct DNA sequencing of all exons and long range PCR were performed in polyposis patients, whereas regular PCR and allele specific PCR (AS-PCR) followed by RFLP were performed in LS patients. The biallelic nature of the mutations was confirmed by AS-PCR. From 7 polyposis patients, one was negative for mutations, one presented the p.Gly396Asp variant in a monoallelic state and 5 exhibited biallelic mutations as follows: 3 with the p.Tyr179Cys allele and a pathogenic mutation in the other allele (one deletion, one duplication and one splice site mutation); one homozygote for the p.Arg241Trp mutation and one homozygote for a >4.2 Kb deletion encompassing exons 4-16. For the later, deletion breakpoints were refined to base pair level through aCGH analysis followed by sequencing. The identified breakpoints were located within intron 3 and 146 bp after the 3' end of the gene (c.348+33_*64+146del4285), with the presence on an AluJr element adjacent to the distal breakpoint. For the LS patients, none of them presented this large deletion or the p.Tyr179Cys variant, while one carried the p.Gly396Asp mutation. This patient exhibit in the other allele a polymorphic AluY insertion in intron 15, which has been described to increase the levels of 8-oxyG:A in DNA and is associated with type 2 diabetes mellitus. However, since this insertion has not been studied in the context of colorectal cancer, segregation studies would be important to verify its role in the etiology of this disease. In silico analysis of the MUTYH locus revealed a high Alu density that may predispose this region to other Alu-mediated rearrangements and deletions. In conclusion, we have identified the first large deletion in MUTYH gene and shown that investigation of this sort of mutation may be important to identify causative mutations in MAP patients.

1239F

Analytical Resolution of p53 Alteration in the Clinical Context. *Y.G. Yue, S. Kadam, J.T. Brandt, I. Wulur, T.R. Holzer, A.D. Fulford, J. Ting, T.D. Barber, C. Reinhard.* Translational Science - Hum Gent, Eli Lilly and Company, Indianapolis, IN.

Accurate measurement of genetic variation in the p53 gene (TP53) is critical in predicting patient outcome and developing tailored therapeutics. A commonly-used measurement of p53 function relies on detection of nuclear accumulation of the p53 protein by means of immunohistochemistry (IHC). Most missense mutations are known to cause nuclear p53 protein accumulation and IHC results have been associated with clinical outcome in several cancer types. However, the concordance between IHC and mutation assays varies considerably among different studies, even within the same cancer type. The disconnect between DNA alteration and nuclear protein accumulation leaves an uncertainty in the true frequency of p53 alterations in cancer, and limits the utility of p53 status in clinical studies. In this study, we sought to provide resolution of p53 functionality measurement by comparing IHC with DNA-level alterations in 48 NSCLC clinical samples. Based on IHC data from FFPE samples, 29/48 (60.4%) were found positive in p53 alteration. TP53 sequencing, using the corresponding FF samples, identified a higher rate of mutation, 32/48 (66.7%). Compared with published data, we observed an unusually high level (10/48 or 20.8%) of truncating mutations (nonsense, frame-shift, and splice site mutations) that accounted for 31.34% of all mutations found in this study. As expected, 20/22 (90.9%) of tumors carrying missense mutations were IHC-positive, confirming the link between structural mutation and p53 nuclear accumulation. In contrast, 9/10 (90%) of truncating mutations were found in IHC-negative tumors. While this is consistent with the literature, the high frequency of truncating mutations explained the relatively low concordance rate between IHC and mutation, and highlights an inherent shortcoming of IHC. Including 8 IHC-positive tumors with no detectable p53 mutation, the frequency of p53 alteration in NSCLC reached 40/48 (83.3%), much higher than any reports to date. In summary, our findings suggest that overall p53 mutation frequency might be underreported and that altered p53 functionality could be more prevalent in lung cancer.

1240F

Dermatofibrosarcoma protuberans in neurofibromatosis type 1 (NF1). *D. Viskochil[1], L. Randall[2].* 1) Dept Pediatrics, Div Med Gen, Univ Utah, Salt Lake City, UT; 2) Huntsman Cancer Institute, University of Utah, Salt Lake City, UT.

Neurofibromatosis type 1 (NF1) is associated with malignant peripheral nerve sheath tumors (MPNST); however, it is also associated with multiple other types of sarcomas. We present an individual with NF1 who had dermatofibrosarcoma protuberans. The patient was followed for a clinical diagnosis of NF1, and was noted to have left sphenoid wing dysplasia. A fullness over his left temple was noted at 4 years of age, and it enlarged over a few months as a firm mass with only mild tenderness to deep palpation. Given an increase in size with mild tenderness, it was resected under the premise that it was a plexiform neurofibroma. Pathology demonstrated a dermatofibrosarcoma protuberans mixed with giant cell fibroblastoma. The mass was well-cicumscribed but pathology review determined the margins were microscopically positive. He underwent wide surgical excision and has been followed conservatively since age 6 years, without recurrence. Dermatofibrosarcoma protuberans is a rare pediatric malignancy that if detected in early stages and treated with wide surgical excision has a good prognosis. There has not been a published report of this tumor in an individual with NF1. This case also had elements of mixed giant cell fibroblastoma, which has been reported in two other non-NF1 patients and the tumors both had a t(17;22)(q22;q13). These tumors overexpress platelet-derived growth factor receptors A and B (PDGFRA and PDGFRB), and may be ammenable to imatinib treatment rather than radiation therapy in the event of recurrence.

1241F

Improved sequencing workflow for characterizing melanoma mutations. *S.-C. Hung[1], S. Berosik[1], S. Schneider[1], P. Ma[1], S. Vemula[2], R. Terrill[2], S. Mirza[2], M. Wenz[1].* 1) Life Technologies, 850 Lincoln Centre Dr., Foster City, CA 94404; 2) University of California, San Francisco, Dept of Dermatopathology, 2340 Sutter Street N-461, San Francisco, CA 94143.

Melanoma is an aggressive skin cancer with increasing incidence worldwide; it is a malignant tumor arising from pigment producing melanocytes in the skin, eye, or mucosal surfaces. Based on histopathological and molecular studies, melanoma can be sub-classified into various categories. Melanoma arising from non-chronically sun damaged (non-CSD) skin shows frequent mutations in the *BRAF* gene and no mutations in the *KIT* gene. In contrast, 20% of melanoma on CSD skin have mutations in the *KIT* gene and infrequently harbor mutations in the *BRAF* gene; whereas in two other categories, acral and mucosal melanomas, 10 to 40% of cancers have been shown to have *KIT* mutations or amplifications of this genomic region. To enable the molecular classification of melanoma in research samples, we have employed an improved capillary electrophoresis Sanger sequencing workflow. The sequencing-based mutation detection workflow described here improved the resolution of sequencing data at the 5' end of all amplicons investigated in the *BRAF*, *NRAS* and *KIT* genes. The modified chemistry used in the workflow enhanced the resolution at tandem mixed base positions in the *BRAF* gene. A combined PCR reaction purification and cycle sequencing step enabled a rapid turn-around time and improved the rate of detection for accurate data and information regarding the metastatic melanoma DNA research samples. Further, the workflow described here occurs in the same sample well from PCR amplification to cycle sequencing reaction purification thereby eliminating the transfer of research samples required in standard sequencing workflows and the possibility of transfer errors. As a result of workflow enhancements, the amount of hands-on time was reduced as were consumable costs, and the number of pipetting steps. Taken together, the sequencing-based mutation detection workflow described here simplifies, increases throughput by 40%, and improves accuracy for the identification of melanoma genetic variants.

1242F

Molecular analysis of possible polymorphism risk factors of CDKN1A gene in sporadic colorectal cancer. *n. zali, m. montazer haghigh, r. fatemi, m. vahedi, m. zali.* Shaheed Beheshti University, Research Center for Gastroenterology and Liver Dis, tehran, IS., tehran, Iran.

Background: Colorectal cancer is the third most commonly diagnosed cancer in both men and women. Progressive loss of cell cycle control is an important feature on the colorectal cancer. p21 (CDKN1A/CIP1/WAF1), one of the cyclin-dependent kinase inhibitors, plays a key role in regulating the cell cycle. The aim of this study was to investigate associations of the CDKN1A gene polymorphism (rs1801270) with risk of colorectal cancer (CRC) in an Iranian population. Methods: The study subjects were 57 cases of colorectal cancer and 65 controls. Genomic DNA was extracted using standard salting out method. Genotypes were determined using the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method. Results: Statistical analysis of results indicated that the genotype frequencies of polymorphisms CDKN1A gene in patients and healthy controls are AA (3.1%), AC (9.4%) and CC (87.5%) for control subjects and AA (5.3%), AC (10.5%) and CC (84.2%) for CRC cases respectively. We found no significant difference between studied polymorphism, between healthy control individuals and colorectal cancer patients (P= 0.817). After stratification of genotyping result we found no statistically significant difference between case and control groups (P=863). Conclusion: to our knowledge this is the first study on association of rs1801270 polymorphism with CRC risk in Iranian population. Our findings indicated that there is no association between selected single nucleotide polymorphism in p21 gene and risk of colorectal cancer.

1243F

Prostate Cancer Proteomics: Using antibody-based high-throughput plasma profiling for the discovery of new prostate cancer biomarkers. *R. Karlsson[1], U. Igel[2], H. Grönberg[1], W. Isaacs[3], M. Uhlén[2], J. Schwenk[2], F. Wiklund[1].* 1) Department of Medical Epidemiology and Biostatistics, Karolinska institutet, Stockholm, Sweden; 2) Science for Life Laboratory, Department of Proteomics, School of Biotechnology, KTH Royal Institute of Technology, Stockholm, Sweden; 3) Department of Urology, Johns Hopkins Medical Institutions, Baltimore, Maryland, USA.

Introduction: Prostate cancer (PC) is the most common male cancer in Europe and North America. Twin and family studies support a strong genetic component of the disease, and genome-wide association studies (GWAS) have so far produced more than 30 robust disease-marker associations. A major problem in the diagnosis and treatment of PC is the difficulty in separating cancers of an aggressive subtype, for which immediate and powerful treatment is necessary, from indolent forms, for which the treatment itself may do more harm than good. The main PC biomarker in clinical use today is serum level of prostate-specific antigen (PSA), a protein produced in prostate tissue. PC severity correlates with serum PSA, but the low sensitivity and specificity of the marker lead to many false positive and false negative results. In an attempt to find new biomarkers useful for PC diagnosis and prognosis, we have screened plasma levels of a selection of 80 proteins for associations with aggressive PC in a case-control sample. The proteins were selected based on either differential mRNA expression in tumor tissue (Isaacs, unpublished data), or GWAS associated markers in or near their genes. **Material and methods:** Plasma samples from 368 men (135 controls, 160 localized PC, 73 aggressive PC) were selected from a repository established as part of the large-scale, population-based case-control study Cancer of the Prostate in Sweden (CAPS). Plasma was derived from blood samples taken before any cancer treatment. Protein levels were measured in a multiplex antibody-based suspension bead array. Antibodies were first coupled to color-coded microspheres, which were then added to diluted plasma samples. The antibodies were allowed to hybridize with their specific antigens, and bead-specific biotin fluorescence was then measured in a flow cytometry instrument (Luminex) for all 80 proteins in a single reaction. **Results:** In a preliminary analysis of plasma protein levels and aggressive PC no single protein was significantly associated after correcting for multiple testing. Several of the strongest protein-PC associations were however considered interesting and will be analyzed further. A more thorough analysis of the data will focus on building multivariate models to increase discriminatory power. Correlations with clinical covariates such as Gleason score and TNM stage will also be presented in addition to the analysis of binary disease status already performed.

1244F

Novel target genes of ETV6 in childhood pre-B acute lymphoblastic leukemia. *B. Neveu[1,2], M. Malouf[1,2], S. Langlois[1], D. Sinnett[1,3].* 1) Division of Hemato-Oncology, Research Center Sainte-Justine University Health Center, Montreal, Canada; 2) Department of Biochemistry, Faculty of Medecine, University of Montreal, Montreal, Canada; 3) Department of Pediatrics, Faculty of Medecine, University of Montreal, Montreal, Canada.

Acute lymphoblastic leukemia (ALL) is the most frequent pediatric cancer. A vast array of genetic aberrations have been described in this disease, of which the most frequent is the t(12;21), observed in 25% of pre-B ALL cases. This translocation involves the ETV6 and AML1 genes on chromosomes 12p13 and 21q22, respectively. The resulting ETV6-AML1 fusion chimera cannot induce leukemia alone, suggesting that additional mutations are required for transformation to occur. The expression of the non-rearranged allele of ETV6 is deleted in 75% of t(12;21) positive ALL patients, which indicates that the complete inactivation of ETV6 is a key event in childhood leukemogenesis. Because ETV6 is a transcriptional repressor (member of the ETS family) it is of great interest to identify ETV6 transcriptional targets to understand how overexpression of these targets (due to ETV6 depletion) could impact ALL onset and progression. Few ETV6 targets are currently known. Using genome-wide expression data, we identified 17 genes whose expression is upregulated in the absence of ETV6. Ten of them were validated by qRT-PCR experiments in childhood ALL patients. To determine whether these genes are direct targets of ETV6, we subcloned the corresponding promoter regions in a gene reporter system and transfected three human cell lines, including Jurkat lymphocyte cells. Using this system, the activity of the ELK3, TERF2, CBFA2T3, RAG1, TCFL5, DPYSL2, FAM134B, RASA4 and SERINC5 promoters was downregulated following overexpression of ETV6 (co-transfection experiments). Chromatin immunoprecipitation (ChIP) assays confirmed the presence of ETV6 on these gene promoters. We then highlighted the importance of the Pointed and ETS domains on ETV6-mediated transcriptional repression by using truncated versions of the ETV6 protein. Interestingly, for certain promoters, the presence of a functional ETS domain was not required for repression, suggesting that ETV6 may regulate transcription without direct DNA binding. We conclude that ETV6 can regulate transcriptional repression through different mechanisms, either by direct DNA binding or possibly through its recruitment via other ETS-transcription factors.

1245F

Detection and prioritisation of prostate cancer risk-associated genetic variants in the *KLK4* gene for functional analysis. *F. Lose[1], J. Batra[2], S. Srinivasan[2], S. Chambers[3,4], R.A. Gardiner[5], J. Aitken[4,6], J.A. Clements[2], A.B. Spurdle[1], Australian Prostate Cancer BioResource.* 1) Molecular Cancer Epidemiology, Queensland Institute of Medical Research, Brisbane, Queensland, Australia; 2) Australian Prostate Cancer Research Centre-Queensland, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, Australia; 3) School of Psychology, Griffith University, Brisbane, Queensland, Australia; 4) Viertel Centre for Cancer Research, Cancer Council Queensland, Brisbane, Queensland, Australia; 5) University of Queensland Centre for Clinical Research, Royal Brisbane Hospital, Brisbane, Queensland, Australia; 6) Griffith Health Institute, Griffith University, Brisbane, Queensland, Australia.

Approximately 40% of prostate cancer is estimated to have a genetic component, and to date over 30 single nucleotide polymorphisms (SNPs) have been identified by genome-wide association studies (GWAS) that influence prostate cancer risk. Interestingly, one of the SNPs detected by a prostate cancer GWAS is located downstream of the *KLK3* gene. Thus, our premise is that genetic markers associated with the *KLK3*/*PSA* gene and other *KLK* family members will prove useful predictive markers for prostate cancer and its progression, rather than, or in addition to, serum tests. Investigation of public SNP databases reveal hundreds of validated genetic variants in the *KLK* genes, the majority of which were not covered by the commonly used GWAS chips. We aimed to comprehensively investigate all validated SNPs in the *KLK* genes for an association with prostate cancer susceptibility and prognostic features, and then to investigate the functional significance of associated SNPs. This study focuses on *KLK4*, which has been shown to be upregulated in prostate cancer and bone metastasis, and several SNPs upstream of *KLK4* were marginally statistically significantly associated with prostate cancer risk in the Cancer Genetic Markers of Susceptibility (CGEMS) project. A total of 70 SNPs in the *KLK4* gene (±10kb) were successfully genotyped in our Queensland, Australia prostate cancer-control sample set of approximately 1,300 prostate cancer cases and 1,300 male controls. Logistic regression analyses showed 7 *KLK4* SNPs to be associated with a decreased risk of prostate cancer ($P<0.05$), none of which are represented on commonly used GWAS chips, either directly or by a surrogate SNP ($r^2 > 0.8$). Four of these SNPs are located in the 3' flanking region of the *KLK4* gene (+0.75-3.5kb) and are in high LD with each other ($r^2 > 0.96$). Bioinformatic analyses predicted 3 of these SNPs to alter transcription factor binding sites relevant to prostate cancer. The remaining three *KLK4* SNPs found to be associated with risk of prostate cancer in our study were independent of each other, with two located upstream of *KLK4* (6.5kb and 8.5kb) and one missense variant 3 nucleotides into exon 2, which alters Serine 22 to an Alanine residue. Although the missense variant is not predicted to have any effect on protein structure or function *in silico*, it may still have important effects on KLK4 stability or splicing. Our results indicate that *KLK4* may be another KLK contributing to prostate cancer susceptibility.

1246F

Integrated molecular profiling of adult and pediatric pilocytic astrocytoma through single nucleotide polymorphism array and gene expression analysis. *M. Shirinian[1,*], A.M. Fontebasso[2,*], D.A. Khuong Quang[2], D.T.W. Jones[3], K. Jacob[1], N. Gerges[1], A. Klekner[4], A. Montpetit[5], A. Nantel[6], S. Albrecht[7], S. Pfister[3], N. Jabado[1,2,8].* 1) Human Genetics, McGill University, Montreal, Canada; 2) Experimental Medicine, McGill Universirty, Montreal, Canada; 3) Division of Molecular Genetics, German Cancer Research Center, Heidelberg, Germany; 4) Department of Neurosurgery, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary,; 5) McGill University and Genome Quebec Innovation Centre,; 6) Biotechnology Research Institute, National Research Council of Canada; 7) Department of Pathology, Montreal Children's Hospital, McGill University Health Centre (MUHC); 8) Department of Hematology & Oncology, Montreal Children's Hospital, McGill University Health Centre (MUHC).

Pilocytic Astrocytoma (PA) is a Grade I Astrocytoma according to the WHO classification and is the most common brain tumor in children. Accounting for 23% of all pediatric brain tumors, PA occurs predominantly in infratentorial regions of the brain in pediatric patients and comprises about 80-85% of all tumors of the cerebellum in children. Recent studies have demonstrated key differences in the gene expression profiles of adult and pediatric glioblastoma multiforme (GBM), but molecular mechanisms important in PA tumorigenesis specific to adult and pediatric patients are largely uncharacterized. As such, we sought to profile key genetic characteristics of adult and pediatric PA tumors utilizing single nucleotide polymorphism (SNP) arrays. In addition, utilizing large-scale microarray-based gene expression profiling of adult and pediatric PA, we have characterized specific molecular mechanisms of putative importance in adult versus pediatric PA tumorigenesis. Upon integration, gene expression profiles can be correlated with genomic alterations that may better profile PA tumors on the basis of patient age, neuroanatomical location and genotype. We have demonstrated, through SNP array analysis, that whole chromosome gain occurs more frequently in adult versus pediatric PA, and that chromosomal gain occurred more frequently in infratentorial brain regions in adult PA patients than pediatric PA patients. Gene expression profiling revealed distinct groups upon unsupervised clustering; namely distinguishing the gene expression profiles of pediatric and adult PA, as well as on a neuroanatomical basis. Such differences in global gene expression may have root in the large-scale genomic changes that distinguish PA with respect to age and brain location. We believe that integrated array-based analyses may allow us to more accurately profile adult and pediatric tumors and design more personalized patient therapy in clinic.

1247F

Identification of functional polymorphisms at the 4q21 locus associated with modification of breast cancer risk in BRCA2 mutation carriers. *Y. Hamdi[1], P. Soucy[1], D. Goldgar[2], B.J Feng[2], G. Reimnitz[1], M. Tranchant[1], T. Pastinen[3], P. Cassart[4], O. Ouimet[4], D. Sinnett[4], C. Verny-Pierre[5], L. Barjhoux[5], D. Stoppa-Lyonnet[5], O. GEMO Study Collaborators[5], O. Sinilnikova[5], J. Simard[1].* 1) Canada Research Chair in Oncogenetics, Department of Molecular Medecine, Faculty of Medecine, Laval University, CHUQ Research Center/CHUL , Ste foy, Quebec, Canada; 2) Department of Dermatology, Health Care, University of Utah, U.S; 3) Department of Human Genetics, McGill University, Montreal, Canada; 4) Division of Hematology-Oncology, Research Center Sainte-Justine University Health center, University of Montreal, Montreal, Canada, H3T 1C5; 5) INSERM U1052, CNRS UMR5286, Université Lyon 1, Cancer Research Center of Lyon, and Unité Mixte de Génétique Constitutionnelle des Cancers Fréquents, Centre Hospitalier Universitaire de Lyon / Centre Léon Bérard, Lyon, France.

Individuals with pathogenic mutations in BRCA1 and BRCA2 genes are known to be at a substantially increased risk of breast cancer throughout their lives. Recent studies have provided evidence of additional genetic risk factors that may considerably modify the risk of developing the disease. Genome Wide Association Studies have shown that a considerable number of these genetic variants reside in the regulatory regions of candidate genes. Also, expression profiling and genome wide mapping studies have shown that strong heritable factors govern differences in gene expression levels within mammalian species. Here we wanted to evaluate the impact of regulatory polymorphisms of candidate genes on their expression levels and assessing their association with breast cancer risk in BRCA1 and BRCA2 mutation carriers. ABRAXAS (FAM175A), located at the 4q21 locus, is a selected candidate gene that encodes for a protein interacting directly with BRCA1 for its recruitment at double-strand DNA breaks. Its promoter region was sequenced in 60 unrelated Caucasian individuals and the functional impact of the identified variants was thereafter assessed using gene reporter assays. Two of these variants were characterized in more details: rs72672798, a rare variant, that significantly decreases the transcriptional activity of this gene and rs530648 that tags major promoter haplotypes. In parallel, we used data from a high density allelic expression analysis of quantitative measurements of allelic expression (AE) to identify SNPs at this locus that are associated with differential AE as previously described. [Pastinen T, Nat Rev Genet. 2010]. rs1494961 is one of the variants shown using this approach to be associated with differential AE. This SNP is located on the same 4q21 locus, in a neighbouring gene, Hel308, a Helicase involved in DNA repair and replication. The two common SNPs, rs1494961 and rs530648, were evaluated for an association with breast cancer risk in approximately 2380 BRCA1 and BRCA2 mutation carriers from the GEMO study (GEnetic MOdifiers of cancer risk in BRCA1/2 mutation carriers). Interestingly, the minor alleles of rs1494961 and rs530648 were significantly associated with a reduced risk of breast cancer (HR, 0.85 and 0.81; P= 0.03 and 0.02 respectively) in BRCA2 but not BRCA1 mutation carriers. We are currently validating these findings by genotyping these two SNPs, as well as other variants at that same locus, in a larger number of BRCA1/2 carriers.

1248F

Genotoxic agents alter the tridimensional nuclear organization of telomeres and the expression profile of shelterin genes. *N. Bastien[1], O. Samassekou[1], D. Lichtensztejn[2], J. Yan[1], S. Mai[2], R. Drouin[1].* 1) Genetics, pediatrics, 3001 12e Avenue, Université de Sherbrooke, Sherbrooke, PQ, j1H 5N4 Canada; 2) Manitoba Institute of Cell Biology, University of Manitoba, 675 McDermot Avenue, Winnipeg, MB, Canada R3E 0V9.

Telomeres are found at chromosome ends. When these ends are not protected by telomeric capping, the telomeres are recognized as double-strand breaks and the consequent recruitment of DNA repair machinery may affect cellular fate. Due to their DNA structure, telomeres are more sensitive to DNA damage, and they might present different outcomes following exposure from different genotoxic agents. For instance, telomeres repair the 8-oxo-7,8-dihydroguanine following hydrogen peroxide treatment whereas they do not remove the cyclobutane pyrimidine dimers after UVB exposure. To study the effects of three DNA-damaging agents on telomere structure, we exposed normal human primary fibroblasts to hydrogen peroxide, gamma radiation, or UVB rays. Parameters of nuclear architecture of telomeres and the expression profile of shelterin genes were assessed just after the exposure to one of the genotoxic agents over a 24-hour period. First, we studied the telomere nuclear architecture by using 3D quantitative fluorescence in situ hybridization. Hydrogen peroxide treatment induced a significant reduction of the number of telomere signals. For gamma irradiation, the nuclear volume was positively correlated to the number of telomere signals and negatively correlated to telomere average intensity. UVB exposure led to a significant increase of telomeric aggregates and disruption of telomere nuclear shape by forming an ellipsoid instead of circular shape. Next, the study of the expression profile of telomere shelterin genes, following exposure of these different DNA damage agents, mainly showed an up regulation of POT1, TERF1 and TERF2IP after hydrogen peroxide treatment. Also, UVB and gamma exposure led to an up regulation of TERF2IP and POT1, respectively. Moreover, we observed an up regulation of ATM, ATR, MRE11, Ku80, p21 following exposure to hydrogen peroxide. In conclusion, certain DNA-damaging events differently alter telomere structure by acting on nuclear telomere architecture and the expression profile of telomere shelterin genes. Furthermore, the shelterin genes might be involved in response and repair of DNA damage. The disruption of telomere structure due to acute insults is another pathway that affects cell homeostasis.

1249F

The Fanconi anemia pathway regulates telomeric recombination and telomere DNA synthesis in ALT-immortalized human cells. *H. Root[1,2], M. Komosa[1], F. Al Azri[1,3], M.S. Meyn[1,2,3,4].* 1) Dept Gen & Genome Biol, Hosp Sick Children, Toronto, ON, Canada; 2) Dept of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 3) Institute of Medical Science, University of Toronto, Toronto, ON, Canada; 4) Dept of Paediatrics, University of Toronto, Toronto, ON, Canada.

Fanconi anemia (FA) is an inherited disorder characterized by bone marrow failure, congenital malformations and cancer. FA proteins help maintain genome integrity by playing multiple roles in DNA replication and repair. To understand FA protein function we are studying their involvement in recombination-dependent ALT telomere maintenance. We find that FANCD2, FANCJ, and FA core complex proteins form nuclear foci that colocalize with telomeric foci in ALT-dependent, but not telomerase-positive or primary cells. FANCM, a DNA translocase that recruits the FA core complex to sites of DNA damage and promotes replication fork stability, also frequently colocalizes with telomeric foci in ALT cells. FA proteins colocalize with telomeric DNA primarily within ALT-associated PML bodies (APBs), subnuclear domains that contain high amounts of extrachromosomal telomeric repeat DNA (ECTR). FANCD2 depletion leads to rapid, ALT-specific alterations in telomere homeostasis, including increased telomere dysfunction-induced foci, telomere entanglements, fragile telomeres, and telomere recombination events. FANCD2 depletion leads to marked increases in ECTR DNA synthesis, with FANCD2-depleted cells containing up to 40x more telomeric repeat DNA than the average control cell. Co-depletion of FANCD2 with BLM, but not RAD51, suppresses these telomere phenotypes.

Depletion of FANCJ has the opposite effect of FANCD2 depletion, with FANCJ-depleted cells exhibiting a significant decrease in both the number and intensity of telomeric DNA foci. Co-depletion of FANCJ with FANCD2 partially suppresses amplification of ECTR DNA in FANCD2-depleted cells, suggesting that FANCJ is also required for ECTR DNA synthesis. Interestingly, FANCM depletion also results in increased telomeric DNA synthesis, however this does not appear to be mediated by FANCD2, as FANCD2 continues to localize to telomeric foci in FANCM depleted ALT cells. This suggests that FANCM and FANCD2 may be acting independently to restrain ECTR DNA synthesis. Our results support a model in which FANCD2 and FANCM restrain recombination and/or amplification of telomeric DNA, while BLM and FANCJ DNA helicases promote these processes. We propose that FA proteins do not solely function in a single linear pathway, but rather, work both together and independently to coordinately facilitate genetic recombination and recovery of stalled replication forks.

1250F

Specific TP53 mutations are associated with chromosomal rearrangements, telomere length changes and remodeling of telomere nuclear architecture. *O. Samassekou[1], N. Bastien[1], D. Lichtensztejn[2], J. Yan[1], R. Drouin[1], S. Mai[2].* 1) Dept Med Gen, Sherbrooke Univ (CHUS), Sherbrooke, PQ, Canada; 2) Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, MB, Canada.

The TP53 mutations are the most common mutations in human cancers and among them the TP53-R175H and TP53-R273H mutations are most frequent. These mutations generally occur during tumor development and are thought to promote genomic instability, with impact on patient's outcome. In order to characterize the effect of specific TP53 mutant on genomic instability, we studied four isogenic lines of LoVo cells, derived from colon carcinoma. The three LoVo cell lines with specific mutation of TP53, TP53-A143V, TP53-R175H and TP53-R273H, and wild-type TP53 were maintained in culture up to twenty population doublings. Using M-FISH, 3D Q-FISH on interphase, and Q-FISH on metaphase chromosomes, we described chromosomal rearrangements, changes in 3D nuclear telomeric architecture and in the length of individual telomeres. We found that LoVo cells expressing mutant TP53-R175H displayed the highest level of chromosomal instability among the LoVo cell lines. This instability was characterized by numerical and structural chromosomal abnormalities. Furthermore, we observed that mutants TP53-R175H and TP53-A143V showed more alterations of their 3D nuclear telomeric architecture than both the mutant TP53-R273H and the wild type. These alterations were mainly characterized by the presence of a substantial number of telomeric aggregates and a change in telomere positions. Telomere length analysis revealed an increase in telomere length in the mutants TP53-R175H and TP53-A143V. Moreover, we noted an association between some chromosomal abnormalities and telomere elongation in the mutant TP53-R175H. Taken together, our results indicate that in the LoVo isogenic cell model, the mutation TP53-R175H is more likely to cause higher levels of genomic instability than the other TP53 mutations. We propose that the type of TP53 mutations associated with the genetic background of a cancer cell is a major determinant of the TP53-dependent genomic instability.

1251F

Mechanisms of maintaining telomere lengths at specific chromosomes in leukemia. *J. Yan[1], J. Lamoureux[1], E. Bouchard[1], J. Herbert[2], H. Li[3], S. Huang[3].* 1) Division of Genetics/Dept Pediatrics, Univ de Sherbrooke, Sherbrooke, PQ, Canada; 2) Banque des Cellules Leucémiques, University of Montreal, Montreal. PQ, Canada; 3) Center for Stem Cell Research and Application, Huazhong University of Science and Technology, Wuhan, Hubei, China.

Leukemia is a neoplasia often characterized by the cellular proliferation of affected cell lineage and specific chromosome abnormalities. As most types of cancers, the telomere lengths in leukemia cells are shorter than normal blood cells. We studied the telomere length on each chromosome in chronic myeloid leukemia (CML) at chronic phase (CP) and acute T lymphoblastic leukemia (T-ALL) using Q-FISH and telomere restriction fragments (TRF). We found there were specific individual telomere elongation profile, or a clonal event, associated with these leukemia samples. The longest telomeres in both leukemia types reached more than 23 Kb, which were correlated specifically with certain chromosome arms as detected by the Q-FISH and the TRF. To understand what mechanism are responsible for the maintenance of the telomere length in these leukemia samples, we examined telomerase activity by real time PCR and a hallmark of alternative lengthening of telomeres (ALT), in terms of extra-chromosome telomeric repeat (ECTR) with 2D gel electrophoresis. Our results showed that the ALT mechanism is involved in these leukemia samples in a higher frequency (~46%) in CML than in T-ALL (12.5%) and the general rate (~15%) that was estimated previously from different types of tumors. Moreover, we found that some of the ALT positive samples were associated with high telomerase activity, suggesting the coexistence of telomerase and ALT maintains for telomere length maintenance. Since activated telomerase was observed in a high rate of T-ALL and CML at the late stage, blastic crisis phase (BP), whereas positive ALT was observed in a high rate in CML at CP, we propose a model that the ALT mechanism might be used at the early stage of leukemogenesis and followed by the telomerase activation to alternately maintain telomere length. This telomere length maintenance model is most likely driven by clonal selection. Therefore, cells of a specific cancer may use different mechanisms to maintain their telomere length depending on disease stage or their mutation status. It is important to take into account in the development of anti telomerase therapeutics that the presence of the ALT mechanism in cancer cells must cause treatment resistance.

1252F

Integrative molecular profiling and regulatory modeling of the Glioblastoma multiforme genome, epigenome, and transcriptome. *S. Li[1], M. Setty[2], P. Zumbo[1], C. Leslie[2], C. Mason[1].* 1) Weill Cornell Medical College, New York, NY; 2) Memorial Sloan-Kettering Cancer Center, New York, NY.

High-throughput, next generation sequencing (NGS) provides rapid and detailed molecular information on nucleic acids from biological samples in a matter of days. RNA-seq is an application of NGS that provides efficient and accurate ways to measure changes in the transcriptome, and it also enables many other types of analysis on the genome that have no yet been utilized in a systematic framework. Here we provide a comprehensive RNA-seq data annotation and analysis package that can integrate data from (T)ranscripts, (E)xons, (A)lleles, (B)ases, poly-(A)denyltation sites, and (G)enes (BEGAT) to annotate the transcriptome information and count reads. After alignment, BEGAT annotates mapped reads with genes, exons and novel transcriptionally active regions (TARs). It will also annotate junction reads that mapped to a database of all possible exon-exon combination. Then, BEGAT annotates reads with poly-A tails by trimmed 3' poly A bases or 5' poly T bases, and identifies the alternative cleavage sites and miRNA targeting site at 3'UTR. Furthermore, all un-mapped reads after every other step will be iteratively trimmed, mapped to the genome again. They will be annotated as fusion genes, which can be identified for novel gene findings with single-end or paired-end data. BEGAT is also capable of aiding in molecular diagnostics and automated tissue source prediction as well. To show our algorithms applicability, we generated miRNA, mRNA, and methylation sequencing data from Glioblastoma multiforme (GBM) patients' tissue, as well as the corresponding cultured tumorspheres (5 tissue/tumorspheres pairs). We used BEGAT to identify specific expression patterns and fusions for the mRNA and miRNA transcriptome between the two primary tumors and their cultured cells. We also demonstrate BEGAT's ability to be integrated with a lasso regression model for delineating the regulators of tumor expression dynamics, which include data on copy number variance, DNA methylation level, and DNase hypersensitive sites (DHS). Together, these tools enable us to build a gene regulatory network for transcription of different GBM types. Further computational analysis will focus on further improving the model of transcriptional regulatory networks to help better classify GBM subtype, and to apply the model to other cancer types from other large-scale sequencing efforts.

1253F

Added value of SNP probes to a CGH chromosomal microarray analysis in clinical diagnostics: experience from a comprehensive high-resolution exon targeted aCGH plus SNP array and pure SNP arrays. *J. Wiszniewska[1,2], S-H.L Kang[1,2], C.A. Bacino[1,2,3], W. Bi[1,2], S. Al Masri[2], P.A. Ward[1,2], C.M. Eng[1,2], C. Shaw[1,2], P. Stankiewicz[1,2], J.R. Lupski[1,2,3], A.L. Beaudet[1,2,3], S.W. Cheung[1,2], A. Patel[1,2].* 1) Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Medical Genetics Laboratories, Baylor College of Medicine, Houston, TX; 3) Dept of Pediatrics, Baylor College of Medicine, Houston, TX.

Chromosomal Microarray Analysis (CMA) is a powerful method widely used for the detection of copy-number variation (CNV) in patients with intellectual disabilities and other clinical abnormalities. A variety of array platforms have been used for the analysis of genomic imbalances, however, only Single Nucleotide Polymorphisms (SNPs) platforms offer the ability to detect copy neutral contiguous regions of absence of heterozygosity (AOH) as in cases of uniparental disomy or consanguinity. Our laboratory tested 603 clinical samples using Illumina Human610-Quad or HumanOmni1-Quad arrays that contain over 610k and 1M SNPs, respectively. We detected clinically significant CNVs in ~10.5% of cases. At least one contiguous region of AOH greater than 5Mb was detected in ~29% of patients including eight cases of uniparental disomy (isodisomy of chromosomes 1, 2, 15, and 18, and segmental isodisomy of chromosomes 11 and 22). Notably, eleven cases had a proportion of AOH that was consistent with matings between first degree relatives. The current resolution of the commercial Illumina arrays does not allow the detection of intragenic deletions or duplications in most disease associated genes. To implement a comprehensive test for both CNVs and AOHs, we have enhanced our custom Agilent high-resolution oligo array with exon coverage of 1714 OMIM genes with 120k SNPs to supplement CNV genomotyping with genotyping data. We tested 581 clinical cases using the combined comprehensive array (CMA HR+SNP) and we detected contiguous regions of AOH greater than 10Mb in ~4.8% of patients, including UPD 9, 14, 15, and 16 as well as two cases consistent with incestuous matings. The higher detection rate of AOH by Illumina array reflects a sampling bias, i.e. many samples were sent for Illumina array analysis to identify regions of AOH in known consanguineous families and assess potential genes/loci responsible for autosomal recessive syndromes. Twenty eight cases with AOH regions detected by comprehensive array were also reflex tested to Illumina SNP array and the results showed high concordance of AOH calls despite the significant difference in the SNP content between the two platforms. Introduction of the CMA HR+SNP array for clinical diagnostics provides an opportunity to benefit from both oligo (exonic resolution) and SNP (chromosome segregation) array technologies enabling optimal genome resolution of potential pathogenic variation and better patient care at lower cost.

1254F

Parental origin of mosaic trisomy 9 or mosaic partial trisomy 9 using SNP microarray analysis. *S.L. Zimmerman, T.A. Smolarek.* Human Gen, Cincinnati Children's Hosp Med, Cincinnati, OH.

Abnormalities of chromosome 9, including unbalanced structural rearrangements and aneuploidy in both mosaic and non-mosaic states are more frequently identified due to the use of single nucleotide polymorphism (SNP) microarray analysis. Common clinical features of mosaic trisomy chromosome 9 anomalies, which are more or less severe depending on the specific region and mosaic state, include skeletal, central nervous system, cardiac and craniofacial abnormalities. Six cases with chromosome 9 aberrations were analyzed using Illumina SNP microarray analysis, standard karyotype, and/or fluorescence in situ hybridization (FISH) analysis. Results from the SNP array analysis identified mosaic abnormalities that were initially missed by standard karyotype analysis. Genotype analysis of child and parental samples was done to determine the parent of origin. Two cases had mosaic partial trisomy 9p, which were maternal in origin. One case had mosaic trisomy 9 with an additional marker chromosome that was derived from chromosome 9 was paternal in origin. Complex inheritance patterns with mitotic recombination events were identified in the three remaining cases. Both of the cases with mosaic trisomy 9 were maternal in origin, while the final case, which was a partial trisomy 9q was shown to be paternal in origin. In summary, of the six cases, 67% (4/6) were maternal in origin while 34% (2/6) were paternal in origin. These results indicate that SNP microarray is a powerful tool to discover low level mosaicism and to provide insight into the mechanisms underlying unusual and complex patterns of inheritance.

1255F

Identification of a novel interstitial deletion of chromosome 5 by molecular cytogenetic analysis: a de novo del(5)(q22.3q31.2). *S. Lee[1,4], H. Chae[1,4], M. Kim[1,4], Y. Kim[1,4], I.Y. Park[2], J.C. Shin[2], J. Lee[3], J. Son[4].* 1) Laboratory Medicine, The Catholic University of Korea, Seoul, Korea; 2) Obstetrics and Gynecology, The Catholic University of Korea, Seoul, Korea; 3) Pediatrics, The Catholic University of Korea, Seoul, korea; 4) Catholic Genetic Laboratory Center, The Catholic University of Korea, Seoul, Korea.

We report here on a case with a de novo interstitial deletion of region 5q22.3 to 5q31.2. The prenatal sonogram showed that the fetus had clenched hands and club feet, and the maternal serum marker was normal. The baby was born by cesarean section due to placenta abruption at 35 weeks, 4 days of gestation. The clinical findings in this case included brachycephaly, a high forehead, hypertelorism with prominent eyes, low-set ears, clenched hands, club feet, a prominent coccyx with hair, ambiguous genitalia, inguinal hernia, heart defect and severe failure to thrive. G-banded chromosome analysis revealed interstitial deletion of the long arm of chromosome 5 without delineation. Subsequent high resolution multicolor banding for chromosome 5 and array comparative genomic hybridization analysis defined that a 22.6 Mb interstitial deletion containing about 146 genes was distal to 5q22, with breakpoints at 5q22.3 and 5q31.2. Compared with the previous reports, this case had a more severe phenotype. This case illustrates that molecular techniques might be helpful to accurately confirm the 5q interstitial deletions detected by cytogenetic analysis. Molecular studies need to be performed on these patients to establish the genotype-phenotype correlation and to understand the role and influence of genes in this region of chromosome 5.

1256F

Recurrence of cat-eye syndrome secondary to parental somatic mosaicism. *M. Mathieu[1], G. Morin[1], S. Kanafani[2], K. Braun[3], B. Demeer[1], L. Lichtenberger[1], A. Receveur[2], G. Jedraszak[1], J. Andrieux[4], H. Copin[2].* 1) Clinical Genetic Unit, Amiens University Hospital, Amiens, France; 2) Cytogenetics Laboratory, Amiens University Hospital, Amiens, France; 3) Pediatric Endocrinology, Amiens University Hospital, Amiens, France; 4) Molecular Genetics Laboratory, Jeanne de Flandres Hospital, Lille, France.

Cat-eye syndrome, also named Schmid-Fraccaro syndrome (OMIM N° 115470) is a non exceptional cytogenetic disease occurring in 1 / 50 000 to 1 / 150 000 living birth. The first clinical description was made by Haab in 1878. The cytogenetic anomaly was discovered by Schachenmann in 1965. The name cat-eye syndrome was first suggested by Gerald in 1968. We report on the observation of 2 brothers presenting the association of an anal atresia, preauriculat tags and isolated growth hormone deficiency. On cerebral MRI, they both had an hypoplastic intrasellar anterior pituitary, but an ectopic neurohypophysis. Their ophthalmologic examination showed no iris coloboma but an external palsy named Duane anomaly. Their father was asymptomatic. Their mother presented bilateral preauricular pit. But she had no history of anal atresia or short stature. On blood karyotype, the 2 boys had a supernumerary marker derivative of the chromosome 22 on all the analysed cells. Their father had normal blood karyotype. Their mother presented only on few lymphocytes (5%) the same supernumerary marker.The array-CGH confirmed that the size of this marker was classical of cat-eye syndrome. This observation is original if we consider the recurrence secondary to parental somatic mosaicism, the Duane anomaly and the presence of growth hormone deficiency.

1257F

An analysis pipeline for detecting copy number variations with a low false discovery rate in microarray data. *D.-A. Clevert[1,2], A. Mitterecker[1], A. Mayr[1], G. Klambauer[1], M. Tuefferd[3], A. De Bondt[3], W. Talloen[3], H. Göhlmann[3], S. Hochreiter[1].* 1) Institute of Bioinformatics, Johannes Kepler University Linz, Linz, Austria; 2) Department of Nephrology and Internal Intensive Care, Charité University Medicine, Berlin, Germany; 3) Johnson & Johnson Pharmaceutical Research & Development, a Division of Janssen Pharmaceutica, Beerse, Belgium.

Motivation: A low false discovery rate (FDR) at the detection of copy-number aberrations (CNAs) in microarray data ensures sufficient detection power and prevents failures in CNA-disease association studies. A high FDR means many falsely discovered aberrations, which are not associated with the disease, though correction for multiple testing must take them into account. Thus, a high FDR not only decreases the discovery power of studies but also the significance level of the remaining discoveries after correction for multiple testing. **Methods:** We obtain a low FDR at the detection of CNAs in microarray data by a probabilistic latent variable model, called "cn.FARMS". The model is optimized by Bayesian maximum a posteriori approach, where a Laplace prior prefers models, which represent the null hypothesis of observing a constant copy number 2 for all samples. The posterior can only deviate from this prior by strong (deviation from copy number 2 intensities) and consistent signals in the data, which hints at a CNA - the alternative hypothesis. The information gain of the posterior over the prior gives the informative/non-informative (I/NI) call that serves as a filter for CNA candidate regions. I/NI call filtering reduces the FDR, because a region with a large I/NI call is unlikely to be a falsely detected CNA, which would neither have strong nor consistent measurements. It can be shown that the I/NI call filter applied to null hypotheses of the association study is independent of the test statistic which in turn guarantees that a type I error rate control by correction for multiple testing is still possible after filtering. I/NI-calls perform well for the usually rare CNAs that are seen at few samples only, where variance-based filtering approaches fail. **Results:** cn.FARMS clearly outperformed prevalent methods for CNA detection with respect to sensitivity and especially with respect to FDR on different HapMap benchmark data sets. **Availability:** The software cn.FARMS is publicly available as an R package at Bioconductor and at http://www.bioinf.jku.at/software/cnfarms/cnfarms.html.

1258F

Comparison of different reference genes used for qPCR-based CNV quantification. *N. Fang, A. Missel, C. Beckmann, U. Deutsch.* QIAGEN, Hilden, Germany.

Copy Number Variant (CNVs), the change of the DNA copy number in the genome, has been recently shown to be a widely-spread phenomenon that affects about 10-20% of the human genome. The occurrence of the CNVs has been associated with various diseases such as autism, autoimmune disorders, and cancer. The most commonly used molecular biology tools for discovery of CNVs are array and next-generation sequencing (NGS). These two high-throughput methods can discover multiple potential CNVs, which normally need to be validated with an independent method. Once validated, the confirmed CNVs can also be examined in a large number of samples to identify the statistically significant association of the CNV and phenotype. Quantitative PCR (qPCR), with its ease of use, sensitivity, and scalability, is often the method of choice for CNV validation and association studies. Relative quantification principle is used for this application: first, a reference gene, whose copy number is presumed to be constant in different genomes, has to be defined. The copy number of the genes of interest (GOIs) is then calculated based on the Ct difference of GOI and reference gene among different samples. Since the consistent copy number of the reference gene is essential for the qPCR-based CNV quantification, we evaluated the reliability of commonly-used single copy reference genes such as RNaseP, as well as other candidates. Our results suggest that, compared to single copy genes, stable multi-copy regions can serve as a more sensitive and reliable CNV quantification reference. We also demonstrate more reliable CNV quantification with the REST software, which takes different qPCR efficiency into consideration and performs statistical analysis of the qPCR data.

1259F

CNVs detection from targeted sequencing of genes associated with congenital heart defects. *Y. Lai[1], A. Postma[5], T. Rahman[2], J. Laros[1], Y. Ariyurek[1], S. Sperling[3], S. Klaassen[4], J. Goodship[2], P. 't Hoen[1].* 1) Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands; 2) Institute of Human Genetics, Newcastle University, Central Parkway, Newcastle upon Tyne, UK; 3) Group Cardiovascular Genetics, Department of Vertebrate Genomics, Max Planck Institute for Molecular Genetics, Berlin, Germany; 4) Max Delbrück Center for Molecular Medicine, Berlin, Germany; 5) Heart Failure Research Center, Academic Medical Center Amsterdam, Netherlands.

Copy number variations (CNVs) in the genome are an important source of genetic variability and underlie many disease phenotypes. If a gene residing in a copy number variable region is dosage sensitive, this can affect its gene expression level. For instance when one has only one functional copy of a gene, which may affect the abundance of the protein to support its normal function. Previous studies showed that numerous genes encode transcription factors play an important role in regulating heart formation. We hypothesize that haploinsufficiency of genes related with heart development is the reason for various severity of congenital heart defects. Haploinsufficiency of genes can be due to copy number change of dosage-sensitive genes or unmasking of recessive alleles by single base substitutions or short indels of the functional copy. Here we focus on the aspect of copy-number alterations within the disease cohort. Most current CNVs detection methods are designed for whole genome resequencing data and are able to identify large (>10 kb) or small (10 bp) indels (insertions or deletions). The objective is to discover medium sized deletions from exome sequencing data. Within the EU-sponsored HeartRepair project, we targeted exons of ~400 genes associated with congenital heart defects and sequence the targeted regions to high depth for ~160 patient samples. From this data, we compiled a matrix of reads that can be aligned uniquely to the targeted regions in samples passing minimum coverage criteria (>80% of targeted regions with coverage >20X) and normalized the matrix to the total number of reads on targets. We calculated the coefficient of variation on the depth of coverage for each targeted region to assess variability among samples, assuming that copy number polymorphic regions will show higher dispersion of the coverage across samples. ChrX can be seen as a copy number polymorphism between male and female samples. We used the 119 targeted regions on ChrX as bench mark for calling copy number variable regions and could clearly detect the higher coefficient of variation in the complete data set compared to data sets containing females or males only. 117 copy number polymorphic regions were detected in the 4200 targeted autosomal regions, of which two are overlapping with known CNVs in DGV database. We further assess the validity of the detected copy number polymorphic regions by checking the proportion of unbalanced heterozygous calls.

1260F

Complex genomic aberrations can be the cause of variable phenotypes of 22q11.2 deletion or duplication syndrome. *D. Li, M. Buch, M. Tekin, YS. Fan.* University of Miami, Miller School of Medicine, Miami, FL.

It is well known that the phenotypes of patients with DiGeorge syndrome can be extremely variable from near normal to severe developmental disabilities including congenital heart defects and risk of psychological problems such as schizophrenia. The causes of lack of genotype-phenotype correlation are not well understood. Prior to the clinical use of array CGH, deletion or duplication in the 22q11.2 region was detected by FISH, and therefore changes in the genome other than the 22q11.2 region was barely known. Array CGH studies have made it possible to reveal the imbalances genome-wide, and the findings of copy number changes other than the 22q11.2 microdeletion or duplication may explain the complexity of the genotype-phenotype correlations in these patients. By array CGH, we have detected copy number changes in the 22q11.2 region in 16 (11 deletions; 5 duplications) of 1292 cases referred for intellectual disabilities and/or congenital anomalies. We observed complex genomic imbalances in 2 of the 16 cases. The first case was a baby female of 15 days of age with tetrology of Fallot revealed by ultrasound. Array CGH showed a 2.47 Mb deletion in the 22q11.2 DiGeorge region and a 1.56 Mb deletion in the Xp22.31 region involving multiple genes including the STS gene which causes X-linked recessive ichthyosis when deleted or mutated. The second case was a baby boy of 15 days of age also with an abnormal ultrasound showing complex congenital heart defects. In this baby, array CGH detected multiple pathogenic copy number alterations, including a 2.84Mb duplication in the 22q11.2 DiGeorge region, a 605kb duplication in the 15q13.3 region involving the CHRNA7 gene, as well as a 209 kb deletion in 16p13.2 region involving the A2BP1 gene. Copy number changes of CHRNA are associated with mental retardation, autism and schizophrenia. Deletion of A2BP1 has been reported in patients with mental retardation, autism and seizure. Our observations have provided evidence that complex genomic changes are not rare in DiGeorge patients and they may contribute to the extremely variable phenotypes in this disease. Also, the additional changes such as duplication of CHRNA and deletion of A2BP1 may add additional risk for psychological diseases in the patient. Continuing follow-up and collection of detailed clinical information in these 2 patients may help our understanding on the complicated phenotypes of the DiGeorge or 22q11.2 microdeletion/duplication syndrome.

1261F

A de novo balanced chromosomal translocation disrupting a copy of the hominoid-specific *TBC1D3* gene embedded in a CNV region in a patient with developmental pituitary defects and Attention-Deficit/Hyperactivity Disorder (ADHD). *K. Machini[1], B. Duriez[1], Y. Rakover[2], P. Duquesnoy[1], F. Dastot-Le Moal[1,3], N. Collot[3], S. Amselem[1,3].* 1) Institut National de la Santé et de la Recherche Médicale (INSERM) U.933, Université Pierre et Marie Curie-Paris 6, Paris, France; 2) Pediatric Endocrinology, Haemek Hospital, Afula 18101, Israel; 3) Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpital Armand-Trousseau, Paris, France.

Over the last few years, accumulating data on genome sequences has illustrated the major role of copy number variation (CNV) regions in genome architecture and evolution. Most importantly, such regions are frequently associated with sporadic, Mendelian or complex diseases in humans. However, while deletions, duplications or inversions frequently occur in segmental duplications or CNV regions, no example of chromosomal translocation interrupting these sequences has been reported to date.

In the present study we mapped the breakpoints of a de novo translocation in a region of segmental duplication of chromosome 17. Moreover, we showed that this balanced translocation disrupts one copy of the hominoid-specific gene *TBC1D3*, which is absent in lower species and present in multiple copies in the human genome and has been shown to regulate the highly conserved EGF pathway in humans. The patient presented with anterior pituitary hypoplasia and hormonal deficiencies (GH, TSH and ACTH), a condition known as Combined Pituitary Hormone Deficiency (CPHD). The molecular basis of this disorder has been determined only in a small proportion of patients with the identification of mutations in a limited number of genes, mainly through a candidate-gene approach relying on mouse models. This chromosomal rearrangement did not affect the gene copy number or the transcript level of *TBC1D3* and no chimeric transcripts were detected in the patient's lymphoblastoid cell line. It is, therefore, highly likely that the translocation affects gene expression only in the pituitary and possibly in a specific developmental window.

Besides CPHD, the patient was diagnosed with Attention-Deficit/Hyperactivity Disorder (ADHD). Interestingly, recent studies support a potential role of CNVs in ADHD, and rearrangements in the very same region of chromosome 17 (17q12) have been shown to confer risk of developing autism spectrum disorders.

Overall, our findings illustrate that chromosomal translocations disrupting CNV regions can indeed cause a human disease. Most importantly, this study indicates that hominoid-specific genes, such as *TBC1D3*, are highly likely to play key roles in developmental pathways and, if affected, contribute to human pathology.

1262F

Diagnosis of Skeletal Muscular Atrophy through use of droplet digital PCR. *J. Regan, P. Belgrader, K. Ness, B. Hindson, S. Saxonov.* QuantaLife, Pleasanton, CA.

Skeletal Muscular Atrophy (SMA) is the most common cause of genetically determined neonatal death. One out of every 60 individuals is a carrier for SMA, which affects one in every 1000 births. It is an autosomal recessive disease characterized by progressive motor neuron degeneration. The disease is caused by loss of the SMN1 gene, whereas the severity of the disease, from early infant death to a normal lifespan with mild weakness, is associated with the copy number of SMN2 genes that are able to partially compensate for SMN1 loss. Although qPCR and multiplex ligation dependent probe amplification (MLPA) are traditionally used to diagnosis SMA, neither of these technologies offer sufficient precision to measure SMN1 and SMN2 copy number reliably. For this study, droplet digital PCR (ddPCR) was used to assess SMN1 and SMN2 copy number in over 30 samples from the Coriell repository, including six positive controls. ddPCR was found to measure copy number states for the two SMN genes accurately and reproducibly. In addition, these estimates were verified via a direct SMN1-SMN2 duplex assay which was used to measure the ratio of SMN1 to SMN2.

1263F

Complex genetics of radial ray deficiencies: Screening of a cohort of 50 patients. *S. Vergult[1], A.J.M. Hoogeboom[2], E.K. Bijlsma[2], M. Jongmans[3], C. Thiel[4], J. Verheij[5], A. Perez-Aytes[6], H. Van Esch[7], A. Küchler[8], D. Barge-Schaapveld[9], Y. Sznajer[10], G. Mortier[11], B. Menten[1].* 1) Center for Medical Genetics Ghent, Ghent, Belgium; 2) Department of Plastic, Reconstructive and Hand Surgery, Erasmus MC, University Medical Center, Rotterdam, The Netherlands; 3) Department of Human Genetics, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; 4) Institute of Human Genetics, University Hospital Erlangen, Erlangen, Germany; 5) Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; 6) Dismorfología y Genética Reproductiva, Grupo de Investigación en Perinatología, Instituto de Investigación Sanitaria, Fundación Hospital La Fe, Valencia, Spain; 7) Center for Human Genetics, Leuven, Belgium; 8) Institut für Humangenetik, Universitätsklinikum Essen, Essen, Germany; 9) Department of Clinical Genetics, Academic Medical Centre, UVA, Amsterdam, The Netherlands; 10) Pediatric Clinical Genetics, Hopital Universitaire des Enfants Reine Fabiola, Center for Human Genetics, Université Libre de Bruxelles, Brussels, Belgium; 11) Department for Medical Genetics, Antwerp, Belgium.

Radial ray deficiencies (RRDs) are characterized by unilateral or bilateral absence of varying portions of the radius and thumb. Both isolated and syndromic forms have been described. Although for some of the syndromes the causal genes have been identified e.g. *TBX5* (Holt-Oram Syndrome), the genetics of RRDs remain complex. Given this complexity we searched for genomic aberrations in 50 patients with (non-) syndromic forms of RRDs by means of molecular karyotyping. In >10% an aberration was detected. We identified known aberrations such as the 1q21.1 microduplication and the 16p13.11 microduplication. Next to these, some rare copy number variations were revealed of which two were of high interest: a deletion at chromosome band 7p22.1 and one at chromosome band 10q24.3. The 7p22.1 deletion contains the *RAC1* gene. Conditional knockout mice of *RAC1* exhibit truncated fore- and hindlimbs (Wu *et al.*, 2008), making it an interesting candidate gene for RRDs. Unfortunately, sequencing analysis of *RAC1* in our patient cohort did not reveal any additional causal mutations. Since it cannot be excluded that a recessive allele is unmasked by the 7p22.1 deletion, capture sequencing is being performed for the other allele in this patient. The 10q24.3 deletion contains only one gene, *FBXW4*. This gene is of high interest since in mice insertions in the orthologue dactylin gene give rise to dactylaplasia. In humans duplication of this region is associated with Split Hand Foot Malformation (SHFM3). How these duplications cause SHFM3 is still not known. Since several genes in the vicinity have a role in limb development, it has already been suggested that these duplications may disrupt the interaction between cis-regulatory elements and their target genes, resulting in SHFM. In our patient it could also be possible that the 10q24.3 deletion gives rise to RRDs by deletion of one or more regulatory regions. For this, six CNEs (conserved non-coding elements) were selected. Sequence analysis of these CNEs revealed one mutation in our cohort, which was absent in 96 control samples. Although this substitution was found in a highly conserved region, the phyloP score suggests a rather low impact. Sequence analysis of the exons of *FBXW4* in our cohort did not reveal causal mutations. This is the first microarray study in a larger cohort of patients with RRDs. Some interesting aberrations were detected, which we will further discuss and elaborate on the genotype-phenotype correlation.

1264F

Copy Number Variation Determination with Blood vs. Saliva Sourced DNA. *D.B. Willmot, R. Elumalai.* Genomics, Agilent Technologies, Wilmington , DE.

Array based comparative genomic hybridization is a widely used technology with many clinical and research applications for measuring copy number variation. Aside from tumors, most DNA samples are extracted from blood. The objective of this study is to compare copy number determinations using DNA extracted from blood samples vs. DNA from saliva. Saliva can be collected without a phlebotomist, more safely, and is more acceptable to many - especially children. Newer DNA isolation kits for saliva report yields as high as 55 ug per 1.0 ml collection tube with intactness comparable to blood sourced DNA. Agilent microarrays with one million features were hybridized with DNA from six individuals (blood vs. saliva DNA). Oragene OG-510 kits from DNA Genotek were used followed by a double RNase digestion. Labeling was with Agilent ULS non-enzymatic labeling. The six individuals consisted of two families with the parents and one offspring each. Parent-offspring comparisons provided differential aberrations for tracking the consistency between the DNA sources. Analysis was performed with Agilent Genomic Workbench Lite version 6.5 with differential aberration reports finding relatively minor differences arising by chance or from the DNA sources. We will show graphically and by tabulation that saliva is a viable source of DNA for array based CGH.

1265F

Evolution of Chromosome Abnormality databases from mid70s to the present. *D. Borgaonkar.* LNG,NIA,NIH, Bethesda, MD, Wilmington, DE.

The first edition of Chromosomal Variation in Man was sublitted for publication to the Johns Hopkins Press in December 1974 and was published the following year.The 8th edition was published by John Wiley/Liss in 1997. Online access to the database material has been provided through the website www.wiley.com/borgaonkar since 1998 to date. This work was and remains the most extensive databse of reference material on all kinds of human chromosomal variants, polymorphisms and anomalies. Plans are underway to publish an ebook. This book should be of use to health professionals, family members and others interested in chromosome science and its role in health and disease. Given the effort put in the last 3 1/2 decades in maintaining the database, the parallel advances made in computer technology, and the methodology of studying chromosomes, it would be of interest to seek input from users. We had demonstrated this database at the International Congress in Washington in 1991. However, the wisdom at that time was not to proceed with offering it Online! Howthe world has changed!

1266F

Automating image detection for Molecular Combing: bringing the FSHD test to routine diagnostics laboratories. *P. Walrafen[1], K. Nguyen[2,3], M. Pierret[1], R. Bernard[2,3], C. Thiberville[1], C. Chaix[2,3], E. Renard[1], S. Attarian[2,4], Y. Ghomchi[1], A. Bensimon[1], J. Pouget[2,4], E. Conseiller[1], N. Lévy[2,3].* 1) Genomic Vision, Paris, France; 2) AP-HM, Département de Génétique Médicale - Hôpital d'enfants de la Timone, Marseille, France; 3) Université de la Méditerranée, INSERM UMR_S 910 “Génétique médicale et génomique fonctionnelle”, Marseille, France; 4) AP-HM, Service de Neurologie, centre de référence des maladies neuromusculaires - Hôpital de la Timone, Marseille, France.

Facioscapulohumeral dystrophy (FSHD) is among the most frequent inherited muscular disorders. Clinical symptoms range from mild to severe, the most severe forms involving full-time use of a wheelchair from early adulthood. The transmission is autosomal dominant, but de novo cases are frequent, essentially related to somatic mosaicism in the index case or a healthy / mild symptomatic parent. The current standard for molecular genetic testing involves a combination of several restriction digests, pulsefield gel electrophoresis and Southern blots, a complex and tedious procedure which sometimes suffers from a lack of accuracy. Interpreting the results is indeed made difficult by the genetic characteristics of the disease: testing involves the quantification of a 3.3 kb CNV with copy numbers varying from 1 to 100, pathogenic alleles having 10 repeats or less. The disease-related locus at the chromosome 4q subtelomere shows almost perfect homology with the chr 10q subtelomere. Besides, the pathology is uniquely associated with one of two common haplotypes of chr 4q characterized by kb-scale differences downstream of the repeat array. Finally, rearrangements are relatively frequent, involving either exchanges between chromosomes 4 and 10, deletion of sequences neighboring the repeat array, or more complex rearrangements. We have published a new approach for molecular testing of FSHD based on Molecular Combing. This technology enables single-molecule visualization through fluorescence microscopy of genetic loci at high resolution (~1 kb) over very large regions (several hundreds of kb). Although the results of this initial study clearly showed the advantages of the Molecular Combing test for reliability and ease of interpretation, the comparison with Southern blotting in terms of hands-on time was less favorable, mainly due to operator-performed image analysis. To enable wide use of the test for routine diagnostics in laboratories, we have developed software which automates the analysis step, and considerably reduces operator time. We have also simplified the test design to make the analysis straightforward. We have tested a cohort of patients including prenatal samples, at an increasing rate, which is now compatible with the typical needs of a laboratory performing molecular testing for FSHD. Interestingly, the approach could be extended to other Molecular Combing tests, thus broadening potential applications for this novel diagnostics technology.

1267F

Subtelomeric rearrangements in a cohort of 50 patients with severe mental retardation and dysmorphism diagnosed by fluorescence in situ hybridization and chromosomal microarray analysis. *I. Ben Abdallah Bouhjar[1], H. Elghezal[1], N. Soyah[2], S. Mougou[1], H. Ben Khelifa[1], M. Gribaa[1], D. H'mida[1], A. Saad[1], PRF. Tunisian Network On MR[3].* 1) Cytogenetics and Reproductive Biology Department , Farhat Hached University Teaching Hospital , Tunisia, SOUSSE, Tunisia; 2) Paediatric Department, Farhat Hached University Teaching Hospital, Sousse, Tunisia; 3) Ministry of Higher Education and Research, Tunisia.

About 5-10% of patients with dysmorphisms, severe mental retardation, and normal standard karyotype are affected by subtelomeric chromosome rearrangements. Sequence homology between different chromosomes and variability between homologs make these regions more susceptible to breakage and reunion. We analyzed the telomeric regions of 50 of these patients, selected with strict clinical criteria described by De Vries et al. Fluorescence in situ hybridization, using commercial probes, was performed according to the manufacturer's instructions (TelVysion, Vysis®, Abbott Park, IL). Ten subtelomeric rearrangements were identified (20%). Six had a unique anomaly (del(1)(p36.2), del (2)(q37.2), del(10)(q26.2), dup(10)(q26.2), del (22)(q13.2) and del (6)(p25.2)), there are de novo imbalances. Two subjects had double segmental imbalances inherited from a balanced parent (der(3)t(-3;2)(p26.3;q37.2)mat and der(3)t(X;3) (q27.3;p26.3)mat). For more characterization of rearranged genes and breakpoints, we analyzed by comparative genomic hybridization array the DNA of subjects with subtelomeric anomalies. The 44,000 Agilent oligonucleotides array was used according to the manufacturer's instructions (Agilent Human Genome CGH Microarray Kit 44K, Agilent Technologies®, Santa Clara, CA). This study provides further evidence for the plasticity of subtelomeric regions, which often results in cryptic rearrangements, and recommends stringent criteria for selecting patient candidates to telomere analysis and to establish a precise phenotype genotype correlation and to provide genetic counselling to the parents.

1268F

Human embryonic stem cells and in situ acquisition of chromosomal imbalance: a case of trisomy 20 in the HD90 line carrying a germline VHL mutation. *F. Pellestor[1], F. Becker[2], C. Monzo[2], J. De Vos[2], P. Sarda[1], G. Lefort[1].* 1) Medical and Chromosomal Genetics, Hopital ADV, CHRU Montpellier, Montpellier, France; 2) Research Institute of Biotherapy, Hopital St Eloi, CHRU Montpellier, Montpellier, France.

The occurrence of numerical chromosomal abnormalities is a major problem in cultures of human embryonic stem cells (hESC), since extra or missing chromosomes can compromise cell viability or lead to adaptive advantages in culture. The early detection of these numerical abnormalities is essential to avoid the replacement of normal cell by unbalanced cells in culture. We report the results of classical and molecular cytogenetic analysis performed on the hES cell line HD90, at different passages in long-term culture, and the identification of trisomy 20. The HD90 line was derived from the inner cell mass of a 5 day-old-preimplantation-blastocyst stage embryo harbouring a germline Von Hippel Lindau (VHL) mutation. Cytogenetic analysis was performed using standard methods and R-banding. FISH using both centromeric and painting probes specific for chromosome 20 were used for chromosomal detection on interphase nuclei. R-band karyotyping of HD90 cells was done at passage 26. A minimum of 30 metaphase spreads were analysed and all indicated the presence of trisomy 20. The defect was confirmed by dual colour FISH in a mimimum of 100 interphase cells from passages 12 and 23. No trisomic cells were observed at the earlier passage 6, whereas the trisomy 20 was detected in 17% of the cells at passage 7. This indicates that the chromosomal abnormality was progressively acquired during long-term culture. Chromosomal abnormalities in hESC have previously been reported, especially trisomy 12 and trisomy 17 which appear to give a proliferative growth advantage in hESC cultures. A similar proliferative advantage has been observed in the HD90 line carrying trisomy 20. The presence of the VHL mutation could promote chromosomal instability and aneuploidy, by impairing spindle checkpoint function. The early detection of these cells by cytogenetic analysis is essential, since trisomic cells could replace the normal cell population in a few passages. Although molecular methods such as micro-arrays offer high resolution in terms of detecting genetic changes, karyotyping and interphase FISH are more sensitive methods for identifying small cell populations with clonal chromosomal abnormalities. Consequently, regular chromosomal testing is required to ensure the integrity of embryonic stem cell lines. Supported by the Fondation Pour La Recherche Médicale and the Agence de la Biomédecine.

1269F

Spectral Karyotyping for Identification of Constitutional Chromosomal Abnormalities at a National Reference Laboratory. *B.T. Wang, A. Anguiano, S. Wang, F.Z. Boyar, L.W. Mahon, M.M. El Naggar, P.H. Kohn, M.H. Haddadin, V. Sulcova, A. Sbeiti, M. Ayad, T. Sahoo, B.J. White, C.M. Strom.* Cytogenetics Dept, Quest Diagnostics Nichols Inst, San Juan Capistrano, CA.

Spectral karyotyping is a diagnostic tool that allows visualization of chromosomes in different colors using the FISH technology and a spectral imaging system. To assess the value of spectral karyotyping analysis for identifying constitutional supernumerary marker chromosomes or derivative chromosomes at a national reference laboratory, we reviewed the results of 179 consecutive clinical samples (31 prenatal and 148 postnatal) submitted for spectral karyotyping. Over 90% of the cases were requested to identify either supernumerary marker chromosomes or chromosomal exchange material detected by G-banded chromosome analysis. We also reviewed clinical indications of those cases with marker chromosomes in which chromosomal origin was identified by spectral karyotyping. Our results showed that spectral karyotyping identified the chromosomal origin of marker chromosomes or the source of derivative chromosomal material in 158 (88%) of the 179 clinical cases; the identification rate was slightly higher for postnatal (89%) compared to prenatal (84%) cases. Cases in which the origin could not be identified had either a small marker chromosome present at a very low level of mosaicism (<10%), or contained very little euchromatic material. Supplemental FISH analysis confirmed the spectral karyotyping results in all 158 cases. Clinical indications for prenatal cases were mainly for marker identification after amniocentesis. For postnatal cases, the primary indications were developmental delay and multiple congenital anomalies (MCA). The most frequently encountered markers were of chromosome 15 origin for satellited chromosomes, and chromosomes 2 and 16 for non-satellited chromosomes. We were able to obtain pertinent clinical information for 47% (41/88) of cases with an identified marker chromosome. We conclude that spectral karyotyping is sufficiently reliable for use and provides a valuable diagnostic tool for establishing the origin of supernumerary marker chromosomes or derivative chromosomal material that cannot be identified with standard cytogenetic techniques.

1270F

The Developmental Genome Anatomy Project (DGAP): Annotating the Genome by Cytogenetic and Sequencing Approaches. *A.M. Lindgren[1], M.E. Talkowski[2,3], C. Hanscom[2], C. Chiang[2], C. Ernst[2,3], S. Ahsan[1], B.B. Currall[1], L. Yuan[1], S. Lachke[4], I. Saadi[4], D.J. Harris[5], R.L. Maas[4], B.J. Quade[1], J.F. Gusella[2,3], C.C. Morton[1].* 1) Depts. of Ob/Gyn and Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 2) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 3) Depts. of Genetics and Neurology, Harvard Medical School, Boston, MA; 4) Dept. of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 5) Children's Hospital Boston, Harvard Medical School, Boston, MA.

The Developmental Genome Anatomy Project (DGAP, dgap.harvard.edu) is a collaborative endeavor to identify genes critical in human development and disease. Balanced chromosomal rearrangements are the biological resource for gene discovery in DGAP as they may indicate the location of disrupted or dysregulated genes that lead to an abnormal phenotype. DGAP analyzes the correlation between genotype and phenotype through FISH-based breakpoint localization, various sequencing methods, candidate gene identification and functional analysis in model organisms. Of 235 cases enrolled to date, breakpoints are FISH mapped in 88 cases, 116 of which are localized to a single clone. Seventy-six breakpoint sequences are determined in 36 cases and 57 disrupted genes identified for which 24 animal models have been evaluated. Notable cases under active investigation include DGAP100 [46,X,t(X;5)(p11.3;q35.2)], a nonverbal 16 year-old female with septo-optic dysplasia, cleft palate, severe myopia, neuromuscular scoliosis, hearing impairment, and a history of seizures. KDM6A, a histone 3 lysine 27 demethylase, is disrupted at Xp11.3, and qRT-PCR reveals ~50% reduction in KDM6A expression compared to control lymphoblast cell lines, suggesting haploinsufficiency of KDM6A is pathogenetic in the phenotype. Zebrafish knockdowns are underway and preliminary analyses show craniofacial anomalies. DGAP120 [46,XY,t(6;11)(q24.3;q21)] is a 12 year-old male with low-to-mid frequency sensorineural hearing loss, intermittent exotropia and craniofacial defects; C6ORF103 is disrupted at 6q24.3. DGAP191 [46,XY,t(5;7)(q14.3;q21.3)], a 3 year-old male, has sensorineural hearing loss, mental retardation, hypotonia and seizures. Although no genes are directly disrupted, the 5q14.3 breakpoint is ~500 kb upstream of MEF2C and the 7q21.3 breakpoint is 2.86 kb upstream of COL1A2. Normal expression of MEF2C and over-expression of COL1A2, as determined by qRT-PCR, suggest dysregulation of COL1A2 as etiologic in the phenotype. Chromosomal rearrangements remain a rich resource for identifying genes and regulatory elements underlying human disease and traits. In conjunction with development of affordable sequencing methods, the study of balanced chromosome rearrangements in phenotypically abnormal individuals is imperative in rapid annotation of the human genome.

1271F

A new case of azoospermic male with 46,XY,t(1;21)(p11;p12) karyotype. *E.O. Ote[1], T. Turunc[2], O. Ozer[1], Z. Yilmaz[1], F.I. Sahin[1].* 1) Department of Medical Genetics, Baskent University Faculty of Medicine, Ankara, Turkey; 2) Department of Urology, Baskent University Faculty of Medicine, Ankara, Turkey.

Infertile men have an increased prevalence of chromosome abnormalities compared to phenotypically normal newborns. Translocations involving acrocentric chromosomes regardless of the partner chromosome integrate with male meiosis. Here we report a 32 years old azoospermic man with a history of infertility for seven years. The patient consulted the infertility clinic and was evaluated for testicular biopsy. Hormone levels were detected as FSH: 7.5 mIU/ml, LH: 4.3 mIU/ml, total testosterone: 3.11 ng/ml, all in the normal range. The patient was also referred to our department for cytogenetic analysis which resulted in a 46,XY,t(1;21)(p11;p12) karyotype, pedigree analysis, although cytogenetic analysis was not performed, revealed recurrent abortions in mother and sister of the proband suggesting a familial translocation. The patient was informed about the result during genetic counseling. Patients with similar karyotypes were reported previously. The mechanism underlying the relation between translocations involving acrocentric chromosomes and male infertility is not clear yet. However, it has been postulated that interaction between the quadrivalent in pachytene of male meiosis and XY body might be the explanatory mechanism.

1272F

Accurate detection of copy number variations in next generation sequencing data by a latent variable model. *G. Klambauer, D.A. Clevert, A. Mayr, K. Schwarzbauer, A. Mitterecker, S. Hochreiter.* Institute of Bioinformatics, Johannes Kepler University Linz, Linz, Upper Austria, Austria.

The quantitative analysis of next generation sequencing (NGS) data like the detection of copy number variations (CNVs) is still challenging. Current methods detect CNVs as changes of read densities along chromosomes, therefore they are prone to a high false discovery rate (FDR) because of technological or genomic read count variations, even after GC correction. A high FDR means many wrongly detected CNVs that are not associated with the disease considered in a study, though correction for multiple testing must take them into account and thereby decreases the study's discovery power. We propose “Copy Number estimation by a Mixture Of PoissonS” (cn.MOPS) for CNV detection from NGS data, which constructs a model across samples at each genomic position, therefore it is not affected by read count variations along chromosomes. In a Bayesian framework, cn.MOPS decomposes read variations across samples into integer copy numbers and noise by its mixture components and Poisson distributions, respectively. The more the data drives the posterior away from a Dirichlet prior corresponding to copy number two, the more likely the data is caused by a CNV, and, the larger is the informative/non-informative (I/NI) call. cn.MOPS detects a CNV in the DNA of an individual by a region with large I/NI calls. I/NI call based CNV detection gurantees a low FDR because wrong detections are less likely for large I/NI calls. We compare cn.MOPS with the five most popular CNV detection methods for NGS data at three benchmark data sets: (1) artificial, (2) NGS data from a male HapMap individual with implanted CNVs from the X chromosome, and (3) the HapMap phase 2 individuals with known CNVs. At all benchmark data sets cn.MOPS outperformed its five competitors with respect to precision (1-FDR) and recall both at gains and losses.

1273F

Cytogenetic study of couples with infertility and recurrent miscarriage. *O.L.de. Moraes[1], F.C. Vinhaes[1], M.T.O. Cardoso[2], L.F.P. Bravin[2], M. Yoshino[2], M.S. Cordoba[2], S.M.do Couto[2], G. Tenser[2].* 1) Centro Universitário de Brasília, Brasilia, Distrito Federal, Brazil; 2) Núcleo de Genética, Hospital de Apoio de Brasília, Brasilia, Distrito Federal, Brazil.

Infertility is defined as the absence of pregnancy for a year or more in a young, healthy, and sexually active couple, with no use of contraceptive measures. It may result from a variety of factors, including both male and female partner. In approximately 20% of infertile couples the etiology of infertility is not known, despite clinical investigations, and in some cases the origin of infertility may be due to chromosomal or genetic factors. Abnormal karyotypes may explain infertility or recurrent pregnancy loss. When anomalies occur in germline cells, abnormal chromosomes segregate in gametes and may be transmitted to offspring. Balanced translocations (paternal or maternal) may interfere on pairing or disjunction of chromosomes during meiosis, forming unbalanced gametes. Couples who report recurrent miscarriage have a higher risk of being structural chromosome abnormalities carriers, particularly reciprocal translocations and inversions. This study aimed to perform cytogenetic analysis in couples with a history of infertility or recurrent miscarriage. Patients included in this study are from a public hospital in Brasilia, Brazil. Cytogenetic analysis was performed using the method of temporary culture of peripheral lymphocytes described by Moorhead et al., (1960) and the analysis of chromosomal aberrations was performed using the G and C-banding techniques. The 67 patients included in this study, from November 2009 to June 2010, had a history of reproductive problems, and an average age of 34 years old. Among these patients, 65.7% presented general causes of infertility and 34.3% a history of recurrent miscarriage. Cytogenetic study revealed normal karyotype in 88.1% of these patients, and abnormal karyotype in 11.9%, whereas 75% were female patients. Regarding chromosomal abnormalities, four patients (50%) carried a balanced translocation; two patients (25%), derivative chromosomes; one patient (12.5%) presented a balanced translocation and an inversion on chromosome 9, and one patient (12.5%) had sex chromosome mosaicism. The present study demonstrates the importance of cytogenetic analysis in elucidating the infertility and recurrent miscarriage etiology, and enables healthcare professionals to properly conduct genetic counseling, allowing couples to make correct decisions about their reproductive life.

1274F

Case reports: A rare chromosomal translocation in woman with recurrent miscarriages. *V. STOIAN[1], D. MIERLA[2], V. RADOI[2,3].* 1) University Bucharest, Faculty of Biology , Bucharest,; 2) Life Memorial Hospital, Bucharest, Romania; 3) Genetic Department, UMF Carol Davila, Bucharest.

Background: Carriers of translocations may have an increased risk of an unbalanced progeny due to imbalances and delays in meiosis. Case: A 38 year old patient was referred to the Genetic Department of Life Memorial Hospital because of her recurrent miscarriages and plurimalformative fetus in history, for genetic counseling. She had three previous pregnancies, each pregnancies ended in abortion. The cytogenetic analysis using GTG banding technique showed that the woman is a carrier of balanced reciprocal translocation involving chromosomes 13 and 16; 46,XX,t(13;16)(q33.3;p12.2), while her husband's karyotype was found to be normal. Therefore, cytogenetic tests are recommended for patients with a history of abortion, especially in women with a higher age to identify chromosomal alterations. Conclusion: To our knowledge, no translocation with such breakpoints t(13;16)(q33.3;p12.2) has been described previously in the women with recurrent miscarriages. Key words: t(13;16), infertility, miscarriage, translocations.

1275F

Variant Rett syndrome in a girl with a pericentric X-chromosome inversion leading to overexpression of the MECP2 gene in the absence of gene duplication. *F. Lopes[1], A. Silva-Fernandes[1], S. Moura[1], S. Pereira[1], B. Costa[1], B. Ylstra[2], J. Weiss[3], T. Temudo[1, 4], T. Lourenço[4], J. Vieira[5], P. Maciel[1].* 1) ICVS/3B's, Health Sciences School - University of Minho, Braga, Braga, Portugal; 2) Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands; 3) Department of Clinical Genetics, VU University Medical Centre, Amsterdam, The Netherlands; 4) Unidade de Neuropediatria, Serviço de Pediatria, Hospital Geral de Santo António, Porto, Portugal; 5) Hospital D. Estefânia, Lisbon, Portugal.

Mutation in the MECP2 gene account for up to 80% of the classic Rett syndrome (RTT) phenotype in girls. The genotype of patients without one of these mutations has also been investigated and some cases with gross rearrangements involving the MECP2 gene were found, usually large deletions. In boys, MECP2 duplication is the rare cause of a severe neurologic disease with mental retardation, microcephaly and epilepsy; MECP2 duplication has also been described in a girl with a speech preserved variant of RTT. Overexpression of the normal MeCP2 has also been shown to cause a progressive neurological disorder in a mouse model. We report the case of a girl with the features of variant Rett syndrome in whom we found no mutations in the MECP2 gene by direct sequencing, nor changes in exon dosage (no deletions or duplications) by Robust Dosage PCR. Karyotype analysis, however, revealed a pericentric inversion in the X chromosome (46, X, inv (X)(p22.1q28). This patient had a balanced pattern of X inactivation. Given the location of the breakpoints, we investigated the expression levels of the MECP2 and CDKL5 genes. Intriguingly, she displayed an overexpression of the MECP2 gene at the mRNA level (mean fold change: 2,55±0,38) in comparison to a group of control individuals; the expression of the CDKL5 gene was similar to that of controls (mean fold change: 0,98±0,10). We performed FISH analysis to test the hypothesis that the inversion could have displaced the MECP2 promoter or its 5' regulatory region, separating it from the coding region; however, this was not the case. aCGH analysis using Agilent 180K custom array platform revealed no microdeletions or microduplications in the breakpoint regions that could explain the overexpression of the mRNA. Methylation analysis revealed a hypomethylation of MECP2 gene, which is consistent with the gene overexpression. The X-chromosome inversion detected in the patient leads to hypomethylation of the MECP2 gene and consequent overexpression of this gene. This results in clinical manifestations similar to those of RTT syndrome. We are currently exploring in further detail the mechanisms of MECP2 methylation and expression changes. Independently of the mechanisms, we propose that de-regulation of MECP2 expression may underlie disease in other RTT-like patients who test negative for MECP2 mutations. MECP2 expression may underlie disease in other RTT-like patients who test negative for MECP2 mutations.

1276F

Next generation genomic microarrays and custom FISH probes for molecular cytogenetic analysis designed by *ab initio* sequence analysis. *S.N. Dorman[1], B.C. Shirley[2], N.G. Caminsky[1], E.J. Mucaki[1], W.A. Khan[3], L. Guo[4], J.H.M. Knoll[3,4], P.K. Rogan[1,2,4].* 1) Department of Biochemistry, University of Western Ontario, London, Ontario, Canada; 2) Department of Computer Science, University of Western Ontario, London, Ontario, Canada; 3) Department of Pathology, University of Western Ontario, London, Ontario, Canada; 4) Cytognomix Inc.

Array comparative genomic hybridization (aCGH) and fluorescence *in-situ* hybridization (FISH) are recommended as first-line tests for detection of chromosomal abnormalities in patients with congenital disorders and malignancies. Current aCGH platforms exhibit high coefficients of variation and low reproducibility in raw intensities, due to the use of blocking DNA, which is contaminated with single copy sequences. To improve the performance and define the content of FISH probes and microarrays, we have implemented an *ab initio* method (US Pat No 7,734,424) which locates genomic sequence intervals without repeat-masking. Our 44K *ab initio* aCGH microarray has lower coefficients of variation relative to Agilent's 4x44K platform ($p \leq 0.001$). Genotypes have been analyzed for other design parameters including proximity of the closest multicopy sequence, divergence of these repeats and probe length. Array performance metrics are based on Mendelian inheritance of CNVs and probe reproducibility are based on replicate data from YRU and CEU *HapMap* trios. Short single copy (scFISH) probes based on *ab initio* designs improve detection of subtle disease-causing abnormalities for genes not covered or too small to be accurately detected by recombinant BAC probes. The *ab initio* design method includes divergent repetitive elements that increase the length of scFISH probes, especially in intervals that are truncated by conventional repeat-masking. Chromosomal hybridization at high stringency produces the expected metaphase hybridization pattern. scFISH probes have been synthesized for *CDKN2A, TP53, KRAS, ERBB2* and *FLCN*, and validation for *CDKN2A* and *FLCN* probes is complete. A genome-wide set of scFISH probes has been designed, covering approximately 98% of all *ab initio* single copy intervals, and allowing potential production of scFISH probes for all unique genes (ie. 90% of known genes). These scFISH probes characterize chromosomal abnormalities in tumours at a resolution equivalent to Southern blot analysis and can be used to validate small acquired rearrangements seen by aCGH.

1277F

PLACE OF CGH-ARRAY ANALYSIS IN THE INVESTIGATION OF CONSTITUTIONAL CYTOGENETIC ABNORMALITIES: EXAMPLE OF THREE PHENOTYPICALLY RELATED CASES. *H. Hannachi[1], S. Mougou[1], M. Kammoun[1], I. Benabdallah[1], W. Fathallah[1], N. Kahloul[4], N. Gaddour[2], D. H'mida[1], H. El Ghazel[1], D. Sanlaville[3], A. Saad[1].* 1) Cytogenetics Molecular Genetic, CHU Faraht Hached, Sousse, Tunisia; 2) Psychiatric department, Fattouma Bourguiba University Teaching Hospital Monastir- Tunisia; 3) Constitutional cytogenetics department, Lyon- France; 4) Pediatric department Regional Hospital Kairouan- Tunisia.

Array comparative genomic hybridization (CGH-array) has revolutionized the cytogenetic diagnosis of patients with learning disabilities, mental retardation, dysmorphic features and multiple anomalies. We report in this work three patients with common clinical features including in particular, moderate mental retardation, severe delay in language, some autistic features and mild facial dysmorphism. Standard karyotype revealed a derived chromosome 22 in two cases, while the third one showed a normal karyotype. CGH-array investigations revealed a terminal 22q microdeletion of at least 1Mb in size, in all these patients. This loss was checked by Fluorescent In Situ Hybridization (FISH) using three telomeric probes: Totel Vysion MIX3 (tel3p, tel3q and tel22q) from Vysis, MD Digeorge « TUPLE (22q11)/22q13 (SHANK3) from Kreatech and MD Digeorge « TUPLE » (22q11)/22q13(ARSA) from Vysis. The use of FISH technique revealed that only the patients with der(22) showed actually a loss of genetic material. Considering the related phenotypic spectrum of these cases the result of this checking test in the third patient was unexpected and the suspected deletion could have been a technical artifact. In fact, telomeres have always posed a problem at the interpretation stage of results in the CGH-array technology due to their sensitivity to alteration during DNA extraction or marking stages. Our work justifies once again the importance of preliminary standard karyotyping, in spite of its low resolution, and the necessity to check by another independent technique any chromosome aberration detected by CGH-array and suspected as causative.

1278F

A microdeletion in 3p24.2 including a portion of the retinoic acid receptor beta (RARB) gene in a boy with autism spectrum disorder. *X. Li, K. Button, D. Witt.* Dept Genetics, Kaiser Permanente San Jose Medical Ctr, San Jose, CA.

Retinoid (vitamin A and its analogues) signaling is transduced by 2 families of nuclear receptors, retinoic acid receptor (RAR) and retinoid X receptor (RXR), which form RXR/RAR heterodimers. There are 3 retinoic acid receptors, alpha (RARA), beta (RARB), and gamma (RARG). Retinoid signaling is important in morphogenesis of the mammalian brain. Retinoid dysregulation has been implicated in the etiology of schizophrenia and other neuropsychiatric disorders. Here we report a microdeletion in 3p24.2 including a portion of the RARB gene in a boy with autism spectrum disorder. The patient is a 12 year male who resents with symptoms consistent with a diagnosis of Pervasive Developmental Disorder, Not Otherwise Specified (PDD-NOS). He has a history of normal developmental milestones but has always been extremely uncoordinated. He exhibits preoccupation with certain interests and objects. There has been a long history of significant delay in the development of peer relationships and reciprocal social interactions. He demonstrates little insight into typical social and peer relationships. He is high functioning intellectually (above age level in terms of verbal abilities) but his adaptive living skills are significantly delayed. Fragile X test was normal. Array comparative genomic hybridization (CGH) (Agilent/ISCA 8x60K) revealed an interstitial deletion of 967.08 Kb within band 3p24.2 at genomic coordinates chr3:4,543,416-25,510,500. The deleted region of 3p encompasses sequences between the THRB and RARB genes and a portion of the RARB including the 5'UTR and exons 1-2 (UCSC HG Build 18/ Ensembl). Parental array CGH studies showed the same deletion in the father. Father reports he has “dyslexia” and received special education and treatment for ADHD in childhood. There is likely a family history of ADHD in extended relatives. Clinical evaluation of the father has not been performed. Deletions of the RXRA gene at 9q34 have been suggested to be associated with schizophrenia. Studies have shown that retinoids are involved in controlling the function of the dopaminergic mesolimbic pathway and defects in retinoic acid signaling may contribute to autism and schizophrenia.

1279F

Design, validation and clinical routine implementation of custom a-CGH in the diagnosis of patients with psychomotor development delay and/or mild-severe mental retardation. *J. NEVADO[1,2], E. VALLESPIN[1,2], M. PALOMARES[1,2], M.A. MORI[1,2], S. GARCIA-MIÑAUR[1,2], F. SANTOS[1,2], F. GARCIA-SANTIAGO[1,2], E. MANSILLA[1,2], L. FERNANDEZ[1,2], A. DELICADO[1,2], P. LAPUNZINA[1,2].* 1) INGEMM (HULP), Madrid, Spain; 2) CIBERER, Madrid, Spain.

Background: The use of array-CGH in clinical routine is already a common practice in many countries. We report the design, validation and implementation for diagnostic of a custom genome-wide high resolution oligonucleotide aCGH-platform (KaryoArray® v3.0), for the study of patients with psychomotor development delay and/or mild to severe mental retardation. Methods: 1) For validation purposes we blindly tested 120 samples. Genomic imbalances had previously been detected in 62 of the 120 samples by karyotyping, FISH or MLPA. The remaining 50 samples were sexmatched samples from healthy individuals. 2) To implement this tool, a prospective study of 100 strictly selected patients (patients had: karyotype, molecular study of FRAXA and MLPA of subtelomere regions and recurrent-genomic-disorders done previously, with normal results) with psychomotor development delay and/or deficit cognitive were carried out. Results: Design was made selecting 60-mer oligonucleotide features from Agilent's eArray_v4.0 probe library in a custom format of 8x60K (KaryoArray®), covering more than 360 microdeleción/microduplicación syndromes, as well as telomeric/centromeric regions with a resolution from 1 kb in the regions of interest, with an average density of 7 Kb. The average density of the probe coverage is 43Kb. Interestingly within validation process, KaryoArray® reveals new genomic imbalances associated with well-defined disorders, such as trisomies and classical del/dup syndromes, in 9/62 cases. In addition, the implementation process identified at least one causal genomic imbalance responsible for its phenotype in ~30% of patients with psychomotor development delay and/or deficit cognitive. Conclusion: The implementation of KaryoArray® as the first-tier test in patients with unexplained development delay/intellectual disability and multiple congenital anomalies is cost-effective and offers a much higher diagnostic yield than karyotype and/or currently commercial platforms for this selected cohort of patients. In addition, this tool may it reveal that additional genomic imbalances could co-exist in patients with trisomies and classical del/dup syndromes, suggesting that chromosomal microarray analysis may also be also indicated in these individuals. Granted by Redes/FIBHULP08.

1280F

Clinical utility of metaphase FISH and/or chromosome studies following the identification of terminal or subcentromeric deletions identified by chromosomal microarray testing (CMA). *C.J. Packard, J.C. Hodge, N.L. Hoppman-Chaney, E.C. Thorland.* Lab Medicine and Pathology, Mayo Clinic, Rochester, MN.

CMA testing has emerged as a first-tier test for the diagnosis of patients with developmental delay/MR, autism, and multiple congenital anomalies. Current guidelines recommend confirmation of clinically relevant copy number changes (CNCs) by a second method (FISH, MLPA, QPCR, conventional chromosomes, etc.). Based on our confirmatory testing experience, primarily using FISH, our laboratory has never failed to confirm a CNC, calling into question the utility of additional testing purely for confirmatory purposes. However, CMA testing does not provide insight into the chromosomal structure or mechanism underlying an observed CNC. Therefore, additional follow-up testing by either metaphase FISH or conventional chromosome studies has clinical utility when an unbalanced translocation or inversion recombinant is suspected based on the observation of concurrent terminal deletions and duplications. Such rearrangements have significant recurrence risks if inherited from parents who are balanced rearrangement carriers. To address the utility of follow-up metaphase FISH or conventional chromosome studies in cases where terminal or subcentromeric deletions (deletions of array probes directly adjacent to the centromere) are observed, we reviewed the results of ~12,000 patients tested through clinical CMA studies. Of these ~12,000 studies, 137 apparently simple terminal deletions and 11 subcentromeric deletions were identified. Analysis of the follow-up studies performed in these cases demonstrated that 10/137 (7.3%) apparently simple terminal deletions identified by CMA testing were actually more complex chromosomal rearrangements with potentially significant recurrence risks. Of these 10 cases, 5 patients had an unbalanced translocation and 5 patients had a ring chromosome. In addition, 5/11 (45.5%) cases with a subcentromeric deletion demonstrated a more complex chromosomal rearrangement. Subcentromeric deletions of acrocentric chromosomes were more commonly associated with complex rearrangements (4/5 cases) as compared to non-acrocentric chromosomes (1/6 cases). In conclusion, the yield of additional metaphase FISH and/or conventional chromosomes studies to detect a more complex rearrangement justifies routine follow-up when CMA testing identifies terminal or subcentromeric deletions. However, confirmatory tests with methods that do not provide structural information (MLPA, QPCR, etc.) are largely unnecessary.

1281F

Identification of Genomic Copy Number Abnormalities Associated with Childhood Apraxia of Speech (CAS). *G. Raca[1], JJ. Laffin[1,2], GM. Rice[1,2,3], KJ. Jakielski[4], EA. Strand[5], LD. Shriberg[2,3].* 1) UW Cytogenetic Services, State Laboratory of Hygiene, Madison, WI; 2) University of Wisconsin-Madison, WI; 3) Waisman Center, Madison, WI; 4) Augustana College, Rock Island, IL; 5) Department of Neurology, Mayo Clinic, Rochester, MN.

Developmental speech-language disorders are a heterogeneous group of conditions that show high heritability, but very little is known about their genetic causes. We hypothesize that for a subset of patients with Childhood Apraxia of Speech (CAS) copy number abnormalities of speech-related genes could be responsible for the abnormal phenotype. Our study uses high resolution array comparative genomic hybridization (aCGH) analysis to identify novel chromosomal regions and candidate genes associated with CAS. Twenty-three phenotypically well-characterized patients with CAS were tested to date, using custom-designed high density oligonucleotide arrays (Roche NimbleGen Systems Inc., Madison, WI). These arrays provide increased coverage for the known apraxia-associated genomic regions (FOXP2, chromosomal regions 4q35.2, 16q23.2, and others), combined with a median interprobe distance of approximately 6kb for the rest of the genome. aCGH testing of the 23 patients detected one case with a 562-kb deletion of chromosome 16p11.2, with breakpoints at 29,537,669-30,099,8220 (NCBI Build 36.1; hg18). Microdeletions and microduplications of the 16p11.2 region were initially identified in patients with autism and autism related disorders, however, they were later shown to be associated with broad and variable phenotypes. Although non-specific speech and language delay has been described as a common clinical feature of the 16p11.2 deletion, we describe the first case of this deletion in a patient with a confirmed diagnosis of CAS. Evaluation by a speech pathologist may be warranted for all patients with 16p11.2 deletion, to determine if the associated speech delay meets the diagnostic criteria for CAS. Besides the 16p11.2 deletion, likely-pathogenic copy number changes were also observed at several candidate chromosomal regions, including 2p14, 2q24.1, 2q31.2, 5q35.1, 6p11.2p12.1, 12q24.23, 14q23.2 and 16p13.2. Our preliminary studies show that aCGH can detect deletions and duplications associated with CAS, and that genomic copy number abnormalities may play a role in susceptibility to this pediatric motor speech disorder.

1282F

Parallel Detection of Copy Number Variation and Loss of Heterozygosity with CGH Microarrays. *C. Shaw, J. Geoghegan, X. Zhang, E. Rorem, T. Richmond, D. Raterman, R. Selzer.* Roche NimbleGen, Inc, Madison, WI.

High resolution array CGH has been widely used to investigate DNA copy number variation (CNV) associated with complex disorders. Disease association studies have become increasingly focused on copy number variants (CNVs), and several recent reports show links between CNVs and schizophrenia, autism, and cancer, among others. However, many copy neutral events undetected by conventional array CGH, including copy number neutral loss of heterozygosity (LOH) and uniparental disomy (UPD), also contribute to disease phenotypes. Regions of the genome showing (LOH)are usually identified through the analysis of single nucleotide polymorphisms (SNPs). In normal samples, SNPs detected across the genome are randomly heterozygous or homozygous indicating that one copy of each chromosome was contributed by each parent. In contrast,only homozygous SNPs are detected in regions of LOH indicating that these regions were derived from only one parent. Until recently, cytogenetic research needing both high resolution, high quality copy number analysis and LOH detection required two separate array platforms. We use a combination microarray that detects both CNVs using standard CGH probes and LOH regions using probes that respond to common SNPs. Two probes are used to detect each SNP: one probe matches the reference genome and another probe matches a common SNP, similar to our comparative genomic sequencing platform for haploid organisms. Using our patented probe design strategy, the detection of over 700,000 common SNPs (MAF >0.3) were tested to ensure only the best responding probes were added to the array CGH/LOH designs. To accommodate the evolving field of disease association research, we also propose a method by which multiple design files with nested regions of interest and variable probe density can be used to extract only the desired data from a single array. This option allows for reanalysis of retrospective data as additional regions of interest in disease association research emerge, or as increased resolution in existing regions of interest is required. Data demonstrate high resolution and high sensitivity detection of both CNVs and LOH using a standard array CGH workflow. Samples with known regions of UPD were used to assess the performance of this new design with a resolution for detecting LOH at approximately 1 Mb.

1283F

Analysis of 24 Cell Lines of Mosaicism by The Affymetrix Genome-Wide Human SNP Array 6.0. *Z. TANG[1,2], N. Gerry[1], D. Berlin[1], A. MacMillan[1], M. Wineburg[1], B. Frederick[1], L. Toji[1], G. Toruner[2], C. Beiswanger[1].* 1) Coriell Institute for Medical Research, Camden, NJ; 2) Institute of Genomic Medicine, UMDNJ-NJ Medical School, Newark, NJ.

Conventional cytogenetic technique, such as G-banded karyotyping, analyzes chromosomal changes within individual cells. Therefore, it can identify an underlying mosaicism without burden, provided that enough cells are analyzed, especially in low-level mosaicisms. An obvious disadvantage of this technique is that it is time-consuming. Newly developed molecular cytogenetic array-based techniques, e.g. array-based comparative genomic hybridization (aCGH), utilize DNA isolated from various types of specimen to explore the copy number variations (CNVs) throughout the whole genome. The current generation of arrays has a much higher resolution and shorter turnaround time compared to conventional cytogenetic technique, but their application for detection of mosaicism has not been validated. In this study, 24 cell lines of known mosaic status at levels of 6% to 81% as previously detected by G-banded karyotyping were tested by using the Affymetrix Genome-Wide Human SNP Array 6.0 (SNP array). The microarray results were analyzed by using the Chromosomal Analysis Suite (ChAS) software. Our analysis has yielded the following observations. 1. Although this SNP array is not designed for detection of mosaicism, 10 out of 24 cell lines have shown specific patterns of Allele Difference or SmoothSignal, indicating a presence of mosaicism; 2. The SNP array detected all CNVs that had been observed by karyotyping in each cell line; 3. In 6 cell lines, clinically relevant cryptic microdeletions or microduplications were detected by the SNP array only; 4. The SNP array results provided more accurate information about the breakpoints of all CNVs; 5. The SNP array detected one or more loss of heterozygosity larger than 5 Mb in 10 cell lines; 6. The SNP array results provided useful information for further investigation of abnormal chromosomes with additional material of unknown origin. In conclusion, the SNP array is a useful tool for the characterization of CNVs in cell lines, while the application of this technique for detection of mosaicism is still a challenge.

1284F

Chromosomal heterochromatic variations involved in reproductive failure. *D. MIERLA[1], V. STOIAN[2].* 1) Life Memorial Hospital, Bucharest , Romania; 2) University Bucharest, Faculty of BiologyBucharest , Romania.

BACKGROUND Cytogenetic heteromorphisms are described as heritable variations at specific chromosomal regions without a proven impact on phenotype. The purpose of this study was to investigate the effects of chromosomal polymorphic variations involved in reproductive failure. We have studied the heterochromatic region polymorphism and pericentric inversions in infertile couple. METHODS During the period from January 2008 to April 2011, 1809 infertile patients who had received in our hospital, were investigate for this retrospective study, and the frequency of chromosomal polymorphic variations was calculated. The control group consists of 1116 fetuses investigated by amniocentesis between January 2009 and April 2011. Chromosomes from cultured peripheral blood lymphocytes and amniotic fluid were analyzed using GiemsaTrypsin-Giemsa (GTG) banding. The results of the two groups were compared. RESULTS Of 1809 infertile people were submitted for cytogenetic investigation 92.53% had normal karyotype and 7.47% showed chromosome polymorphisms, in the control group while the incidence was 94.17%(normal karyotype) and 5.82% (chromosome polymorphisms). CONCLUSIONS Chromosomal polymorphic variations appear to have no adverse effects phenotypic nor clinical, nor of any apparent association with fetal wastag. The statistically significantly higher incidence of heterochromatic variations found in infertile individuals stresses on the need to evaluate their role in infertility and subfertility.

1285F

Genome-Wide Analysis of Genomic Stability in Human Induced Pluripotent Stem Cells (iPSCs). *D.S. Berlin[1], M. Bucan[2], R. Allen[1], A. MacMillan[1], N.P. Gerry[1], Z. Tang[1], M.A. Keller[1,3], C. Tarn[1], K. Fecenko-Tacka[1].* 1) Coriell Institute for Medical Research, Camden, NJ; 2) University of Pennsylvania, Philadelphia, PA; 3) American Red Cross, Philadelphia, PA.

The NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research recently began offering human iPSCs to the biomedical research community. These iPSC lines, generated by multiple investigators worldwide, are expanded and characterized at Coriell prior to distribution through our online catalog (www.ccr.coriell.org). Recently, several publications have shown that both embryonic stem cells and human iPSCs are susceptible to genomic instability. We hypothesized that the number of chromosomal aberrations (either gross aberrations detectable by karyotyping, or copy number variations detectable by chromosomal microarray analysis) would increase with increased cell passaging. We evaluated the chromosomal integrity of two human iPSC lines submitted to the NIGMS Repository by testing the lines at three time points representing different cell passage numbers. For each subject, the fibroblast cell line from which the iPSCs were derived was used as a reference. G-banded karyotyping was performed on the parental fibroblasts and in the iPSC lines at 3 time points. Initial analysis of one iPSC line showed a normal 46,XY karotype at early passage, and mosaicism for an additional Y chromosome (47,XYY[6]/46,XY[19]) after 8 additional passages. All cells analyzed after 12 more passages had an extra chromosome Y chromosome and a derivative X chromosome that most likely resulted from a translocation between chromosomes X and 7 (47,der(X)t(X;7)(p22.1;p13),YY). Fluorescence *in situ* hybridization (FISH) is being employed to further investigate the chromosomal stability of these cells. Additionally, fibroblast- and iPSC-derived genomic DNA is being genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0 platform. The genotyping data will be analyzed using a suite of CNV-calling algorithms. We will present chromosomal microarray and karyotyping data as they pertain to assessing iPSC genomic stability in a biobank setting.

1286F

Shorter telomeres on chromosome 1 demonstrated in 11 older individuals with mild cognitive impairment and Down syndrome using linear measurements in microns. *E. Jenkins[1], L. Ye[1], S. Krinsky-McHale[2], W. Zigman[2], N. Schupf[3, 4], W. Silverman[5, 6].* 1) Dept Hum Genetics, NYS Inst Basic Res Dev Disab, Staten Island, NY; 2) Department of Psychology, NYS Inst Basic Res Dev Disab, Staten Island, NY; 3) Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University, New York, NY; 4) Departments of Epidemiology and Psychology, Columbia University, New York; 5) The Department of Behavioral Psychology, Kennedy-Krieger Institute, Baltimore, MD; 6) Department of Psychiatry and Behavioral Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

We have reported previously that people with Down syndrome(DS) and dementia or mild cognitive impairment (MCI) have shorter telomeres (chromosome ends with highly conserved TTAGGG repeats) than their healthy age- and sex-matched peers (Jenkins et al., 2006, 2008a, http://www.ashg.org/cgi-bin/2010/ashg10s), using quantitative light intensity analyses (MetaSystems) of PNA FISH telomere probes. Since our last report, we have studied an additional 11 individuals with MCI/DS who exhibited greater loss of PNA telomere probe signals than their sex- and age-matched peers (Jenkins et al., 2008b). We now have a total of 15 individuals, thereby increasing the strength of evidence that reduced telomere size and signal number are biomarkers for the detection of MCI in older individuals with DS. We have recently identified a “new” measure that allows comparison of telomere length in microns and have found shorter chromosome 1 telomere physical lengths in short-term T lymphocyte cultures from 11/11 individuals with MCI ($p < 10\text{-}6$), versus 11 sex- and age-matched cognitively healthy peers. Importantly, there were no overlaps between telomere lengths from people with MCI versus peer controls with DS using this method of measurement, suggesting that it may be particularly useful for verifying clinical status. We also found that the time needed to measure chromosome 1 telomeres in microns was comparable to, or even slightly less than the other methods that we have been using to quantify telomere length. This indicates that linear length measurement is as practical as other alternatives with the possible exception of interphase measurements that have required significantly less time to find and upload versus whole metaphases. It should be mentioned that it is theoretically possible to use either a fluorescence or bright-field microscope without expensive software to obtain both linear measurements and signal counts. In conclusion, we have now established that shorter as well as “missing” telomeres can be used as a biomarker for MCI in people with DS, and that current techniques providing direct linear measurements of telomere length can be completed as rapidly as alternative measures and may provide better differentiation among individuals varying in dementia status(MCI, dementia or their absence). This work was supported in part by NYS OPWDD, Alzh. Assoc. grants IIRG-07-60558, IIRG-99-1598, IIRG-96-077;by NIH grants P01 HD35897, R01 HD37425, R01 AG014673,and R01 AG14771.

1287F

Genomic imbalances in patients with syndromal ocular developmental anomalies. *A. Delahaye[1, 2, 3], P. Bitoun[4], S. Drunat[5], M. Gérard-Blanluet[5], N. Chassaing[6], A. Toutain[7], A. Verloes[3, 5], F. Gatelais[8], M. Legendre[9], L. Faivre[10], S. Passemard[3, 5], A. Aboura[5], S. Kaltenbach[5], S. Quentin[11], AC. Tabet[5], S. Amselem[9], J. Elion[3, 5, 12], P. Gressens[3], E. Pipiras[1, 2, 3], B. Benzacken[1, 2, 3, 5].* 1) Cytogenetics Laboratory, APHP-Jean Verdier Hospital, Bondy, France; 2) Université Paris-Nord, Paris 13, UFR SMBH, Bobigny, France ;; 3) Inserm, U676, Paris, France; 4) AP-HP-Hôpital Jean Verdier, Consultation de génétique médicale, Service de Pédiatrie, Bondy, France; 5) AP-HP, Hôpital Robert Debré, Département de Génétique ; Univ Paris Diderot, Sorbonne Paris Cité, Paris, France; 6) CHU Toulouse, Hopital Purpan, Service de Génétique Médicale ; UPS III EA4555, Toulouse, France; 7) CHU de Tours, Hôpital Bretonneau, Département de Génétique, Tours,; 8) CHU d'Angers, Service d'endocrinologie diabétologie pédiatrique, Angers, France; 9) Inserm, U933 ; Université Paris 6 ; AP-HP, Hôpital Armand-Trousseau, Paris, France; 10) CHU de Dijon, Centre de Génétique, Dijon, France; 11) AP-HP, Hôpital Saint-Louis, Plateforme génomique, Institut Universitaire d'Hématologie ; Univ Paris Diderot, Sorbonne Paris Cité, Paris, France; 12) Inserm U763, Paris, France.

Background: Chromosomal abnormalities are found in 7.7% to 10% of newborns with eye anomalies associated with other malformations. Introduction of array technologies has revealed that rearrangements below the resolution of standard or high resolution karyotyping are exceedingly common.

Objective: Sixty five patients with unexplained ocular developmental anomalies associated to another congenital malformation and/or mental retardation were analyzed using oligonucleotide array-CGH (Agilent).

Results: In 4 patients, clinically relevant deletions encompassing genes already known to be involved in eye development (*FOXC1* and *OTX2*) were identified. In 4 other patients we found pathogenic deletions not classically associated with abnormal eye morphogenesis: del(17)(p13.3p13.3), del(10)(p14p15.3) and del(16)(p11.2p11.2). We also detected two additional copy number variations of uncertain pathogenicity. Rearranged segments ranged in size from 0.11 to 5.57 Mb.

Conclusion: These results show that array-CGH provides a high diagnostic yield in patients with syndromal ocular developmental anomalies and point to novel chromosomal regions associated with these conditions. Besides their importance for diagnosis and genetic counseling, these data may pave the way to the identification of genes involved in eye development.

1288F

De Novo Intrachromosomal Xp22 Deletion and Xq28 Duplication including HCCS and MECP2 in a Female with Microphthalmia, Glaucoma, Cataracts, Cleft Palate, Seizures and Global Developmental Delay. *I.E. Amarillo[1], B.F. Crandall[2], P.N. Rao[1], F. Quintero-Rivera[1].* 1) Clinical Cytogenetics Lab, Dept of Pathology and Lab Medicine, UCLA, Los Angeles, CA; 2) UCLA Prenatal Diagnosis Center, Los Angeles, CA.

X-chromosome rearrangements are the underlying cause of many human genetic disorders. In females, clinical manifestations of a X-linked disorder are complicated by skewed inactivation of the X-chromosome. Here we report on a 16-year old female followed since birth, due to congenital bilateral microphthalmia, glaucoma, cataract and nystagmus(Peter's anomaly), height and head circumference between 5-10% centile, dysmorphic features with preauricular pits, rolled helices and prominent antihelices, short and broad neck, hypomelanosis of Ito all over the body, except on the face, complex partial seizures, pituitary insufficiency with hypothyroidism and growth hormone deficiency, ventricular tachycardia, and global developmental delay. Standard chromosome studies identified extra chromosomal material of unknown origin at Xp22 leading to a terminal deletion which extended distally, from the STS (Xp22.3) locus to the Xpter. Further characterization by chromosomal microarray analysis not only fine-mapped the deletion breakpoints (12.7 Mb; Xp22.2-p22.33; 109805-12859892 hg18; 49 RefSeq genes) but also identified the unknown material as an extra copy of Xq27.2-q28 region (13.2 Mb; 141297566-154582680 hg18;123 RefSeq genes), as confirmed by metaphase FISH. Parental karyotyping and FISH analyses are normal. X-inactivation studies are ongoing. Patients with Xq28 duplication involving MECP2 , usually present with a Rett syndrome variant phenotype with preserved speech variant , infantile hypotonia, severe mental retardation, progressive spasticity, recurrent respiratory infections, progressive cerebellar degenerative changes and seizures. Patients with Xp deletions involving the STS locus and the telomeric adjacent genes present with short stature and some degree of skeletal abnormalities and learning disability. In addition, HCCS (Xp22.2) associated with micropthalmia with linear skin defects, MLS syndrome, an X-linked dominant trait with male lethality, is also deleted in our patient. To our knowledge, this is the first report of a female patient with concomitant Xp22 microdeletion and Xq28 microduplication. The data presented here highlights not only the importance of metaphase FISH post-microarray results but also improves our understanding of the phenotype of female patients with such chromosomal rearrangements. Finally, these findings provide insights in the genetic counseling of patients with X-linked aberrations.

1289F

Rapid screening of people exposed to ionizing radiation for cytomolecular alteration de novo. *S. Moosavi[1], S. Shabani[1], M. Tondar[1], F. Mojtahedi[1], Z. Ghanbari[1], H. Dargahi[2], F. Heshmati[3].* 1) AST Cytomolecular Diaprognostic Lab, Department of Allied Health, Tehran University of Medical Science (TUMS), Tehran, Iran; 2) Department of Allied Health, Tehran University of Medical Science (TUMS), Tehran, Iran; 3) Department of Laboratory Science, Tehran University of Medical Science (TUMS), Tehran, Iran.

Energy released from ionizing radiation is a source for treat and remove tumors. It also can ironically be the most important etiologic factor in activating proto oncogenes deactivating tumor suppressor gene as well as malfunctioning DNA repair systems leading to quantitative and qualitative aberrations in cell chromosomes. Prompt chromosomal analysis of tissues exposed to radiation is a crucial factor in identifying and prognosing tumor and subsequently in effective treatment and control of tumorigenesis and metastasis. Thus, a rapid cytomolecular screening test with high sensitivity and specificity could be very fruitful to study chromosomal radiosensitivity. 38 PB samples were analyzed at G2 phase using solid-staining chromosomal banding and whole chromosomal painting FISH. Of 38 samples, 14 had 10 % or more various structural aberrations in lymphocytic chromosomes, 8 had 4-8 %, and rest had no structural aberration. We have tested samples with chromosomal breakages by whole chromosomes painting FISH for reconfirmation, but we only obtained numerical information through this technique. Nonetheless, significant relation ($p < 0.05$) existed between duration of radiation exposure and number of aberration in chromosomes. This suggests that longer duration of exposure (>2years) could have profound effect on structure of chromosomes which can be detected efficiently by solid-staining chromosomal banding and whole chromosomal painting FISH.

1290F

Phenylbutyrate therapy for pyruvate dehydrogenase deficiency. *R. Ferriero[1], E. Lamantea[2], B. Lee[3,4], M. Zeviani[2], N. Brunetti-Pierri[1,5].* 1) Telethon Institute of Genetics and Medicine, Naples, Italy; 2) Besta Neurologic Institute, Milan, Italy; 3) Dept. Molecular and Human Genetics, Baylor College of Medicine, Houston,TX; 4) Howard Hughes Medical Institute, Baylor College of Medicine, Houston,TX; 5) Dept. Pediatrics, Federico II University of Naples, Italy.

Deficiency of pyruvate dehydrogenase complex (PDHC) is the most common disorder leading to lactic acidemia. Phosphorylation of specific serine residues of the E1-alpha subunit of the PDHC by pyruvate dehydrogenase kinase (PDK) inactivates the enzyme, whereas dephosphorylation restores PDHC activity. We recently found that phenylbutyrate prevents phosphorylation of the E1-alpha subunit of the branched-chain ketoacid dehydrogenase complex (BCKDC) and reduces plasma concentrations of neurotoxic branched chain amino acids in patients with maple syrup urine disease (MSUD), due to the deficiency of BCKDC. We hypothesized that, similarly to BCKDC, phenylbutyrate enhances PDHC enzymatic activity by increasing the portion of unphosphorylated enzyme. To test this hypothesis, we treated wild-type human fibroblasts at different concentrations of phenylbutyrate and found that it reduces the levels of phosphorylated E1-alpha as compared to untreated cells. To investigate the effect of phenylbutyrate in vivo, we administered phenylbutyrate to C57B6 wild-type mice and we detected a significant increase in Pdhc enzyme activity and a reduction of phosphorylated E1-alpha subunit in brains and muscles as compared to saline treated mice. Being a drug already approved for human use, phenylbutyrate has great potential for increasing the residual enzymatic activity of PDHC and to improve the clinical phenotype of PDHC deficiency.

1291F

Novel mutations in the GCDH gene. *F. Ghasemi[1], O. Aryani[1], M. Houshmand[1,2].* 1) Special Medical Center, Tehran, Iran; 2) National Institute for Genetic Engineering & Biotechnology, Tehran, Iran.

Glutaric aciduria type I (GA-1) is an autosomal recessive disorder caused by a deficiency of glutaryl-CoA dehydrogenase(GCDH), a mitochondrial enzyme involved in the catabolism pathway of lysine, hydroxylysine and tryptophan. It results in elevation of glutaric acid and related metabolites in blood and urine . Affected patients often present with macrocephaly at birth or shortly after, dystonia, many times resembling seizures at the first episode, with acute basal ganglia injury.The GCDH gene has been mapped to chromosome 19p13. More than 150 GCDH gene mutations have been reported in populations around the world. The aim of this study was the mutational analyses of the GCDH gene in the patients with GA-1. These were carried out on five Iranian unrelated GA-1 patients by PCR and sequencing all 11 exons and the exon/intron borders of the GCDH gene. Homozygote mutations were found in all patients. Two have been reported in the literature previously as follows: c.541G > C (p.E181Q) and c.1205G>A (p.R402Q). Two patients had the first mutation. The other alterations have not been reported before. One was a T to C transition (c.536T>C) in exon 6, causing a leucine at position 179 to be changed to a proline (p.L179P) . Another was leucine substituted by phenylalanine at position 302 (p.L302F) due to a C to T transition (c.904C>T) in exon 8. Both parents of the patients having these novel changes were heterozygote . These two mutations were not found in 50 control subjects. It is thought these novel mutations disrupt the protein structure and were the cause of GA-1.

1292F

Comparison of expression profiles of children born before and after maternal bariatric biliopancreatic diversion surgery. *F. Guenard[1, 2], P. Marceau[3], K. Cianflone[4], Y. Deshaies[4], J.G. Kral[5], M.-C. Vohl[1, 2].* 1) Institute of Nutraceuticals and Functional Foods (INAF), Laval University, Quebec, PQ, Canada; 2) Department of Food Science and Nutrition, Laval University, Quebec, PQ, Canada; 3) Department of Medicine and Laval Hospital Research Center (IUCPQ), Quebec, PQ, Canada; 4) Department of Surgery, Faculty of Medicine, Laval University, Quebec, PQ, Canada; 5) Department of Surgery, State University of New York Downstate Medical Center, Brooklyn, New York, USA.

Obesity is increasing rapidly in children and adolescents worldwide. Parental obesity contributes substantially through genetic and environmental influences. Gestational obesity and diabetes mellitus predispose offspring to lifelong obesity and its comorbidities. We demonstrated that children born after maternal surgical (AMS) weight loss were less obese and exhibited improved cardiometabolic risk profiles compared to their siblings born before maternal surgery (BMS). This study correlates childrens' mRNA expression levels in peripheral blood mononuclear cells (PBMCs) and pathway regulation with their mother's pre- and postoperative status. A cohort of 25 BMS and 21 AMS children and their 20 different mothers was recruited. Fasting plasma glucose, insulin and lipid levels and other phenotypic differences between BMS and AMS children were measured. Expression analysis in PBMCs was conducted using the Illumina HumanHT-12 v4 Expression BeadChip. The FlexArray software and Lumi algorithm were used for expression data analysis and normalization. The SAM algorithm was used to assess differences between AMS children and BMS children. The Ingenuity Pathway Analysis System was used to visualize gene expression and biological pathways to identify potentially altered biological functions. Lower fasting insulin levels were found in AMS compared to BMS children ($p = 0.02$). Whole-genome mRNA expression analysis identified more than 600 transcripts differentially expressed in AMS compared to BMS children ($p \leq 0.05$; fold change: ≥ 1.2 or ≤ 0.83). Pathway analyses revealed that differentially expressed genes were associated with cell development, cell growth and proliferation, cellular function and maintenance, cell-to-cell signaling and interaction, inflammatory response, immune cell trafficking and cell-mediated immune response. Our study demonstrates an impact of maternal weight loss on insulin levels and gene expression profiles, which may partially explain the lower prevalence of obesity and improved cardiometabolic risk profile in children born after maternal weight-loss surgery.

1293F

Accuracy of diagnostic tests in Niemann-Pick type C disease. *R. Hartung, H. Runz, M. Beck, E. Mengel.* Childrens Hospital, University Medical Center Mainz, Mainz, Germany.

Background Niemann-Pick Disease Type C (NPC) is a lysosomal storage disorder caused by an intracellular transport defect. A mutation in one of the 2 genes (NPC1/NPC2) is leading to a progressive accumulation of cholesterols and glycolipids. Clinically patients are suffering from hepatosplenomegaly, supranuclear gaze palsy and neurodegeneration with an onset between 0 and >60 years. Due to the lack of one accurate diagnostic test it is suspected that a large number of un-/misdiagnosed patients is existing. Aim of this study is to evaluate the accuracy of specific tests to find an optimal diagnostic pathway. Methods We analyzed the data of 40 unrelated patients regarding Splenomegaly, Supranuclear gaze palsy, Chitotriosidase, Filipin Staining, Bone marrow biopsy and Genetic mutations in the NPC1 and NPC2 gene by direct sequenzing. Pathological values are: Splenomegaly: >2 Standard deviations Supranuclear gaze palsy: saccadic eye movement or gaze palsy Chitotriosidase: >200 mU/ml (>100 mU/ml with heterozygous mutation in the Chito1-Gene) Filipin Staining (Cholesterolaccumulation in fibroblasts): classic or variant Bone marrow biopsy: Confirmation of Foam cells Genetic mutation: 2 mutations (1 mutation undefinite, cannot be evaluated without clinical symptoms) Results Splenomegaly (35 pat): 34 (97 %) pathological, 1 (3 %) normal Supranuclear gaze palsy (28 pat): 22 (79 %) pathological, 6 (21 %) normal Chitotriosidase (38 pat): 30 (79 %) elevated, 5 (13 %) not significant elevated, 3 (8 %) unevaluable due to a homozygous mutation in the Chito1-Gene Filipin Staining (25 pat): 25 (100 %) pathological; 20 (80 %) classic, 5 (20 %) variant Bone marrow biopsy (15 pat): 14 (93 %) pathological, 1 (7 %) normal Genetic mutation (29 pat): 21 (72 %) pathological (2 mutations), 4 (14 %) undefinite (1 mutation), 4 (14 %) no mutation detected Conclusions Of the different diagnostic tests the Filipin staining showed the best accuracy. All patients had a pathological result. But it should be taken into account that a variant staining can exist in other LSDs or after longer cultivation of fibroblasts. A negative Filipin test excludes NPC. The splenomegaly is to evaluate similar. The bone marrow biopsy shows Foam cells with high accuracy, but due to the invasive nature of the test it was often performed in inconclusive cases. So a selection bias cannot be excluded. The often proposed mutation analysis could give a definite result in 72 % only.

1294F

Mutation screening of two genes involved in intracellular Vitamin B_{12} metabolism genes by high resolution melting analysis (HRMA). *M.L. Illson[1], Q. Huang[2], L. Dempsy Nunez[1], A. Brebner[3], B.M. Gilfix[4], D. Watkins[1], D.S. Rosenblatt[1], C.T. Wittwer[5].* 1) Department of Human Genetics, McGill University, Montreal, Canada; 2) Department of Biomedical Sciences, School of Life Sciences, Xiamen University, Xiamen, Fujian, China; 3) Department of Biology, McGill University, Montreal, Canada; 4) Division of Medical Biochemistry, Royal Victoria Hospital and McGill University, Montreal, Canada; 5) Department of Pathology, University of Utah Medical Center, Salt Lake City, UT USA.

Intracellular vitamin B_{12} (cobalamin) transport and metabolism require the products of at least 10 genes. Some of the inherited diseases involving the cobalamin pathway cannot be distinguished through simple biochemical assays. Time consuming and expensive testing methods such as somatic cell studies and sequencing have traditionally been used for precise diagnosis. HRMA was tested as an alternative screen to identify variants in the cobalamin pathway: *MMACHC*, responsible for the *cblC* disorder and *MUT*, responsible for classic methylmalonic aciduria. A total of 16 coding exons and flanking introns were amplified by PCR (fragments sizes 145-359 bps) and melted in the presence of a saturating dye. Screening of 96 normal DNA samples revealed 6 common polymorphisms, and confirmed that melting curves (max. 4 melting domains) were accurately predicted by uMelt^{SM} and uView^{SM}, (http://www.dna.utah.edu/). A blinded study of 197 whole genome amplified (WGA) patient samples compared HRMA results to sequencing. Melting after PCR identified all heterozygous variants. While homozygotes could be detected in exons containing multiple domains, single domain exons required mixing with known PCR products before re-melting. 241 heterozygous (114 different) and 69 homozygous (48 different) mutations were found. The sensitivity and specificity of variant detection by melting were both >99%. Errors were either clerical or resulted from allele bias secondary to WGA. Unlabeled probes and snapback primers were used to genotype common variants. Batch analysis (many samples, one exon) was easier to interpret than patient-centric analysis (fewer samples, many exons), although both were successful. Having demonstrated the efficacy of HRMA for the recessively inherited genes of the B_{12} pathway, 16 additional coding exons and flanking introns have been optimized for two genes implicated in vitamin B_{12}-responsive methylmalonic aciduria- *MMAA* and *MMAB* (fragments sizes 131-357 bps). Screening of 96 normal DNA samples confirmed melting curve predictions and revealed 5 common polymorphisms. HRMA provides a cost effective, simple and rapid screening method facilitating clinical diagnosis of overlapping phenotypes for genes in the vitamin B_{12} pathway.

1295F

Methylenetetrahydrofolate reductase and methionine synthase reductase deficiency affect behaviour and alter choline metabolism in cerebellum and hippocampus of mice. *N.M. Jadavji[1], L. Deng[1], O. Malysheva[2], M.A. Caudill[2], R. Rozen[1].* 1) Departments of Human Genetics and Paediatrics, McGill University, Montreal, Canada; 2) Division of Nutritional Sciences, Cornell University, Ithaca, NY.

Homocystinuria, an inborn error of homocysteine metabolism, can be caused by mutations in methylenetetrahydrofolate reductase (MTHFR) or methionine synthase reductase (MTRR). MTHFR generates 5-methyltetrahydrofolate, the methyl donor in the remethylation of homocysteine to methionine by methionine synthase. MTRR is involved in maintaining methionine synthase in its active state. Betaine, a metabolite of choline, serves as an alternate methyl donor for homocysteine remethylation, particularly when folate metabolism is disrupted. Since individuals with homocystinuria present with gait and motor abnormalities, mental retardation, seizures, as well as severe developmental delays, we investigated the effects of MTHFR or MTRR deficiency on behaviour and neurobiology of the cerebellum and hippocampus in adult mice. Male mice of 3 genotypes for Mthfr and Mtrr (+/+, +/-, -/-) were tested on motor, anxiety, exploratory and cognitive tasks. Global DNA methylation in cerebellum was measured using thin layer chromatography. Choline acetyltransferase (ChAT) immunoreactive protein and choline metabolites were assessed in cerebellum and hippocampus. Mthfr-/- and Mtrr-/- animals exhibited impaired gait, decreased anxiety, increased exploratory behaviour and impairments in short-term memory. Mthfr-/- and Mtrr-/- mice also had decreased global DNA methylation in cerebellum, and increased protein levels of ChAT in cerebellum and hippocampus. Choline metabolite analysis revealed decreased levels of acetylcholine and betaine in hippocampus of Mthfr-/- and Mtrr-/- animals. Mthfr-/- animals also had decreased levels of betaine in cerebellum and Mtrr-/- animals had decreased levels of choline in cerebellum and hippocampus. Our results suggest that behavioural and biochemical anomalies in these forms of homocystinuria may be due to altered methylation or compensatory disturbances in choline metabolism, and highlight the important role of MTHFR and MTRR in brain development, maturation and normal function. Supported by: Canadian Institutes of Health Research.

1296F

Spatial and temporal characterization of Single Minded 1 (*SIM1*) enhancers. *M.J. Kim[1,2], C. Vaisse[3], N. Ahituv[1,2].* 1) Department of Bioengineering and Therapeutic Sciences, UCSF, San Francisco, CA, USA; 2) Institute for Human Genetics, UCSF, San Francisco, CA, USA; 3) Diabetes Center and Department of Medicine, UCSF, San Francisco, CA, USA.

Obesity leads to an increased risk for a variety of health issues, most notably cardiovascular disease, type 2 diabetes, cancer, arthritis, sleep apnea and liver disease. The Single Minded 1 (*SIM1*) gene is involved in the development of the paraventricular and supraoptic nuclei of the hypothalamus, both regions having essential roles in regulating food intake. In addition, *SIM1* was shown to be important for the long-term regulation of energy homeostasis in adult mice. Rare coding mutations in *SIM1* have been found to be more prevalent in severely obese individuals than in lean controls. In addition, haploinsufficiency of *SIM1* in both humans and mice leads to severe obesity, suggesting that altered expression of *SIM1* by way of regulatory elements, such as enhancers, can also predispose individuals to obesity. To further explore the role of *SIM1* regulatory elements in obesity susceptibility, we have used comparative genomics and an *in vivo* transgenic enhancer assay in zebrafish to identify and characterize noncoding enhancers in the *SIM1* genomic region in a spatial and temporal manner. Of the eighteen tested sequences, three were found to have hypothalamus-specific enhancer activity. Due to the dual role of *SIM1* in hypothalamus development and in adult energy homeostasis, the activity of these three enhancers was annotated from embryonic to adult age. We observed that the tissue-specific enhancer activity of these enhancers changed over time, suggesting that the developmental and physiological expression of *SIM1* may be regulated by different enhancer elements. Combined, these results provide candidate gene regulatory sequences in which nucleotide variation in humans could lead to obesity susceptibility and demonstrate that enhancers can change their spatial activity over time.

1297F

Ethylmalonic encephalopathy: case report of a long-surviving patient with a mild course and atypical neurologic presentation. *A. Kwan[1], T.M. Cowan[1], K. Cusmano-Ozog[1], P. Rinaldo[3], V. Tiranti[2], G.E. Enns[1].* 1) Dept Pediatrics, Stanford Univ Med Sch, Stanford, CA; 2) Unit of Molecular Neurogenetics IRCCS Foundation Neurological Institute “C.Besta”, Milano, Italy; 3) Dept Laboratory Medicine and Pathology, Biochemical Genetics Laboratory, Mayo Clinic College of Medicine.

Ethylmalonic encephalopathy (EE) is a rare, recessive metabolic disorder with characteristic biochemical findings, MRI abnormalities and mutations in the ETHE1 gene at 19q13. Typical clinical features include chronic diarrhea, petechiae, orthostatic acrocyanosis and neurologic symptoms which present within the first year of life. The majority of patients have severe developmental delay with death in early childhood in over half of cases reported. We present a patient who is still alive at 27 years. She had chronic diarrhea in childhood. Acrocyanosis was noted at 5 years. Neurologic symptoms did not present until 10-12 years of age with complaints of headaches. At 16, she was noted to have decreased strength in her lower extremities, poor coordination, spasticity, brisks reflexes and a positive Babinski sign. Early gross motor milestones were normal but fine motor skills and language were delayed. IQ was 58 at the age of 7 years. She completed high school in special education. MRIs at 10 and 14 years were normal. Biochemical findings included elevated pyruvate, ethylmalonic acid, glutaric acid, methylsuccinic acid and butyrylglycine in urine organic acids. Acylcarnitine profile showed elevations of C4, C5, C8, and C10. She has a homozygous deletion of the entire ETHE1 gene. We present the oldest living patient with EE and propose that the phenotypic spectrum of EE may be wider than previously described. We recommend that the diagnosis be considered, and mutation analysis performed, in any patient with chronic diarrhea, acrocyanosis, developmental delay and later onset neurologic symptoms, especially if characteristic biochemical findings are present.

1298F

Infantile sialic acid storage disease: Two unrelated Inuit cases homozygous for a common novel *SLC17A5* mutation. *MA. Lines[1], T. Rupar[2], B. Baskin[3], P. Ray[3], D. Grynspan[1], J. Michaud[1], MT. Geraghty[1].* 1) Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada; 2) Child and Parent Resource Institute, London, Canada; 3) The Hospital for Sick Children, Toronto, Canada.

Infantile Sialic Acid Storage Disease (ISSD) is a clinically severe lysosomal storage disease characterised by accumulation of free sialic acid in multiple tissues. ISSD is allelic with Salla disease, a nonlethal, primarily neurological disorder; the gene for both conditions, *SLC17A5*, encodes an anionic monosaccharide transporter. Salla disease is common in Finland due to the presence of a specific Finnish founder mutation. Other mutations of *SLC17A5* have been described in ISSD patients from a variety of ancestral populations. Here, we describe particulars of two cases of ISSD detected in the Inuit. Both patients presented with nonimmune fetal hydrops and proceeded to follow a severe, rapidly fatal perinatal course despite maximal supportive treatment. Autopsy confirmed a picture of widespread lysosomal storage, with deposition of material negative for Oil-Red-O and Periodic Acid-Schiff stains in multiple tissues from both children. Enzymatic studies showed a marked (tenfold), unexplained, increase in sialidase activity, with lesser elevations in the activities of several other lysosomal enzymes. *SLC17A5* sequencing showed both children, whose parents were unrelated and non-consanguineous, to be homozygous for a novel mutation (c.526-2A>G) situated within the splice acceptor site of exon 4. RT-PCR studies showed skipping of exon 4, predicted to result in a loss of reading frame. As far as we are aware, these constitute the first reported cases of ISSD amongst the Inuit, raising the question of a common founder mutation in this population. Furthermore, if confirmed in further ISSD cases, the marked induction of sialidase activity seen here may prove useful in the clinical diagnosis of this condition.

1299F

Atypical presentations - Fabry disease in a 12 year old female and 55 year old male. *J. MacKenzie[1,4], I. Young[2,4], A. Jin[3,4], J. Garland[3,4], M.T. Geraghty[1,5].* 1) Dept Pediatrics, Kingston General Hosp, Kingston, ON; 2) Dept Pathology, Kingston General Hosp, Kingston, ON; 3) Dept Medicine, Kingston General Hosp, Kingston, ON; 4) Queens University, Kingston, ON; 5) Dept Pediatrics, CHEO, University of Ottawa, ON, Canada.

Fabry disease, due to the deficiency of alpha galactosidase A, results in the accumulation of globotriaosylceramide throughout the body. The clinical presentation includes onset of acroparesthesias, hypohydrosis, angiokeratomas, proteinuria, and abdominal symptoms in childhood or adolescence which progresses to accelerated kidney function decline, cardiac decompensation and neurologic involvement by the third to fifth decade in males. Due to variable X inactivation, females typically have milder disease although some can present with features indistinguishable from males. The average delay in diagnosis is at least 10 years from presentation in spite of patients accessing numerous physicians. First, we report a 12 year old female referred for corneal verticillata found at a routine optometry exam. Although healthy, her activities had been limited by pain in her extremities characterized as intense and burning, exacerbated by heat, cold and intercurrent illness. On examination, angiokeratomas were noted on the right leg and buttock in a linear distribution attributed to trauma 2 years previously. Her mother was asymptomatic except for corneal findings similar to her daughter. Alpha galactosidase A activity was 2 per cent in both and a c.680G>A (p.Arg227Gln) mutation was identified in GLA. Monitoring of renal and cardiac function and MRI have been normal in both over 2 years without enzyme replacement. Second we describe, a 55 yr old male presented to an inherited arrhythmia clinic with left sided hypertrophic cardiomyopathy. He had a brother with the same finding. A cardiomyopathy gene panel revealed a c.729G>C (p.Leu243Phe) change in GLA and 1 per cent residual enzyme activity in the patient. At evaluation, he was on hemodialysis for 3 years, had unexplained acroparasthesias in his hands and feet since 13 years of age, hypohydrosis, and episodes of expressive aphasia. A renal biopsy demonstrated ultrastructural findings diagnostic of Fabry disease. These two cases highlight diverse presentations of Fabry disease and the delay in diagnosis often seen with genetic conditions. This is increasingly relevant with the development of specific therapy. This challenges educators to adopt learning strategies that focus on problem solving and the development of metacognitive learning skills as opposed to recall of distinct rare disorders. By reducing the interval from presentation to intervention, we can reach our ultimate goal of improving health outcomes.

1300F

Transdifferentiation of Patient Fibroblasts for the Study of Tissue Specific Mitochondrial Respiratory Chain Disorders. *M. Menezes[1,2], L. Riley[1], S. Cooper[2,3], D. Thorburn[4], J. Christodoulou[1,2].* 1) Genetic Metabolic Disorders Research Unit, Western Sydney Genetics Program, The Children's Hospital at Westmead , Sydney, NSW, Australia; 2) Discipline of Paediatrics & Child Health, Faculty of Medicine, University of Sydney, Sydney, NSW, Australia; 3) Neuromuscular Research Institute, The Children's Hospital at Westmead, Sydney, NSW, Australia; 4) Mitochondrial Disorders Research Unit, Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, Victoria, Australia.

Mitochondrial respiratory chain (RC) disorders are among the most common inborn errors of metabolism with an estimated incidence of 1 in 5000 births. Diagnosis usually relies on detection of altered activities of one or more of the RC complexes. However tissue specific expression of enzymatic defects, where RC enzyme deficiencies can be detected in muscle or liver but do not manifest in fibroblasts, complicates diagnosis. For patients with muscle specific RC defects, transdifferentiation of patient fibroblasts into muscle cells using a MyoD lentiviral vector could improve the accuracy and usefulness of genetic counselling, and potentially facilitate prenatal testing. Previously we identified a novel pathogenic mutation (c.156C>G; p.F52L) in YARS2, in a family with two children affected by myopathy, lactic acidosis and sideroblastic anemia (MLASA). YARS2 encodes mitochondrial tyrosyl tRNA-synthetase. The YARS2 defect, which leads to decreased mitochondrial protein synthesis and RC dysfunction, was a classic example of tissue-specific manifestation of mitochondrial RC dysfunction. We investigated the applicability of MyoD-mediated myogenesis of cultured fibroblasts from two YARS2 mutant RC patients and age-matched controls with normal fibroblast RC enzyme function. Enzymatic analysis of MyoD-derived myotubes revealed a significant reduction in patient complex IV enzyme activity, compared with the non-diseased controls, whereas results in patient fibroblasts were equivocal. Moreover, analysis of the five RC complexes by Western blot demonstrated a distinct reduction in the protein levels of complexes I and IV in patient myotubes compared with the non-diseased samples. We subsequently screened a cohort of patients with a similar phenotype, which led to identification of another three patients with different YARS2 variations. For the additional YARS2 variations, recombinant proteins have been produced and dimerization and aminoacylation activity of YARS2 is being investigated. RC enzyme assays will be performed on MyoD derived myotubes from these additional patients to study whether unmasking can be achieved in other patients. Our study will establish whether in vitro myogenesis of fibroblasts has broad application for diagnosis of mitochondrial RC dysfunction, and provides crucial insight into the basis for tissue-specific manifestation of mitochondrial RC disorders.

1301F

A new patient with the cblD cobalamin disorder presenting with high homocysteine. *J.A.J Raiman[1], I.R Miousse[2], D. Watkins[2], D.S Rosenblatt[2,3].* 1) Division of Clinical & Metabolic Genetics, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Human Genetics, McGill University, Montreal, Canada; 3) Division of Medical Genetics, McGill University Health Center, Montreal, Canada.

Derivatives of cobalamin (vitamin B12) are essential cofactors for the remethylation of homocysteine to methionine, catalyzed by methionine synthase, and the conversion of methylmalonyl-CoA to succinyl-CoA, catalyzed by methylmalonyl-CoA mutase. A number of inborn errors have been identified that impair conversion of exogenous cobalamin to its metabolically active derivatives, methylcobalamin and adenosylcobalamin. The patient was a 5-year-old girl of East Indian origin with a history of global developmental delay and autistic features. Her parents are first cousins. She initially came to medical attention at 18 months with speech and language delay, and was found to have elevated serum homocysteine and decreased methionine. At five years there was evidence of delay in gross and fine motor skills, autistic features and mental retardation. Serum homocysteine levels were elevated (48mmol/l), methionine 30 mmol/l with normal methylmalonic acid. MRI investigation indicated atrophy of frontal lobes and corpus callosum. Treatment with hydroxocobalamin IM resulted in normalization of biochemical parameters (a reduction of homocystine to 18mmol/l) but was discontinued by the parents after 3 months due to administration difficulties. She was subsequently treated with betaine, resulting in variable homocystine levels between 30-40 mmol/l. Studies of cultured patient fibroblasts showed decreased incorporation of label from methyltetrahydrofolate into cellular macromolecules, indicating decreased function of methionine synthase. Function of methylmalonyl-CoA mutase was normal. Synthesis of methylcobalamin from exogenous cyanocobalamin was decreased, with normal adenosylcobalamin synthesis. Somatic cell complementation identified the disorder in the patient as cblD variant 1. Sequencing of the MMADHC gene resulted in identification of a homozygous missense mutation, c.746A>G (p.Tyr249Cys). This mutation has been reported previously in the heterozygous state in another patient with cblD variant 1. This patient represents only the fourth case of cblD variant 1 to be reported.

1302F

Response to creatine supplementation in 2 boys with X-linked creatine transporter deficiency with residual creatine peak on MR spectroscopy (MRS). *D. Renaud[1], A. Boetticher[2], E. Wirrell[1].* 1) Div Child/Adolescent Neurology, Mayo Clinic, Rochester, MN; 2) Noran Neurological Clinic, Minneapolis, MN.

Background: X-linked creatine transporter (SLC6A8) deficiency presents with developmental delay, seizures and behavioral disturbance in boys due to cerebral creatine deficiency. Oral creatine supplementation generally has not resulted in an improvement in neurological symptoms.

Case reports: The first boy presented at age 12 years with autism, developmental delay and seizures. A hemizygous c.1222_1224del TTC (p.408del) mutation was detected. The second boy presented at age 3 with mild global developmental delay with significant speech impairment. A novel hemizygous c.238 T>C (p.Y80H) variant was found in the SLC6A8 gene. MRS for both boys revealed a significantly decreased but not absent creatine peak. In view of the small residual creatine peak on MRS, treatment with oral creatine monohydrate supplementation was initiated.

Results: Both boys demonstrated an improvement in developmental abilities, particularly speech, on creatine supplementation. In addition, the first boy became seizure-free and had a resolution of abnormal involuntary movements. Despite a clinical response to creatine supplementation in both boys, the size of the MRS peak remained unchanged on serial MRS studies.

Conclusion: Boys with X-linked creatine transporter deficiency with a residual creatine peak on MRS may benefit from supplementation with oral creatine monohydrate.

1303F

Hereditary renal hypouricemia caused by deletion in human urate transporter 1. *I. Sebesta[1,2], B. Stiburkova[2], K. Ichida[3], M. Hosoyadama[4].* 1) Institute of Clinical Biochemistry;; 2) Institute of Inherited Metabolic Disorders,Charles University,First Faculty of Medicine,Prague,Czech Republic; 3) Department of Pathophysiology,Tokyo University of Pharmacy and Life Sciences,Tokyo,Japan; 4) Division of Pharmacotherapeutics, Faculty of Pharmacy,Keio University, Tokyo,Japan.

Introduction: Hereditary renal hypouricemia is an inborn error of renal transport of uric acid. Two types were recently distinguished: a) renal hypouricemia type 1 (OMIM #220150), caused by the defects in the SLC22A12 gene which encodes the human urate transporter 1 (hURAT1) and b) renal hypouricemia type 2 (OMIM #612076), caused by the defects in the SLC2A9 gene, coding GLUT9 transporter. This disorder is characterized by two biochemical markers: hypouricemia (less than 119 μmo/l) and increased fractional excretion of uric acid (more than 10%). Most patients are asymptomatic, but some may form urolithiasis and /or be predisposed to exercise-induced acute renal failure. To date, the cases with mutations in hURAT1 have been reported in East Asia only. Over one hundred cases were identified in Japan and several patients in Korea. Methods: Uric acid was quantified by enzymic method. The analysis of hURAT1 was performed as previously described (Ichida K. et al. J.Am.Soc. Nephrol. 2004;15:164-73). 5-year-old boy with unexplained hypouricemia and urolithiasis was referred to our department for the detailed purine metabolic investigation. At the age of two months ultrasound investigation revealed renal calculi of 5-6 millimeters in size on the right side. Very small calculi was found also in the lower part of the right kidney. Results: Using our diagnostic approach to unexplained hypouricemia we have found repeated hypouricemia (53-108 umol/l) and increased fractional excretion of uric acid (24.5-84.2 %) in this Czech patient. The value of hypoxanhine and xanhine were within normal limits, thus the possible genetic defect of xanthinoxidase was excluded. No other secondary causes of hyperuricosuric hypouricemia such as Wilson diseases, Fanconi syndrome or drug-induced tubulopathy were found. Subsequent mutational analysis revealed novel heterozygous 9bp deletion (1242-1250delGCTGGCAGG) in hURAT1 transporter. Conclusions: First Czech patient with defect in hURAT1 was detected. Our finding shows that this disorder is present also in Caucasian population. Patients with unexplained hypouricemia need detailed purine metabolic investigations. (supported by grant VZMSM0021620806, Czech Republic).

1304F

Effects of PPARG, APOE, ACE, LPL, IL-6 and AT1R gene variants on development of metabolic syndrome. *J. Sertic[1,2], J. Lovric[2], T. Bozina[2], N. Bozina[1], B. Jelakovic[3], Z. Reiner[4].* 1) Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Croatia; 2) Department of Medical Chemistry, Biochemistry and Clinical Chemistry, School of Medicine, University of Zagreb, Croatia; 3) Department of Internal Medicine, Division of Nephrology and Arterial Hypertension; University Hospital Centre Zagreb, Croatia; 4) Department of Internal Medicine, Division of Metabolic Diseases; University Hospital Centre, Zagreb, Croatia.

Background: Metabolic syndrome (MS) is a cluster of risk factors including hypertension, abdominal obesity, dyslipidemia and hyperglycemia. The contribution of genetic factors to the development of MS has been widely recognized, but the contribution of genes has not yet been fully clarified. We investigated the possible role of gene polymorphisms of PPARG (Pro12Ala), ApoE (, 2, , 3, , 4), LPL (P+/-), IL-6 (-174G>C), ACE (I/D) and AT1R (1166A>C) in MS. Methods: Genotyping of PPARG, LPL, IL-6, ACE and AT1R was performed by PCR-RFLP and APOE by real-time PCR in a group of 263 patients and 176 controls. Associations of alleles and genotypes with biological and clinical variables were performed using independent t-tests or $\chi 2$ where appropriate and UNPHASED-3.0.10. Results: In comparison to females, males had higher BMI and waist circumference, higher triglycerides and glucose levels and lower HDL. Males had significantly more often high blood pressure. Age accounted for the differences in glucose levels and HDL. In female group associations were found for: LPL and ACE with MS (p=0.04); PPARG and LPL with blood pressure, (p=0.04); LPL with cholesterol and LDL (p=0.01 and p=0.05 respectively). Significant gene interactions observed between: APOE and PPARG, ACE and APOE were associated with BMI (p=0.01 and p=0.05 respectively) ; LPL and PPRG were associated with triglycerides (p=0.03). For males we found associations of: LPL variants with MS (p=0.02), BMI (p=0.002) and waist circumference (p=0.008); PPARG and APOE with BMI (p=0.05); IL-6 with CRP (p=0.02). Significant gene-gene interactions observed between: PPARG and AT1R were associated with blood pressure (p=0.05); PPARG and APOE with triglycerides (p=0.02); PPARG and APOE, PPARG and IL6 (p=0.03), ACE and APOE (p=0.0002) with cholesterol PPARG and LPL (p=0.003) PPARG and IL6 (p=0.06) with HDL; PPARG and IL6 (p=0.01), ACE and APOE (p=0.04), AT1R and ACE (p=0.04) with LDL; PPARG and APOE, LPL and ACE (p=0.01), AT1R and ACE (p=0.06) with CRP. Conclusion: Gene variants of PPARG, APOE, LPL, ACE, AT1R and IL-6 could be susceptibility factors of obesity, lipid status, and glucose intolerance contributing to the development of MS.

1305F

LONG CHAIN ACYL-CoA DEHYDROGENASE DEFICIENCY: A NEW INBORN ERROR of METABOLISM MANIFESTING as CONGENITAL SURFACTANT DEFICIENCY. *J. Vockley[1], K. Suhrie[1], A. Karunanidhi[1], W. Mohsen[1], M. Reyes-Mugica[2].* 1) Pediatrics, Children's Hosp of Pittsburgh of UPMC, Pittsburgh, PA; 2) Department of Pathology Chidren's Hospital of Pittsburgh of UPMC 4401 Penn Ave., Floor B Pittsburgh, PA 15224.

BACKGROUND: The physiologic role of long chain acyl-CoA dehydrogenase (LCAD) has long remained elusive. LCAD is expressed in type II pneumocytes, leading us to hypothesize that a patient with LCAD deficiency would present with congenital surfactant deficiency. OBJECTIVE: Identify a case of LCAD deficiency in patients with a phenotype of congenital surfactant deficiency. DESIGN/METHODS: Term infants with unexplained respiratory distress were evaluated for known causes of congenital surfactant deficiency and confirmed to have a normal genotype. Lung tissue from patient and controls was evaluated using routine light microscopy for histologic evaluation, electron microscopy for lamellar body structure, and detection of LCAD antigen expression by immunostaining and confocal microscopy. Genetic studies were performed. RESULTS: One patient was identified. EM of lung tissue demonstrated eccentric deposits within the lamellar bodies. LCAD antigen was absent in patient lung tissue. Genomic DNA sequencing of patient DNA revealed a homozygous base pair change involving intron 6, causing a splicing error. At six months of age, a liver biopsy demonstrated accumulation of cis-3,4-methylene-heptanoylcarnitine, a likely derivative from an LCAD substrate. CONCLUSIONS: This is the first report of LCAD deficiency. The patient presented with a unique phenotype of congenital surfactant deficiency suggesting that LCAD has a very unique synthetic function rather than a catabolic function as predicted by the other members of the acyl dehydrogenase family of enzymes.

1306F

Multicopy Suppressors Screening in *Saccharomyces cereviseae* Lacking Aspartate-glutamate Carriers. *P. Wongkittichote[1], LT. Jensen[2], S. Tungpradapkul[2], D. Wattanasirichaigoon[3].* 1) Graduate Program in Molecular Medicine, Faculty of Science, Mahidol University, Bangkok, Thailand; 2) Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok, Thailand; 3) Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

Citrin deficiency is an autosomal recessive disorder caused by mutations in *SLC25A13*, which encodes citrin, a member of mitochondrial carrier family. Together with its homologue, aralar, they act as aspartate-glutamate carriers, functioning in the export aspartate from the mitochondrial matrix to the cytosol. Citrin deficiency leads to two phenotypes: neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD, OMIM#605814) and adult-onset type II citrullinemia (CTLN2, OMIM#603471). Currently the only effective treatment of citrin deficiency is liver transplantation. However the limited number of donors as well as the cost and complications of liver transplantation restricts its use as a therapy for citrin deficiency. In order to gain more insight into the pathogenesis of citrin deficiency and to identify new therapeutic targets, we employed the baker's yeast (*Saccharomyces cereviseae*) as a model system to examine defects associated with citrin deficiency. *S. cerevisiae AGC1* is a functional homologue of citrin and yeast lacking *AGC1* (*agc1*Δ) are unable to utilize acetate as a carbon source and the growth of *agc1*Δ yeast on acetate medium can be restored by the introduction of human citrin. In this study we searched for genes that when over-expressed could bypass the growth defect associated with loss of *AGC1*. This multicopy suppressor screen selected for *agc1*Δ transformants with the restored ability to utilize acetate. From approximately 10,000 transformants we identified 24 colonies that were able to utilize acetate as a carbon source, indicating that the genes present on the multi-copy plasmids may be able to compensate for loss of *AGC1*. We are currently working to identify the genes present on these multi-copy suppressors of *agc1*Δ cells. Investigation of how increased expression of the suppressor genes is able to bypass the need for *AGC1* may provide insight into metabolic pathways affected by citrin deficiency in human cells and could possible lead to new avenues of research toward the treatment of citrin deficiency.

1307F

Carnitine Palmitoyltransferase II (CPT II) Deficiency - Clinical phenotypes, Mutations and Polymorphisms. *B.Z. Yang[1], S.A. Greene[1], N. McNeill[1], J.M. Pascual[2], J.H. Ding[1].* 1) Inst Metabolic Disease, Baylor Res Inst, Dallas, TX; 2) UT Southwestern Medical Center.

Carnitine palmitoyltransferase II (CPT II) deficiency, one of the most commonly inherited disorders of fatty acid oxidation defect, is clinically heterogeneous and has three different forms: the neonatal, infantile and adult form. In order to investigate the molecular basis of these three phenotypes, patients with CPT II deficiency have been studied and 82 patients were discovered to have mutation(s) and/or polymorphisms through genetic testing. Genomic DNA was isolated from whole blood or cultured skin fibroblasts. All exons and flanking splice junction of the CPT II gene were amplified from patient`s genomic DNA by using specific intronic primers. The PCR products were purified and bi-directional sequencing of PCR products was performed. Carnitine palmitoyltransferase activities were assayed as palmitoyl L-[methyl- ^{14}C] carnitine formed from L-[methyl- ^{14}C] carnitine and palmitoyl-CoA by using lymphocytes or fibroblasts. A total of thirty different mutations in the CPT II gene have been identified in 82 patients who harbored one or more mutations and/or polymorphisms. Among them, the previously reported common mutation, S113L, was only found in 43 out of 164 of the variant alleles. Three (V245P, R350C, and E645fs) of the 30 mutations were confined only to individual families, whereas the remaining 27 mutations were identified in multiple families. Interestingly, heterozygotes are commonly considered CPT II mutation carriers are expected to remain asymptomatic. However, heterozygotes harboring an additional amino acid-changing polymorphism (in compound heterozygosity) were at risk of developing clinical manifestations when exposed to precipitating factors such as infectious illness, fasting, and/or prolonged exercise. These compound heterozygotes exhibited lower CPT II activity (<40% residual activity) than heterozygotes (>55% residual activity). Our data indicate that CPT II deficiency phenotype correlates closely with residual CPT II activity and that compound heterozygosity represents a disease variant associated with latent onset that manifests upon conditions of increased muscle catabolism.

1308F

Acute Intermittent Porphyria: A Severely Affected Knock-in Mouse that Mimics the Human Homozygous Dominant Phenotype. *M. Yasuda[1], C. Yu[1], J. Zhang[1], S. Clavero[1], W. Edelmann[2], L. Gan[1], J.D. Phillips[3], R.J. Desnick[1].* 1) Gen & Genomic Sci, Mount Sinai Sch Med, New York, NY; 2) Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY; 3) Hematology, Internal Medicine, University of Utah, Salt Lake City, UT.

Acute Intermittent Porphyria (AIP), the most common acute hepatic porphyria, is an autosomal dominant inborn error of heme biosynthesis due to half-normal hydroxymethylbilane synthase (HMBS) activity. In heterozygous patients, life-threatening acute neurological attacks are triggered by porphyrinogenic drugs, fasting, and hormonal changes that induce heme biosynthesis. In contrast, children with homozygous dominant AIP (HD-AIP) have a severe, early-onset neurologic phenotype, including ataxia and psychomotor retardation. To investigate the nature of their constitutive, severe manifestations, efforts were directed to generate knock-in mice that mimic human HD-AIP. Two mouse lines were generated carrying the HMBS missense mutations R167Q or R173Q, which were identified in human HD-AIP patients. These mutations expressed ~7.5% and ~1.5% of wild-type HMBS activity *in vitro*, respectively. Homozygous R173Q mice were embryonic lethals, while ~65% of the homozygous R167Q mice survived to adulthood and had a normal life span. They had ~5% mean hepatic and erythrocytic HMBS activities, early-onset ataxia, and abnormal motor coordination and locomotion, including impaired rotarod performance, dystonic postures, and action tremors. The mice had constitutively elevated plasma and urinary +- aminolevulinic acid (ALA) and porphobilinogen (PBG) levels (increased ~5 to 7 fold and ~70 to 100 fold, respectively) that were further increased by stimuli that induce heme biosynthesis, including pregnenolone-l6(-carbonitrile and fasting. Liver and brain heme content were ~85% and 50-75% of normal, respectively. Despite their marked neurological phenotype, brain histology was grossly normal. ALA and PBG were markedly elevated in their cerebral spinal fluid (CSF) and central nervous system (CNS) tissues. Of note, the `inducible' AIP mouse model previously generated by Lindberg et al. (Nat Genet 12:195, 1996) did not accumulate porphyrin precursors in their CSF or CNS tissues following phenobarbital induction, despite markedly elevated liver and plasma ALA and PBG levels. These studies suggest that: 1) ALA and PBG do not readily cross the blood brain barrier (BBB) or blood CSF barrier (BCSFB) and 2) the severe neurological phenotype of the HD-AIP children results from the endogenous overproduction of porphyrin precursors in their neuronal tissues and/or neuronal heme deficiency.

1309F

Formation of the multienzyme complex of the de-novo purine synthesis in adenylosuccinate lyase deficiency and AICA-ribosiduria. *M. Zikanova, V. Baresova, V. Skopova, S. Kmoch.* Institute of Inherited Metabolic Disorders, Charles University, First Faculty of Medicine, Prague, Czech Republic.

De-novo purine synthesis (DNPS) requires 10 enzymatic steps to generate inosine monophosphate (IMP), and is catalyzed by 6 enzymes, 3 of which are multifunctional in eukaryotes. Dysfunction of DNPS is represented by genetic defects of individual DNPS enzymes. Two inherited metabolic diseases, adenylosuccinate lyase (ADSL) deficiency and AICA-ribosiduria (aminoimidazolcarboxamide riboside-transformylase/IMP cyclohydrolase (ATIC) deficiency), both leading to severe neurological involvement, are known. In presented study we tested the formation of the multienzyme complex of DNPS - purinosome - in skin fibroblasts obtained from patients with severe and moderate form of ADSL deficiency and with AICA-ribosiduria. We performed immunofluorescence labeling and subsequent analysis of confocal microscopy data of DNPS proteins produced by cells cultured in purine depleted conditions. Firstly the combination of enzymes from the beginning (PPAT) and the end (ATIC) of DNPS was tested, and almost no formation of purinosome was observed in the defect cells. When compared to control fibroblasts, PPAT protein formed clusters but only in reduced rate and ATIC protein remained diffuse in the patient fibroblasts. Secondly we were interested in changes of purinosome formation by the ADSL and ATIC proteins and we observed that the nature of both enzymes remain diffuse in patient cells, suggesting no purinosome formation. Futhermore, when compared to the control fibroblasts, the amount of the produced enzymes was reduced especially in AICA-ribosiduria and the severe form of ADSL deficiency. Finally PPAT and ADSL proteins were colocalized and we observed strong reduction of purinosome formation in patient with severe defect of ADSL, while the reduction was only slight in AICA-ribosiduria and in moderate form of ADSL deficiency. The control fibroblasts formed the complex very strongly in all tested enzyme combinations. We observed correlation between purinosome formation and severity of the disease. It suggest that genetic defects of DNPS affect not only activity and structure of the individual enzyme, but, in the dependency of the relevance of the disease, also the functionality of the DNPS pathway by purinosome complex destabilization. Pathogenic mechanisms leading to neurological involvement are unknown in both diseases, but it is clear that intervention into purinosome formation may represent a new pharmacological target for therapeutic intervention.

1310F

Cell selection in mosaic cell lines from females with Menkes syndrome. *L.B. Møller[1], M. Lenartowicz[2], T.G. Jensen[3].* 1) Department of Applied Human Genetics, Kennedy Center, Gl. Landevej 7, 2600 Glostrup, Denmark; 2) Department of Genetics and Evolution, Institute of Zoology, Jagiellonian University, Ingardena 6, 30-060 Krakow, Poland; 3) Department of Human Genetics, University of Aarhus, Denmark.

Background: Menkes Disease (MD) is a rare X-linked recessive fatal neurodegenerative disorder caused by mutations in the ATP7A gene, and most patients are males. Female carriers are mosaics of wild type and mutant cells due to the random X inactivation, and they are rarely affected. In most of the asymptomatic carriers, the mutant X-chromosomes has been found to be preferential silencing, but preferential silencing of the normal X-chromosome has been observed in affected females. Purpose: The purpose of this study is to test the possibility that the copper status during the foetal development might affect the resultant X-inactivation pattern in the female carrier. Methods: Cell selection was analyzed by exposing mosaic cell cultures obtained from female carriers, to different copper concentrations. The percentage activity of the two different X-chromosomes; the chromosome containing the ATP7A mutation versus the chromosome with normal ATP7A sequence was obtained by investigation of the X-inactivation pattern using the human androgen-receptor gene methylation assay (HUMAR). DNA from an affected male from the same family was included to reveal the allelic AR variant on the mutant X-chromosome. Results: For the first time we demonstrate that cells that express the normal ATP7A gene have a selective growth advantage in media with high copper concentrations, whereas cells that express the mutant ATP7A gene have a selective growth advantage in media with no copper. Conclusions: Giving pregnant heterozygous females supplemental copper in order to favour skewed X-inactivation of the affected X-chromosome in the heterozygous daughters, should be considered.

1311F

Involvement of islet expressed micro RNA regulation in T2D susceptibility risk. *M. van de Bunt[1], K.J. Gaulton[2], I. Moran[3], P.R.V. Johnson[1,4], A.L. Gloyn[1], J. Ferrer[3], M.I. McCarthy[1,2].* 1) Oxford Centre for Diabetes Endocrinology & Metabolism, University of Oxford, Oxford, United Kingdom; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; 3) Genomic Programming of Beta Cells Laboratory, Institut d'Investigacions Biomèdiques August Pi I Sunyer, Barcelona, Spain; 4) Nuffield Department of Surgery, University of Oxford, Oxford, UK.

Genome-wide association (GWA) studies have identified multiple common variants associated with type 2 diabetes (T2D). Most of these T2D-associated loci map to non-coding regions, and understanding the functional mechanisms through which they influence disease risk has proved challenging. In this study, we aimed to establish whether the transcriptional targets of miRNAs highly-expressed in human pancreatic islets, a critical tissue in T2D pathogenesis, play a role in T2D susceptibility. To establish the miRNA expression profile in human pancreatic islets, we sequenced an islet preparation (purity > 90%) on an Illumina GAII platform. In 17.7 million raw reads, we identified 396 mature miRNAs expressed in islets. Two highly abundant transcripts (mir-143, mir-375) predominated within the islet expression profile, representing 40% of all miRNA aligned reads. It has previously been shown that expression of mir-375 affects glucose-stimulated insulin secretion, whilst mir-30d, also amongst the top 10, has been involved in the regulation of insulin gene expression. To determine if the mRNA targets of the most highly expressed islet miRNAs showed enrichment in T2D association signals, we defined a set of islet miRNA target genes (n=2237) based on prediction using TargetScan for the 29 miRNAs representing 90% of the miRNA reads. We used MAGENTA to determine whether this set of islet miRNA target genes was enriched for variants with significant T2D association in the DIAGRAM GWA meta-analysis (8130 cases / 38,987controls). When compared against 2572 pathway-related gene sets (from GO, KEGG, Ingenuity and Panther), the islet miRNA target set emerged, following false discovery rate correction, as the most significantly enriched set (P= 0.028 for the 5% most significant gene scores). This result was unchanged (p=0.026) when the islet miRNA target gene set (n=1783) was restricted to genes with mRNA-seq expression in human islets. Equivalent analyses using miRNA expression lists from 3 other tissues resulted in no enrichment for T2D GWA signals, providing further evidence that the enrichment has a biological basis. In conclusion, we have established the miRNA profile of human islets and and implicated islet miRNA-regulated transcripts in Type 2 diabetes predisposition.

1312F

A novel mutation in the *MT-ATP8* gene associated with cerebellar atrophy, retinitis pigmentosa, polineuropathy, and hearing loss. *A. Blazquez[1,2], M. Moran[1,2], A. Moreno[1,3], A. Delmiro[1,2], L. Rufian[1,2], H. Rivera[1,2], J. Arenas[1,2], M.A. Martin[1,2].* 1) Mitochondrial diseases laboratory, `12 de Octubre' Hospital. Biomedical Research Institute (i+12), Madrid, Spain; 2) Spanish Biomedical Research Center for Rare Diseases (CIBERER); 3) Neurology Dept, `Morales Messeguer' Hospital, Murcia, Spain.

Background: Mitochondrial respiratory chain (MRC) disorders are caused by mutations in mitochondrial DNA (mtDNA) or nuclear DNA genes. Most of mtDNA disease-causing mutations occur in heteroplasmy. Two structural proteins of ATP synthase (MRC complex V) are encoded by mtDNA (*MT-ATP6* and *MT-ATP8* genes). Common mutations in the *MT-ATP6* gene, particularly, m.8993T>G are associated with MILS (Maternal Inherited Leigh Syndrome) or NARP (Neuropathy, Ataxia, Retinitis Pigmentosa) depending on mutant load. Conversely, only 3 mutations have been reported in the *MT-ATP8* gene so far, associated with cardiomyopathy and neuropathy. Recently, a fatal disorder in a 10 years old child displaying an encephalopathy with leukodystrophy, seizures, and congenital deafness was described. We report on a novel mitochondrial DNA (mtDNA) mutation in the *MT-ATP8* gene associated with NARP and deafness. **Patient**: A 34 year-old female patient was diagnosed at chilhood of ureteral duplication. At the age of 16 she presented with short stature, and severe bilateral hearing loss. Neurological examination showed generalized hyperreflexia and muscle hypotrophy. Brain MRI displayed marked cerebellar atrophy, and white matter symmetric diffuse lesions in thalamus and periventricular area. Electrophysiologic studies revealed a moderate axonal sensitive polyneuropathy. In addition, she currently suffers from migraine and retinitis pigmentosa. **Methods and Results**: Muscle did not show ragged-red or COX negative fibers. MRC complexes activities were normal except for complex V (30% of reduction). Common molecular lesions of mtDNA were discarded by southern blot, real time PCR, and minisequencing. By complete mtDNA sequencing, a change m.8391G>A (p.W9X) in the *MT-ATP8* gene was identified, which was not annotated in a number of mtDNA variation databases. Muscle heteroplasmy was 84% using Bioanalyzer 2100 (Agilent). Mutation was absent in blood from asymptomatic proband's mother, sister and maternal aunt, as well as in muscle DNA from 150 controls. **Conclusions**: Some evidences for the pathogenic role of the novel mtDNA change are: i) diminished complex V activity, ii) heteroplasmy, iii) a non-sense variation, and iv) absence in both controls and SNPs databases. The mutation is thought to be *de novo* as it was not found in 3 asymptomatic maternal relatives. This case contributes to expand the phenotypic variability already observed in the few patients reported with *MT-ATP8* gene mutations.

1313F

Normal urinary methylmalonic acid (MMA) does not rule out *SUCLA2*-related Mitochondrial DNA Depletion Syndrome. *D.C. Buhas[1], D. D'Agostino[1], N. Braverman[2].* 1) Medical Genetics, Montreal Children's Hospital, Montreal, Quebec, Canada; 2) Human Genetics, Montreal Children's Hospital Research Institute, Montreal, Quebec, Canada.

Background: Succinyl CoA synthase is a mitochondrial matrix enzyme that catalyzes the reverse reaction from succinyl-CoA to succinate in the TCA cycle and converts ADP or GDP to ATP or GTP respectively. It is composed of two subunits: (-subunit coded by SUCLG1 and a) -subunit encoded by SUCLA2. Patients with deficient activity of the succinyl-CoA synthase due to SUCLA2 mutations present with severe hypotonia in early infancy, sensorineural hearing loss, Leigh disease, dystonia and progressive muscle weakness with mtDNA depletion in muscle. From 21 patients reported, 16 originate from an inbred Faroe Island community, 3 were Italians and 2 of Muslim origin. Case presentation: An 8 month-old French-Canadian female was evaluated for congenital bilateral sensorineural deafness, severe hypotonia and global developmental delay. Brain MRI showed increased T2 signal intensity in the caudate and lentiform nuclei, internal and external capsules and myelination delay. The plasma lactate was repeatedly increased (6.3, 4.5 and 4mmol/l, normal <2.5) with high alanine (534 umol/l, normal <396). Further evaluation revealed high C3 (2.89 umol/l, normal <0.63) and C4-DC (0.6 umol/l, normal <0.48) on acylcarnitine profile. The urine organic acids showed mild elevations of citric acid cycle intermediates (alpha-ketoglutarate 1004 umol/mmol creatinine, normal <429; fumarate 11 umol/mmol creatinine, normal <10), 3-methylglutaconate (15 umol/mmol creatinine, normal <12), 3-hydroxyisovalerate 226 umol/mmol creatinine (normal <119). There was a mild elevation of methylcitrate (17 umol/mmol creatinine, normal <11) but the MMA was normal: 11 umol/mmol creatinine (normal <15), even in a repeat sample. However, the plasma MMA showed a small increase: 2435 nmol/l (normal <500). The clinical features as well as the high lactate, mild elevation of plasma MMA, acylcarnitines and urinary organic acid profile pointed to a deficiency in Succinyl CoA synthase. The SUCLA2 gene sequencing revealed the common Faroese mutation c.534+1 as well as a new variant c.985A>G, which is predicted by PolyPhen-2 as being deleterious. Treatment with thiamine and riboflavin was started. Conclusions: We report the first North-American patient with SUCLA2 defect. This report underscores also the importance of considering this disorder even if the urinary organic acids do not identify an elevation of MMA but the clinical and the other metabolic investigations are compatible with this diagnosis.

1314F

The use of Next Generation Sequencing for the Development of the Mitochondria Disorder Clinical Assays. *S. Dames[1, 3], T. Wayman[1], J. Stocks[1], J. Durtschi[1], M. Singleton[1], P. Ridge[1], K. Eilbeck[3], R. Mao[1, 2].* 1) ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, USA; 2) Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT, USA; 3) Department of Biomedical Informatics, University of Utah School of Medicine, Salt Lake City, UT, USA.

Background: Mitochondrial disorders involve mutations in the mitochondrial genome (mtDNA) and human nuclear genes. Since the condition is highly variable and presents with non-specific clinical features, patients with mitochondrial disorders are under-diagnosed. Current clinical tests such as examining lactic acid concentration or oxidative phosphorylation may be ambiguous or falsely negative. Sanger sequencing of the mtDNA and some nuclear genes is available, but is expensive and the sensitivity is low. We have developed a next generation sequencing (NGS) assay that includes both mtDNA and a 128-gene nuclear panel for selected mitochondrial disorders using the Illumina HiSeq 2000. **Methods**: mtDNA is enriched by long range PCR (LRPCR) and the 128-gene panel is enriched using the RainDance 1000 platform (1,304 amplicons). Libraries are constricted with a Beckman SPRI-TE platform, indexed, and 100-base, single-end sequenced. Bioinformatic analysis is performed using multiple alignment and variant detection programs. All clinically associated variants are Sanger-confirmed. **Results**: Analysis of mtDNA NGS has displayed 100% concordance inter/intra between runs based on Sanger verification using clinically associated samples. Preliminary analysis of the 128-gene panel displays very high (~98%) concordance intra/inter run. Differences in concordance between NGS runs for the 128-gene panel are attributed to variant allele frequency outliers. We are in the process of validating selected nonsynonymous and synonymous variants to determine optimal coverage, allele frequency, quality score, and variant calling metrics. **Conclusions**: NGS is a cost-effective and sensitive method for detecting low levels of heteroplasmy in mtDNA. NGS allows for the sequencing of multiple genes that is either technically daunting or cost prohibitive by Sanger sequencing. The reproducibly and sensitivity of the mitochondrial disorder assay is 100% when detecting single nucleotide polymorphisms and short deletion/insertion polymorphisms. Large deletions are difficult to detect using our current sequencing methods. To detect large deletions or insertions, an array-based method is performed concurrently. Critical for all NGS assays will be the development of bioinformatics tools that provide curated information for reported annotations, internal variant databases, and the ability to represent the reported information in a format that is easily reviewed by clinicians and medical directors.

1315F

Succinyl-CoA Ligase Deficiency in the Mouse is a Model for Mitochondrial Disease with mtDNA Depletion. *B.H. Graham[1], T.R. Donti[1], N.K. Hawkins[1], M. Ge[1], K.W. Eldin[2].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; 2) Dept. Pathology and Immunology, Baylor College of Medicine, Houston, TX, USA.

Over the past few years, mitochondrial DNA (mtDNA) depletion has been implicated in several mitochondrial diseases. Mutations in subunits of Succinyl-CoA Ligase (Synthetase), a component of the citric acid cycle, have been associated with mitochondrial encephalomyopathy and mitochondrial DNA depletion. In humans, mutations in SUCLA2, encoding the ADP-specific beta subunit, and SUCLG1, encoding the alpha subunit, have been identified as causes of mitochondrial disease with mtDNA depletion. The goal of this project is to develop model systems of Sucinyl-CoA Ligase deficiency to study disease pathogenesis and biology of mtDNA maintenance. A gene trap allele of Sucla2 ($Sucla2^{SA}$) geo) has been isolated in mouse embryonic stem (ES) cells and used to generate transgenic animals. $Sucla2^{SA}$) geo homozygotes exhibit recessive lethality with most mutants dying late in gestation (e18.5). Rare stillborn homozygote pups exhibit significantly elevated levels of methylmalonic acid (MMA) and varying degrees of mtDNA depletion. Histological analysis of mutant placenta reveals increased mineralization and mutant embryos are approximately 25% smaller than wild type littermates. Mutant placenta and embryonic brain, heart and muscle show varying degrees of mtDNA depletion (20-60%), while there is no appreciable mtDNA depletion in mutant liver. Mouse embryonic fibroblasts (MEFs) derived from e12.5 embryos show a 50% reduction in mtDNA content after five passages for mutant compared to wild type. The mtDNA depletion in MEFs and mutant tissues is functionally significant as indicated by reduced steady state levels of COXI by western and by respiratory chain deficiencies. Ongoing and future studies include performing complementation experiments in cells to determine the structural requirements of Sucla2 for mtDNA maintenance as well as expression of affinity-tagged Sucla2 in mutant MEFs to identify interacting proteins by pulldown and mass spectrometry. This mouse model of Succinyl-CoA ligase deficiency and mtDNA depletion will provide insights into the pathogenesis of mitochondrial diseases with mtDNA depletion and into the biology of mtDNA maintenance as well as facilitate the exploration of novel therapeutic strategies.

1316F

Relationship of mitochondrial heteroplasmy and variation with childhood obesity in European and African Americans. *S.F.A. Grant[1,2,3], J.T. Glessner[1], J.P. Bradfield[1], J. Zhao[3], J.E. Tirone[4], R.I. Berkowitz[5,6], H. Hakonarson[1,2,3], N. Sondheimer[4].* 1) Center for Applied Genomics, Children's Hospital of Philadelphia Research Institute, Philadelphia, Pennsylvania 19104, USA; 2) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, USA; 3) Division of Human Genetics, Children's Hospital of Philadelphia Research Institute, Philadelphia, Pennsylvania 19104, USA; 4) Division of Biochemical Genetics, Children's Hospital of Philadelphia Research Institute, Philadelphia, Pennsylvania 19104, USA; 5) Behavioral Health Center and Department of Child and Adolescent Psychiatry, The Children's Hospital of Philadelphia, Philadelphia PA 19104, USA; 6) Center for Weight and Eating Disorders, Department of Psychiatry, University of Pennsylvania, Philadelphia PA 19104, USA.

It has been suggested that the mitochondria genome plays a role in the pathogenesis of obesity. We examined the influence of mitochondrial genotype and heteroplasmy in a large pediatric case-control population. Single nucleotide polymorphism genotyping arrays accurately determine mitochondrial polymorphisms and heteroplasmy. We queried a large existing genome wide genotyped childhood obesity dataset consisting of two different ethnicities: 1,080 European American (EA) cases (BMI$\geq$ 95th percentile) and 2,500 lean controls (BMI<50th percentile) plus 1,479 African American (AA) cases and 1,575 lean controls. Association was not observed between childhood obesity and any of the 138 mitochondrial polymorphisms in either population. We also found no observable differences in gross heteroplasmy between the obese and non-obese groups. Finally, we analyzed the quantitative mitochondrial genotype calls generated, whether they exceeded the heteroplasmy threshold or not. With this more lenient test, we found six positions with a significant difference between EA cases and controls (P<1x10^{-4}). However, when evaluated in the AA data set, no differences were noted at these sites, suggesting that our initial observations were due to chance rather than a meaningful relationship to childhood obesity. It is unlikely that common mitochondrial polymorphisms or heteroplasmy play a role in common childhood obesity.

1317F

Metabolic interventions reduce mitochondrial dysfunction in a neuronal model of MELAS. *V. Procaccio[1,2], V. Desquiret-Dumas[1,2], N. Gueguen[1,2], M. Barth[1], A. Chevrollier[1,2], P. Guardiola[3], S. Hancock[4], D.C. Wallace[4], P. Amati-Bonneau[1,2], D. Henrion[2], D. Bonneau[1,2], P. Reynier[1,2].* 1) Biochemistry and Genetics Dept, Univ Angers, Angers, France; 2) UMR INSERM, U771-CNRS6214, F-49000 Angers, France; 3) Department of Hematology, Angers University Hospital, F-49000, France; 4) Center of Mitochondrial and Epigenomic Medicine; University of Pennsylvania, Philadelphia, PA, USA.

The 3243 A>G variant in the mitochondrial tRNALeu (UUR) gene is one of the most common mitochondrial DNA (mtDNA) mutation. Phenotypic manifestations depend mainly on the ratio of mutant to normal mtDNA copies (heteroplasmy). High percentage of mutant mtDNA is associated with the severe and life-threatening neurological syndrome known as MELAS (Myopathy, Encephalopathy, Lactic Acidosis, and Stroke-like episodes). This mutation primarily affects brain and blood vessels, but the pathophysiology is still poorly understood in neuronal or vascular cells. We have created a series of cybrid cell lines with the nuclear background of a neuroblastoma cell line (SHSY-5Y) and patient fibroblast mitochondria carrying the 3243A>G mutation at different mutant loads (control cells, 70%, 90% and 100% mutant loads). This cellular model should substantially help us to clarify the genetics and pathophysiology of the MELAS disease. We have first investigated the impact on cellular metabolism and mitochondrial respiratory chain in cybrid cells. The introduction of the 3243 A>G mutation in neuronal cells induces a metabolic switch towards glycolysis and severe defects in respiratory chain activity and assembly, highly dependent on the mutant load. As an attempt to compensate the biochemical defects in mutant cells, we used different strategies. Low glucose medium in cultured cells which significantly shifted the 100% mutant towards wildtype reaching 90% mutant and restored respiratory chain complexes assembly and activity. In addition, nitric oxide (NO) metabolism was also significantly altered in mutant neuronal cells, and treatment with arginine, a NO donor, improved significantly complex I activity. Indeed, L-arginine has been proposed as a treatment for MELAS 3243 A>G syndrome patients during the acute stroke phase or long term use. Metabolic interventions such as arginine therapy appear as a promising therapeutic strategy for MELAS patients.

1318F

Renal manifestations in mitochondrial cytopathies : about a series of 55 patients. *M. Rio[1], O. Boyer[2], AS. Lebre[1], P. Niaudet[2], A. Rotig[3], A. Munnich[1].* 1) Departement de Génétique, Hosp Necker-Enfants Malades, Paris, France; 2) Service de Néphrologie Pédiatrique, Hosp Necker-Enfants Malades, Paris, France; 3) INSERM 781, Hosp Necker-Enfants Malades, Paris, France.

Mitochondrial cytopathies are a clinically heterogeneous group of diseases characterized by defect of oxydative phosphorylation (OXPHOS). These disorders can affect several organs or tissues. Renal involvement has been occasionally reported. We report here a series of 55 patients with mitochondrial diseases associated with renal involvement. Age at onset of mitochondrial diseases ranged from birth to 41 years. Renal manifestations were the first sign of the mitochondrial disorder in only three patients. In most cases, renal disease was associated with neurological or multisystemic symptoms. The commonest renal manifestation was a proximal tubulopathy observed in 32/55 patients. Other renal manifestations were nephrotic syndrome and tubulo-interstitial nephropathy. Molecular genetic studies revealed mitochondrial DNA mutations in 5 patients, including 3 point mutations (mt3243A>G, mt13514A>G, mt9185T>C), and 2 large deletions. Mutations in nuclear gene were identified in 15 patients. Among them, four patients had complex III deficiency due to nuclear *BCS1L* mutations and presented with early onset lactic acidosis, tubulopathy, liver involvement, neurological symptoms, and failure to thrive. Five patients with a neonatal multisystemic disorder and tubulopathy showed mtDNA depletion in muscle and *RRM2B* mutation. Two patients had mutations in genes involved in mitochondrial protein translation (*TRMU* and *MRPL3*). Four patients had a primary defect of coenzyme Q10 biosynthesis, related with mutations in *COQ2* and *PDSS2* genes. Finally, 35 patients had variable OXPHOS deficiency with no molecular basis yet identified. In conclusion, renal involvement is an infrequent but possible manifestation of mitochondrial cytopathies and is not associated with a specific OXPHOS deficiency either specific nuclear or mitochondrial mutations being identified in this series of patients.

1319F

Exome-analysis reveals new genes for mitochondrial disorders of early childhood. *A. Suomalainen[1], T. Tyni[1,2], P. Isohanni[1,2], J. Elo[1], P. Ellonen[3], H. Pihko[2], H. Tyynismaa[1].* 1) Molecular Neurology, Biomedicum-Helsinki, University of Helsinki, Helsinki, Finland; 2) Helsinki University Central Hospital, Hospital of Children and Adolescents; 3) Institute of Molecular Medicine Finland, FIMM, Helsinki, Finland.

Mitochondrial dysfunction is the most common cause of inherited metabolic disorders of childhood. The early-onset disorders typically show a severe, progressive disease course and are often fatal. In spite of increasing knowledge on the molecular features of mitochondrial disorders, majority of these children still lack genetic diagnosis. We have collected a wide patient material of early-onset cardiomyopathies and mitochondrial encephalomyopathies. We applied whole-exome sequencing analysis to search for genetic background of these disorders. We utilized an in-house data mining pipeline to analysis of the typically over 60.000 individual single-nucleotide variants in one individual. We applied serial filters to enable identification of changes affecting conserved positions in proteins that are predicted to be targeted to mitochondria. Currently, we have identified mutations in two novel autosomal recessive genes encoding mitochondrial proteins. The first one caused cardiomyopathy in a sibpair; one died before one year of age upon acute progressive cardiac decompensation, induced by respiratory infection, whereas her sister manifested cardiomyopathy before one year of age, but with slower progression, as she is currently a teenager. The second family had two children, both conceived after fertilization treatment, who both developed a progressive mitochondrial encephalopathy soon after birth, leading to their death around one year of age. We showed that the identified defects in the genes, not previously associated with any disease, cause dysfunctional mitochondrial translation, with severe consequences to respiratory chain proteins. Mitochondrial translation defects are emerging as an important cause of both wide-spread and tissue-specific respiratory chain disorders of early childhood.

1320F

Large-scale deletions on 22q13.33 and 12q24.33 detected in patients with mitochondrial disorders. *M. Tesarova[1], A. Vondrackova[1], K. Vesela[1], K. Vinsova[1], V. Stranecky[2], T. Honzik[1], H. Hansikova[1], J. Zeman[1].* 1) Department of Pediatrics and Adolescent Medicine, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague 2, Czech Republic; 2) Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague 2, Czech Republic.

Mitochondrial disorders represent a group of clinically and genetically heterogeneous diseases whose molecular-genetic diagnosis is challenging. The standard diagnostic approach is direct DNA sequencing of candidate genes whose list is steadily growing. Nevertheless, this technique does not enable to detect large heterozygous deletions. To avoid this, gene-specific MLPA analyses or custom oligonucleotide array-based CGH are used. Alternatively, SNP arrays with high marker density may be utilized. The **aim** of this study is to analyze large-scale deletions in group of 15 patients with mitochondrial disorder of unknown etiology. **Methods:** Genome-Wide Human SNP 6.0 array (Affymetrix) was used for genotyping of DNA isolated from leucocytes of patients. Detected deletions were confirmed by TaqMan Copy Number Assays (Applied Biosystems). **Results:** In 20% of patients, large deletions as a cause of the mitochondrial disease were found. In 2 of them, 175-kb, 87-kb resp. heterozygous deletions on 22q13.33 affecting *SCO2* and *TYMP* genes were detected. Missense point mutations in *TYMP*, *SCO2* genes resp. were identified by direct sequencing on the other allele. Patient 1 with MNGIE phenotype inherited c.261G>T in *TYMP* from father and 175-kb deletion from mother. A novel mutation c.667G>A in SCO2 resulting in substitution p.Asp223Asn was found on maternal allele of patient 2 in combination with 87-kb deletion inherited from father. Interestingly, 175-kb deletion on 22q13.33 partially overlaps with two described copy-number variations (CNV; variation_5192 and variation_4139). According to Database of Genomic Variants (Iafrate et al, 2004), these two CNVs spanning *TYMP* and *SCO2* genes occurs with 7% frequency in control samples. In patient 3, homozygous 5-kb deletion on 12q24.33 affecting exon 4 of *PUS1* gene was revealed. Additionally, clinical phenotype of patient 2 and patient 3 did not fully correspond with symptoms usually observed in other *SCO2* and *PUS1* patients resp. **Conclusions:** Genome-Wide Human SNP 6.0 Array allowing detection of deletions larger than 700 bp was successfully used to determine genetic diagnosis in 3 out of 15 patients. Copy-number variations occur frequently in human genome based on the results of whole-genome analyses and they may overlap with many genes associated with clinical phenotypes including mitochondrial disorders. *Supported by research projects MSM0021620806, 1M520 and grants IGA NS 10581-3/ 2009, IGA NS 10561-3/2009, GAUK 28410.*.

1321F

A new mouse model for the R653Q variant of MTHFD1, a trifunctional folate enzyme. *K.E. Christensen[1], L. Deng[1], C. Perugino[1], O.V. Malysheva[2], E. Arning[3], T. Bottiglieri[3], N.I. Krupenko[4], M.A. Caudill[2], R.E. MacKenzie[5], R. Rozen[1].* 1) Depts. of Human Genetics and Pediatrics, McGill University, Montreal, Quebec, Canada; 2) Division of Nutritional Sciences, Cornell University, Ithaca, NY; 3) Baylor Institute of Metabolic Disease, Dallas, TX; 4) Dept. of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC; 5) Dept. of Biochemistry, McGill University, Montreal, Quebec, Canada.

The MTHFD1 c.1958 G→A (R653Q) polymorphism, an amino acid substitution in the synthetase domain of the trifunctional folate enzyme MTHFD1, has been implicated as a risk factor for common disorders such as birth defects or cancer. MTHFD1 produces folate derivatives for several critical one-carbon transfer reactions including nucleotide synthesis and methylation reactions. We have previously shown that the R653Q mutation destabilizes the synthetase domain and reduces the production of formylTHF by this enzyme. This variant is likely to have its biggest impact in rapidly proliferating tissues, such as developing embryos, because formylTHF is required for the de novo synthesis of purines. To investigate the effect of synthetase deficiency on embryonic development, we have recently generated a new mouse model for synthetase deficiency. In this mouse, a null mutation has been introduced into the synthetase domain of the Mthfd1 gene that inactivates the synthetase leaving the other two activities intact. The Mthfd1 S-/- genotype is embryonic lethal; no Mthfd1 S-/- embryos have been observed beyond gestational day 10.5 and those found were severely delayed and abnormal. The synthetase-deficient (Mthfd1 S+/-) mice are healthy, appear to reproduce normally and should be a good animal model for humans with the MTHFD1 1958AA genotype. Proper expression of the mutant protein has been confirmed by enzyme assay and immunoblot; only synthetase activity differs between the wild type and heterozygous (Mthfd1 S+/-) mice. Preliminary metabolic studies suggest that folate and choline metabolism are disrupted in non-fasted Mthfd1 S+/- males fed standard laboratory chow. The proportion of 10-formylTHF in plasma is decreased ~40% (formylTHF/total folates, p=0.044). The same trend was observed in liver. Additionally, in liver, the amount of glycerophosphocholine, a phosphatidylcholine catabolite that can be used to generate free choline, is reduced ~20% (p=0.053). This observation has implications for cellular methylation as choline becomes an important alternate methyl donor when folate-dependent methylation is compromised. Pilot studies have revealed developmental delays and heart defects in offspring. Experiments are underway to investigate these effects, and to examine the influence of diet on phenotype.

1322F

Loss of the Prader-Willi Syndrome Candidate Gene Magel2 impairs leptin signaling in mice. *R.E. Mercer[1], R. Wevrick[1], W.F. Colmers[2].* 1) Medical Genetics, University of Alberta, Edmonton, Alberta, Canada; 2) Pharmacology, University of Alberta, Edmonton, Alberta, Canada.

Prader-Willi Syndrome (PWS) is the most common genetic cause of severe obesity. People with PWS, who are missing several imprinted genes on chromosome 15 including MAGEL2, have significant alterations in energy homeostasis and body composition associated with hypothalamic dysfunction. MAGEL2 has previously been demonstrated to have very high levels of expression within the hypothalamus, and the known involvement of the hypothalamus in energy homeostasis led us to investigate the leptin system in transgenic mice lacking Magel2, referred to as Magel2-null. Similar to PWS, Magel2-null mice are underweight prior to weaning, but show increased weight gain and increased adiposity as adults. Adult (20-week old) Magel2-null mice fail to respond to an intraperitoneal (IP) injection of leptin (2.5mg/kg) by reducing 24h food intake, in contrast to control mice where a significant reduction in 24h food-intake is seen. This leptin insensitivity precedes the development of obesity, as even 6-week old Magel2-null mice, comparable in weight to controls, also do not respond to IP leptin injection. To study leptin signaling in the hypothalamus of Magel2-null mice, we crossed them to transgenic mice expressing GFP in neurons that express the signaling form of the leptin receptor, and then used patch-clamp recordings in hypothalamic brain slice preparations to study responses of GFP-expressing neurons to the bath application of leptin (100 nM). In the arcuate nucleus (ARC), a region of the hypothalamus possessing first-order leptin-responsive neurons, different populations of neurons are either excited or inhibited by leptin, as seen in de- or hyperpolarizations, respectively, of neuronal membrane potential. While recordings from GFP-expressing neurons from control mice show substantial populations of both cell types, no leptin-excited neurons were found in the ARC of Magel2-null mice, suggesting a critical role for Magel2 in the coupling of the leptin receptor to excitatory neuronal activity. These results reinforce our hypothesis that reduced leptin responsiveness contributes to the energy balance deficits seen in Magel2-null mice. Further, our results suggest a critical role for Magel2 in leptin excited neurons in the ARC, which are mostly pro-opiomelanocortin (POMC) expressing neurons. We plan further investigation of the melanocortin system in these mice.

1323F

Clinicopathological and genetical features of autophagic vacuolar myopathies characterized by autophagic vacuoles with sarcolemmal features (AVSF). *K. Sugie[1,2], H. Komaki[2], T. Kurashige[3], D. Kaneda[4], A. Kimura[5], M. Matsumoto[3], S. Ueno[1], I. Nishino[2].* 1) Department of Neurology, Nara Medical University, Kashihara, Nara, Japan; 2) National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan; 3) Department of Clinical Neuroscience and Therapeutics, Hiroshima University Graduate School, Hiroshima, Japan; 4) Department of Neurology, Osaka Red Cross Hospital, Osaka, Japan; 5) Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan.

Introduction: Danon disease (DD), an X-linked dominant cardiomyopathy and myopathy, is caused by primary LAMP-2 deficiency. X-linked myopathy with excessive autophagy (XMEA) is known to be caused by VMA21 gene mutations. DD, XMEA and related autophagic vacuolar myopathies (AVM) are pathologically characterized by autophagic vacuoles with sarcolemmal features (AVSF). AVSF delineate a group of at least five clinically different myopathies, including DD and XMEA. However, the clinicopathological and genetical features of these AVM are not well established. Patients and Methods: We studied 51 DD patients (27 men and 24 women) from 17 families, 3 XMEA patients, 2 infantile AVM patients, 7 X-linked congenital AVM patients, and 1 adult-onset AVM with multi-organ involvement patient. Clinicopathological and genetical features were based on review of clinical histories and skeletal muscle specimens. Results: All DD patients had LAMP-2 gene mutaions. Myopathy and cardiomyopathy were evident in all DD patients. Hypertrophic cardiomyopathy (HCM) was documented in most men, while dilated cardiomyopathy was more common among women. Cardiomyopathy was the most important prognostic factor and the main cause of death in DD. All XMEA patients had VMA21 gene mutations. XMEA patients showed slowly progressive myopathy and no cardiomyopathy. Both infantile AVM patients had no cardiomyopathy. Only 1 of the 7 patients with congenital AVM had HCM. The adult AVM patient developed HCM with arrhythmias. Pathologically, AVSF in all AVM expressed virtually all sarcolemmal proteins, in addition to acetylcholinesterase, on their vacuolar membranes. Multilayered basal lamina and complement C5b-9 deposition along the vacuolar membranes were present in XMEA, infantile AVM, congenital AVM and adult AVM. These findings suggested that they had a similar pathomechanism. In addition, the locus for infantile AVM and congenital AVM was suggested to be the same region with XMEA. Therefore, these diseases could even be allelic as for each other. Conclusion: These AVM may be primarily due to lysosomal dysfunctions in consideration of both genetically diagnosable DD and XMEA. In addition, because infantile AVM and congenital AVM are pathologically and genetically similar to XMEA, these three diseases could even be allelic as for each other. Furthermore, there will most likely be other diseases in these AVM and the list of these AVM will probably expand rapidly.

1324F

Clinical Findings and Molecular Analysis of the GALT gene in Filipino patients with Classical Galactosemia. *C.L.T. Silao[1,2], D.M. Canson[1], K.N. Hernandez[1], S.C. Estrada[1,2].* 1) Inst Human Genetics, Manila, Philippines; 2) Department of Pediatrics, University of the Philippines-Philippine General Hospital, Manila, Philippines.

INTRODUCTION: Galactosemia is caused by enzyme deficiencies involved in galactose metabolism one of which is the deficiency of galactose-1-phosphate uridyltransferase (GALT). This study describes the clinical presentation and the results of the GALT gene analysis of seven (7) unrelated Filipino patients diagnosed with Classical Galactosemia. METHODS: Clinical data were collected from charts of Classical Galactosemia patients who were either referred or were detected via neonatal screening from 1996 to May 2011. DNA from the patients were PCR-amplified and sequenced following standard protocols. RESULTS: All patients presented with elevated galactose metabolites and varying degrees of GALT activity. Those detected by newborn screening had early diagnosis and management thus were spared of the disease complications. Those who did not benefit from a screen, were diagnosed at one month of age. They presented with feeding problems, failure to thrive and liver enlargement. Reversal of the acute complications followed with proper management. All patients are presently doing well and are developmentally at par. The results of the GALT gene analysis will be presented. CONCLUSION: The importance of early detection, immediate institution of the proper management and molecular diagnosis in the medical management and genetic counseling of the patient and their families cannot be overemphasized.

1325F

Observations on the Molecular Basis of Sucrase-Isomaltase Deficiency. *Z. Wu, S. Uhrich, C.R. Scott.* Pediatrics, University of Washington, Seattle, WA.

Congenital sucrase-isomaltase deficiency (CSID) is a disorder that affects the ability to digest natural carbohydrates with clinical symptoms of flatulence, diarrhea and malnutrition. CSID is caused by mutations in the sucrase-isomaltase gene, and the condition is inherited in an autosomal recessive pattern. The surcrase-isomaltase gene is 100 kb in size and consists of 1827 amino acids and includes isomaltase and sucrase domains. We have tested thirty-six samples from affected persons worldwide. Twenty-four different mutations were indentified in the sucrase-isomaltase gene. We have identified twenty novel variations: eighteen missense mutations p.W105C, p.Q307X, p.P348L, p.D500E, p.D536V, p.E613X, p.L741P, p.R774G, p.E801X, p.F875S, p.W931R, p.W931X, p.R1124X, p.I1378S, p.Y1417X, p.C1531Y, p.T1606I, p.G1760V; one splicing error IVS10 -2 a→g; and one deletion c.489delT. Analysis of the mutations reveals that: (1) Four mutations were detected more frequently than others with p.G1073D, p.V577G, p.F1745C and p.R1124X having frequencies of 0.28, 0.15, 0.11 and 0.06 respectively; assuming Hardy-Weinberg equilibriums, 84% of affected patients should have one or two of these mutations [p2+2pq]. (2) The frequency of mutations occurring in the sucrase domain was greater than in the isomaltase domain (57% vs. 43%), although the isomaltase domain is larger (1007 amino acids) than the sucrase domain (820 amino acids). For patients suspected of CSID, molecular testing may be a less expensive diagnostic approach than intestinal biopsy and enzyme analysis.

1326F

The Heritability of Metabolic Profiles in Preterm Twins. *K.K. Ryckman[1], S.L. Berberich[2], O.A. Shchelochkov[1], S. Copeland[3], J.C. Murray[1].* 1) Dept Pediatrics, University of Iowa, Iowa City, IA; 2) State Hygienic Laboratory, University of Iowa, Iowa City, IA; 3) Health Resources and Services Administration, Rockville, MD.

BACKGROUND: Understanding the genetic and metabolic profiles of neonatal diseases has the potential to impact diagnosis and clinical treatment of neonates. Infants born preterm (<37 weeks) have different genetic and metabolic profiles than term infants; however, the heritability of these metabolic profiles has not been previously characterized, particularly in preterm infants. Measurements taken during routine newborn screening provide a snapshot of the metabolic profile at birth and can be used to determine the heritability. Understanding the genetics factors contributing to the metabolic profiles from newborn screening will aid in the optimization of newborn screening tests and establish associations with metabolic disorders acquired during childhood. OBJECTIVE: To examine metabolic biomarkers in preterm twins at birth and determine the genetic heritability of each measurement. METHODS: This study included 27 monozygotic and 54 dizygotic twin pairs that were born preterm. Measurements of 9 amino acids, 8 acylcarnitines, 17-hydroxprogesterone (17-OHP) and thyroid stimulating hormone (TSH) were obtained from the Iowa Neonatal Metabolic Screening Program for specimens collected between 24 and 72 hours after birth and in infants that had not received a transfusion at the time of specimen collection. Heritability was estimated using multilevel mixed-effects linear regression adjusting for year of sample collection and major change in the assay lot. RESULTS: The highest heritability were found for the essential amino acids including leucine (h^2=0.68), methionine (h^2=0.80) and valine (h^2=0.57). Other groups of metabolites had a wide range of heritability including the non-essential amino acids (h^2=0.16-0.78), short chain (h^2=0.13-0.51), medium chain (h^2=0.15-0.46) and long chain (h^2=0.16-0.24) acylcarnitines. The heritability of TSH (h^2=0.70) was consistent with what is reported in the literature for adult serum TSH and the heritability of 17-OHP was h^2=0.17. CONCLUSIONS: We have identified high heritability for many metabolic factors as measured at birth, particularly essential amino acids and TSH in preterm twins. This implicates that genetic factors regulate variation in metabolites at birth and understanding these factors will help to optimize newborn screening tests as well as in the understanding of metabolic diseases during childhood. Ongoing studies are underway to replicate these findings examining a larger independent cohort of twins.

1327F

Guanidinoacetate methyltransferase (GAMT) deficiency presenting as a late-onset refractory seizure disorder and mental retardation in sibs. *A. Levtova[1], L. Carmant[2], M. Lambert[1], V. Désilets[1].* 1) Medical Genetics, CHU Sainte-Justine, University of Montreal, Montreal, Quebec, Canada; 2) Pediatric neurology, CHU Sainte-Justine, University of Montreal, Montreal, Quebec, Canada.

Introduction: Guanidinoacetate methyltransferase (GAMT) deficiency is a disorder of creatine synthesis resulting in depletion of the brain creatine pool. Case report: We describe two siblings with newly diagnosed guanidinoacetate methyltransferase (GAMT) deficiency. Patient 1, a 6 y.o. girl, had normal milestones until age 2 with subsequent developmental slowing, though without frank regression, particularly affecting language. Her epilepsy began at age 5 with staring, myoclonic and atonic (head nodding) episodes whose frequency escalated over an 8-month period. She then had a tonic-clonic convulsion followed by non-convulsive status. A brain MRI showed delayed myelinisation in the bifrontal and mesiotemporal regions. She has multiple daily seizures despite trials of multiple anticonvulsants; her development is mildly to moderately delayed. Patient 2, a 13 y.o. boy whom we evaluated as part of his sister's investigation, had first presented to the neurology clinic at 10 months with an isolated motor delay; he was then lost to follow-up until the onset of similar complex partial with an atonic component as well as tonic-clonic seizures at age 4. At this point, he had significant global developmental delay. His epilepsy has been refractory to multiple agents; vagus nerve stimulator placement was also not effective. Brain MRI was normal at the ages of 5 and 11. Liquid chromatography/mass spectrometry detection showed low-normal creatine (12 and 22 umol/mmol creatinine in patients 1 and 2, respectively) and high guanidinoacetate (880 and 527 umol/mmol creatinine, respectively). Patient 2's GAMT activity on fibroblasts was undetectable. Brain MRI with spectroscopy for patient 1 and GAMT sequencing for patient 2 are pending. Creatine monohydrate treatment was begun in both children (400 mg/kg/day) and will be escalated as needed. Future steps include dietary arginine restriction and ornithine supplementation. Conclusion: Despite undetectable GAMT activity in Patient 2, these sibs stand apart from most known cases of GAMT deficiency in that their developmental delay has been somewhat milder, but especially in that, in both cases, non-specific developmental delay preceded the onset of epilepsy by several years. As there is evidence that early diagnosis of GAMT deficiency may lead to dramatically improved outcomes, this highlights the importance of measuring urinary creatine and guanidinoacetate in children with nonsyndromic developmental delay.

1328F

The Clinical Spectrum of Pancreatitis in Organic Acidemias. *H. Anderson[1,2], I.S. Fortgang[2], M.S. Petrescu[2], T.C. Narumanchi[1,2], A. Cunningham[1,2].* 1) Hayward Gen Ctr, Tulane Univ Med Ctr, New Orleans, LA; 2) Pediatroc Department, Tulane Univ Med Cntr, New Orleans, LA.

Pancreatitis is a rare complication attributed to organic acidemias. No known pathophysiologic mechanism has been identified and no clear therapeutic recommendations exist. Treatment of pancreatitis involves avoidance of enteral nutrition complicates management of organic acidemias in which avoidance of catabolism is paramount. We describe 3 cases of pancreatitis in patients with organic acidemias illustrating the wide clinical spectrum of pancreatic abnormalities. Cases: Case A was a 12-year old boy with recurrent pancreatitis but no other problems noted who was transferred to tertiary care from an outlying hospital with severe metabolic acidosis and loss of consciousness. He had been fasted for several days for pancreatitis receiving only intravenous dextrose for nutrition. Organic acid analysis revealed significantly elevated propionic acid and 3-OH-propionic acid. He expired of brainstem herniation; his mother, who was pregnant, delivered a healthy male who also has an attenuated form of propionic acidemia (asymptomatic with diagnostic organic acids). Case B is a 6-year old Vietnamese boy with methylmalonic acidemia diagnosed in infancy when he presented with lethargy and metabolic acidosis. He had been managed with protein restriction, metabolic formula, oral carnitine and IM OH-cobalamin without recurrent exacerbations until age 5 years when he presented with intractable abdominal pain and elevated lipase/amylase. Abdominal imaging revealed splenic vein thrombosis and pseudocyst measuring 1.4 cm x 2.8cm. At age 6 years, he had a recurrence of abdominal pain associated with elevated lipase and the pseudocsyt size had doubled to 2cm x 6 cm. Case C is a 5-year old girl with methylmalonic acidemia diagnosed in infancy with severe metabolic acidosis who had yearly severe metabolic exacerbations and presented with abdominal pain and nausea with elevated lipase and amylase but no metabolic exacerbation. Conservative care with gut rest and optimization of parenteral calories (15% dextrose) including amino acids has been successful in abating two episodes of pancreatitis without inducing catabolic crisis. Pancreatitis is a rarely reported complication of many organic acidemias and can be the presenting symptom in mild/attenuated forms. Early detection is important and avoidance of catabolism in management is critical to successful outcomes. Clinical severity and exacerbations do not seem to correlate with incidence of pancreatitis which may be recurrent.

1329F

Identification of novel biochemical abnormalities in organic acidemias through a GC-MS based metabolomics approach. *H. Vernon[1], Y. Sandlers[2], R.I. Kelley[2], D. Valle[1].* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Department of Neurogenetics, Kennedy Krieger Institute, Baltimore, MD.

We applied a GC-MS based metabolomics approach to investigate biochemical abnormalities in energy metabolism in Isovaleric Acidemia (IVA), Methylmalonic Acidemia (MMA) and Propionic Acidemia (PA). We began with a non-targeted GC-MS approach to screen plasma samples from 4 patients with MMA and 4 patients with PKU on diet (as a control population) and identified 95 individual metabolites seen in all 8 individuals. We selected for further study target metabolites that were related to energy metabolism and showed a difference between the MMA and control (PKU) populations. We then studied six Krebs cycle intermediates by GC-MS isotope dilution in plasma samples from 4 patients with IVA, 5 patients with MMA, 6 patients with PA, and 6 control (PKU) patients. We found abnormal profiles of plasma Krebs cycle metabolites in MMA, PA, and IVA patients compared with controls, and cohorts with MMA, PA, and IVA showed individually different profiles. Patients with PA had significantly higher malate (12.9 uM vs. 5.8 uM, P-value 0.0008), fumarate (3.0 vs. 1.5 uM, P-value 7.1E-05), and succinate (8.26 uM vs. 4.56 uM, P-value 0.03) compared to controls, but not 2-ketoglutarate, isocitrate or citrate. Patients with MMA had significantly higher malate (11.0 uM vs. 5.8 uM, P-value 0.05), but not of the other metabolites, however the level of fumarate trended in the same direction as in the PA cohort (3.1 vs 1.5 uM) but did not reach significance because of an outlier in the MMA cohort. Patients with IVA had slightly lower levels of isocitrate compared to controls, but normal levels of the other metabolites. We also used these methods to track metabolic aberrations in a single patient with MMA during different clinical states and found that the level of fumarate appeared to correlate with the severity of illness, with fumarate highest during hospitalization requiring an ICU setting (5.2 uM), moderate when hospitalized in a regular pediatric setting requiring minimal medical intervention (4.0 uM), and lowest when measured twice as an outpatient (3.07 uM and 2.73 uM). Our results indicate that MMA, PA and IVA cause fundamental metabolic disturbances not detected by traditional metabolic testing, which largely focuses on measurements of immediate upstream and downstream metabolites. We propose that a better understanding of the ways by which MMA, PA, and IVA disrupt the Krebs cycle will lead to improved targeted treatments.

1330F

Characterization of surfactant secretion in alveolar type II cells cultured from PEX7 hypomorphic mice: does plasmalogen deficiency lead to surfactant abnormalities? *X. He[1], W. Cui[2], S. Jiralerspong[2], I. Mandeville[2], S. Steinberg[3], A. Moser[3], N. Braverman[1,2].* 1) Department of Human Genetics, McGill University, Montreal, QC, Canada; 2) Montreal Children Hospital Research Institute, Montreal, QC, Canada; 3) Kennedy Krieger Institute, Department of Neurogenetics, Baltimore, MD, USA.

Rhizomelic chondrodysplasia punctata (RCDP) is commonly caused by defects in the peroxisome transporter, PEX7. Clinical features include skeletal dysplasia, cataracts, mental retardation and chronic pulmonary compromise that ultimately causes death. These features result from plasmalogen (PL) deficiency, a specialized class of membrane phospholipids whose functions are largely unknown. Relevantly, PLs are integral components of surfactant, secreted by alveolar type II (ATII) cells. To study RCDP and PLs, we generated Pex7 deficient mouse models, which showed abnormalities in late lung development (see W. Cui et al, abstract on lung abnormalities in the Pex7 mouse model). We hypothesized that ATII cells cultured from the Pex7 hypomorphs produce abnormal surfactant and that this contributes to the chronic lung disease in patients. To test this hypothesis, we will stimulate surfactant secretion in primary ATII cells cultured from these mice using drugs that stimulate the three kinases essential to the surfactant secretion pathway: protein kinase A by terbutaline, protein kinase C by ATP and TPA, and CaMK by ionomycin. We will then evaluate surfactant protein and lipid composition and relative amounts pre and post stimulation. In preliminary experiments, we have characterized the synthesis and secretion of surfactant proteins in ATII cells cultured from wild type neonatal (P7-P10) mouse lungs using fibroblasts as negative controls and A549 human carcinoma lung cells as positive controls. Immunofluorescence assays using antiserum to surfactant proteins A, B, C, and D confirms the intracellular localization of these proteins during synthesis, and immunoblotting shows secretion of these surfactant proteins in cell culture media. In subsequent assays, we will obtain lipid profiles by tandem mass spectrometry to characterize amounts of PLs and other surfactant lipids and then evaluate all of these parameters in our Pex7 deficient mouse models. Finally, we will treat the primary ATII cells with a plasmalogen precursor to determine if we can rescue the abnormalities detected.

1331F

Increased incidence of Zellweger syndrome in French-Canadians of Saguenay-Lac-St-Jean due to a founder mutation in *PEX6*. *S. Levesque[1], C. Morin[2,6], S.-P. Guay[3], J. Villeneuve[2], P. Marquis[5], L. Bouchard[3,7], S. Steinberg[4], K. Dewar[5], N. Braverman[1].* 1) Dept of Medical genetics, Montreal Children`s Hospital, Montreal, QC, Canada; 2) Dept of Pediatrics, Centre Hospitalier de Chicoutimi, Saguenay, QC, Canada; 3) ECOGENE-21, Centre Hospitalier de Chicoutimi, Saguenay, QC, Canada; 4) Dept of Neurogenetics, Kennedy-Krieger Institute, Balimore, MD; 5) Genome Quebec Innovation Center, McGill University, Montreal, QC, Canada; 6) Dept of Pediatrics, Université de Sherbrooke, Sherbrooke, QC, Canada; 7) Dept of Biochemistry, Université de Sherbrooke, Sherbrooke, QC, Canada.

Peroxisome biogenesis disorders show defective peroxisome assembly, division and/or impaired importation of proteins inside the organelle and include Zellweger syndrome (ZS). Patients show dysmorphic features and usually present in the neonatal period with central hypotonia, seizure and multi-organ dysfunction. Neither the clinical presentation nor specialized biochemical assays distinguish between the 12 causative PEX genes. Overall, ZS spectrum has an estimated incidence of 1 in 50,000 births. For decades, clinicians have been suspecting an increased incidence of ZS in French-Canadians of the Saguenay-Lac-St-Jean region (SLSJ), located in the northern-eastern part of province of Quebec. This has remained unproven until now and molecular basis of these ZS cases remained unsolved. Increased incidence related to founder effect has been described for several disorders in SLSJ, including Tyrosinemia type I. Therefore, we hypothesized that if an increased incidence of ZS is indeed present in SLSJ, it is likely due to a founder effect and a low allelic complexity should be expected. We first reviewed the suspected ZS cases diagnosed in SLSJ between 1990 and 2010. During that period, 5 cases of ZS were suspected based on clinical presentation with additional laboratory supportive evidence, either plasma very long chain fatty acid level or liver biopsy. All cases were of French-Canadian descent, presented in the neonatal period with severe hypotonia, and deceased within the first year of life (0.5 months - 6 months). Sanger sequencing was then performed in one patient for known human PEX genes (13), and coding and flanking intronic sequences were analyzed for sequence variations. Among these, a homozygous mutation (c.802_815del) was found in the exon 1 of *PEX6*, which has been previously described in ZS. DNA samples of the 4 remaining patients (if available) and their parents were obtained and PEX6 exon 1 was sequenced. All patients were homozygous for the c.802_815del mutation. Parental heterozygous state was confirmed for all cases. Using these definitive ZS patients, we estimated the incidence in SLSJ to 1 case per 12,191 live births, with calculated carrier frequency of 1 in 55. In conclusion, we showed evidence of founder effect for ZS in the French-Canadian of SLSJ by the identification of a single causal mutation within *PEX6*. To our knowledge, this is the highest reported incidence of ZS worldwide.

1332F

Single nucleotide polymorphisms in the promoter region of ADIPOQ are associated with adiponectin level measured at first trimester in pregnant women. *M-F. Hivert[1], M. Lacroix[1], M. Doyon[1], M-C. Battista[1], P. Perron[1], D. Brisson[2], L. Bouchard[1].* 1) Université de Sherbrooke, Sherbrooke, QC., Canada; 2) Université de Montréal, Montréal, Qc., Canada.

Purpose: Adiponectin is exclusively produced by adipocytes and is suspected to act as an insulin sensitizer. In pregnant women, low circulating adiponectin levels at 1st trimester have been associated with increase risk of developing gestational diabetes mellitus (GDM). Expression of the gene encoding for adiponectin (ADIPOQ) is decrease in adipose tissue of pregnant women developing GDM, suggesting that adiponectin could be involved in insulin resistance pathways leading to GDM. A few genetic variants located in or around ADIPOQ have been associated with adiponectin levels in non-pregnant women, but it is unknown whether those variants are also associated with circulating adiponectin concentration in the pregnant state. Methods: This study includes 2 cohorts of pregnant women recruited in Sherbrooke (n=167) and in Chicoutimi (n =179), Québec, Canada. Women were recruited at 1st trimester and followed over the course of their pregnancy. Anthropometric data were measured with standardized procedures. Blood was collected at 1st trimester to assess adiponectin levels (RIA;Millipore). DNA was extracted from circulating white blood cells; single nucleotide polymorphisms (SNPs) were genotyped on a RT-PCR 7500Fast using TaqMan probes (Applied Biosystems Inc). Regression models were performed using additive genetic models to predict adiponectin level (log-transformed). Results: In Sherbrooke, mean age of the participants was 28.4±4.4 years old, mean BMI at 1st trimester was 25.7±5.9 kg/m^2, and mean adiponectin level was 8.7±6.0 µg/mL. In Chicoutimi, mean age was 28.5±3.9 years old, mean BMI at 1st trimester was 25.4±5.4 kg/m^2 and mean adiponectin level was 13.3±5.1 µg/mL. Three SNPs previously known to be involved in regulatory regions of ADIPOQ were genotyped: rs17300539 and rs822387 in the promoter (both in strong LD), and rs6773957 in the 3'UTR. In the promoter region, presence of the minor allele (A) at rs17300539 was associated with higher level of adiponectin at first trimester (p=0.02 when pooling both cohorts). Similar results were obtained for minor allele at rs822387 (p= 0.02). The SNP located in the 3'UTR was not significantly associated with adiponectin levels. Conclusion: SNPs located in the promoter region of ADIPOQ are associated with adiponectin levels at first trimester in pregnant women from European descent. Future studies will help to determine whether those variants influence adiponectin levels later during pregnancy and development of GDM.

1333F

Significant association of ADRB2 Glu27Gln polymorphism with triglyceride level in Tongans. *I. Naka[1], R. Kimura[2], N. Nishida[3], T. Inaoka[4], Y. Matsumura[5], J. Ohashi[1].* 1) Doctoral Program in Biomedical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Ibaraki, Japan; 2) Transdisciplinary Research Organization for Subtropics and Island Studies, University of the Ryukyus, Okinawa, Japan; 3) Research Center for Hepatitis and Immunology, International Medical Center of Japan Konodai Hospital, Ichikawa, Chiba, Japan; 4) Department of Environmental Sociology, Faculty of Agriculture, Saga University, Saga, Japan; 5) Faculty of Health Care, Kiryu University, Gunma, Japan.

To investigate whether the) 2 adrenergic receptor gene (*ADRB2*) polymorphisms play a role in the regulation of lipid mobilization, energy expenditure and glycogen breakdown, the associations of non-synonymous single nucleotide polymorphisms (SNPs) in the coding region of *ADRB2*, Gly16Arg (rs1042713) and Glu27Gln (rs1042714), and in the 5' leader cistron, 5'LC-Arg19Cys (rs1042711), with total-cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride levels were examined in 142 adult subjects in Tonga. A multiple regression analysis adjusted for age, sex, and body mass index (BMI) revealed that a copy of 27Glu significantly increased serum triglyceride by 0.866 mmol/l (P-value = 0.0379). No other significant associations were observed. Although the detected association of 27Glu with triglyceride level was not highly significant, the present result is consistent with previous studies in various ethnic groups (Ishiyama-Shigemoto et al., 1999; Ehrenborg et al., 2000; Iwamoto et al., 2001; Macho-Azcarate et al., 2002; Isaza et al., 2005; Yoshida et al., 2009). Our results suggest that *ADRB2* Glu27Gln polymorphism is associated with serum triglyceride level independent of BMI in Tongans.

1334F

Genetic polymorphism of -1297 T/C on Interleukin-18 may predispose gallstone patients to cholecystectomy. *H. Yang[1], S. Shih[2,3], T. Chang[1], H. Wang[2,3], K. Hu[2,3], C. Chang[2,3], C. Chang[2,3], C. Hung[2,3], H. Chan[1], M. Lin[1], Y. Lee[1,4,5].* 1) Medical Research, Mackay Memorial Hospital, Taipei, Taiwan; 2) Department of Internal Medicine, Mackay Memorial Hospital, Taipei, Taiwan; 3) Mackay Medicine, Nursing and Management College, Taipei, Taiwan; 4) Department of Pediatrics, Mackay Memorial Hospital, Taipei, Taiwan; 5) Department of Pediatrics, Taipei Medical University, Taipei, Taiwan.

Individuals with gallstones are often detected in health check by ultrasonography and most of them remain asymptomatic. These asymptomatic ones will tune to symptomatic gallstone disease (GSD) with acute, severe, intermittent abdominal pain, or other complications in 1 to 2% per year. GSD is a complex disease resulting from interaction between genetic and environmental risk factors. During past decades, the incidence and prevalence of GSD have increased in Taiwan. Its overall prevalence was 5% including asymptomatic and symptomatic subjects in a community-based study. IL18 is proinflammatory and able to induce production of INF-. from activated T lymphocytes and natural killer cells and secretion of proinflammatory cytokines. IL18 induces INF-. production in the presence of interleukin-12 but Th2 signaling in an IL4 dependent manner. Genetic mapping of lithogenic susceptible genes also contain immune-related factors including INF-. . The amount of INF-. in the liver correlates with IL18 suggests a critical role of IL18 in Th1 mediated intrahepatic immune responses. Therefore, IL18 may involve in gallstone induced inflammation response by regulating Th1 or Th2 signaling. A number of single nucleotide polymorphisms (SNPs) in the promoter region of IL18 gene are associated with differential levels of gene transcription, protein production, and diseases. For example, -607 C/A and -1297 T/C SNPs can influence transcription factor binding and have an impact on IL18 gene activity in autoimmune diseases. Some of these polymorphisms have been investigated in several autoimmune, inflammatory or metabolic syndromes. The present study was to examine whether specific IL18 gene SNPs, including -1297 T/C and -607 C/A and their haplotypes were associated with GSD.In conclusion, we found an association between IL18 -1297 T/C SNP and cholecystectomy in patients with GSD. Genotype T/T and allele T conferred a risk of cholecystectomy, however, carrier C and haplotype AC rendered protection against this complication. We propose that polymorphisms of IL18 might promote inflammatory response in a gallbladder stones leading to severe inflammatory responses.

1335F

A prediction model for gout using genetic polymorphisms of ten loci associated with serum levels of uric acid. *A. Taniguchi[1], W. Urano[1], E. Inoue[1], C. Sekita[1], Y. Koseki[1], N. Ichikawa[1], N. Kamatani[2], H. Yamanaka[1].* 1) Inst Rheumatology, Tokyo Women's Medical Univ, Tokyo, Japan; 2) Laboratory for Statistical Analysis, Center for Genomic Medicine, Institute of Physical and Chemical Research.

Objective: Gout is the most common disorder with inflammatory arthritis. Sustained hyperuricemia introduced crystallization of monosodium urate crystals within joints and eventual development of gouty arthritis. Gout is a multifactorial disease that is caused by the simultaneous action of multiple genetic and environmental factors. Recent genome-wide association studies using Caucasian or Japanese samples have provided evidence that polymorphisms on several genes were associated with serum levels of uric acid and/or the development of gout. In the present study, we validated the associations of these 11 genes using Japanese patients with gout and attempted to construct a prediction model for gout using genetic polymorphisms and environmental factors. Patients and Methods: Subjects were Japanese male patients with gout (n = 153) and male controls (n = 532). The genotypes of 12 polymorphisms on the ten genes, PDZK1, GCKR, ABCG2, LRRC16A, LRP2, SLC2A9, SLC16A9, SLC17A1, SLC22A11, and SLC22A12, were determined using the TaqMan method (Applied Biosystems, Foster city, CA) and allele frequency of each polymorphism was compared between gout patients and controls. Then, each risk allele was allocated weighted score from 0 to 3 according to P value and odds ratio. Genotype score of individual was calculated by summing the weighted scores. A prediction model for gout using C statistics was constructed by incorporating genotype score of the 12 polymorphisms, adjusted for age, body mass index, serum levels of triglyceride, and eGFR. Results: Nine loci showed P value less than 0.05, and 5 of these 9 loci showed significant association by the Bonferroni correction. The minimum genotype score was 7 in gout, and the maximum was 22. The genotype score was significantly higher among patients with gout (16.4±2.3) than those of controls (14.6±2.4) (P<0.0001). The OR of gout increased accordingly across the genotype score. When we compared the OR of gout between the individuals with the lowest score and those with highest score, 27-fold increased risk of developing gout was shown. The C statistic using the weighted genotype score was 0.78, indicating that models including genetic and non-genetic factors may be useful to predict gout. Conclusion: Our data suggest that a model incorporate the weighted genotype scores and environmental factors is useful for prediction of gout in Japanese males.

1336F

Genome wide expression profiling in the Smith-Lemli-Opitz syndrome (SLOS). *R. Steiner[1], J.-B. Roullet[1], C. Wassif[2], F.D. Porter[2], Q. Yang[1], S. Impey[1].* 1) Pediatrics, Oregon Hlth Sci Univ, Portland, OR; 2) Eunice Kennedy Shriver NICHD, Bethesda, MD.

Background: SLOS is caused by inactivating mutations of the DHCR7 gene leading to cholesterol deficiency. It is characterized by a broad phenotypic spectrum typically including congenital malformations and mental retardation. SLOS pathophysiology remains poorly understood and there is no effective treatment. Objectives: to characterize the molecular changes caused by DHCR7 deficiency. Specifically, to obtain baseline gene expression profiles in affected and non-affected cells. Methods: Genome wide expression profiling (RNA-Seq) was performed using skin fibroblasts isolated from a patient with severe SLOS and neural stem cells (NSC) isolated from Dhcr7-/- mice (E18). Results: The SLOS fibroblast gene expression profile showed down regulation of genes involved in mitochondrial function, calcium homeostasis, cytoskeleton, lysosomal function, signaling (caveolin1, Na+/K+ ATPase), folate metabolism and defense against oxidative stress. Genes related to apoptosis, ubiquitination and extracellular matrix synthesis were upregulated. Such expression profile was not observed in Dhcr7-/- NSCs. In these cells, stem-cell marker nestin and cell-replication genes were down-regulated whereas genes implicated in synaptic transmission, signaling, and cell differentiation were upregulated, suggesting abnormal NSC-to-neuron differentiation in vivo. Conclusion: Cell biology is profoundly altered in SLOS. Future studies with cell lines from other patients and from NSC-derived neurons are needed to confirm and expand these findings.

1337F

Surface plasmon resonance - mass spectrometry to identify novel binding partners in intracellular vitamin B_{12} metabolism. *M. Plesa[1,2], M.A. Hancock[3], J.C. Deme[1], D. Watkins[2], D.S. Rosenblatt[2], J.W. Coulton[1].* 1) Microbiology and Immunology, McGill University, Montreal, Quebec, Canada; 2) Department of Human Genetics; 3) Sheldon Biotechnology Centre.

Human MMACHC and MMADHC are soluble proteins involved in the intracellular metabolism of vitamin B_{12}. They are required to generate essential cofactors necessary for activity of two key enzymes: methionine synthase and methylmalonyl CoA mutase. Defects in the genes encoding these proteins result in the *cblC* and *cblD* disorders. Recently, we predicted and validated specific interactions between MMACHC and MMADHC. To identify other potential MMACHC binding partners, we have combined classical fractionation with surface plasmon resonance (SPR) and mass spectrometry (MS). Compared to traditional ligand fishing approaches that include yeast two hybrid and co-immunoprecipitations, SPR-MS offers increased accuracy, sensitivity, and efficiency. Soluble fibroblast lysates were separated by size exclusion chromatography and the individual fractions screened for binding against MMACHC that was immobilized as purified bait on SPR surfaces. Positive hits, species that may be previously unrecognized binding partners, were further fractionated by ion-exchange chromatography and then re-screened for MMACHC-binding activity by SPR. Optimization of the interface between SPR for microrecovery of bound unknowns and MS facilitates identification of novel MMACHC binding partners. Ultimately, we anticipate that SPR-MS directs identification of novel therapeutic candidates for rare, inborn errors of vitamin B_{12} metabolism.

1338F

A Genome Wide Association Study Identifies 31 Genetic Loci Associated with Human Serum Metabolites. *J. Kettunen[1,2], T. Tukiainen[1,3,4,5], A.P. Sarin[1,2], A. Ortega-Alonso[6], E. Tikkanen[1,2], L.P. Lyytikäinen[7], A.J. Kangas[5], P. Soininen[5,8], P. Würtz[1,3,5], K. Silander[1,2], D.M. Dick[9], R.J. Rose[10,11], M.J. Savolainen[12], J. Viikari[13], M. Kähönen[14], T. Lehtimäki[7], N.B. Freimer[15], M.I. McCarthy[16,17], A. Jula[2], J. Eriksson[18,19,20,21], O.T. Raitakari[22], V. Salomaa[2], J. Kaprio[1,23,24], M.R. Järvelin[3,25,26], L. Peltonen[1], M. Perola[1,2,27], M. Ala-Korpela[5,8,12], A. Palotie[1,28,29,30].* 1) Institute for Molecular Medicine Finland, Helsinki, Finland; 2) Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland; 3) Department of Epidemiology and Biostatistics, Imperial College London, London, United Kingdom; 4) Department of Biomedical Engineering and Computational Science, School of Science, Aalto University, Finland; 5) Computational Medicine Research Group, Institute of Clinical Medicine, University of Oulu and Biocenter Oulu, Oulu, Finland; 6) Department of Physiology, Finnish Twin Cohort Study, Department of Public Health, University of Helsinki, Helsinki, Finland; 7) Department of Clinical Chemistry, University of Tampere and Tampere University Hospital, Tampere, Finland; 8) NMR Metabonomics Laboratory, Department of Biosciences, University of Eastern Finland, Kuopio, Finland; 9) Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, Virginia, USA; 10) Department of Psychology, University of Helsinki, Finland; 11) Department of Psychological and Brain Sciences, Indiana University, Bloomington, USA; 12) Department of Internal Medicine, Clinical Research Center, University of Oulu, Oulu, Finland; 13) Department of Medicine, University of Turku and Turku Iniversity Hospital, Turku, Finland; 14) Department of Clinical Physiology, University of Tampere and Tampere University Hospital, Tampere, Finland; 15) Center for Neurobehavioral Genetics, Semel Institute for Neuroscience and Human Behavior, University of California Los Angeles, Los Angeles, CA, USA; 16) Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, UK; 17) Wellcome Trust Centre for Human Genetics, University of Oxford, Headington, Oxford, UK; 18) Unit of Chronic Disease Epidemiology and Prevention, National Institute for Health and Welfare, Finland; 19) Department of General Practice and Primary Health Care, University of Helsinki, Finland; 20) Helsinki University Central Hospital, Unit of General Practice, Helsinki, Finland; 21) Folkhälsan Research Centre, Helsinki, Finland; 22) Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku and Department of Clinical Physiology, University of Turku and Turku University Hospital, Turku, Finland; 23) Department of Mental Health and Alcohol Abuse Services, National Institute for Health and Welfare, Helsinki, Finland; 24) Department of Public Health, University of Helsinki, Helsinki, Finland; 25) MRC Health Protection Agency (HPA) Centre for Environment and Health, Imperial College London, London UK; 26) Institute of Health Sciences, University of Oulu, Oulu, Finland; 27) Estonian Genome Centre, University of Tartu, Tartu, Estonia; 28) Department of Human Genetics, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK; 29) Department of Medical Genetics, University of Helsinki and the Helsinki University Hospital, Helsinki, Finland; 30) The Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA.

Over a hundred genetic loci have been associated with metabolic traits such as lipids, glucose and diagnostic biomarkers. In this study, we estimate the heritabilities in 657 twin pairs of circulating levels of 216 metabolic traits identified using serum NMR metabonomic screening techniques. We also test for associations between the metabonomic measurements and 7.7 million genetic markers, directly genotyped or imputed using 1000 genomes reference panel, in 8330 randomly ascertained Finnish individuals from five population cohorts. Our genome wide association analysis reveals 33 independent genomic markers from 31 genetic loci associated with one or more metabolites using a stringent level of statistical significance ($p < 2.31*10^{-10}$). To our knowledge, 15 of the loci have not been associated with metabolic traits before. Our novel findings include association with albumin locus and various lipoprotein measures, four other lipid or lipoprotein associations, an association with citrate ion and nine associations with different amino acids, including six variants associating with amino acids recently linked with risk of developing type 2 diabetes. Using an independent cohort, we find that these 33 variants together explain up to 40% of the genetic variance in an individual metabolic measure. We show that both genetic and phenotypic fine mapping lead to substantial increases in statistical power and bring us in close proximity with the metabolic processes modified by these loci. Thus, our study elucidates novel genetic information and pathways in human metabolism, underpinning genetic influence of metabolic biomarkers informative for cardiovascular disease and diabetes risk assessment.

1339F

Long-term outcome and intervention of urea cycle disorders in Japan. *J. Kido[1], K. Nakamura[1], H. Mitsubuchi[1], T. Ohura[2], M. Takayanagi[3], M. Matsuo[4], M. Yoshino[5], Y. Shigematsu[6], T. Yorifuji[7], M. Kasahara[8], R. Horikawa[9], F. Endo[1].* 1) Department of Pediatrics, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan; 2) Division of Pediatrics, Sendai City Hospital, Sendai, Japan; 3) Division of Emergency and General Medicine, Chiba Children's Hospital, Chiba, Japan; 4) Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan; 5) Department of Pediatrics and Child Health, Kurume University School of Medicine, Kurume, Japan; 6) School of Nursing, Faculty of Medical Sciences, University of Fukui, Fukui 910-1193, Japan; 7) Division of Pediatric Endocrinology and Metabolism, Osaka City General Hospital, Osaka, Japan; 8) Department of Transplantation Surgery, National Center for Child Health and Development, Tokyo, Japan; 9) Department of Endocrinology and Metabolism, National Center for Child Health and Development, Tokyo, Japan.

Urea cycle disorders (UCDs) are one of the most frequently inherited metabolic diseases in Japan, with an estimated prevalence of 1 per 50,000 live births. Here, we investigated the clinical manifestations and prognosis of 177 patients with UCDs evaluated and treated from January 1999 to March 2009 to study more effective treatment and prognosis in patients. These included 75 cases of neonatal-onset UCDs and 91 cases of late-onset UCDs. The most common disorder in UCDs was ornithine transcarbamylase deficiency (OTCD), which accounted for 116 out of 177 patients. This result is similar to a previous study performed between 1978 and 1995 in Japan: OTCD accounted for about two thirds of the total number of UCD cases. We studied the relationship between prognosis and the peak blood ammonia level at the onset in 151 UCD patients. Compared with a previous survey conducted in Japan, we found that more patients whose peak blood ammonia levels were greater than 360 μmol/l survived without any mental retardation. The 5-year survival rate of patients with OTCD improved to 64% for those with the neonatal-onset type and to 91% for those with the late-onset type. We hypothesize that the increased survival is due to earlier diagnosis and better treatments that are now available in Japan. It is very important to diagnose and treat UCDs, especially OTCD, when the blood ammonia levels in patients are low. The outcome in patients with low blood ammonia levels was better than in patients with high blood ammonia levels.

1340F

Knockdown of upstream stimulatory factor 1 leads to a beneficial metabolic profile in mice. *P-P. Laurila[1,2], J. Soronen[2], E. Kaiharju[2], C. Ehnholm[2], V. Olkkonen[3], M. Jauhiainen[2], L. Peltonen[1,2,4].* 1) Dept of Medical Genetics, University of Helsinki, Helsinki, Finland; 2) Public Health Genomics Unit, National Institute for Health and Welfare, Helsinki, Finland; 3) Minerva Research Institute, University of Helsinki, Helsinki, Finland; 4) The Wellcome Trust Sanger Institute, Cambridge, UK.

Metabolic derangements in the body are tightly linked with cardiovascular disease. We have earlier established the association between genetic variants of USF1 with familial combined hyperlipidemia and its component traits, TG and cholesterol in Finnish families. In the current study we have conducted a detailed analysis of the effects of USF1 on metabolic parameters using both in vivo and in vitro models. We generated a congenic strain of Usf1 knockout (-/-) (11 males, 29 females) mice which along with their +/- and +/+ (27 M, 31 F) littermates were fed with Western diet rich in TG and cholesterol for 5 months. The Usf1 -/- mice displayed both elevated cholesterol ($p<0.001$) and phospholipids in their plasma. In human hepatoma cells (HuH7), using USF1 targeting siRNAs, we showed that APOA1 transcription and secretion were elevated in USF1 silenced cells in line with the animal model. USF1 overexpression also inhibited the activity of a 2000 bp APOA1 promoter-linked luciferase ($p<0.05$), raising the possibility of APOA1 being a direct target gene of USF1. The Usf1 -/- mice had lower plasma total and VLDL TG as compared to their +/+ littermates, which is due to increased lipolysis. This was evidenced by -/- mice having higher activity and expression ($p<0.01$) of lipoprotein lipase in the liver, muscle, and adipose tissue which was studied using the Affymetrix microarrays. We observed that despite having increased food consumption and being physically less active than +/+ mice, the Usf1 -/- mice were actually protected from obesity during Western diet and also showed an increased sensitivity to insulin during insulin tolerance test. Expression analysis from their adipose tissue revealed an increased expression of complexes of the mitochondrial respiratory chain ($p<0.001$) which was evidenced by both increased VO2 and VCO2, suggesting a higher overall metabolic rate for the -/- mice. These changes were not observed at an early age, but after 5 months of Western diet. Our study establishes the critical role of USF1 at the crossroads between metabolic and cardiovascular homeostasis. Since the loss-of-function of USF1 caused a favorable blood lipid phenotype and protection from obesity during Western diet, we hypothesize that its knockdown effect in appropriate tissues has favorable cardiovascular and metabolic effects.

1341F

Heterozygous mutation in the X chromosomal NDUFA1 gene in an oligosymptomatic 5-year old girl with complex I deficiency. *O. Bodamer[1], J. Mayr[2], T.B. Haack[3,4], F. Zimmermann[2], H. Prokisch[3,4], C. Rauscher[2], J. Koch[2], W. Sperl[2].* 1) Dept Human Genetics, Univ Miami Miller Sch Medicine, Miami, FL; 2) Dept.Pediatrics, Paracelsus Medical University, Salzburg, Austria; 3) Institute of Human Genetics, Helmholtz Zentrum Munich, Germany; 4) Institute of Human Genetics, Technical University Munich, Germany.

Background: Respiratory chain enzymes consist of multiple subunits encoded either by the mitochondrial or by the nuclear genome. Recently the first X-chromosomal mutations in complex I deficient males have been described. Heterozygous female carriers did not appear to be clinically affected. **Case report:** Here, we report a now 5 year-old girl with age appropriate psychomotor development and essentially normal muscle function. Her past medical history was unremarkable with the exception of recurrent episodes of vomiting and somnolence during episodes of inter-current infections. She is the only child of non-consangineous Caucasian parents. The father suffers from diabetes mellitus type 1 of juvenile onset, the mother is reportedly healthy. Her initial work-up at 11 months of age revealed a moderate lactic acidosis (3-4 mmol/L; norm: < 2.7 mmol/L) and a markedly increased betahydroxybutyrate/acetoacetate (BOH/AA) ratio (7.9; norm: < 2.6) following a 12 hour fast. **Biochemical and genetic investigations:** Biochemical investigation of a muscle biopsy at 15 months of age revealed a deficiency in complex I activity in fresh, unfrozen mitochondria (complex I /citrate synthase activities 0.05; norm: 0.14-0.35). Mutation screening of all structural subunits of complex I identified a heterozygous mutation c.94G>C, p.Gly32Arg in the X-chromosomal NDUFA1 gene. Analysis of the cDNA showed that 72 % of the expressed mRNA was mutated in the muscle biopsy sample. Investigation of the X-inactivation pattern demonstrated that 74 % of the paternally inherited allele was active in the muscle. The mutation was not detectable in either maternal or paternal blood. **Summary and conclusions:** Mutations in X-chromosomal mitochondrial genes pose a particular diagnostic challenge in oligo-symptomatic females. The clinical phenotype may be atypical and sometimes misleading in affected females due to tissue specific X-chromosomal inactivation and expression of proteins. In fact to the best of our knowledge our report is the first of a X-chromosomally inherited respiratory chain defect in a heterozygous female.

1342F

Circulating Collections: Ethics and the Institutional and Social Locations of Resources for Specimen-Based Human Genome Research. *J. Radin[1], J. Brown[2].* 1) History and Sociology of Science, University of Pennsylvania, Philadelphia, PA; 2) Anthropology, University of Pennsylvania, Philadelphia, PA.

This poster identifies the diverse kinds of collections available for genomic research, the similarly diverse ethical concerns that condition their use, and visualizes the paths that these collections trace as they circulate through different institutional contexts.

With the refinement and cost reduction of genomic techniques an increasingly diffuse and diverse array of human biomaterials are now available for analysis. These include ancient materials, those accumulated decades ago, and those being collected today. However, the context of origin of these specimens may mitigate or constrain the use of such materials as they circulate through different institutional locations. Commentators concerned with the appropriate use of human specimens in the context of genomic research often fail to distinguish between samples collected for forensic, biomedical, anthropological, or other purposes. They also frequently overlook the relative age of collections. At the same time, the kinds of questions that contemporary researchers are asking often blend one or more category. For example, biological anthropologists have begun to ask questions about chronic and infectious disease and some in medical genetics have become interested in forensics.

With this poster, we provide a resource for parsing the varied ethical and regulatory requirements that affect human tissues dependent on both the circumstances of their collection and the institutional location of their subsequent genomic interpretation.

1343F

Freedom to directly access to genetic tests on internet: a part of the right to innovation? *E. Rial-Sebbag[1], H. Howard[2], P. Borry[3], A. Cambon-Thomsen[1].* 1) UMR U 1027, Inserm, Université de Toulouse - Université Paul Sabatier -Toulouse III Faculté de médecine. 37 allées Jules Guesde F-31073 Toulouse Cedex 7 France; 2) Institute of Biomedical Ethics, University of Basel, Switzerland; 3) Centre for Biomedical Ethics and Law, University of Leuven, Belgium & Department of Clinical Genetics and Medical Humanities, VU University Medical Centre Amsterdam, The Netherlands.

The ethical, legal and social literature on direct-to-consumer genetic tests (DTC-GT) traditionally emphasizes the protection of individuals against risks, which are often related to the lack of information delivered by the companies selling them. Less has been written regarding the application of the principle of freedom of people in accessing innovations (DTC-GT can be considered as innovations). The right to freedom has been described in several international human rights texts and traditionally applies to rights such as freedom of movement or of expression. Nevertheless freedom is translated in health practice through the implementation of two different principles: autonomy of people to gather information on their health and right to benefit from new innovations. The later is emerging from various social claims (European Charter of Patients' Rights Active Citizenship Network. Rome, November 2002) and is implicitly referred to by European legislations and jurisprudence. This new right would be, on the one hand, limited by States `duties such as the promotion of a high level of protection for health (which implies a control of this new practice), but on the other hand, will have to be built on the current legislation (freedom and autonomy would be used in support of a court decision). Public policies, as well as courts, will have to challenge this new right in the near future. We will then analyse this new claim together with the European legal framework within the context of DTCGT in order to: 1/Assess the possibility of the emergence of a new fundamental right: the right to innovation, 2/ Evaluate the consequences of such a new right, in particular with regards to the construction of a pro-active right while regulation is currently intended to restrict freedom in the name of public health argument.

1344F

A research activity for ethical, legal, and social implications of personal genomics in Japan. *T. Shirai[1], J. Minari[1], A. Nomura[1], K. Kato[1,2,3].* 1) Inst. Res. Humanities, Kyoto University, Kyoto, Japan; 2) Grad. Sch. Biostudies, Kyoto University, Kyoto, Japan; 3) Inst. Integrated Cell-Mat. Sci., Kyoto University, Kyoto, Japan.

In 2010, a large-scale project, “Support for Genome Research” started in Japan. This 5 years' project is funded by the Ministry of Education, Culture, Sports, Science and Technology (MEXT). The aim is to comprehensively support the genome researchers funded by MEXT, and to advance and promote genome research in Japan. The project has 3 support programs; large-scale genome data production program, medical genome science program, and bioinformatics analysis program. These programs perform sequencing and/or data analysis for genome researchers who are selected from among applicants.

Our unit, “Research Unit for the ELSI of Genomics” is a part of the project. The unit's vision is to establish research governance in genome research. Here, “research governance” refers to a mechanism in which scientific research is conducted within the social context based on decision making by researchers and other people from different fields. Under this vision, we cooperate with genome researchers and tackle with ethical, legal, and social implications (ELSIs) of genome research.

The range of their implications has expanded with dramatic developments of genome research, including issues regarding the use of human samples and a new generation sequencer, and other matters about DTC-genetic testing, synthetic biology stem-cell research and public outreach. Among these topics, we are currently focusing on issues associated with personal genome research, since this is the highest priority for one in the medical genome science program of the project.

For this, we have mainly conducted 2 activities. One is preparing and revising a model informed consent document which is used in medical genome science program. This activity contains not only making a document but also making a policy of the program. During this process, we researched and considered various issues such as data sharing, biobanking, return of results, and use of previously collected samples, cooperating with scientists and medical researchers of the program. The other is the creation of a diagram of issues on using the human samples for personal genome researches. For making the diagram, we listed the issues, and categorized them into 5 groups; informed consent, handling of data and samples, IRB, return of results, and working environment of researchers. We also made summarized reports having more detailed information about each issue.

1345F

"My46:" an innovative web-based tool for management of results return from exome and whole genome sequencing studies. *H.K. Tabor[1, 2], A. Hartzler[2], J. Crouch[1], E. Kuwana[2], M.J. McMillin[2, 3], J. Stock[3], J. Yu[2], K.M. Dent[4], N. Anderson[5, 6], J. Swanson[7, 8], M. Bamshad[2, 9].* 1) Ctr Pediatric Bioethics, Seattle Children's Hosp, Seattle, WA; 2) Department of Pediatrics, University of Washington, Seattle, WA; 3) Seattle Children's Hospital, Seattle, WA; 4) Department of Pediatrics, University of Utah, Salt Lake City, UT; 5) Department of Biomedical Informatics, University of Washington, Seattle, WA; 6) Institute of Translational Health Sciences, University of Washington, Seattle, WA; 7) National Children's Study Orange County Vanguard Center, Irvine, CA; 8) Department of Pediatrics, University of California, Irvine, CA; 9) Department of Genome Sciences, University of Washington, Seattle, WA.

Exome sequencing (ES) and whole genome sequencing (WGS) are powerful new tools for gene discovery that are rapidly and broadly being applied in human genetics research. ES/WGS have the potential to identify all variants that influence health-related traits, including those of clinical and/or personal utility, thereby challenging existing approaches for return of results. As a feasibility study for the National Children's Study, we are developing a web-based interactive tool that allows research participants to manage return of results from WGS. "My46" has four key modules: 1) a "learn the basics" section about the genome, including an overview of the type of results that might be returned and their potential impact, 2) a tool for selecting preferences for return of results, 3) a platform for reporting results and 4) an instrument for conducting surveys. The educational module is targeted toward participants with limited knowledge of genetics, genomics and/or genetic testing. The preferences tool allows participants to choose which broad categories of results they do or do not want to receive: the disease(s) studied, carrier status for recessive disorders, complex health-related traits, and medication responses. Participants receive available results only for traits in the categories they have selected and can modify their preferences at any time. The results reporting module provides a succinct summary of the utility of each result that can be shared with health care providers. The survey module facilitates the conduct of empirical research, assessing correlations with participant result preferences, impact of results on a psychological and health care utilization outcomes, and participant understanding. Each module can be customized to fit researchers' specific needs and the population being studied. "My46" has several important features for large-scale results management in ES/WGS studies. It provides participants secure access to their individual genetic results to review at their convenience. The design is scalable allowing results to be returned as they become available over time. "My46" also includes a feature that facilitates direct interaction with a genetic counselor via telephone or live streaming video, extending the role of counselors as the amount of genetic information available to research participants increases. These features make "My46" an attractive tool for managing the return of individual research results in ES/WGS studies.

1346F

Genetic research with descendants of Australia's First Peoples. *S. van Holst Pellekaan, A. Wilton.* Biotechnology and Biomolecular Sciences D26, University of New South Wales, Sydney, NSW, Australia.

Over nineteen years, with substantial ongoing consultation and discussion, genetic research has proceeded slowly with a small number of Aboriginal communities in western New South Wales, Australia. Participants are descendants of the Paakintji (Barkindji), Ngiyambaa and associated language groups, whose recent history has followed a familiar pattern of colonization marked by dispossession and sad experience. Initially genetic research was ancestry focused, the results of which augment a rich archaeological heritage, marking cause for celebration of survival and resilience. In the current era of genomic research the opportunity for a health and wellbeing focus with the communities has begun with their ongoing consent, but some issues remain unresolved, constraining the ability to extend into other communities. Poor research practices of the past left a legacy of distrust that obstructs useful dialogue between researchers and Aboriginal Australians. The reasons for distrust are understandable and in the case of genetic research, touch on issues of identity, privacy and the perceived threat to `Aboriginality'. Control over their own research is desirable but is difficult to implement for some projects, especially without adequate funding to provide training or employment. The use of information for community purposes, the sharing of data with other researchers and a perceived distinction between ancestry related and health related genetic research are among the concerns not currently resolved. For example, we are currently unable to share raw data files from a genome wide SNP study with other researchers, limiting the value of published results. The continued resistance to genetic research by many Aboriginal Australian groups has resulted in a paucity of information about the genetic diversity in Australia compared to other studied populations. While the reasons for resistance can be understood, there is a risk that this will lead to increased disadvantage for Aboriginal Australians. Results from genome wide studies in other populations will be used as references to inform future health policies, strategies and treatments, but important information from the Australians will be excluded unless their collaboration in more genetic projects eventuates. There is much still to be learned about how genetic diversity interacts with different environmental and lifestyle conditions.

1347F

Feasibility of informed consent for whole genome sequencing in the National Children's Study: participant understanding and informational needs. *J. Yu[1], H.K. Tabor[1,2], K. Lakes[3,4], P. Flodman[3,4], K. Gutzman[5,6], B. Specker[5,6], J. Swanson[3,4], M.J. Bamshad[1,7].* 1) Department of Pediatrics, University of Washington, Seattle, WA; 2) Treuman Katz Center for Pediatric Bioethics, Seattle Children's Hospital, Seattle, WA; 3) Department of Pediatrics, University of California, Irvine, CA; 4) National Children's Study Orange County Vanguard Center, Irvine, CA; 5) Ethel Austin Martin Program in Human Nutrition, South Dakota State University, Brookings, SD; 6) National Children's Study South Dakota State University Study Center, Brookings, SD; 7) Department of Genome Sciences, University of Washington, Seattle, WA.

Whole genome sequencing (WGS) and exome sequencing (ES) are transformative new tools for gene discovery in human and medical genetics research. These technologies challenge existing models for informed consent because of the volume of sequence data generated and the number of potentially clinically significant results identified. There are no empirical data on how best to explain to participants during the informed consent process the nature of WGS/ES, the kinds of results that might be identified, the potential benefits and risks of those results, or the potential risks to privacy of sharing WGS/ES data. Therefore, researchers, IRBs, and policy makers have little guidance on how to approach informed consent for WGS/ES research. We developed and tested an informed consent form and protocol for use in a feasibility study for WGS in the National Children's Study, a multisite population-based longitudinal birth cohort study of children and their parents. The informed consent included statements describing WGS, the large volume of data generated by WGS, the identifiability of whole genome data, the indefinite storage of samples and data, and NIH data sharing requirements. It also included an option to be re-contacted about possible future results. A total of 100 parent-child trios are being recruited into the WGS study and will participate in the informed consent process. Parents' subjective and objective understandings of the research protocol are assessed using a modified version of the Quality of Informed Consent (QuIC), a standardized tool for evaluating the effectiveness of the informed consent process. This tool assesses parents' knowledge of information presented in the consent process; their self-perceived understanding of the information presented; and their report of factors that facilitate or impede WGS research participation. We will present the results from the pilot phase of this study and share examples of the language that were used to meet the standards for informed consent. Moreover, the results from this first test of our WGS consent will be useful for the development and implementation of informed consent for other WGS/ES studies.

1348F

Evaluating the Status of State-Level Public Health Genetics Activities in the United States: A Decade of Declining Public Health Genetics Leadership. *A.K. Yu, S.M. Au.* Hawaii Department of Health Genetics Program, Honolulu, HI.

In 2010, the Hawai`i Department of Health Genetics Program did a survey to evaluate state-level public health genetics (PHG) activities in the United States. The survey was modeled after a pilot study conducted by the Coalition of State Genetics Coordinators (SGCs) in 2000. Similar in scope, the 2010 SGC survey aimed to collect and disseminate information about state-level PHG activities, with results to be used to help shape future education, policy, research and funding decisions. SGCs from each of the 50 states, the District of Columbia, Puerto Rico, Guam and the US Virgin Islands were identified by phone/email and then invited to participate. As of May 2011, 89% (n=48) of the SGCs had completed the survey. Each survey was done by phone interview with results entered into an online database (SurveyMonkey). Prior to data analysis, each SGC was sent a summary of their responses to review for inaccuracies. Responses were then de-identified and coded into SPSS for analysis. Survey data revealed that SGCs had dissimilar training/education. While 54% of SGCs report having some graduate level genetics education or higher, ~21% of SGCs report having no formal genetics education at all. Comparing the workforces, the number of full-time (≥ 35hrs/wk) SGCs has decreased from 53% to 29% since 2000, with the majority of the part-time SGCs fulfilling other responsibilities, such as newborn bloodspot/hearing screening coordinator. The number of SGCs working <10hrs/wk has also increased from 34% to 52%. As well, 29% of SGCs report having no standalone PHG program. With increased opportunities and ongoing efforts over the past decade to integrate genomics into public health research and practice worldwide, it would be reasonable to hypothesize that the level of PHG activities in the US should be increased. However, the survey data appears to present a contrasting picture. The data affirms the conclusion that the level of support for PHG activities has decreased greatly since 2000 and appears hindered by disappearing state PHG infrastructures. This includes a lack of strong and consistent leadership at the SGC level; a lack of dedicated funding and a lack of resources to ensure appropriate genetics education/training of state PHG staff. We conclude that without an improvement in these areas, the US will encounter a “translational bottleneck”, preventing the successful integration of promising genetic discoveries into public health activities.

1349F

Parental Attitudes Towards Participation in a Pediatric Genetic Repository. *S.I. Ziniel[1,2], J.G. Amatruda[3], E.D. Harris[3], C.M. Clinton[3], S.K. Savage[4], N.L. Huntington[1,5], R.C. Green[6,7], I.A. Holm[1,3,8].* 1) Department of Pediatrics, Harvard Medical School, Boston, MA; 2) Clinical Research Program, Children's Hospital Boston, Boston, MA; 3) Division of Genetics and Program in Genomics, Children's Hospital Boston, Boston, MA; 4) Children's Hospital Informatics Program, Children's Hospital Boston, MA; 5) Division of General Pediatrics, Children's Hospital Boston, Boston, MA; 6) Partners Center for Personalized Genetic Medicine, Boston, MA; 7) Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 8) The Manton Center for Orphan Disease Research, Children's Hospital Boston, Boston, MA.

Objective: Little is known about factors that influence parental interest in enrolling themselves and their children in a pediatric genetic repository. Children's Hospital Boston (CHB) has initiated a large pediatric genetic repository, The Gene Partnership, in which individual research results will be returned to participants. The goal of this study was to better understand the views of parents of children at CHB towards participation in such a genetic repository. Here we report on factors that influenced parental enrollment in the hypothetical genetic research repository. **Methods:** We designed a survey to assess the interest of parent in enrolling themselves and their children in a hypothetical genetic research repository, and their preferences for return of return of research results on themselves and their children. The survey was mailed to a sample of 6872 parents of patients at CHB. **Results:** Preliminary analysis of data on over 800 respondents showed that 77% of respondents would enroll themselves in the genetic registry and about 75% would enroll at least one of their children. 85% of parents were concordant with their choice to enroll or not enroll themselves and their child. Ninety-eight percent of parents with 2 or more children would make the same choice with regard to participation in a pediatric genetic repository across all of their children. Using logistic regression, parents who were more likely to sign themselves up in a hypothetical genetic registry were older, female, white, of higher income, and had reached a lower education level. They were more likely to have participated in research in the past, have a child with a genetic condition, feel that their participation would contribute to society, and trust that their confidentially would be maintained. Hierarchical logistic regression models showed that the same factors predicted if a parent would enroll their child in a hypothetical genetic registry. Factors that were not important in whether a parent enrolled themselves or their child included prior genetic testing, and anticipated benefits from the research. **Conclusions:** Characteristics of the parents' child did not influence whether or not a parent would enroll their child in a genetic registry. Parents with a positive view of research were overall more likely to participate in research.

1350F

Consistently inconsistent - DTC results of identical twins from three companies and implications for service provision. *K. Barlow-Stewart[1,2], C. Cormick[3].* 1) Ctr for Genetics Education, Sydney, NSW, Australia; 2) Sydney Medical School, University of Sydney, Sydney, NSW, Australia; 3) Public Awareness and Community Engagement, Department of Innovation, Industry, Science and Research, Canberra, ACT, Australia.

With the $1,000 full genome testing imminent, it is useful to examine what has been learned from the experience of direct to consumer (DTC) genetic testing and how consumers and health professionals have reacted to these services. Consumers in Australia have access to DTC genetic testing from several overseas companies, whose laboratories are based in the US (23andMe and Navigenics) and Iceland (DeCODEme). Anecdotal evidence suggests that some consumers who are early adopters of DTC are sending their DNA out of the country to be tested but are then bringing the masses of data generated by these genome wide scans to health professionals including genetics service providers or their primary care providers in Australia for assistance with interpretation and advice, while others prefer to rely on the interpretation provided by the companies alone. Vital to both these approaches is the veracity of the data. Two 50-year-old twin brothers undertook DTC testing with the three companies to allow a comparison not only of the differences in interpretation between the DNA data by the different companies, but how the DNA of two different individuals with near-identical DNA was interpreted by the same company. Through a diary of reactions that was kept before,during and after the testing process, key findings relate to how the data was interpreted by each individual, similarities and differences, and how the inconsistent between company (but within company consistent) results impacted upon levels of trust in genetic testing in general. A key question related to behavioural changes that might be made based on interpretations of data, and how different interpretations affected this. A complimentary study was conducted with clinical geneticists and genetic counsellors in New South Wales (the most populous Australian State) regarding the use of DTC testing over the last two years by current or new patients; possible reasons for its use; relevance and informativeness of the results; and how many primary care providers or other health professionals had sought advice where their patients had had DTC testing. The aim was to gain insight from both the user and clinician end, and examine if there were gaps between how each approached the DTC process and data obtained.

1351F

An overview of the genetic testing offer in Europe. *M. Jovanovic[1], E. Dequeker[2], A. Mondelaers[2], M. Morris[2], J. Cassiman[2], S. Aymé[1].* 1) Orphanet, INSERM SC11, 96 rue Didot, 75014 Paris, France; 2) EuroGentest, Gasthuisberg O&N, Herestraat 49, Box 602, 3000 Leuven, Belgium.

Genetic tests are now offered internationally, through both public and private sector genetic testing services. Physicians prescribing these tests and biologists receiving the samples need to know which tests are available, where they are performed and whether identified laboratories meet quality standards.

To fulfill this need, http://www.orpha.net was launched fourteen years ago to set up a database of medical laboratories in the field of rare diseases. Data was collected in 1 country in 1997, 15 in 2003, 26 in 2006 and 36 in 2011, with resources from the EC DG Public Health. In collaboration with the EuroGentest Network of Excellence, information on quality management has been added to the Orphanet database over the past five years.

Information on genetic testing in Orphanet can be searched by disease name or by gene (symbol or name in English) as well as by laboratory or by professional. The information provided on laboratories includes data on quality management. Information is freely accessible online, and access to all data can be granted upon request. Currently, 1,049 laboratories offering tests for 1,764 genes are registered. The test offer differs greatly from one large country to another: Germany (1,400 genes), France (1,070 genes), Spain (947 genes), Netherlands (763 genes), Italy (753 genes), United-Kingdom (507 genes). The test offer in medium and small-sized countries ranges from 1 to 344 genes.

This situation explains the large cross-border flow of specimens, highlighting the need to provide access to services in other countries when necessary, especially for very rare diseases. According to available data, only testing for Cystic fibrosis is provided by every country. The distribution of this test offer will be presented.

1352F

The UK Genetic Testing Network, its role and achievements. *M. Kroese, J. Westwood, P. Farndon, J. Deller, S. Stenhouse, S. Mohammed, F. Stewart, J. Hoyle, T. Turtiainen, UK Genetic Testing Network.* UK Genetic Testing Network National Specialised Commissioning Team 2nd floor, Southside 105 Victoria Street London SW1E 6QT.

This session describes the UK Genetic Testing Network (UKGTN) organisation and the `Gene Dossier' genetic test evaluation process. A brief review of the UKGTN genetic test evaluation experience and the development of testing criteria will be presented. In addition, examples of UKGTN policy developments and other outputs will be described. The Department of Health (England) supported the establishment of the UKGTN in 2002. A fundamental principle underpinning the UKGTN is that of geographical equity of access for patients and their families who require genetics advice, diagnosis and management via local clinical genetics centres. It advises the National Health Service (NHS) on genetic testing for inherited disorders. It aims to ensure the provision of high quality equitable genetic testing services for all NHS patients across the whole of the UK. This involves evaluating new genetics tests through its Gene Dossier process and making recommendations to commissioners on new NHS clinical genetics services. The UKGTN also provides an advisory role to the Department of Health on national policies relevant to clinical genetics services. The UKGTN covers a range of activities which are summarised below: • Assessing applications from laboratories to become members. All member laboratories must fulfil defined criteria including quality criteria. • Evaluates new tests for scientific validity and clinical utility that member laboratories wish to provide on a national basis and makes recommendations to NHS commissioners. • Inform national policies for clinical genetics services • Provides an information source including the online and open access database of national testing services that member laboratories provide. The UKGTN has completed a significant work programme during 2004-2010 including 269 dossiers evaluated, 203 new genetic tests recommended for NHS provision and 216 testing criteria developed. The testing criteria are a unique feature of the Gene Dossier process, as they identify the key features of the disorder and aim to ensure clinically appropriate genetic testing. From a healthcare purchaser perspective testing criteria provide assurance on the clinically appropriate use of diagnostic resources.

1353F

Guidelines for evaluating genetic associations for use in direct-to-consumer personal genetic analysis. *S. Wu, G.M. Benton, J.Y. Tung, A.B. Chowdry, J.L. Mountain, B.T. Naughton.* 23andMe, Inc., Mountain View, CA.

Crowd-sourced, free, and online tools mingle alongside commercial and non-profit ventures now offering genetic testing and analysis to individuals. Groups that offer information about how genetics may influence health and physical traits base their services on the scientific literature, primarily genome-wide association studies (GWAS), but how they evaluate and report on this literature can vary. The standards for evidence used by different groups can range from very permissive to quite stringent, balancing different needs for validity, stability, flexibility, scalability, and relevancy to the state of research. 23andMe has developed and evolved a robust set of guidelines for evaluating genetic associations reported in the literature. These guidelines account for sources of error and bias -- including confirmation bias, multiple hypothesis testing, population stratification, and type I error -- by considering parameters such as sample size, existence of replications, effect size, and correction for multiple hypotheses. In addition, the guidelines address broader issues raised by reporting on research primarily focused on populations of European descent and on associations with different levels of evidence to a diverse consumer audience. We describe lessons learned from several years of evaluating genetic associations in the context of connecting individuals to their genetic information.

1354F

Paternalism vs. Autonomy: Who should be the gatekeeper for genomic testing results? *S. Adam[1], P.H. Birch[1], A. Townsend[2], Z. Lohn[1], F. Rousseau[3], J.M. Friedman[1].* 1) Med Gen, C & WH, University of British Columbia, Vancouver, BC, Canada; 2) Centre for Applied Ethics, University of British Columbia, Vancouver, BC, Canada; 3) Medical Biology, Université Laval, Montreal, PQ, Canada.

Clinical testing with Whole Genome Sequencing (WGS) may uncover unexpected genetic changes with important medical or social implications. No clinical guidelines have been proposed for disclosure/nondisclosure of these incidental findings thus the need for stakeholders to discuss these issues is paramount. The experience and knowledge of genetics professionals and the general public regarding WGS are important, diverse and complementary. We therefore conducted two focus groups, one with 10 genetics professionals (geneticists, genetic counsellors and laboratory personnel), and a second with 10 members of the public (from diverse educational backgrounds, with little knowledge of WGS). Qualitative analysis of the transcripts has yielded important and surprising themes. Genetics professionals focussed on the type of results that might arise and felt that the certainty, complexity, and reliability of the data should all play a role in the disclosure decision. They were keen to find ways to be blind to incidental findings so as to have less to disclose. Much of this related to beneficence and their concerns about the risk of creating the “worried well.” In contrast, the lay group was united in feeling that everything the patient wished to know should be disclosed. They expressed this as an issue of autonomy: “...an element of control that I don't want anybody having other than me.” Not everyone felt that they would want the information but they agreed that it should be personal choice. There was inter-group agreement that pre- and post-test counselling and discussion was critical: Lay individuals discussed the need for educational material but geneticists also considered practical issues such as limited human and financial resources. In addition, both groups identified issues around family members, confidentiality and discrimination as important and possibly problematic. The genetics group appeared willing to use these and other issues such as insurance considerations and the likelihood of patient actionability as reasons to limit the incidental findings communicated to patients. The public on the other hand, perceived this as paternalistic and felt strongly that it was their fundamental right to have access to that information. Our findings suggest that the public values autonomy above all else whereas genetics professionals are concerned that this approach has potential for harm. Wider consultation and study are needed to reconcile this dichotomy.

1355F

Are U.S. Doctors Receiving DTC Genetic Tests Results from Their Patients? *V.L. Bonham[1], C. Clark[1], L.A. Cooper[2], W.G. Feero[3,4].* 1) Social and Behavioral Research Branch, NHGRI/NIH, Bethesda, MD; 2) Johns Hopkins University School of Medicine, Department of Medicine, Baltimore, MD; 3) Maine Dartmouth Family Medicine Residency, Augusta, ME; 4) Genomic Healthcare Branch, NHGRI/NIH, Bethesda Maryland.

Background: Since 2005, the number of health-related direct-to-consumer (DTC) genetic tests has increased dramatically. Little is known about how often United States primary care physicians (PCPs) receive DTC genetic test reports and use the results in clinical care. **Methods:** We analyzed data from The Health Professionals' Genetics Education Needs Exploration Survey(HPGENE), a national survey of 787 general internists fielded from April to December 2010 (45% response rate), to examine the frequency with which PCPs received genetic test results in the preceding year, characteristics of PCPs who received these results, the extent to which DTC results were incorporated into clinical care, and the association of physicians' participatory decision-making (PDM) style with receipt of DTC results. **Results:** Of the 787 physicians that responded to the survey 505 were men and 479 were older than 45 years of age. Over half of the PCPs that received DTC test results worked in group or staff model practices (33%) and academic health centers (22%). Nineteen percent of respondents reported receiving DTC genetic test results from at least one patient in the preceding year. A higher proportion of female than male physicians reported seeing patients who presented DTC genetic test reports (24% vs. 16%, $p<0.05$). Physicians who had genetics training in residency (31%) and those who rated their knowledge of genetics as excellent, very good, or good (24%) were more likely to receive test results than physicians who had no training in residency (17%) and those who reported fair or poor knowledge (15 %), both p-values <0.01. Among physicians who received genetic test results, 59% discussed the results with the patients, 46% reviewed and placed the results in the patients' medical records, 14% ordered additional tests, 10% incorporated the results into their treatment plans, and 7% referred the patient to a genetic specialist. Physicians' PDM style score was not associated with their use of patients' DTC test results in clinical care. **Conclusions:** A substantial minority of general internists are receiving patients' DTC genetic test results. Physician gender, practice setting, genetics training during residency, and knowledge of genetics are positively associated with receiving genetic test results. Importantly, physicians are incorporating this data into the medical record and clinical decision making.

1356F

Dutch difficulties with carrier status information: 25 years of health policy. *M.C. Cornel[1,2], S. Jans[1,2], C.G. van El[1,2], P. Lakeman[1], L. Henneman[1,2], G. de Wert[2,3].* 1) Clinical Genetics/EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, Netherlands; 2) Center for Society and Genomics, Nijmegen, Netherlands; 3) Department of Health, Ethics and Society, Maastricht University, Maastricht, The Netherlands.

Introduction More than two decades ago the Dutch government started asking advice on carrier screening for autosomal recessive disorders, such as hemoglobinopathies (HbP) and cystic fibrosis (CF). So far, carrier screening is not offered in regular Dutch health care, unlike many other countries. The neonatal screening program from 2007 onwards does generate carrier status information for hundreds of families each year, a challenge to physicians not used to this. We investigated the reasons for Dutch reluctance in carrier screening policy. *Methods* We performed a socio-technical analysis (2007) based on previous pilot studies, a Witness seminar (2010), reconstructing recent history with actors involved in these last decades, and surveyed midwives and general practitioners (2010). *Results* Our findings in 2007 showed that technical (im)possibilities and the lack of facilities and services played a role, especially the absence of a preconceptional health care setting and the deficiency of genetic knowledge among health care professionals. The target group of parents-to-be was unaware and no support from government and public health organisations existed. Yet societal acceptability was likely. The witness seminar showed that carrier screening in general never appeared high on the policy agenda. A requested advisory report on HbP screening showed a low prevalence, and indicated fear of stigma and low awareness among the target group as reasons not to implement HbP screening. The advice was followed by the ministry, but debated by professionals who argued both for neonatal screening and carrier screening. The registration of ethnicity in health care (to allow for targeted carrier screening) remained sensitive, more than 50 years after the persecution of ethnic minorities in the Second World War. The involvement of representatives of ethnic minority target groups is very important for further policy making on screening for HbP. Health care workers see the professional standards of their colleagues as relevant: they would not offer carrier testing if their colleagues do not offer it either. Furthermore, they experience the lack of support from health authorities as an obstacle. *The future* Complexities related to carrier status information remain a challenge in Dutch health care. It remains to be seeen whether carrier screening will be considered a valuable complementary strategy in the Netherlands.

1357F

Building Genomics Infrastructure in Africa: Ethical and Policy Implications. *E. Peprah, C. Rotimi.* Center for Research on Genomics and Global Health National Human Genome Research Institute, National Institutes of Health Bethesda, MD.

Evidence supporting the potential for genomics to inform the health and history of African people is growing. This growing potential has brought the need for genomics research in Africa to the forefront. As suggested by others, we also believe that genomics has the potential to significantly contribute to the scientific development of the continent if the existing global inequity in the application of genomic science is reduced or eliminated. Encouragingly, several new initiatives are starting to address the gap in genomics research in Africa. These initiatives including the Malaria Genomic Epidemiology Network, the Africa America Diabetes Mellitus Study, the Africa TB Genetics Consortium, Armauer Hansen Research Institute which are characterizing the genetics of infectious and noninfectious disease in African populations. Plant and animal genomics are being investigated at multiple research centers in Africa including the International Livestock Research Institute in Kenya. These ongoing initiatives are funded by several different entities including the Wellcome Trust, Swedish International Development Cooperation Agency, the National Institutes of Health (NIH), World Health Organization (WHO) and non-governmental organizations. In addition, major new genomic initiatives have been funded and are in the process of implementation. These including Human Heredity and Health in Africa co-funded by the NIH and the Wellcome Trust, the Grand Challenges of Canada, the WHO Research Catalyst Platform and the Southern African Human Genome Project Initiative; these initiatives promise to build genomic research capacity in Africa to enable African scientists to become major players in genomic research. As genomics research grows in Africa, there is pressing need to address the local cultural, ethical and legal issues that are likely to be unique to African populations in the design, implementation, analysis and interpretation of results. For example, it has been noted that the existing international policies on sample and data sharing may not necessarily be adequate for developing scientists who are not likely to have the robust research infrastructure. Other issues include how to conduct culturally sensitive community engagements. This presentation will discuss the strategies that have worked and those that have not. It will also offer new insights into some of the issues that have arisen from genomic/genetic studies in African populations.

1358F

Thailand national plan for prevention and care of birth defects. *P. Wasant.* Dept Ped/Div Med Gen, Siriraj Hosp/Mahidol Univ, Bangkok, Thailand.

Introduction: Birth defects are common and important public health problems causing health and economical burden to patients and their families including government long - term budget. Presently, there is no clear - cut public health policy in care and prevention of newborns/children with birth defects in Thailand; while there had been increased awareness in several developed countries for many years. As for developing countries e.g. Philippines and Malaysia already started their Birth defects registry (BDR) and prevention program such as folate supplementation for pregnant women and women of child-bearing age in past couple of years. It is a well-known fact that Birth defects registry is proven and beneficial in setting up government health policies in order to decrease the incidence of birth defects and provide care and prevention for children with birth defects in a holistic manner. **Objectives:** Thailand Task Force on Prevention and care of Birth Defects & Disabilities is working to establish Birth Defects Registry (BDR) to help set up government health policies in order to decrease the incidence of birth defects and provide care and prevention for children with birth defects in a holistic manner. **Materials & Methods:** Thailand Birth Defects Registry (BDR) Working Group had initiated the action plan to develop the BDR including preventive measure and educational program since 2008. The Working Group consist of experts in at least 8 medical schools, had organized the Annual `National Forum on Birth defects and Disabilities' in July 2009, 2010 and 2011; to educate Thai physicians and public health personnels regarding birth defects and disabilities. The Working Group presently, “Thailand Task Force on Prevention and Care of Birth Defects & Disabilities” just received requested funding from Thailand Health Promotion Foundation (ThailHealth) for their goals to accomplish within 3 years (2011-2013). The workshops supported by Thailand Health Promotion Foundation (ThaiHealth) from July to September 2010 successfully produced 1. Strategic mapping 2. Master plan and 3. Care map for 5 chosen common birth defects (Down Syndrome, Neural Tube Defects, Cleft lip/palate, Duchenne Muscular Dystrophy) to start with. **Summary:** The Birth Defects Registry in Thailand is being developed.

1359F

The attitudes and opinions of genetic researchers and clinicians towards the regulation of direct-to-consumer genetic testing. *C.A. Wicklund, S.M. O'Neill, M.G. Hayes, P.M. Kaushik.* Northwestern University, Center for Genetic Medicine, Chicago, IL.

The availability of direct-to-consumer genetic testing has generated much debate in the last few years, in particular the tests available for common complex diseases, which are based on the results of genome-wide association studies. Clinical validity and the regulation of these tests by the FDA and CLIA have been key issues in this debate. To date, no studies have been carried out to assess the attitudes of genetic researchers and clinicians towards the current regulations. In addition, no studies have been performed to determine if there is a sense of concern or responsibility amongst researchers regarding their research data being used to develop these tests. The purpose of this study was to assess genetic researchers' and clinicians' attitudes towards regulation and to determine if concern/responsibility towards the use of their data exists. A survey was developed to assess attitudes towards these two topics and sent electronically to all members of the American Society of Human Genetics (~7000). The overall response rate was 23.3% (n=1631). Of the 1486 respondents who have been involved in research, 23.4% were concerned that their data was being used, 43.2% felt responsible for knowing how their data was being used, and 40.6% would restrict the use of their data if they could. Of the 1517 participants, the majority agreed or strongly agreed (93.6%) that the regulation of DTC genetic tests is important, and 51.9% disagreed or strongly disagreed that current regulations are sufficient. The majority agreed that the DTC genetic testing companies, the government, medical professionals, researchers, and the public should have an input in the development of regulation policies. The majority (67.7%) agreed or strongly agreed that the involvement of a healthcare provider should be required when using DTC genetic tests. Logistic regression analysis showed that clinical experience, involvement in GWAS, and gender were all independent predictors of participants' opinions towards regulation. Individuals without clinical experience, those who have been involved in GWAS, and male participants were less cautious about the regulation of DTC genetic tests. This study suggests that there is concern among some researchers regarding their data being used to develop DTC genetic tests. It also suggests that genetic researchers and clinicians consider current regulations to be insufficient, and would like to have an input in the development of regulation policies.

1360F

***"I'm still going to be me; I'm not going to be anyone else"* Firsthand accounts of predictive genetic testing in adolescents for adult onset conditions.** *C. Mand[1,2,3], L. Gillam[3,4,5], R.E. Duncan[2,3,6,7], M.B. Delatycki[2,3,8].* 1) Department of Medicine, Monash University, Melbourne, VIC, Australia; 2) The Bruce Lefroy Centre for Genetic Health Research, Royal Children's Hospital, Parkville, VIC, Australia; 3) Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, VIC Australia; 4) Children's Bioethics Centre, Royal Children's Hospital, Parkville, VIC, Australia; 5) Centre for Health and Society, University of Melbourne, Melbourne, VIC, Australia; 6) The Centre for Adolescent Health, Royal Children's Hospital, Parkville, VIC, Australia; 7) Department of Paediatrics, University of Melbourne, VIC, Australia; 8) Clinical Genetics, Austin Health, Melbourne, VIC, Australia.

Background: Technological advances have allowed asymptomatic individuals at risk for genetic disease to undergo predictive testing (PT) to discover if they will develop the condition later in life. PT is routinely offered to adults at risk for adult onset conditions (eg: Huntington disease). More controversial is the PT of minors where no effective treatment or prevention exists. The discourse concerning PT in minors is lively, yet remains largely speculative, with a scarcity of empirical evidence. While sporadic reports of PT in minors and the rise of the `mature minor' doctrine have led to greater flexibility in recommendations, most maintain a general position against testing in minors for conditions without effective medical intervention. Aim: To explore young peoples' experiences of undergoing PT for an adult onset condition, using a qualitative methodology Methods: Interviews with 9 young people who had, as minors, PT for adult onset conditions (3 neurogenetic; 6 familial cancers). Transcripts underwent thematic analysis. Results: A range of harms and benefits were reported in association with the entire PT experience, encompassing the period prior to requesting PT, through the preparative process, receiving a result and the aftermath. Harms were not limited to those who received a gene-positive result. Participants described harms associated with being refused a test as well as with the often protracted predictive testing process. Overall, participants indicated that these harms were significantly outweighed by the benefit they each had derived from PT. Regardless of result, all participants described benefits of PT, and in particular raised the difficulty of living with uncertainty prior to PT, and the relief which greater certainty, in the form of a PT result, brought them. Themes included the impact of living at risk, preconceived ideas about what the result would be, defeated expectations regarding the testing process, and the impact of a PT test. Conclusions: These data add to the breadth and complexity of the discourse concerning PT in minors highlighting factors previously underrepresented in the literature. For the first time, this study gives voice to the adolescents at the centre of the debate, inviting review of whether current guidelines have reached the correct balance. The data highlight the need for further study of outcomes of PT in minors to ensure guidelines are based on empirical evidence and not simply on theoretical analysis.

1361F

Patient's and health care professional's attitude regarding preimplantation genetic diagnosis in cancer genetics. *S. Côté[1,2], B.M. Knoppers[3,4,5], P. Hamet[1,6], C. Bouffard[7].* 1) Centre hospitalier de l'université de Montréal, Département de médecine, Service de médecine génique, Canada; 2) Département des sciences biomédiacles, Faculté de médecine, Université de Montréal, Montréal, Canada; 3) Centre de génomique et politiques, McGill University, Montréal, Canada; 4) Chaire de recherche du Canada en Droit et Médecine, Université McGill, Montréal, Canada; 5) Département de génétique humaine de l'Université McGill, Montréal, Canada; 6) Chaire de recherche du Canada, Génomique Prédictive, Université de Montréal, Montréal, Canada; 7) Service de génétique, Département de Pédiatrie, Faculté de Médecine et des Sciences de la santé de l'Université de Sherbrooke, Canada.

Introduction: An important motivation for patients when considering cancer genetic testing in familial cancer is to learn about children's risk. Despite this fact, there are important gaps regarding information about alternatives in familial planning, such as preimplantation genetic diagnosis (PGD). Method: Review and comparative analysis of the literature. Reviewed18 articles dealing with PGD in cancer genetics (PubMed, Ovid): 12 focused on patient's attitude toward PGD and 3 on health care professionals Results: These studies are not only rare, but their results show important differences. The percentage of patient's acceptability of PGD varies from study to study (90% to 30%). Studies investigating the attitude of health care professionals specialized in medical genetics show a threshold of acceptability around 80% concerning early-onset cancer genetic syndromes. In this study, the health care professionals attitude's toward acceptability drops to 13% when dealing with a late onset cancer genetic syndrome. Others studies, involving obstetrician/gynecologists conclude that the lack of knowledge about PGD is responsible for the rate of referral to genetics clinics. Conclusion: Carriers of mutations in cancer predisposition genes rarely choose PGD, perhaps because they not receive enough information about reproductive options. Current data from the literature is insufficient to clearly identify ethical, organizational and informational problems regarding PGD in familial cancer context.

1362F

Report from a patient support group for rare diseases in Japan: our experiences on 3.11 earthquake/tsunami disaster. *A. Nakaoka, J. Koizumi.* SORD (The Supporting Organization for Patients with Rare Diseases), Kyoto, Kyoto, Japan.

A non-profit organization SORD (the Supporting Organization for Patients with Rare Diseases) was launched in 2008 by a woman with rare disease and her supporters. Since only 344 diseases are specified as intractable diseases and funded by the government for research in Japan today, there was a need to establish an environment where rare disease research will be performed more actively. We have been working on 3 major tasks: 1.To support communication between patients and establishment of a patient organization with our social networking service and collect clinical data 2.To collect clinical data from overseas and coordinate the cooperation between Japanese patients and patients overseas 3.To establish a bank for genome information and iPS cells of rare diseases SORD has been connecting patients to patients in Japan, providing clinical information from overseas to them and providing the genome information and iPS cells of Japanese patients to researchers in the world. The Patient-driven iPS Genome Information Bank Project (PRiG Project) was launched in October, 2010. So far iPS cells of 3 cases have been established and the genome analysis completed. Our future plan is to provide the iPS cells and genome information to researchers who are willing to study them. On March 11th, 2011, massive earthquake and tsunami attacked Japan and inflicted damage we have never experienced in Japan before. Although SORD started to provide aid for the devastated patients right after the quake, we found it extremely difficult to reach and provide aid for them. Patients were isolated and left with inadequate care without even being recognized. Most of them have lost connections with support groups or other patients under such disaster. Furthermore the patients' current conditions have not been assessed adequately due to the absence of specialists and the insufficient disaster countermeasure, while they need doctors with high expertise and knowledge and the collaboration of clinicians in various areas. It seems necessary to reorganize the current support system for patients with rare diseases in Japan. Also it is our responsibility to share this tragic experience with other health professionals and patient support organizations worldwide to encourage the discussion about the disaster countermeasure which will not leave the patients alone. We would like to walk forward together with patients and researchers in the world and be there for patients to live a hopeful life.

1363F

Multidisciplinary analysis of emotions in the context of prenatal diagnosis of Down's syndrome and termination of pregnancy: the perspective of couples and health professionals concerned. *A.A. BROUSSIN DUCOS.* Ethique appliquée, Université de Sherbrooke, Sherbrooke, Québec, Canada.

The prenatal diagnosis(PND) is used to estimate the risk and confirm the presence of fetal anomalies. It offers couples the ability to:1- Anticipate the constraints, support necessary, sociocultural representations of the handicaps; 2-Evaluate the future sufferings. In the case of a PND of Down's syndrome(DS), the doctor announces the irreversible nature of the anomaly and associated handicaps. The couples are faced with the options to terminate or not the pregnancy. That's why, those faced with a PND of DS are confronted with negative emotions. If couples choose to abort, they and the Health professionals(HPs) are confronted with the symbolic violence of feticide which lead to psychological distress. PURPOSE:To identify,understand the emotional consequences induced among the couples/HPs concerned by the PND of DS and the termination of pregnancy(TP) in this context METHODE:A Literature review on the : 1-various aspects of the PND of DS; 2-emotions felt by the French, Canadian american couples/HPs concerned, allowed us to developed a conceptual grid analysis RESULTS: This analysis enables us to identify the emotions, their role and consequences on the well-being of couples/HPs concerned. The results can be viewed from various perspectives: A PND of DS and a TP generate various emotions,such as: guilt, shame, anger, hate, regret, fear, anguish, remorse, denial and sadness.An analysis of emotions allows the identification of psychological/physical damages caused by their over-expression Clinical:A categorization grids containing the emotions and the personalities who experience them should help psychologists to identify those who run a significant risk of developing psychological distress Sociopolitical:Acomparative analysis of the emotions experienced by couples/HPs from different countries demonstrates that the emotions,their intensity differ according to societal values and public health organization Ethical:The couples disinvest emotionally the fetus for decision-making. However, the feticide causes the emergence of hidden feelings, generates trauma, casts doubts on the well founded of the TP decision. Those affected grasp the importance to consider the emotions during the decision processes and the importance of the compassion and empathy CONCLUSION:The study of emotions can improve care for those affected by these PND/TP and provide intellectual input/support for the creation of codes of conduct and health policies in the context of PND of DS.

1364F

Diagnostic Misconception Underlies Some Preferences for Return of Genetic Research Results from a DNA Repository. *R. Green[1], S. Ziniel[2], N. Huntington[2], E. Harris[2], S. Savage[2], W. Wolf[2], J. Amatruda[2], I. Holm[2].* 1) Partners Center for Personalized Genetic Medicine, Division of Genetics, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 2) Division of Genetics, Children's Hospital Boston and Harvard Medical School, Boston, MA.

Background: "Therapeutic misconception" occurs when research subjects fail to appreciate distinctions between research using study medication and standard clinical treatment. In the controversial area of returning incidental research results to individuals who contribute to DNA repositories, hypothetical surveys consistently reveal that a majority of subjects prefer return of research results. In a survey exploring preferences for return of research results, we hypothesized that respondents who misconceive the personal utility of a DNA repository are more likely to prefer return of research results. **Methods**: Following initial focus groups, a 46 question survey asking about knowledge, attitudes and preferences for return of research results was constructed, refined through additional focus groups and mailed to 6872 parents of children registered as patients at Children's Hospital Boston. Some parents were previously consented to be part of the Gene Partnership (GP) DNA repository. **Results**: Of surveys sent, 941 (13.4%) were returned and used for preliminary analyses. Parents were 41.4 (SD 8.2) years of age, 87.5 % female, 74.4% White, 11.6% African American and 63.7% college graduates. When asked whether participating in a DNA research bank could "directly" help them or their children, 64.8% and 74.7% "Strongly" or "Somewhat" agreed with this misconception, respectively. When asked if they would want to receive genetic research results about themselves and their children, 85.6% responded "Definitely" or "Probably" about themselves and 88.7 % about their children. Persons who misconceived the personal utility of the repository for themselves were 2.3 times (95% CI 1.6,3.3, $p < 0.001$) more likely to prefer receiving genetic research results about themselves. Misconception about the utility of the repository for their children were 2.0 times more likely to prefer receiving results about their children (95% CI 1.4,3.3, $p < 0.001$). These associations were not significantly different between individuals who had been previously consented for GP and those who were not. **Conclusion**: These survey data support and extend impressions from our focus groups that some parents of pediatric patients conflate the research agenda of a DNA repository with hopes that there will be direct medical benefit for themselves or their children. This "diagnostic misconception" may help explain the broad appetite of biobank donors for return of individual genetic research results.

1365F

Genomic Medicine and Health Policy in Mexico: a future perspective in research and application. *P. Oliva-Sanchez, G. Saruwatari, J. Siqueiros, A. Arellano.* ELSI - Ctr, National Inst Genomic Med, Mexico, Mexico.

Objective. To explore the perspectives of Mexican health researchers on genomic medicine (GM) and its consequences in the decision-making process regarding the health policy in Mexico. The research took place at the 2nd National Conference of Genomic Medicine. This Conference was celebrated in Mexico City in October 25 - 27, 2006. Materials and Methods. A grounded theory qualitative research was conducted. Punctual interviews based on four questions were applied to collect data. Results. All researchers that were interviewed agreed on the idea that in developing countries there is no applicability of genomic medicine and that so far, GM is a basic and experimental science. Researchers didn't envision an impact of GM in public health in the near future. For its applicability, the interviewed said that it is necessary to generate a financial policy in order to support research towards defining the contribution of GM to the solution of the public health problems of our country. Some researchers believe that the 3P's of GM (Predictive, Personalized, Preventive) have generated "inflated expectations" according to the real benefits and its impact on public health. Opinions were divided among those who believed that GM is the new `paradigm' of public health and those who thought that GM is just part of a set of tools for public health, along with epidemiology and clinical trials. Discussion. To improve the health conditions of Mexicans, GM must come part of the government programs for human development and social protection. This, under the perspective that it will help to prioritize the National Health Spent. GM must be understood as a new tool in the resolution of the public health problems but we need a realistic view.

1366F

NETwork: A New Tool for Education and Empowering Participation in Translational Research. *S. Terry[1], K. Baxter[2], L. Horn[3], A. Krokosky[4], A. Mills[5], J. King[6], P. Terry[7].* 1) President & CEO, Genetic Alliance, Washington DC; 2) Translational Research & Policy Coordinator, Genetic Alliance, Washington DC; 3) Director of the Genetic Alliance BioBank, Genetic Alliance, Washington DC; 4) Assistant Director of Genetics Resources & Services, Washington DC; 5) Translational Research Intern, Genetic Alliance, Washington DC; 6) Biomedical Research Intern, Genetic Alliance, Washington DC; 7) Principal, Scientia Advisors, Cambridge, MA.

Models are important tools for making complex processes understandable. The current drug development model, which depicts drug development as a linear pipeline, grossly oversimplifies a nuanced process and cannot accurately represent the complexities of the real tasks involved. This oversimplification hinders, rather than helps, the translational research enterprise.

A more accurate depiction of the drug development process is needed, and Genetic Alliance has developed a wiring diagram describing the process as a complex system and not the traditional linear pipeline. This provides an alternative structure around which resources, both human and material, can coalesce, for greater participation in translational research. This wiring diagram has been converted into a web-based tool, Navigating the Ecosystem of Translational Research (NETwork). NETwork educates users about the drug development process and indicates key areas where stakeholders, including basic researchers, industry, advocacy leaders, and research participants, can meaningfully engage in translational research.

In creating NETwork, we have organized many tools that currently exist, including peer-reviewed publications, webinars, courses, partnership opportunities and more. The resources that have been included in NETwork come from a variety of organizations, including government organizations, academic institutions and non-profits, including advocacy organizations. We have also created tools as needed to fill gaps in the NETwork.

NETwork was first introduced to the community at the Genetic Alliance Annual Conference (June 2011). A number of organizations and individual stakeholders have also provided feedback on the concept, content, and design of NETwork. NETwork is continually updated to increase the number of available resources, keep the content current and relevant, and reflect feedback received from members of the community. NETwork is a framework for education and collaboration within the drug development process and the translational research enterprise. It is only by working together that we will see advances in translational medicine.

1367F

Assessment of a pharmacogenomic marker panel in a population taking multiple medications derived from electronic medical records. *M.T. Oetjens[1], J.C. Denny[2,3], A.R. Baker[1], H.H. Dilks[1], M.A. Basford[4], E. Bowton[4], N.A. Restrepo[1], V.M. Youngblood[1], J.M. Pulley[4], D.M. Roden[2, 4, 5], D.R. Masys[3], M.D. Ritchie[1,6], D.C. Crawford[1,6].* 1) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Department of Medicine, Vanderbilt University Nashville, TN; 3) Department of Biomedical Informatics, Vanderbilt University, Nashville, TN; 4) Office of Personalized Medicine, Vanderbilt University, Nashville, TN; 5) Department of Pharmacology, Vanderbilt University, Nashville, TN; 6) Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN.

Inter-individual variability in pharmacokinetics is thought to be a major contributor to variable drug response. Altered sensitivity to multiple drug classes can be expected given the pleiotropic effect of some pharmacogenomic variants. However, important genetic variants in absorption, distribution, metabolism, and excretion (ADME) genes are often rare (minor allele frequency or MAF <5%) or located in difficult-to-assay regions of the genome. We used the Illumina ADME Core Panel, a fixed content panel of 184 pharmacogenomic-related markers in 34 genes, to genotype DNA samples from 320 individuals on five or more classes of medications which have known pharmacogenomic indications (a total of 12 medications) given these individuals might be a potential target for pharmacogenomic panel utilization in a clinical setting. Samples were obtained from BioVU, the Vanderbilt repository linking DNA extracted from discarded blood samples to deidentified electronic medical records. All subjects were European American, 45% were female, and the mean body mass index was 29.7 kg/m2. After removing low quality samples (n=14), the call rates were 100% for the remaining samples. A total of 66 variants were monomorphic, 35 were very rare (MAF<0.0001), and *SLCO1B3* rs7311358 deviated from Hardy Weinberg Equilibrium (p<1x10-5). We abstracted allele frequencies from public repositories (such as the International HapMap Project and PharmGKB) as well as the literature for markers targeted by the ADME Core Panel to compare to this population exhibiting high polypharmacy rates. We tested for differences between allele frequencies in this clinic sample compared with reference sample allele frequencies. Preliminary results suggest that ~30% of ADME targeted markers do not have reference allele frequency data in HapMap or the literature. Out of 130 comparisons that could be made, one variant, *CYP2D6* rs1080985, was significantly different between our sample set and the reference frequencies (Pearson's chi-square; p=5.65x10-7). *CYP2D6* rs1080985 is in the promoter region of the gene, and this variant has been associated with variable response to the anti-Alzheimer drug donepezil. This pilot study suggests that ADME Core Panel performs well on DNA extracted from discarded blood samples from a clinic population. Further research is needed to understand the relationship between *CYP2D6* rs1080985 and the use of multiple medications.

1368F

Development of a High-Throughput Microdroplet-based Targeted Next-Generation Sequencing and its application to HLA and ADME gene panel sequencing. *J.A. Brayer, D.R. Link, J. Olson, L. Deming, A. Lennhoff, M. Weart, S. Kellett.* RainDance Technologies, Lexington, MA., Select a Country.

We describe the technical details of a new automation system, called the ThunderStorm, designed to perform PCR amplification of up to 20,000 reactions on each of 96 samples in a single 24-hour run. Samples are combined with PCR primer libraries and single molecule selection of specific regions of the genome is performed in picoliter droplets. To evaluate the performance of the ThunderStorm system, we evaluated a set of 16 HapMap samples against two defined content libraries (RainDance HLASeq™ and ADMESeq™ Extended Research Screening Panels). The HLA panel targets 3.8 MB of the HLA super locus and simultaneously amplifies >240 genes including all of the Class I, II and III cell surface antigen-presenting proteins. The ADME panel targets 241 genes involved with compound pharmacokinetics including CYP2D6, CYP2C19 and ERCC1. We also will discuss the relationship these two panels have in cancer research and immune response. In total 96 samples were processed, 16 samples x 2 libraries x 3 replicates per sample. All 96 samples were processed in a single run in less than 24 hours. The samples were barcoded and subsequently sequenced on an Illumina HiSeq2000. We will describe the uniformity and coverage of the sequence data across often difficult to sequence genes with significant homology. We will also describe the system's performance in detection known and novel variants across the genomic regions of interest.

1369F

Association of CYP2D6 polymorphisms and disease-free survival of Thai Breast cancer patients who received adjuvant tamoxifen. *M. Chamnanphol[1], T. Jantararoungtong[1], S. Santon[1], K. Pechatanan[2], E. Sirachainan[2], T. Sirisinha[2], T. Ativitavas[2], R. Panvichian[2], V. Ratanatharathorn[2], W. Chantratita[3], C. Sukasem[1].* 1) Laboratory for Pharmacogenomics and Personalized Medicine, Department of Pathology, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; 2) Division of Medical Oncology, Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; 3) Unit of Virology and Molecular Microbiology, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

Objective: To investigate the influence of CYP2D6 variant coverage on genotype-phenotype relationships and the disease-free survival (DFS) of Adjuvant tamoxifen efficacy in adjuvant treatment of breast cancer in Ramathibodi hospital.

Methods: We conducted a case-control study. Peripheral blood DNA samples from 24 patients who had disease recurrence during adjuvant tamoxifen treatment, and from 24 patients who had completed 5 years of tamoxifen without recurrence of breast cancer was genotyped using the AmpliChip CYP450 GeneChip®, Roche that facilitates the classification of individuals by test-ing of 33 alleles. Disease free survival and correlation with genotypes were analyzed by Kaplan-Meier method, and log-rank test.

Results: Median follow up time was 5.6 years. Forty-eight patients were evaluated for DFS. The mean age of the subjects was 50 ±11 years. 62.5% of patients had premenopausal status. The phenotypes frequencies of extensive metabolizer (EM) and intermediate metabolizer (IM) were 70.8% and 29.2 %, respectively.The 3 common allelic frequencies were 43.8% (*10), 36.5 (*1) and 10.4% (*2) .All alleles frequencies observed in this study were concordant with previous re-ports in Asian population. No association between CYP2D6 genotypes and DFS was demonstrat-ed. Nevertheless, an exploratory analysis of DFS in postmenopausal patients according to EM (13 of 34 patients) or IM (5 of 14 patients) phenotypes, the result showed statistically significant shorter DFS in IM phenotype patients (HR, 6.85; 95% CI, 1.48 -31.69; P =0.005). Furthermore, we observed statistically significant shorter DFS of homozygous CYP2D6*10 (3 of 10) when compared among heterozygous CYP2D6*10 (7 of 22) and the remaining genotypes (8 of 16) (P=0.005).We found shorter DFS of IM patients (5 of 14) than homozygous EM (7 of 12) and heterozygous EM patients (6 of 22) (P =0.016).

Conclusions: The CYP2D6*10 (reduced alleles) was a common SNP in our subjects. Postmeno-pausal patients with homozygous *10 or IM have shorter DFS. These group of patients may need genotype testing prior to treatment planning .Our findings do not indicate importance of broad CYP2D6 coverage for accurate phenotype prediction and maximum stratification between me-tabolized status.

1370F

Analysis of association between statin medication, genetic variation and prostate cancer outcomes. *R.J. Hamilton[1], S. Alanee[1], J. Vijai[1], D.J. Gallagher[2], I. Ostrovnaya[1], J. Bhatia[1], M.M. Gaudet[3], S. Fine[1], A. Dutra-Clarke[1], J. Przybylo[1], J.M. Rendleman[1], C. Savage[1], S. Lipkin[4], R.J. Klein[1], P.T. Scardino[1], H. Lilja[1], T. Kirchhoff[1], H.I. Scher[1], J.A. Eastham[1], K. Offit[1].* 1) Memorial Sloan-Kettering Cancer Center, New York, NY; 2) Mater University Hospital, Dublin, Ireland; 3) American Cancer Society, Atlanta, GA; 4) Weill Cornell School of Medicine, New York, NY.

BACKGROUND: The relationship between statin use and prostate cancer risk and outcome remains controversial. Genetic variation in cholesterol and statin metabolism pathways appears to modify the effect of statins on cholesterol and their protective association with some cancers. However, no studies to date have examined this pharmacogenetic interaction in the setting of prostate cancer. METHODS: Blood was collected for DNA extraction from 1491 men (775 Ashkenazi Jewish (AJ); 716 non-Jewish (NAJ)) treated for prostate cancer at Memorial Sloan-Kettering Cancer Center. Statin use at the time of diagnosis was recorded. Associations between statin use and biochemical recurrence, castration-resistant metastasis and prostate cancer-specific survival were analyzed using Cox proportional hazards models adjusted for age, year of diagnosis, PSA, Gleason grade, clinical stage, and treatment. Patients were genotyped for 42 single nucleotide polymorphisms (SNPs) in genes implicated in cholesterol synthesis or statin metabolism. We explored if genotype modified the association between statin use and clinical outcomes. RESULTS: In total, 252 (17%) men were taking a statin at diagnosis. Statin users were diagnosed more recently (median year: 2002 vs. 1998, p<0.001), at lower clinical stages (T1:55% vs. 44%, p=0.002), and with lower PSA (6.4 vs. 7.7 ng/mL, p=0.002). After adjusting for baseline features, statin use was associated with a reduced risk of biochemical recurrence (HR 0.67, 95% CI 0.50-0.88, p=0.004), metastases (HR 0.62, 95% CI 0.39- 0.99, p=0.05) and prostate cancer-specific death (HR 0.30, 95% CI 0.12-0.74, p=0.01). For preliminary genetic analyses we only examined interaction with the statin biochemical recurrence association. AJ and NAJ cohorts were analyzed separately. In the AJ cohort 3 SNPs significantly interacted with the statin-recurrence association at p<0.05, but the interaction was driven by a small number of statin users possessing the rare homozygote genotype. In the NAJ cohort, 2 SNPs in genes involved in statin excretion (ABCG2, SLCO1B1) were nominally significant at p=0.03. CONCLUSIONS: In this cohort, statin use was associated with improved outcomes after treatment, however SNPs involved in statin excretion had only weak modifying effects on the association with disease recurrence. Further study is on-going to explore the statin pathway for potential gene-exposure interactions.

1371F

Computational identification of non synonymous SNPs in human cytochrome P450 for prediction of population pk/pd of cyclophosphamide. *R.M. Labib[1], D. Yassin[2], M. Emam[3].* 1) Research, Children's Cancer Hospital, Giza, Egypt; 2) Clinical Pathology, Children's Cancer Hospital, Cairo, Egypt; 3) Faculty of Pharmacy, Beni Sweif University.

Background The potential pharmacogenetic effects of non synonymous single nucleotide polymorphism (nsSNPs) have long been overlooked. These nsSNPs can lead to amino acid changes that may cause altered drug response/toxicity. Cytochromes P450 (CYPs) are the key enzymes for activating and inactivating many drugs; individual expression levels of CYPs may play a crucial role in drug safety and drug efficacy. Genetic polymorphism in CYPs has been studied in different populations, yet studies on middle -eastern variants have not been widely studied. Cyclophosphamide(CPA), a chemotherapeutic agent used in many types of cancer, a prodrug that needs to be activated in vivo by CYPs. Wide inter-individual variations in Clinical response and toxicities to CPA were shown in different clinical studies. The aim of the present study is to search for presence of variants of CYP genes in Egyptian cancer patients, with a particular focus on their effect on population pharmacokinetic/ pharmacodynamic (pk/pd) pattern of CPA. The correlation between presence of such variants and pk/pd of CPA may aid as a potential tool for personalized drug dosing. Materials and Methods Blood samples from pediatric cancer patients were obtained from Children's Cancer Hospital. The pharmacokinetics of CPA was determined using HPLC-UV/Vis. The nsSNPs in all exons of the CYP genes analysed by RFLP and direct DNA sequencing. Population pk/pd will be analyzed using Nonmem® software. Computational tools will be used for the identification, haplotype analysis and prediction of functional effects of nsSNPs. To identify the potential pharmacogenetic effects of nsSNPs within cytochrome enzyme forming genes responsible for CPA activation and metabolism, all nsSNPs within the cytochrome P450 will be identified computationally. This will help in identifying molecular targets for drug treatment, and conducting individualized pharmacotherapy. Python scripts and established bioinformatics tools will be used to determine which of the nsSNPs in cytochrome P450 may result in a pk/pd variation. Results and Conclusion An on-line database and analysis tool will be created. The analysis tool will aid the discovery of the pharmacogenetic effects of these SNPs within genes associated with CPA activation. This prediction tool of nsSNPs in human CYP genes would be useful as a pharmacodiagnostic tool for the study of inter-individual variation in clinical response and drug toxicity.

1372F

Clinical Validation of Fragment Analysis to Genotype the Androgen Receptor VNTRs.. *A.B. Freeman[1], H. Hou[2], L. Shi[2], Q. Shu[2], B. Epps[2], B. Wilkinson[2], L. O'Brien[1].* 1) Eli Lilly and Company, Dept. of Translational Medicine, Indianapolis, IN 46285; 2) SeqWright, Inc., Houston, TX 77054.

The Androgen Receptor gene (AR, Chr X), encodes a transcription factor that is activated by androgens, such as testosterone, to regulate downstream gene expression. The activating domain in AR exon 1 contains a “CAG” and “GGC” variable number of tandem repeats (VNTR) resulting in glutamine (CAG) or glycine (GGC) repeat stretches in the receptor, respectively. Both VNTRs are known to be inversely associated with androgen activity and diseases such as osteoporosis and cancer. Additionally, fragment analysis (FA) genotyping of the CAG and GGC VNTRs in 268 HapMap DNAs showed that they were in linkage disequilibrium (p≤0.05), although having different allelic distributions, in normal African, Asian, and European populations. Therefore, we sought to validate the FA methodology to assess the AR VNTRs as clinical biomarkers in human patients.

Thirty-three (33) HapMap DNAs representing the most frequent CAG and GGC VNTR repeat alleles were blinded, amplified, and genotyped using FA. Clinical accuracy, precision, specificity, and technical variability were assessed for the FA methodology. Twenty (20) HapMap DNAs were subsequently sequenced to confirm the AR VNTR FA genotyping results.

We observed 100% concordance between past and present AR VNTR FA genotyping results in the HapMap samples. Unlike the GGC VNTR, the FA automated genotyping for the CAG VNTR was highly affected by technical variability, but easily corrected by adjusting the allele bin definitions. Ultimately, sequencing confirmed the FA genotyping results in 100% (20/20) and 95% (19/20) of HapMap samples for the CAG and GGC VNTR, respectively.

In one, random HapMap sample, the GGC VNTR FA and sequencing results conflicted, showing 21 and 20 repeats, respectively. Sequencing that sample revealed a “GGT” insertion 5' upstream of the GGC VNTR which mimicked a 21-repeat GGC amplicon size via FA when it actually contained only 20 GGC repeats. Additional genetic variability located within the AR GGC VNTR region with potential clinical relevance, but undetectable via FA, has also been reported (Bogaert, et al., 2009). Based on these results, we concluded that sequencing the AR GGC VNTR region is the most efficient and all-inclusive clinical genotyping methodology, while FA genotyping of the CAG VNTR region is clinically sufficient.

1373F

Phosphodiesterase (PDED4) gene polymorphism - association with the response to short acting bronchodilators in paediatric asthma patients. *M. Labuda[1], S. Laberge[2], J. Brière[1], D. Bérubé[2], P. Beaulieu[1], T. Pastinen[3], M. Krajinovic[1, 2, 4].* 1) Centre des Recherche, Ste_Justine Hospital, Montreal, PQ, Canada; 2) Departement of Paediatrics, University of Montreal; 3) Departments of Human and Medical Genetics, McGill University and Genome Quebec Innovation Centre; 4) Department of Pharmacology, University of Montreal, Montréal, Quebec, Canada.

Short-acting b2-adrenergic receptor agonists are commonly used as bronchodilators for symptom relief in asthmatics. Activated b2-adrenergic receptor leads to increased cAMP level, an essential bronchodilator. In turn, cAMP induces expression of phosphodiesterases (PDE), enzymes promoting cAMP degradation. The aim of this study was to test whether genetic variants in PDE4D gene affect the response to short acting b2-agonists. Bronchodilator responsiveness was assessed by % change in baseline forced expiratory volume in one second (FEV1) after administration of albuterol. FEV1 % change adjusted for baseline FEV1 values was significantly different between genotypes of PDE4D rs1544791 G/A polymorphism (P=0.006) and -1345 C/T (rs1504982) promoter variation (P=0.03) in patients with airway obstruction (n=93). The association remained significant after inclusion of appropriate covariates into multivariate model (p=0.004 and p=0.02, respectively). Our work identifies new genetic variants implicated in modulation of asthma treatment, one of them (rs1544791) previously associated with asthma phenotype.

1374F

Association analysis between *GIRK2* gene polymorphisms and post-operative analgesic requirements after painful cosmetic surgery. *D. Nishizawa[1], K. Fukuda[2], S. Kasai[1], W. Han[1], J. Hasegawa[1], A. Nishi[1], M. Koga[3], T. Arinami[3], M. Hayashida[4], K. Ikeda[1].* 1) Addictive Substances, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan; 2) Department of Oral Health and Clinical Science, Division of Dental Anesthesiology (Orofacial Pain Center/Suidoubashi Hospital), Tokyo Dental College, Tokyo, Japan; 3) Department of Medical Genetics, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Japan; 4) Department of Anesthesiology, Juntendo University, Tokyo, Japan.

Purpose: Opioids are commonly used as effective analgesics for the treatment of acute and chronic pain. However, considerable individual differences have been widely observed in sensitivity to opioid analgesics, which can lead to wide variability in the dose of opioid analgesics required to achieve adequate pain control. We focused on a G-protein-activated inwardly rectifying potassium (GIRK) channel subunit, GIRK2, that is an important molecule in opioid signal transmission, and examined genetic polymorphisms associated with opioid sensitivity. **Methods**: Subjects were 248 healthy patients who were scheduled to undergo cosmetic orthognathic surgery for mandibular prognathism at Tokyo Dental College Suidoubashi Hospital and provided informed, written consent for the genetics studies. Postoperative pain was managed with a bolus dose of 20 μg fentanyl on demand using a patient-controlled analgesia (PCA) pump when patients felt pain, with a lockout time set at 10 min. Postoperative PCA fentanyl use during the first 24-h postoperative period was recorded, whereas venous blood (10 ml) of the subjects was sampled for preparation of DNA specimens for genotyping with iScan System and the BeadChip Human1M v1.0 or Human1M-Duo v3 (Illumina K.K.). Association was explored by two-stage analysis between the analgesic requirement and single-nucleotide polymorphisms (SNPs) in the whole exon and intron region as well as 5'- and 3'-flanking regions (~10kbp) of the *GIRK2* gene. **Results**: In the first stage analysis, nine SNPs showed strong association with postoperative PCA fentanyl use (μg/kg) during the first 24-h postoperative period ($p<0.05$) among a total of 122 SNPs selected after the linkage disequilibrium analysis. In the second stage analysis, the association was identified to be significant in the rs2835859 SNP within the intron 3 after correction for multiple tests (combined $p=0.000237$). **Conclusion**: Although the mechanism underlying the difference in opioid sensitivity caused by the SNP needs to be clarified in future studies, the results indicate that this SNP could serve as a marker that predicts analgesic requirements. Our findings will provide valuable information for achieving satisfactory pain control and open new avenues for personalized pain treatment.

1375F

Optimizing Efavirenz Treatment by Detection of Pharmacogenetic Markers of CYP2B6 Corrected with Plasma Concentration in HIV-1 Infections. *C. SUKASEM[1], P. PRAPAITHONG[2], E. PASOMSUB[2], C. SRICHUNRUSAMI[1], TR. CRESSEY[3], T. JANTARAROUNGTONG[1], S. SANTHON[1], W. CHANTRATITA[2].* 1) Laboratory for Pharmacogenomics and Personalized Medicine, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; 2) Unit of Virology and Molecular Microbiology, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; 3) Program for HIV Prevention and Treatment (PHPT), Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand.

AIMS Pharmacogenomic studies are contributing to our understanding of interindividual differences in response to antiretroviral drugs. Recent studies have shown that a polymorphism in the CYP2B6 gene is associated with higher plasma Efavirenz (EFV) concentrations and increased central nervous system (CNS) side effects in Thai HIV-1 infections. METHODS We analyzed the genotypes and the allelic frequencies of CYP2B6 genetic polymorphism that are associated with EFV plasma concentration in a group of Thai HIV-1 infected patient. Extensive genotyping of the 9 single nucleotide polymorphisms (SNPs) of CYP2B6 were performed using the TaqMan real-time PCR assay on blood samples from 52 HIV-1 infected Thai patients who had received an EFV-based regimen. EFV plasma concentrations were measured by HPLC. We also analyzed the genotypes and haplotype structure of CYP2B6. RESULTS Our results showed that plasma EFV concentrations displayed high inter-individual variability amongst subjects with concentrations ranging from 42 to 40,307 ng/μl at 12 hours after initiation of treatment. Four SNPs (rs2279343, rs2279345, rs3745274, rs8192719) were significantly associated with EFV plasma concentration (P=0.0056). The composite CYP2B6 of 6 SNPs (516/3003/21563/18492/785/64) genotypes were significantly associated with EFV concentration. CONCLUSIONS In conclusion, our data indicate that the TTTCGT CYP2B6 haplotype may have value in predicting adverse drug reaction from CNS toxicity to EFV with treatment in Thai patients with HIV-1 infection.

1376F

Genetic factors in Bupropion Response and Remission. *A. Tiwari, C. Zai, G. Sajeev, T. Arenovich, D. Mueller, J. Kennedy.* Neuroscience, Centre for Addiction and Mental Health, Toronto, Canada.

There is considerable variability in the rate of response and remission following treatment with antidepressant drugs. Bupropion, a commonly prescribed antidepressant, is a dual norepinephrine and dopamine transporter inhibitor. A total of 532 tagging single nucleotide polymorphisms (SNPs) in 34 candidate genes (monoaminergic pathways and Hypothalamic-pituitary-adrenal axis) were investigated for association with remission and response in patients with major depressive disorder (MDD). These patients were from four GSK funded clinical trials in the USA in which they were treated with bupropion (n=319). Analyses were performed using conditional logistic regression. Gene-wide corrections for multiple testing were carried out. Significant association was observed for remission following treatment with bupropion with a SNP within the serotonin receptor 2A gene (HTR2A, rs2770296, pcorrected=0.02) and marginal evidence for association within the angiotensin converting enzyme gene (ACE, rs8075924, pcorrected= 0.054). Response to bupropion treatment was also significantly associated with a SNP in the dopamine transporter gene (SLC6A3; rs6347, pcorrected= 0.013) and marginally associated with another variant within the vesicular monoamine transporter 2 gene (SLC18A2; rs363225, pcorrected=0.059). These results suggest an important role for HTR2A in remission to bupropion treatment. In accordance with bupropion pharmacology, response may be associated with genetic variation in dopamine and vesicular monoamine transporters.

1377F

Correlated Meta-analysis of Genome-Wide Association Studies of Agonist-mediated Native Platelet Aggregation in African Americans. *R. Qayyum[1], D. Becker[1], D. Vaidya[1], L. Yanek[1], N. Faraday[2], R. Mathias[1,3], L. Becker[1].* 1) Medicine, Johns Hopkins School of Medicine, Baltimore, MD., USA; 2) Anesthesiology, Johns Hopkins School of Medicine, Baltimore, MD., USA; 3) Epidemiology, Johns Hopkins School of Public Health, Baltimore, MD, USA.

While genome-wide association studies (GWAS) of dose-specific agonist-mediated platelet aggregation have been reported, results from an agonist-centered GWAS approach that combines the effect of different agonist doses has not been reported. In this study our aim was to identify novel loci that are associated with native platelet aggregation in African Americans (AA) by combining the results from dose-specific GWAS. Platelet-rich plasma (PRP) was isolated from blood samples obtained from AA participants of the GeneSTAR (N=835) cohort. Optical aggregation was used to measure maximal aggregation in PRP after collagen (C) 2, 5, 10 μg/mL, ADP 2, 10 μmol/L, or epinephrine (E) 2, 10 μmol/L. Genotyping was conducted with Illumina 1M arrays. For each dose, linear mixed models adjusting for family structure, population stratification, age, sex, and cardiovascular risk factors were used to test for association between each SNP and phenotype under an additive model. To adjust for correlation between different doses, we pooled dose-specific GWAS results by performing separate meta-analyses with tetrachoric correlation for each agonist. Adequacy of adjustment for correlation between the doses was confirmed by simulation. We sought replication in European Ancestry participants from GeneSTAR (N=1257). We found 48 SNPs associated with aggregation (with ADP=3, C=46, E=1). A previously reported SNP in PEAR1 gene, rs12041331, was associated with all 3 agonists ($p=6.2 \times 10^{-14}$, 9.4×10^{-10}, 1.1×10^{-15} for ADP, C, and E respectively) at GWAS significance and associated at nominal significance with ADP and E in the replication cohort ($p=1.7 \times 10^{-5}$ & 3.4×10^{-5} respectively). Two SNPs in PPARGC1B, rs17797713 & rs10515634, were associated with ADP ($p=5.5 \times 10^{-10}$ & 9.8×10^{-9}) and another SNP in CNTN4, rs13060035, was associated with C ($p=1.1 \times 10^{-9}$); these SNPs were also significant in the replication cohort ($p = 0.01$, 0.03, & 0.04 respectively). Variants in PPARGC1B have been associated with obesity and in CNTN4 with hypertension in previous studies. In this first GWAS of dose-independent, agonist-mediated, native platelet aggregation, we have discovered, and replicated in an independent sample, three regions associated with platelet aggregation in AA. The elucidation of pathophysiological mechanisms of these associations requires further study and may provide insights into platelet biology and the increased risk of thrombosis in obese or hypertensive individuals.

1378F

Mixture regression analysis on antipsychotics induced weight gain: investigation of the role of serotonergic genes. *V. De Luca, B. Nowrouzi, C.C. Zai, J.L. Kennedy.* Neurogenetics, CAMH, Toronto, ON.

Antipsychotics induced weight gain is a complex trait with a relevant underlying genetic basis. HTR1A, HTR1D, HTR2A, HTR3A, HTR4, SLC6A4, TPH1 and TPH2 polymorphisms were genotyped in 139 schizophrenia patients and inserted as covariates in a mixture regression model of antipsychotic-induced weight gain expressed as percentage of weight change. Mixture analysis decompounded the observed distribution of weight gain into a mixture of two normal theoretical distributions. The DENORMIX program found two components with a mean (SD) of 2.61 (3.73) and 8.65 (8.75) comprising respectively 56+/-37; and 44+/-37; of the sample respectively. We obtained a cut-off point at 8% enabling the sample to be divided into two subgroups in order to assess the weight gainers and non-weight gainers. One-hundred-two subjects (73±37;) were included in the non-weight gainer group and 37 (27±37;) were included in the weight-gainer group. None of the studied polymorphisms were associated with non-weight gainer component under recessive or dominant model. The 5HT receptors did not show any significant interaction when pair-wise combinations were considered. These findings do not suggest a role of genes codifying for elements of the serotonergic system in influencing the weight gain in antipsychotic treatment.

1379F

Failure of metformin to reduce diabetes incidence in carriers of the *SLC22A1* Arg61-Leu160 haplotype. *T.I. Pollin[1, 2], J.C. Florez[3,4,5], S-W. Yee[6], K.A. Jablonski[2], J.B. McAteer[3,4], A. Taylor[4], W.C. Knowler[7], K.M. Giacomini[6], A. Shuldiner[1], DPP Research Group.* 1) Div Endocrinol/Diabetes/Nutrit, Univ Maryland, Baltimore, MD; 2) The George Washington University Biostatistics Center, 6110 Executive Blvd, Suite 750, Rockville, MD 20852; 3) Center for Human Genetic Research and Diabetes Research Center (Diabetes Unit), Massachusetts General Hospital, Boston, Massachusetts; 4) Program in Medical and Population Genetics, Broad Institute, Cambridge, Massachusetts; 5) Department of Medicine, Harvard Medical School, Boston, Massachusetts; 6) University of California San Francisco, San Francisco, CA; 7) Diabetes Epidemiology and Clinical Research Section, National Institute of Diabetes and Digestive and Kidney Diseases, Phoenix, Arizona.

In the Diabetes Prevention Program (DPP), a multicenter, multi-ethnic randomized clinical trial conducted in individuals at risk for type 2 diabetes, the insulin-sensitizing drug metformin (MET) reduced diabetes incidence by 31%, and an intensive lifestyle intervention (ILS, consisting of weight loss and dietary modification) by 58% over an average follow-up period of 3.2 years. We evaluated several candidate genes as predictors of metformin response in the DPP. *SLC22A1* encodes the organic cation transporter family member 1 (OCT1), responsible for hepatic metformin uptake. Individual human *SLC22A1* coding variants, including G401S (rs34130495) and R61C (rs12208357) but not F160L (rs683369), were previously shown to alter cellular metformin uptake and metformin pharmacokinetics. In 2,994 DPP participants (990 on metformin), we evaluated the association of all known *SCL22A1* coding variants with overall minor allele frequency (MAF) ⁄ 1% (6 missense, 1 in-frame deletion and 2 silent) with response to metformin and lifestyle interventions using age-, sex- and ethnicity-adjusted Cox proportional hazards. F160L and G401S were significant effect modifiers (SNPxMET interaction *P*-value = 0.01 and 0.05 respectively) of the relationship between metformin and diabetes risk, but not between ILS and diabetes risk (SNPxILS interaction *P*-value = 0.80 and 0.26 respectively). The 7 missense/deletion variants lie in a region of strong LD and comprise 8 haplotypes with frequencies⁄ 1% in the DPP. We identified two metformin-response associated haplotypes/isoforms, distinguishable from the remaining six haplotypes by the genotypes at R61C and F160L and differing from each other at G401S. The combined risk haplotype (HAP), R61-L160, had a frequency in the DPP sample ranging from 0.039 in African Americans to 0.136 in Caucasians. Carriers of at least one copy of R61-L160 (n=583) appeared non-responsive to MET (hazard ratio [HR]=1.13 [0.76-1.68], *P*= 0.55, $P_{METxHAP}$=0.004) but responsive to ILS (HR=0.41 [0.26-0.66], *P*= 0.0002, $P_{ILSxHAP}$=0.87]), whereas noncarriers (n=2,362) exhibited response to both interventions (MET HR=0.61 [0.49-0.75], $P=4x10^{-6}$; ILS HR=0.46 [0.36-0.58], $P=3 \times 10^{-11}$). Pending replication and functional validation, these results suggest that metformin non-responders could be identified preemptively by genotyping these two SNPs, enhancing the ability to personalize effective prevention and/or treatment of type 2 diabetes or other metformin responsive conditions.

1380F

Combining Nested PatchPCR™ and Nextera™ for High-Throughput Targeted Resequencing. *N.C. Caruccio[1], K.T. Varley[2, 3], D.T. Moore[2], R.C. Bachmeyer[2], R.M. Meyers[2, 3].* 1) Epicentre - An Illumina Company, Madison, WI 53713; 2) Kailos Genetics Inc., Huntsville, AL 35806; 3) HudsonAlpha Institute for Biotechnology, Huntsville, AL 35806.

The ability to sequence hundreds to thousands of targeted genomic regions in parallel, across a large number of patient samples is crucial for adopting next-generation sequencing in clinical practice and research settings. While single-locus PCR can be used to amplify genomic regions from many samples, the number of targeted regions is limited. Conversely, hybridization-based capture methods can target many regions, but sample throughput is limited.

To address these limitations, we have combined Nested PatchPCR™ and Nextera™ library construction to enable targeted sequencing across hundreds of genomic regions (of varying sizes) across large numbers of patient samples to more fully utilize Illumina sequencing capacity for clinical re-sequencing.

We have developed a pharmacogenomics panel targeting genetic variants associated with adverse drug reactions. Using Nested PatchPCR™, 480 genomic regions in 39 genes are specifically amplified in a single tube from 250ng of patient genomic DNA. 85 patient samples were processed in parallel in 96-well plates. High-throughput library construction was enabled using Nextera™ technology to prepare Illumina-compatible sequencing libraries containing patient-specific barcodes. The 480 amplified regions from each of 85 patient samples were pooled and sequenced on a single Illumina GAIIx flowcell. We achieved uniform coverage across the targeted amplicons, with an average of 400x read depth (>50X coverage across 85% of amplicons) and 95% of reads aligning to targeted regions.

The CYP2C19*3 polymorphism was detected in six technical replicates of a poor mephenytoin metabolizing patient and not in six technical replicates of a normal patient, validating variant detection using this approach. High coverage depth provides excellent accuracy for SNP identification, but also indicates there is capacity to process more genomic loci or patient samples. We have developed a larger-scale oncology panel combining Nested PatchPCR™ and Nextera™ to sequence 760 genomic regions covering 63 genes that are frequently mutated in cancer and associated with prognosis or response to therapy. The scale and utility of this approach is broadly applicable to re-sequencing candidate genes or regions in large cohorts of individuals.

1381F

Effects of ABCB1 drug transporter genotypes on antiepileptic drug disposition. *N. Božina[1], M. Lovrić[1], S. Hajnšek[2], D. Sporiš[3], Z. Lalić[1], P. Granić[1].* 1) Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Croatia; 2) Department of Neurology, University Hospital Centre Zagreb; 3) Department of Neurology, University Hospital Dubrava, Zagreb.

Background: 25-30 percent of epileptic patients remain resistant to different antiepileptics. ABC transporters which are responsible for drug transport across intestinal, renal and hepatic epithelial membranes and blood-brain barrier represent significant factors of variability in bioavailability of different drugs. The aim of the study was to evaluate the impact of ABCB1 (coding for P-glycoprotein) variants C1236T, G2677T/A, C3435T on antiepileptic drug disposition. We therefore correlated plasma levels of lamotrigine in mono- and polytherapy (carbamazepine, oxcarbazepine, levetiracetame, phenytoin, phenobarbital, topiramate, valproate) with gene variants. Patients and methods: A total of 222 epileptic patients, aged 16-76 years, were stratified into lamotrigine monotherapy group (n=58), a group receiving lamotrigine plus inductors (n=98), and those receiving inhibitors (n=29) or both (n=37). ABCB1 genotyping (C1236T, G2677T/A), C3435T, was performed by Real-time PCR and PCR-RFLP. Therapeutic drug monitoring was performed by HPLC with diode array detector and immunoassay. Results: A statistically significant correlation was confirmed between lamotrigine concentration and additional drugs (p<0.001), type of epilepsy, GGT, ALT, age and weight. Statistical analysis showed correlation between lamotrigine concentrations and ABCB1 C1236T allele (p=0.005), and genotype (p= 0.021) and G2677T/A genotype (p=0.05). Lamotrigine concentrations in subjects who were T allele carriers were significantly decreased. Haplotype analysis showed that 1236T-2677T-3435C carriers had the lowest dose-corrected lamotrigine level (p<0.001), followed by 1236T-2677T-3435C haplotype (p<0.04). Conclusion: The obtained results show that ABCB1 polymorphisms exert an influence on antiepileptic drug pharmacokinetics and may serve as a pharmacogenetic marker of lamotrigine bioavailability.

1382F

Decreased mucosal expression of barrier genes and vitamin D receptor gene in inflammatory bowel disease. *I. Arijs[1,2], J. Van Der Goten[1], K. Machiels[1], L. Van Lommel[2], I. Cleynen[1], G. De Hertogh[3], K. Van Steen[4], G. Van Assche[1], S. Vermeire[1], F. Schuit[2], P. Rutgeerts[1].* 1) Department of Gastroenterology, KU Leuven, Leuven, Belgium; 2) Gene Expression Unit, Department of Molecular Cell Biology, KU Leuven, Leuven, Belgium; 3) Department of Pathology, KU Leuven, Leuven, Belgium; 4) Department of Electrical Engineering and Computer Science (Montefiore Institute), University of Liège, Liège, Belgium.

INTRODUCTION: In inflammatory bowel disease (IBD), the intestinal epithelial barrier function is thought to be impaired, leading to aberrant exposure to changed luminal microbiota causing chronic mucosal inflammation. Kong et al showed that vitamin D receptor (VDR) plays a role in maintaining mucosal barrier integrity. This study investigated colonic expression of *VDR* and of known barrier genes in active IBD patients, and studied the effect of anti-inflammatory therapy with infliximab (IFX) and mucosal healing on colonic expression of these genes. METHODS: The expression of *VDR* gene and genes related to intestinal epithelial barrier function [IBD candidate susceptibility genes, claudins (CLDNs), mucins (MUCs), tight junction proteins (TJPs), E-cadherin (CDH1)] was investigated in colonic mucosa from 43 anti-TNF naïve IBD patients with active colitis (24 ulcerative colitis (UC) and 19 Crohn's disease (CD)) before and 4-6 weeks after their first IFX infusion and in 6 controls. Response to IFX was defined as complete endoscopic and histologic healing. Total RNA isolated from biopsies was used to analyze gene expression via Affymetrix Human Genome U133Plus2.0 Arrays. Quantitative RT-PCR and immunohistochemistry were used to confirm array data. Data was analyzed with Bioconductor and SPSS software. RESULTS: *VDR* gene expression was >1.5-fold significantly (false discovery rate<5%) decreased in active IBD vs controls. IFX therapy slightly increased *VDR* gene expression in healed mucosa in IBD responders. Gene expression levels of *ABCB1*, *CDH1*, *CFTR*, *CLDN3*, *CLDN8*, *CLDN23*, *CXADR*, *MUC20*, *OCLN*, *SLC22A4*, *SLC22A5*, *TJP1* and *TJP3* were all >1.5-fold significantly decreased in active UC and/or CD vs controls. After IFX therapy, gene expression of *ABCB1*, *CXADR*, *SLC22A5* and *TJP1* remained >1.5-fold significantly decreased after complete healing in UC and/or CD. Colonic expression of the decreased barrier genes after IFX and *VDR* gene significantly ($P_{Spearman's\ rho}$<5%) correlated with villin-1 expression, a gut epithelial cell marker, indicating that the decrease is directly related to epithelial damage. Immunohistochemistry of VDR showed partial restoration of epithelial expression in IBD colon with mucosal healing. CONCLUSION: Our data shows that decreased expression of the barrier genes and the *VDR* gene in IBD colon is directly related to mucosal damage, but a persistent barrier defect even after healing may contribute to an increased risk of relapse of mucosal ulceration.

1383F

Evidence for a heritable response to treatment for asthma. *C. Teerlink[1], M. Hegewald[2], L. Cannon-Albright[1,3].* 1) Dept Internal Medicine, Univ UtahSch Med, Salt Lake City, UT; 2) Department of Pulmonary and Critical Care Medicine, University of Utah and LDS Hospital, Salt Lake City, UT; 3) George E. Wahlen Department of Veterans Affairs Medical Center, Salt Lake City, Utah.

Evidence of familial clustering for a phenotype of interest can provide strong motivation to investigate the underlying genetic contribution. However, assessment of heritability to treatment response for a disease that is itself recognized to have a heritable component can be problematic due to the potential for confounding with the underlying disease heritability. We have applied a strategy to consider the heritability of the response to various asthma medications while correcting for the underlying heritability of asthma. The approach uses the Genealogical Index of Familiality (GIF) to compare the familial clustering of the subset of treated cases to the expected familial clustering for all asthma cases. A significant excess relatedness among the subset of cases provides evidence for familial clustering of the subset beyond the expected clustering for all asthma cases; thereby effectively identifying a subset of asthma cases who share a heritable response to medication. We have applied this strategy to clinically relevant treatment response phenotypes for asthma using genealogy records from the Utah Population Database that have been record-linked to over 15 years of electronic prescription drug data at the University of Utah Health Sciences Data Warehouse. Using ICD-9 codes, we identified 5,458 total asthma cases to serve as controls. We then identified various subsets of this cohort that had prescription drug records consistent with one of several treatment responses to serve as cases. To empirically assess significance we calculated the GIF statistic for each subset and then compared the value of the GIF to the distribution of GIFs collected from 1000 random sets of the controls, matched on place of birth, sex and age. Phenotypes of interest included multiple prescriptions for oral corticosteroids (n=196, case GIF=9.0, control GIF=4.6, p = 0.000); any prescription for corticosteroids (n=616, case GIF=9.6, control GIF=4.8, p=0.000); prescriptions only for inhaled beta-agonists (n=1487, case GIF= 7.6, control GIF=4.8, p=0.000); multiple prescriptions for leukotriene modifiers (n=207, case GIF=10.3, control GIF=4.8, p=0.000); and multiple prescriptions for long-acting beta-agonists (n=526, case GIF=7.6, control GIF=4.9, p=0.000). These results show strong evidence for familial clustering for all of the treatment response outcomes for asthma management, and encourage further investigation into underlying genetic factors.

1384F

Evaluation of pharmacogenetic markers by exome-sequencing of DNA extracted from saliva samples. *R.M. Iwasiow, M. Tayeb.* DNA Genotek, Inc., Ottawa, ON, Canada.

With increasing popularity and advancements in next generation sequencing resulting in decreasing costs it is enabling the use of these technologies for clinical applications. It is becoming feasible to process large numbers of samples for the purpose of genetic analysis. Sequencing large number of samples requires complex and expensive bioinformatics infrastructure. An alternative which can significantly reduce this burden is the use of targeted enrichment to only sequence areas of interest. Using the Agilent SureSelect Human All Exon Kit to capture regions totaling 38MB = 1.22% of human genomic regions significantly reduces the bioinformatics burden. Both next generation sequencing and target enrichment technologies require the collection of reliable, high quality samples. Previously, we have demonstrated that Oragene®•DNA provides an alternative method to blood samples for collecting large amounts of high quality genomic DNA that is suitable for next generation sequencing applications. Oragene•DNA collection devices are designed for non-invasive collection of saliva samples. The devices contain a stabilizing reagent that ensures the sample is of high quality and allows long term storage at ambient temperature. In this study we investigated DNA extracted from saliva collected using Oragene•DNA and paired-end sequenced on the Illumina Genome Analyzer II after target enrichment using the Agilent SureSelect Human All Exon Kit for the purpose of identifying pharmacogenetic markers for drug metabolism. Using the Agilent SureSelect Human All Exon Kit we observed a mean coverage of 98.48% against the targeted sequences at a mean depth of 77x. Paired-end sequencing of the target enriched sample on the Illumina Genome Analyzer II generated a mean lane yield of 6,352,886 kbases of which 46.1% corresponded to exon sequences. The high depth of coverage and depth of sequencing achieved by using DNA from saliva with the Agilent SureSelect Human All Exon Kit and sequencing using paired-end sequencing on a single lane of the Illumina Genome Analyzer II has allowed us to confidently mine the data for SNPs of interest. As such, here we demonstrate the ability to use saliva as a non-invasive DNA sample to investigate pharmacogenetic markers using next generation sequencing technologies.

1385F

Whole exome sequencing study of drug-induced liver injury: the association of rare variants. *Y. Shen[1,7], A. Floratos[1], A. Daly[2], M. Lucena[3], M. Molokhia[4], C. Stephens[3], M. Nelson[5], M. Daly[6], I. Pe'er[1,7].* 1) Center for Computational Biology and Bioinformatics, Columbia University, New York, NY; 2) Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom; 3) Facultad de Medicina, Hospital Universitario Virgen de la Victoria, Campus Universitario s/n, Málaga, Spain; 4) Department of Primary Care & Public Health Sciences, Kings College, London, United Kingdom; 5) GlaxoSmithKline, Research Triangle Park, North Carolina; 6) Harvard Medical School, Boston, Massachusetts; 7) Department of Computer Science, Columbia University, New York, NY.

Drug-Induced Liver Injury (DILI) is an important cause of drug withdrawal from the market, termination of clinical development, and mortality and mobility in developed countries. Amoxicillin-clavulanate (coamoxiclav) is a widely used antibiotic drug and a leading cause of DILI worldwide. Previous studies (Lucena et al 2011) of coamoxiclav liver toxicity established common HLA alleles as risk factor with large effect in European population. However, the clinical utility is limited by poor positive prediction value. Here we hypothesize that rare variants have significant contribution to the susceptibility of DILI. We performed a whole exome sequencing study to search for the association of rare variants. We searched the association of rare variants using both single variant and gene-based group test, and found suggestive evidence of rare variants association in several interesting genes. We are working towards validation and replication of these findings.

1386F

Pharmacogenomic study of side effects for antidepressant treatment options in STAR*D. *S.L. Clark[1], D.E. Adkins[1], K. Aberg[1], J.M. Hettema[2], J.L. McClay[1], R.P. Souza[3], E.J.C.G. van den Oord[1].* 1) Center for Biomarker Research and Personalized Medicine, Virginia Commonwealth University, Richmond, VA; 2) Department of Psychiatry, Virginia Commonwealth University, Richmond, Va; 3) Laboratory of Neurosciences, Universidade Do Extremo Sul Catarinense, Criciuma, SC, Brazil.

Understanding individual differences in susceptibility to antidepressant therapy side effects is essential to optimize the treatment of depression. Clinical trials have suggested that only 50% of patients with uncomplicated major depressive disorder respond to any single antidepressant (Papakostas & Fava, 2009), with inability to tolerate side effects being the most common reason for discontinuing antidepressant therapy (Bull, Hu et al., 2002; Bull, Hunkeler et al., 2002; Maddox, Levi, & Thompson, 1994; Mitchell, 2006). Studies have consistently indicated that antidepressant response is substantially heritable (Franchini, Serretti, Gasperini, & Smeraldi, 1998; Malhotra, Murphy, & Kennedy, 2004; Oreilly, Bogue, & Singh, 1994; Pare & Mack, 1971), suggesting that pharmacogenomic approaches represent a particularly promising avenue toward individualizing antidepressant treatment. Here we perform genome-wide association studies (GWAS) to search for genetic variation affecting the susceptibility to side effects. The analysis sample consisted of 1,439 depression patients, successfully genotyped for 421K SNPs, from the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study. Outcomes included four indicators of side effects: general side effect burden, sexual side effects, dizziness and vision/hearing related side effects. Our criterion for genome-wide significance was a pre-specified threshold ensuring that, on average, only 10% of the significant findings are false discoveries. Thirty-four SNPs satisfied this criterion. The top finding indicated 10 SNPs in SACM1L (p = 4.98x10-7, q =0.023) and MAGI2 (p = 2.76x10-6, q =0.097) whose minor allele effects mediate the effects of bupropion on sexual side effects. Both SACM1L and MAGI2 potentially alter cellular trafficking which has been shown to be associated with sexual dysfunction. Genome-wide significant findings were also found for SNPs in DTWD1 (p = 2.76x10-7, q =0.069), WDFY4 (p = 1.64x10-7, q =0.069) and CHL1 (p = 2.28x10-6, q =0.080). Possible clinical implications of the findings will be discussed. Although our findings require replication and functional validation, this study demonstrates the potential of GWAS to discover genes and pathways that potentially mediate adverse effects of antidepressant medication.

1387F

Association of COX-2 rs20417 with Stroke Risk, Functional outcome and Aspirin resistance. *A. Munshi[1, 2], V. Sharma[1], S. Kaul[3], A. Jyothy[1].* 1) Molecular Biology, Institute of Genetics and Hospital for Genetic Dis, Hyderabad, India; 2) Department of Botanand Microbiology, King Saud University, Riyadh, Saudi Arabia; 3) Nizams Institute of Medical Sciences, Panjagutta, Hyderabad.

Aspirin is the most commonly used antiplatelet drug for treatment of a serious vascular event, most notably stroke and myocardial infarction. However, despite the demonstrated benefit of aspirin, significant fraction of aspirin-treated patients may be resistant to the antiplatelet effects of the drug. A genetic basis for aspirin resistance has been suggested to exist. Therefore, the present study was taken up to investigate the role of -765 G/C polymorphism (rs 20417) in the Cyclooxygenase-2 (COX-2) gene with aspirin resistance in stroke patients. Four hundred and fifty stroke patients and 440 age and sex matched healthy controls were involved in the study. Baseline clinical data were collected and follow-up telephone interviews were conducted with patients at 3 months post event to determine stroke outcome. Blood sample were collected and genotypes determined. Significant difference was observed in the genotype distribution and allele frequency between patients and controls. CC genotype and C allele associated significantly with stroke (p=0.026 and 0.003). A step wise logistic regression analysis confirmed these findings. Further the COX-2 GC genotype showed a significant association with poor stroke outcome and CC genotype with poor outcome as well as death at 3 months from stroke onset. Therefore the carriers of C allele of COX-2 rs 20417 are more prone to aspirin resistance in comparison with non carriers. Further C allele of COX-2 gene is also an important risk factor for ischemic stroke. However, this is a preliminary study and the results need to be confirmed in a larger cohort.

1388F

The AA Genotype of Tumor Necrosis Factor Induced Protein 2 (TNFAIP2) rs8126 Polymorphism is Associated with Increased Mortality in Septic Shock. *S.A. Thair, J.H. Boyd, T.A. Nakada, J.A. Russell, K.R. Walley.* University of British Columbia Critical Care Research Laboratories, Heart + Lung Institute, St. Paul's Hospital, 1081 Burrard Street, Vancouver, BC, Canada V6Z 1Y6.

One of the central mediators of the pathophysiology of septic shock is the transcription factor nuclear factor 2B (NF-2B). We tested the hypothesis that genetic variation in inflammatory mediators of the NF-2B pathway are associated with increased 28-day mortality in patients who have septic shock. Using microarray to measure gene expression under inflammatory conditions we found that TNFAIP2 transcription is greatly increased in multiple settings. We then tested the hypothesis that single nucleotide polymorphisms (SNPs) of TNFAIP2 are associated with altered clinical outcome of septic shock patients. We performed an Armitage trend test using 28-day mortality on 68 SNPs genotyped in the region of TNFAIP2 +/- 50,000 bp in 530 Caucasian patients of the Vasopressin and Septic Shock Trial (VASST). In vitro studies include IL-6 ELISAs on genotyped lymphoblastoid cell lines after inflammatory stimulus, as well as IL-6 ELISAs on Hela cells after knock down and over expression of TNFAIP2. The initial screening analysis of the 68 SNPs identified rs8126 as highly associated with mortality (uncorrected p= 0.007). Using Cox regression we found that septic shock patients with the GG (homozygous minor) genotype of rs8126 had increased 28-day mortality (VASST relative risk 0.73 (95% CI 0.59-0.82, p=0.0065)). In vitro studies of lymphoblastoid cells with the AA (homozygous minor) genotype of rs8176373 (linkage disequilibrium (LD) with rs8126 >0.8) had lower levels of IL-6 production due to inflammatory stimulus than those of the GG genotype (p=0.03). Furthermore, IL-6 ELISAs of knock down and over expression of TNFAIP2 in Hela cells shows a statistically significant decrease in IL-6 levels when TNFAIP2 is knocked down and conversely, a statistically significant increase in IL-6 when TNFAIP2 is over expressed (p=0.005 and p= 0.00003 respectively). We conclude that the GG genotype of TNFAIP2 rs8126 is associated with increased septic shock mortality, possibly because this allele contributes to decreased TNFAIP2 function and subsequently decreased IL-6 and other pro-inflammatory cytokine production.

1389F

Deep Resequencing Identifies Common, Low Frequency and Rare Variants in ADIPOQ Underlying Adiponectin Variation and Provides Insight into its Role in Type 2 Diabetes Susceptibility. *L. Warren[1], L. Li[1], M. Nelson[1], M. Ehm[1], J. Perry[4], C. Palmer[4], T. Frayling[4], V. Mooser[2], J. Whittaker[3], D. Waterworth[2].* 1) Quantitative Sciences, GlaxoSmithKline, Res Triangle Park, NC. USA; 2) Quantitative Sciences, GlaxoSmithKline, Upper Merion, PA, USA; 3) Quantitative Sciences, GlaxoSmithKline, Stevenage, UK; 4) Peninsula College of Medicine and Dentistry University of Exeter, UK.

Resequencing technologies make it possible to investigate the role of genetic variants across the allele frequency spectrum and so dissect the genetic architecture underlying complex trait variation. Here we report a resequencing study of ADIPOQ in 3665 subjects with adiponectin levels from two independent studies. We confirmed previously reported common variant associations and identified multiple novel associations due to low frequency (P=2.2E-17) and very rare amino acid-changing variants (p=0.02). As expected, we found that the effects of common variants were modest, while the less common possessed large effect and the extremely rare variants resulted in extreme phenotypic outliers. This level of insight into the genetic architecture of a gene of medical interest is only possible by large scale resequencing. By analyzing genome-wide genotype and resequence data jointly and via frequentist and bayes variable selection methods, we identified seven independent signals that contribute to adiponectin levels, explaining 6% of the variation. Epidemiological studies have shown an inverse relationship between adiponectin levels and T2D risk, however whether adiponectin is causal or merely a marker of pre-diabetes is not yet known. These SNPs were thus subsequently genotyped in a cohort of 5145 type 2 diabetes (T2D) cases and 6374 controls and tested in a Mendelian randomization framework. None of the SNPs were significantly association with T2D. The lack of association between ADIPOQ SNPs that affect adiponectin levels and T2D suggests that low adiponectin levels do not causally predispose to T2D. This study exemplifies the capability of deep resequencing to elucidate the genetic architecture underlying traits of medical interest. Such knowledge can enhance our understanding of the genetic causes of complex disease and serves to inform drug target discovery and validation.

1390F

Cytochrome oxidase subunits: a pharmacogenetics prediction marker of Accutane therapy. *S. ABADPOUR, A. TAVAKOLI TAMEH, S. MATOO, A. YASARI MAZANDARANI, B. SEDAGHATI KHAYAT, N. HATAMNEJADIAN, M. MAHDAVI, A. EBRAHIMI.* Parseh Medical Genetics Counselling Center, Floor 7, No.75, Royan Alley,Keshavarz Bolv. Tehran, Iran; Tel-Fax: +98 21 88966579, 88996889; E-mail: ae35m@yahoo.com.

Accutane is one of the commonly drugs which is used vastly in therapy of skin disorders. Different people taking Accutane often have very different reactions. These variations in response and side effects are caused by many variables, but genetic variation and metabolism-based drug, herbal, and food interactions are among the most common. Most of variations in genes involved in, drug metabolism with a particular emphasis on improving drug safety in skin disorders area focused on CYP gene families. As part of the inborn system for clearing the body of xenobiotics, the cytochrome P450 oxidases (CYPs) are heavily involved in drug metabolism, and genetic variations in CYPs affect large populations. Molecular studies of CYPs subunits polymorphism can help the physicians to predict the drug responses and reduce unexpected side effects of Accutane. Materials and methods To evaluating of common polymorphisms in CYP2C9, CYP2C19 and CYP2D6 we selected about 50 patients which were under treatment for acne. Clinical examinations and genetic counselling were done and then the patients were divided into three groups including positive drug response, negative drug response and control group. The blood samples were collected, DNA was extracted and common polymorphisms were studied by using PCR, ARMS_PCR and RFLP methods. Result The results revealed that polymorphisms of CYP2C9 and CYP2D6 subunits have the most important effect in prediction of Accutane treatment responses in studied group. KEY WORDS: pharmacogenetics, Accutane, CYP2C9, CYP2C19, CYP2D6.

1391F

Web-based phenotyping for pharmacogenomics research. *K.E. Barnholt[1], A.K. Kiefer[1], T.K. Acquaye[1], R.B. Altman[2], H.L. McLeod[3], J.A. Johnson[4], C.B. Marsh[5], J.Y. Tung[1], J.L. Mountain[1].* 1) 23andMe, Inc., Mountain View, CA; 2) Department of Bioengineering, Stanford University, Stanford, CA; 3) Division of Pharmacotherapy and Experimental Therapeutics, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC; 4) Department of Pharmacotherapy and Translational Research, College of Pharmacy, University of Florida, Gainesville, FL; 5) Division of Pulmonary, Allergy, Critical Care, and Sleep Medicine, Department of Internal Medicine, Ohio State University, Columbus, OH.

Significant barriers to the progress of pharmacogenomics research include the cost and time required to assemble, phenotype and genotype an appropriately-sized cohort. 23andMe's research program uses web-based surveys to gather a broad range of phenotype data from an expanding cohort of genotyped individuals. The primary goal of this study is to develop, assess and implement web-based surveys for the rapid, efficient collection of drug response and toxicity data across three classes of medication: warfarin, proton pump inhibitors (PPIs), and non-steroidal anti-inflammatory drugs (NSAIDs). In the initial phase of this two-part study, we have assessed how well web-based surveys elucidate drug response data by comparing online self-reported data to responses obtained via semi-structured telephone interviews. A set of approximately 120 telephone interviews is currently underway. The second phase will incorporate data from tens of thousands of 23andMe customers to validate known associations between PPIs and the CYP2C19 gene, and between warfarin and NSAIDs and the CYP2C9 gene. The latter phase will also leverage 23andMe's customized genotyping chip to search for novel genetic associations with drug efficacy and toxicity. Our survey objective is to gather high quality data regarding drug name, drug dosage, duration of drug use, side effects, and efficacy while achieving a high completion rate. Telephone interview data from the initial phase have indicated that the greatest challenges center around providing accurate date and dosage information. Based on participant feedback, we have revised the structure and flexibility of surveys to correspond with the type of drug under investigation. For drug classes that encompass a broad range of use patterns, such as NSAIDs, we have implemented a web-based approach with several layers of conditional logic to allow adequate input of the most relevant data. This approach is designed to better meet the needs of responders with both vague and precise recollections of their drug dosage and use duration. These survey revisions are undergoing further assessment. Since web-based surveys can be administered efficiently to millions of individuals, validation of this method of assessing drug response would lead to significant acceleration of pharmacogenomics research. This study is funded in part by NIH grant 1R43HG005807-01.

1392F

Warfarin pharmacogenetic algorithms: why the dose of some patients cannot be explained? *M.R. Botton[1], E. Bandinelli[1], L.E.P. Rohde[2], L.C. Amon[3], M.H. Hutz[1].* 1) Department of Genetics, UFRGS, Porto Alegre, Rio Grande do Sul, Brazil; 2) Cardiology Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil; 3) Internal Medicine Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil.

Warfarin is an oral anticoagulant used for prophylaxis of thromboembolic diseases. Its effect varies among patients. Medicines and genetic profile influence the dose/effect of warfarin. Based on these factors, several algorithms to predict warfarin dose have been developed. The algorithms can predict most of patients' dose, but some patients cannot have their doses explained by these models. Moreover, some studies related the presence of some non-synonymous mutations in VKORC1 gene in patients with warfarin resistance. We have developed an algorithm that includes: body weight, age, use of amlodipine, amiodarone, carbamazepine, beta-blocker, diuretics and polymorphisms in CYP2C9, VKORC1, CYP4F2 and F2 genes. This model explains 63.3% of warfarin dose variation, one of the highest coefficients of determination among those described in the literature. However, some patients had a great difference between predicted dose and observed stable warfarin dose. The aim of this study is to investigate the presence of mutations in VKORC1 gene exons in patients with, at least, 30% higher observed doses when compared to predicted doses. A total of 279 patients of European ancestry on warfarin medication were included to elaborate an algorithm, of which, 13 showed, at least, 30% higher observed warfarin doses. Exons 1, 2 and 3 of VKORC1 gene of these patients were sequenced to detect possible mutations. None non-synonymous mutations were found, but some synonymous mutations were detected: one patient with Arg12Arg mutation in exon 1 and one patient with Leu120Leu mutation in exon 3. These results indicate that mutations that cause warfarin resistance are not the same than those that determine higher observed doses than that predicted by algorithms. In addition, these synonymous mutations may interfere in other unknown processes. Genetic variants in introns or in other genes could also be associated with these predictions. Moreover, environmental factors like diet or even other medicines not reported by patients could be related with algorithm prediction errors in some cases. However, due to the differences in variables included in algorithms described in the literature, different models can have different causes of errors and they must be investigated.

1393F

Meta-analysis of warfarin stable dose in Caucasians. *S. Bourgeois[1], N. Eriksson[2], A. Jorgensen[3], C. Nilsson[4], B. Burkley[5], M. Wadelius[6], M. Pirmohamed[4], A. Rane[7], J. Lindh[7], M.J. Rieder[8], J.K. Burmester[9], M.D. Caldwell[9], T. Mushiroda[10], M. Kubo[10], A. Wu[11], D. Roden[12], D. Crawford[12], M. Wagner[13], P. Svensson[4], S. Scott[14], R. Desnick[14], T.E. Klein[15], Y. Nakamura[10], J.A. Johnson[5], P. Deloukas[1], International Warfarin Pharmacogenetics Consortium.* 1) Wellcome Trust Sanger Inst, Cambridge, United Kingdom; 2) Uppsala Clinical Research Center, Uppsala, Sweden; 3) University of Liverpool, Liverpool, United Kingdom; 4) University Hospital, Malmö, Sweden; 5) University of Florida, Gainesville, USA; 6) Uppsala University, Uppsala, Sweden; 7) Karolinska Institutet, Stockholm, Sweden; 8) University of Washington, Seattle, USA; 9) Marshfield Clinic, Marshfield, USA; 10) RIKEN Center for Genomic Medicine, Yokohama, Japan; 11) University of California, San Francisco, USA; 12) Vanderbilt University, Nashville, USA; 13) University of North Carolina, Chapel Hill, USA; 14) Mount Sinai School of Medicine, New-York, USA; 15) Stanford University, Stanford, USA.

Warfarin is the most widely used oral anticoagulant worldwide, prescribed for thromboembolic disease prophylaxis. Genome-wide association (GWA) studies in Caucasians have identified common variants in VKORC1, CYP2C9, and more recently in CYP4F2 as the main genetic determinants of warfarin stable dose. Models including both known environmental (age, height, weight, amiodarone) and genetic factors explain ~ 49.6% of the observed inter-individual variability in stable dose requirement. In the International Warfarin Pharmacogenetics Consortium we undertook an inverse-variance weighted GWA meta-analysis in 1781 subjects from Europe and North America representing five studies. GWA data sets were imputed against 1000 Genomes and HapMap 3 with Impute v2. We applied study-wise standard QC and genomic control correction. We selected 17 loci (20 SNPs) at p < 10-05 for replication in an independent sample of 2000 Caucasians. Initial analysis in 693 of the replication samples found one SNP to be significant after taking multiple testing in to account. It is a synonymous-coding SNP, rs6042 (p=1.68x10-03, combined p=5.2x10-05), located in the sixth exon of the Factor 7 gene on chromosome 13. Factor 7 is a vitamin K dependent clotting factor whose synthesis in the liver is directly affected by warfarin. The locus harbours two additional biological candidates Factor 10, another clotting factor, and Protein Z, a regulatory factor implicated in the coagulation cascade, both of which are vitamin K dependent. In conclusion, we identified a novel locus influencing warfarin stable dose. A model comprising environmental (age, height, weight, amiodarone), rs9923231 (VKORC1), rs1799853 and rs1057910 (CYP2C9*2 and *3 respectively), rs2108622 (CYP4F2) and rs6042 accounts for 50.3% of warfarin dose variance, thus explaining a further 0.7% of the variance.

1394F

Genome-wide mapping for predictors of cutaneous hypersensitivity reactions associated with anti-epileptic drugs. *G. Cavalleri[1], M. McCormack[1], C. Depondt[6], K. Shianna[2], T. Urban[2], N. Walley[2], E. Chaila[3], G. O'Conner[3], C. Catanario[4], D. Kasperavičiūtė[4], R. Radtke[5], S. Sisodiya[4], D. Goldstein[2], N. Delanty[3].* 1) MCT, Royal College Surgeons, Dublin, Ireland; 2) Center for Human Genome Variation, Duke University; 3) Beaumont Hospital, Dublin, Ireland; 4) Department of Clinical and Experimental Epilepsy, Univer- sity College London, Institute of Neurology, Queen Square; 5) The Department of Medicine, Duke University Medical School; 6) the Department of Neurology, Hôpital Erasme, Université Libre de Bruxelles, Brussels.

Ranging from mild to life threatening, adverse drug reactions are a common feature in the pharmacological treatment of epilepsy. We and others recently illustrated the potential of HLA-A*3101 as a predictor for the full spectrum of hypersensitivity reactions associated with the anti-epileptic drug (AED) carbamazepine. HLA-A*3101 therefore joins HLA-B*1502 as a clinically relevant pharmacogenomic test for epilepsy. However, cutaneous hypersensitivity reactions in AED treatment extend beyond carbamazepine and are also well described for phenytoin, lamotrigine and oxcarbazepine. These drugs are structurally related to carbamazepine in that they contain aromatic rings. Here we report on a case-control genome-wide association study conducted using a European-descent cohort of 90 hypersensitivity cases (due to phenytoin, lamotrigine and oxcarbazepine) and 1500 controls. Applying imputation to enrich for variation across the HLA, we i) explore to what extent HLA-A*3101 acts as a predictor for hypersensitivity reactions beyond carbamazepine and ii) report on mapping results for the wider genome. Results to date suggest the predictive ability of HLA-A*3101 in the development of milder hypersensitivity ADRs is, in European-descent patients, specific to carbamazepine.

1395F

Pharmacogenomic investigations on drug metabolizing enzymes GSTT1, GSTM1 and CYP2B6 in breast cancer. *P. Chaturvedi[1], S. Tulsyan[1], G. Agarwal[2], P. Lal[3], B. Mittal[1].* 1) Medical Genetics, SGPGIMS, Lucknow, Uttar Pradesh, India; 2) Endocrine surgery, SGPGIMS, Lucknow, Uttar Pradesh, India; 3) Radiotherapy, SGPGIMS, Lucknow, Uttar Pradesh, India.

Background: Breast cancer remains a major health problem around the world and its incidence continues to increase. Chemotherapeutic drug responses are genetically determined and the relationship between genotype and drug response is of particular diagnostic value, especially in breast cancer. Polymorphisms in genes encoding Phase I and Phase II drug metabolizing enzymes are important tools in predicting clinical outcomes. Rationale: Glutathione S transferase plays an important role in phase II detoxification of chemotherapeutic drugs. The deficiency is highly correlated with the inability to conjugate glutathione with small molecular weight toxicants, hence drug tolerance. In cyclophosphamide based chemotherapy Cytochrome P450 (CYP2B6) mediates the conversion of cyclophosphamide to 4-hydroxycyclophosphamide. Drug toxicity or efficacy is determined by pharmacogenetic variability in these genes which is one of the possible mechanisms underlying drug metabolism. Aim: To screen genetic variations in Phase I drug metabolizing enzyme cytochrome P450 CYP2B6*9 (rs3745274; Q172H) and Phase II enzyme glutathione-S transferase GSTM1, GSTT1 and their role in predicting clinical outcomes and toxicity profiles. Work Plan: Clinical outcome on survival, toxicity and recurrence in 144 breast cancer patients was followed in this pilot study. The patients were genotyped for GSTM1, GSTT1 and CYP2B6 variatns using PCR-RFLP. Chi square and logistic regression was carried out using SPSS ver.17.0. Findings: Total 144 patients were followed for a minimum period of three years and therapeutic failure in terms of recurrence was noticed in 5 patients. Out of 5, in 1 patient both GSTM1 and GSTT1 were absent and it carried heterozygote allele GT for CYP2B*6. Others either lacked GSTM1 or GSTT1 and two of them were heterozygote for CYP2B*6. In 10 patients, dose delay or dose reduction was noticed due to toxicity in terms of grade IV neutropenia/leucopenia. The frequency for GSTT1 null and GSTM1 null was found to be 20.1% and 39.9% respectively in these patients and CYP2B6*9 showed a minor allele frequency of 38.85%. As it is pilot study, a larger sample size may be more efficient in ascertaining the role of these genes in pharmacogenetic assessments.

1396F

Genetic Association Study between Proopiomelanocortin and Melanocortin-4 Receptor Gene Polymorphisms with Antipsychotic Induced Weight Gain. *N. Chowdhury[1], A. Tiwari[1], R. Souza[1], C. Zai[1], S. Shaikh[1], S. Chen[2], F. Liu[2], J. Lieberman[3], H. Meltzer[4], J. Kennedy[1], D. Müller[1].* 1) Neurogenetics, Centre for Addiction & Mental Health, Toronto, Ontario, Canada; 2) Molecular Neuroscience, Department of Neuroscience, Centre for Addiction and Mental Health, University of Toronto, Toronto, ON, Canada; 3) 3. Department of Psychiatry, College of Physicians and Surgeons, Columbia University and the New York State Psychiatric Institute, New York City, NY, USA; 4) 4. Psychiatric Hospital at Vanderbilt University, Nashville, TN, USA.

Introduction Antipsychotic induced weight gain (AIWG) may result in the metabolic syndrome in schizophrenia patients. A recent genome wide association study of AIWG found the highest main peak in the region of the melanocortin 4 receptor (MC4R) gene, at the downstream marker rs489693. The precursor polypeptide proopiomelanocortin (POMC) has a downstream stimulatory effect on MC4R to regulate food intake and energy expenditure in the hypothalamus. Thus, we investigated the potential role of MC4R and POMC variants with antipsychotic induced weight gain Methods Four MC4R SNPs (rs2229616, rs17782313, rs11872992, rs8087522) and two POMC SNPs (rs1042571, rs6715352) were genotyped in 237 patients who underwent treatment for chronic schizophrenia and were evaluated for AIWG for up to 14 weeks. We compared weight change (%) across genotypic groups using analysis of variance and covariance for three SNPs (r2／ 0.8).Variants were genotyped using ABI TaqMan assays. In the case of a positive association, we investigated in silico and in vitro for functional relevance of the SNP. Results The rs2229616 SNP was monomorphic in our population and thus excluded from analyses. No significant genotypic or allelic associations were found between the MC4R rs11872992, rs17782313 or rs8087522 polymorphisms or the POMC rs1042571 and rs6713532 polymorphisms and weight gain (p > 0.05). However, a subsequent analysis showed that patients of European ancestry who were carriers of the MC4R rs8087522 A-allele (AG+AA) on clozapine gained significantly more weight than non-carriers (p=0.027). Electrophoretic mobility shift assay suggested that the presence of the A-allele may create a transcription factor-binding site. Conclusions In this study, we observed that the rs8087522 SNP of the MC4R gene may be associated with AIWG in schizophrenia patients of European origin. This SNP is present in the promoter, alters transcription factor binding and therefore may affect MC4R expression. This observation warrants replication in an independent sample and for exploration of MC4R and POMC interaction analyses.

1397F

Pharmacogenetic determinants of statin-induced reductions in C-reactive protein. *A.Y. Chu[1], F. Guilianini[1], J. MacFadyen[1], B.J. Barratt[2], F. Nyberg[3], D.I. Chasman[1], P.M Ridker[1].* 1) Preventive Medicine, Brigham and Women's Hospital, Boston, MA; 2) AstraZeneca Research and Development, Adderley Park, United Kingdom; 3) AstraZeneca Research and Development, Mölndal, Sweden.

In clinical trials, reduction in plasma levels of both C-reactive protein (CRP) and LDL-C on statin therapy is associated with decreases in cardiovascular events. However, whether mechanisms of statin-induced CRP reduction differ from statin-induced LDL-C reduction is unknown. Using genome wide data from the Illumina Omni 1M platform on 3,386 individuals of European ancestry randomly allocated to rosuvastatin 20 mg daily in the JUPITER (Justification for the Use of Statins in Primary Prevention) trial, we performed an association study to identify genetic determinants of rosuvastatin-induced CRP response, and compared the results to known pharmacogenetic determinants of LDL-C response previously described in this cohort (manuscript submitted). Similar to other statin trials, both CRP and LDL-C levels were reduced by ~50% after 12 months of therapy. There was no correlation, however, between statin induced LDL-C reduction and statin induced CRP reduction (Spearman's rho=0.05). No associations were detected at a genome wide level ($p<5x10^{-8}$) for rosuvastatin-induced CRP change. None of the SNPs in three genes previously shown on a genomewide basis to be associated with rosuvastatin-induced LDL-C reduction in the JUPITER population (*ABCG2*, *LPA*, and *APOE*) were associated with rosuvastatin-induced changes in CRP. However, among 20 candidate SNPs selected based on prior evidence from genetic analyses of baseline CRP levels (Dehghan, 2011), rosuvastatin-induced changes in CRP were most strongly associated with rs2794520 in *CRP* (mean -3.7% [se 2.0] CRP change per minor allele, $p=6.4x10^{-4}$), and with rs2847281 in *PTPN2* (mean 3.6% [se 1.9] CRP change per minor allele, $p=7.4x10^{-4}$), and remained significant after correction for multiple testing. By contrast, neither the *CRP* nor *PTPN2* variants were associated with LDL-C reduction; sensitivity analyses including LDL-C change as a covariate in regression analyses minimally influenced the association between *CRP* and *PTPN2* with CRP change. In summary, within the JUPITER trial, the major pharmacogenetic determinants of rosuvastatin-induced CRP reduction differ from and are largely independent of the major pharmacogenetic determinants of rosuvastatin-induced LDL-C reduction. This supports the hypothesis that different pathways may mediate the anti-inflammatory and lipid-lowering properties of statin therapy.

1398F

Genome wide association analysis of rheumatoid arthritis patients treated with anti-TNF medication. *M. Coenen[1], M. Umicevic Mirkov[1], J. Wessels[2], S. Vermeulen[9, 1], E. Toonen[1], A. Lee[3], R. Makkinje[1], W. Kievit[4, 8], H. Scheffer[1], T. Jansen[5, 8], E. Dutmer[6, 8], T. Radstake[4], M. van de Laar[7, 8], P. Barrera[4], P. van Riel[4, 8], H. Guchelaar[2], P. Gregersen[3], B. Franke[1].* 1) Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; 2) Department of Clinical Pharmacy & Toxicology, Leiden University Medical Center, Leiden, The Netherlands; 3) The Feinstein Institute for Medical Research, Manhasset, New York, USA; 4) Department of Rheumatology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands,; 5) Department of Rheumatology, Medical Centre Leeuwarden, Leeuwarden, The Netherlands; 6) Department of Rheumatology, Gelderse Vallei Hospital, Ede, The Netherlands; 7) Department of Rheumatology, University Twente & Medisch Spectrum Twente, Enschede, The Netherlands; 8) Dutch Rheumatoid Arthritis Monitoring registry; 9) Department of Epidemiology, Biostatistics and HTA, Radboud University Nijmegen Medical Centre, The Netherlands.

Background Treatment strategies blocking tumour necrosis factor (anti-TNF) have proven very successful in patients with rheumatoid arthritis (RA). However, a relevant subset of patients does not respond for reasons that are unknown. Although there is some indication that genetic variants may influence the response to TNF blockers, there are currently no means of identifying these patients prior to treatment start. **Objective** We aimed at identifying genetic factors predicting anti-TNF treatment outcome in patient with RA using a genome-wide association approach. **Methods** We selected patients with RA treated with antibodies directed against TNF from the Dutch Rheumatoid Arthritis Monitoring (DREAM) registry and the database of Apotheekzorg, which facilitates the Dutch distribution of adalimumab. Disease activity scores (DAS) 28 at baseline and after 14 weeks were available of 872 patients. Single nucleotide polymorphisms (SNPs) were genotyped using the Illumina HumanHap550-Duo BeadChip or the Human660W-Quad. Association analysis using the DAS28 change as outcome was performed using the whole genome association analysis toolset in PLINK. DAS28 at baseline and use of methotrexate were included as covariates. **Results** 516168 SNPs passed quality control. No findings passed the threshold for genome-wide significance ($p\text{-value} \leq 5x10^{-8}$). Eight SNPs showed suggestive association with DAS28 change with a $p\text{-value}<10^{-6}$. Seven of these were located in genes (*ALK, NKAIN3, CETP, CICP10, L3MBTL3, PBX3, RPL18P1*). The top associated gene *ALK* ($p=8.11x10^{-7}$) is involved in apoptosis, one of the working mechanisms of anti-TNF. Pathway analysis, including all SNPs with a p-value $<10^{-4}$, was performed using Ingenuity. This resulted in the identification of four gene networks of which two could be linked to apoptosis. The second largest network linked thirteen of the associated genes to TNF and its downstream signaling pathway. Replication of the top 50 associated SNPs in ~1500 RA patients treated with anti-TNF is ongoing. **Conclusions** The GWA approach is a potent tool for the identification of new candidate biomarkers predicting anti-TNF response. Suggestive findings will be replicated in larger, independent patient cohorts to prove whether the identified SNPs are associated with treatment outcome.

1399F

A Comparison of the Power and False-Positive Rate Protection When Analyzing Pharmacogenetic Data in Clinical Trials Using LOCF and MMRM. *B. Fijal, F. Zhao, C. Mallinckrodt.* Global Statistical Sciences, Eli Lilly, Indianapolis, IN.

Introduction: Within clinical trials, efficacy measurements are typically repeated on subjects throughout the trial. However, subjects frequently drop out of trials for various reasons, such as lack of efficacy, before the trial ends. Two statistical approaches that can be used to compensate for subject dropout are last observation carried forward (LOCF) and mixed models repeated measures (MMRM). The goal of our simulation study was to compare the performance of these two approaches. Methods: The simulation study was set up to mimic neuropsychiatric trials. Three dropout scenarios were examined: 1) all subjects completed the trial, 2) subjects' dropout was not related to the observed efficacy data, or 3) subjects' dropout was related to the observed efficacy data which, in some of the scenarios examined, could be impacted by a gene. The gene was assumed to 1) be bi-allelic, 2) have a minor allele frequency of 30%, and 3) behave in an additive manner. The sample size was 100 unless otherwise indicated. In both the LOCF and MMRM analyses, genotype was analyzed as a continuous variable. The accuracy of the estimate of the impact of the gene was measured by the slope (i.e., the improvement in response for each copy of the “good” responder allele a subject had). Results: When all subjects completed the trial or left the trial for reasons unrelated to the observed efficacy data, both LOCF and MMRM were comparable in terms of power and in controlling the false-positive rate. When dropout was related to the observed efficacy endpoint, MMRM provided adequate false-positive rate control while LOCF inflated the false-positive rate, and, in the scenarios examined here, the inflation increased as the sample size increased. When the gene had an impact on efficacy and it resulted in differential dropout between the genetic groups, MMRM provided an unbiased estimate of the genetic effect while LOCF, in the scenarios examined here, underestimated the impact of the gene. Additionally, MMRM provided better power to detect the genetic effect. Conclusions: Our research provided evidence that, in many situations, MMRM outperforms LOCF analysis by providing adequate false-positive control and unbiased estimates of genetic effects.

1400F

Characterization of the CYP2C19*alleles by using different genotyping and sequencing platforms. *G. Ghaffari, M. Rodriguez, S. Rodriguez, C.Y. Hung, L. Brenton, M. Tekin, M. Morra.* CMGDL, Department of Human Genetics, University of Miami Miller School of Medicine, 1501 NW 10th avenue, BRB-415 (M-860), Miami FL, 33136.

Individuals respond differently to drugs and this depends, at least in part, on individual variation in genes associated with drug metabolism, transport, and their receptors. The cytochrome P450 enzyme CYP2C19 is an important drug-metabolizing enzyme involved with the metabolism of many therapeutic agents, including antidepressants, antiulcers, anticonvulsants, antidiabetic, and antiplatelets drugs such as clopidogrel. To date, more than 38 CYP2C19 SNPs have been reported which are associated with CYP2C19 increased, decreased, or abolished enzyme function, leading to classify individuals into ultra rapid, extensive and poor metabolizers. Different methodologies both PCR- and array-based have been so far used to determine the CYP2C19 gene variation. In our laboratory we have performed studies by using the Illumina's BeadExpress Vera Code ADME Core panel (San Diego, CA) and a GeneMark Dx eSensor XT-8 platform (Carlsbad, CA), targeting the CYP2C19 gene. The Vera Code ADME Core panel covers the CYP2C19*2, *3, *4, *5, *6, *7, *8, *12, *17 and the eSensor XT-8 platform covers the *2, *3, *4, *5, *6, *7, *8, *9, *10, *13, *17 alleles. We have tested multiple DNA samples derived from different collections such as the Coriell Institute (Camden, NJ), ParagonDx samples (Decisive Diagnostics, Jackson, WY) and clinical samples kindly provided by diagnostics laboratories. Results accuracy was determined by cross-matching our data obtained with different platform against the results from other laboratories. Samples reported to carry a *10 or *2 allele have been further subjected to molecular characterization by capillary sequencing of the CYP2C19 gene. Data publicly available at NCBI dbSNP were also used in case that a specific genotypic allele was not available by other means. Our results highlight difficulties in demonstrating accuracy for ultra rare alleles. Also, our work indicates the relevance of using a multi-platform testing approach, in particular capillary gene sequencing as an important tool to be used in confirming uncertain genotypes.

1401F

Impact of HLA-B*1502 Testing for Carbamazepine on Medical Practice. *S.K. Grantham[1,2], S. Katsanis[1].* 1) Duke University Institute for Genome Sciences and Policy, Duke University, Durham, NC; 2) North Carolina State University, Raleigh, NC.

Carbamazepine (Tegretol®) is considered to be a first-line pharmaceutical treatment for epilepsy. Carbamazepine can trigger the potentially fatal skin reactions Stevens-Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN) among those who possess the HLA-B*1502 variant, the risk of which is approximately 1-6:10,000 in those of European descent. The risk is 10 times greater in individuals of Asian ancestry. In December 2007, the FDA directed carbamazepine manufacturers to update package inserts, resulting in a black box warning stating, “studies in patients of Chinese ancestry have found a strong association between the risk of developing SJS/TEN and the presence of HLA-B*1502,” and further asserting, “patients testing positive for the allele should not be treated with Tegretol unless the benefit clearly outweighs the risk.” Assuming adherence to the warning, we postulated two outcomes of this labeling change: a) an increase in pharmacogenetic testing for carbamazepine; or b) a decrease in prescription of carbamazepine. With 200,000 new diagnoses of epilepsy made per year in the U.S., and over 10 million U.S. individuals self-reporting as “Asian,” the market for testing for HLA-B*1502 is expected to be significant. We approached 149 U.S. laboratories designated by the American Society for Histocompatability and Immunogenetics as HLA DNA laboratories for information on testing volume. Of the 149 laboratories, 17 offer HLA-B*1502 testing for carbamazepine. Of the 8 consenting laboratories, the 6 responding to our survey reported negligible requests for HLA-B*1502 testing in comparison with total testing volume and total pharmacogenetic testing volume. Three laboratories reported never receiving requisitions for HLA-B*1502 testing, 2 laboratories reported 0-2 requisitions per month, and 1 laboratory reported 3-19 requests per month. These figures are significantly lower than expected if carbamazepine testing were integrated into routine care for newly diagnosed Asian patients. Our findings demonstrate that the FDA recommendation for pharmacogenetic testing in Asian patients has either been ignored or patients of Asian descent are prescribed medications other than carbamazepine. We suggest that updating the labeling of carbamazepine with pharmacogenetic recommendations dramatically decreased the prescription of this first-line pharmaceutical treatment, highlighting the direct impact that FDA recommendations can have on medical practices.

1402F

Pharmacogenomic variation and implications in African populations, using existing data. *K. Kalideen, R.S. Ramesar.* University of Cape Town , Cape Town , Western Province, South Africa.

Pharmacogenomics, the study of genetic variation and influence on an individual's response to xenobiotic agents, is capable of immediate translation from the bench to the bedside. A constituent of adequate health relies on the optimal prescribing and utilisation of medication for the effective management and treatment of a plethora of diseases. However, a significant proportion of individuals treated with standard doses of a specific medication have differential responses such as adverse drug reactions, no response or a partial response. Efficacy and effectiveness of said medication is of immense importance in Africa, as National health systems do not have the resources to supplement their treatment regimens with alternate and additional pharmaceuticals. It is therefore necessary to investigate methods to optimize existing treatment. Aim: (1) Use existing data to investigate pharmacogenetic variants in African populations compared to their Caucasian counterparts, (2) Create a diversity map of African populations, based on their pharmacogenetic profiles and (3) Investigate the implications of these variants in South African populations in terms of the Burden of Disease and Essential Drugs List in South Africa. Methods: Pharmacogenetic relevant genes were identified and the allelic frequencies of variants within these genes were mined using existing databases (HapMap 3, African Affymetrix SNP6.0 database, 1000 Genomes). Statistical analysis (Fishers Exact test, df = 1, $p>0.05$) was performed for each variant in a population (in comparison to the Caucasian population). The significant variants were then classified and comparedto the South African Burden of Disease and Essential Drugs List. Population genetic analyses (Arelquin 3.3 and Phylip) were performed to estimate the distance between the populations. Results and Discussion: A total of 84 variants in pharmacogenes were significantly different between African populations and the reference Caucasian population. Furthermore, the genetic structure of certain genes differed within the different African populations. In terms of clinical utility, the significant variants in this study, have an impact on the treatment recommended in the South African Essential Drugs List; therefore providing impetus on future research in this area.

1403F

Targeted resequencing and haplotyping of a large panel of ADME genes. *F. Kaper, B. Klotzle, J. Chen, M. Bibikova, M. Ronaghi, J.B. Fan.* Illumina, Inc., San Diego, CA.

Individual response to environmental factors, chemicals and drugs can vary significantly and is frequently associated with polymorphisms in genes involved in absorption, distribution, metabolism and excretion (ADME). In order to predict drug response and drug toxicity in an individual, it is important to determine the genotype of these genes. We have therefore selected a consensus panel of 329 ADME genes, and designed probes to capture all exons in a targeted pull-down assay, followed by sequencing on an Illumina® Genome Analyzer IIx. The targeted region encompasses 1Mb, allowing for multiplexing of 15 samples within one assay, while achieving 30X coverage on one of the 8 lanes of the Genome Analyzer IIx. Optimizations to the pull-down assay and probe design resulted in coverage of 99.7% of the 329 genes. Coverage was quite uniform, with 70% of the genes within 2-fold of average coverage, and 96% within 5-fold of average coverage. In addition, a method was developed to obtain the haplotypes of each gene. This information is clinically relevant as the phenotype may vary depending on whether multiple polymorphisms are located on the same chromosomal copy or on different copies. Genomic DNA was distributed into multiple aliquots, each receiving less than a haploid copy number. The DNA was subsequently amplified using a multiple displacement amplification (MDA) protocol, followed by Infinium® genotyping on an Illumina® 300K HumanCytoSNP-12 BeadChip. In parallel, MDA-amplified DNA was used for next generation sequencing library preparation with targeted pull-down of the ADME genes, as described above. In an initial pilot experiment, we show that all heterozygote alleles were resolved into homozygotes. Per aliquot, approximately 10-20% of the expected ADME genes were detected by sequencing. Furthermore, we were able to determine and confirm the haplotype of the daughter of a father/mother/daughter family trio.

1404F

Pharmacogenetics and cycling: the interactive effects of the ADRB2 A46G SNP and salbutamol on cycling performance. *S. Koch[1], M.J. MacInnis[1], B.C. Sporer[2,3], J.L. Rupert[1], M.S. Koehle[1,4].* 1) School of Human Kinetics, University of British Columbia, Vancouver, British Columbia, Canada; 2) Faculty of Medicine, University of British Columbia, Vancouver, Canada; 3) Canadian Sport Centre Pacific, Vancouver, Canada; 4) Division of Sport Medicine, University of British Columbia, Vancouver, Canada.

Polymorphisms of the adrenergic) 2-receptor gene (ADRB2) are associated with key components of cardiorespiratory function during exercise. Specifically, the G-allele of the A46G SNP (Arg16Gly; rs1042713) results in a substitution of a glycine for an arginine at amino acid 16, which is associated with increased bronchodilation, heart rate, and cardiac output. Asthmatic and non-asthmatic individuals with the G-allele have a greater increase in forced expiratory volume in one second (FEV1) after the inhalation of a short-acting) 2-agonist such as salbutamol (SAL). It is unclear if the influence of the G-allele on change in FEV1 after the inhalation of SAL affects performance in highly trained athletes. **PURPOSE**: 1. To determine if common variants of the adrenergic) 2-receptor influence percent change in FEV1 after the inhalation of SAL in male cyclists. 2. To assess the influence of the A46G SNP on 10-km time trial performance in cyclists after inhaling SAL. **METHODS**: The A46G SNP of the ADRB2 gene was genotyped in 36 unrelated competitive male cyclists aged 19 - 40 years (AA: 4; AG: 14; GG: 17). Athletes performed two simulated 10-km time trial rides on a cycle ergometer 60 min after the inhalation of either 400 µg of SAL or placebo. Medication administration was double-blinded and randomly assigned. The change in FEV1 was assessed immediately before and 30 min after inhalation. Performance was assessed by the time needed to complete the ride. Mixed between-within subject ANOVAs were conducted to assess differences between percent change in FEV1 and cycling performance after the inhalation of SAL or placebo based on an individual's A46G SNP. **RESULTS**: The percent change in FEV1 after the inhalation of SAL was significantly greater than placebo, $F(1,33) = 4.4$, $p = 0.043$, / $p^2 = 0.118$). This was independent of A46G genotype, $F(2,33) = 0.26$, $p = 0.77$, / $p^2 = 0.016$. Furthermore, there was no interaction effect between the A46G SNP and the time needed to complete a time trial after the inhalation of SAL or placebo, $F(2,32) = 0.68$, $p = 0.51$, / $p^2 = 0.01$. No main effect was found between the SAL and the placebo condition, $F(1,32) = 1.4$, $p = 0.71$, / $p^2 = 0.004$). **CONCLUSION**: In competitive male cyclists, FEV1 is improved after the inhalation of SAL (400 µg) regardless of genotype at the ADRB2 A46G SNP. In addition, the A46G SNP did not influence a 10-km time trial performance after the inhalation of SAL in male cyclists.

1405F

Pharmacogenetics of childhood acute lymphoblastic leukemia - asparaginase pathway. *M. Krajinovic[1,2,3], V. Gagné[1], J. Rousseau[1], M. Labuda[1], C. Cyrielle Beaubois[1], D. Sinnett[1,2], C. Laverdière[1,2], A. Moghrabi[1,2], S.E. Sallan[4,5], J.L. Kutok[6], D. Neuberg4[4], L.B. Silverman[4,5].* 1) Research Center, CHU Sainte-Justine, Montreal, QC, Canada; 2) Department of Pediatrics, University of Montreal; 3) Department of Pharmacology, University of Montreal; 4) Department of Pediatric Oncology, Bostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA; 5) Division of Hematology/Oncology, Children's Hospital, Boston, MA; 6) Department of Pathology, Brigham and Women's Hospital, Boston, MA.

Several groups including ours conducted pharmacogenetic studies in acute lymphoblastic leukemia (ALL) and identified variations in several genes that may contribute to variability in treatment responses. This presentation gives a short overview of the present knowledge in ALL pharmacogenetics and presents in more details our recent results on the polymorphisms of asparaginase pathway in ALL. Asparaginase is a standard and critical component in the therapy of childhood ALL .Several enzymes are essential for antileukemic effect of asparaginase such as asparagine synthetase (ASNS), the basic region leucine zipper transcription factors ATF5 and arginosuccinate synthase 1 (ASS1). They have been shown to display variable expression between leukemia cells that are resistant and sensitive to aspraginase. Fourteen polymorphisms in regulatory and coding regions of these genes were investigated for an association with ALL outcome in 336 children diagnosed with ALL and CHU Sainte Justine (discovery cohort). Lower event free survival (EFS) was associated with ATF5T1562C, tandem repeat ASNS polymorphism and derived haplotype, as well as with ASS1 G1343T and G34T substitutions ($p \leq 0.03$). Associations were limited to patients who received E.coli asparaginase. Variations that sustained correction for multiple testing (ATF5T1562C, $p=0.005$, ASNS tandem repeat and related haplotype, $p \leq 0.01$) were subsequently analyzed in replication cohort (Dana Farber Cancer Institute, Boston). The E.coli dependent association of ATF5 T1562 allele with reduced EFS was confirmed ($p=0.01$). Given significant association of ATF5 C1562T (located in 5'UTR) in the test and replication cohort, further analyses focused on the functional assessment of C1562T and remaining polymorphisms in the promoter and 5'UTR of the gene. Gene reporter assay showed that the haplotype tagged by the T1562 had higher promoter activity ($p \leq 0.01$). Remaining regulatory polymorphisms appeared also to affect ATF5 function; two additional high-activity haplotypes were identified ($p \leq 0.02$) and further corroborated by quantitative mRNA analysis in lymphoblastoid cell lines. ATF5- regulated increase in ASNS expression in a response to more efficacious E.coli induced asparagine depletion might explain observed results. The study will further increase the knowledge how to use host genetic variations to tailor therapy of antileukemia drugs.

1406F

Whole Exome Sequencing and Antidepressant Treatment Outcome. *G. Laje, N. Akula, F.J. McMahon.* Human Genetics Branch, Intramural Research Program, NIMH, NIH, USDHHS, Bethesda, MD.

Background: Genome-wide association studies of antidepressant treatment cohorts have not identified common genetic variants that have a large and reproducible impact on treatment outcome. It is possible that rare genetic variants may offer additional insight into the etiology of this complex phenotype. We undertook a whole exome sequencing experiment in individuals selected from the Sequenced Treatment Alternatives to Relieve Depression study (STAR*D).

Methods: In this pilot study we employed an extreme trait strategy to detect genetic variants that may be enriched at the extremes of the antidepressant response distribution. European-ancestry individuals who failed to respond to several treatment s were compared to individuals who experienced a sustained remission of symptoms after typical treatment. Individuals were matched on gender, anxious depression status, treatment adherence, drug and alcohol abuse, and genetic ancestry, determined by a large set of common SNPs. DNA samples were processed with the Agilent SureSelect Human All Exon 50MB kit and sequenced on a SOLiD platform with⁄ 10X coverage. Functional relationships among implicated genes were analyzed with DAVID.

Results: At this time, we only report single nucleotide variants (SNVs). SNVs were initially called using CLC Bio software and an in-house pipeline using BFAST, PICARD and GATK. SNVs with < 4X coverage were excluded from our analysis. Each individual had about 90,000 SNVs. Transition/transversion ratio was 3.04 for known and 2.03 for novel SNVs, and there was >99.9% agreement among common SNPs genotyped on the Illumina OmniExpress Array. There were no overall differences between the groups in the number of known SNVs. However, novel SNVs predicted to be damaging by SIFT were increased in the sustained remitters ($p<0.05$). Genes with damaging SNVs observed in at least 3 out of 4 treatment-resistant cases but no remitters were enriched for rhodopsin-like G protein-coupled receptors (Benjamini $p=0.008$).

Conclusions: The detection of rare variants through high throughput sequencing may provide new insight into the etiology of treatment-resistant depression.

1407F

Association of the *CES1* G143E Polymorphism with Platelet Aggregation and Cardiovascular Outcomes in Patients on Clopidogrel Therapy. *J.P. Lewis[1], K. Ryan[1], Q. Gibson[1], J.R. O'Connell[1], R.B. Horenstein[1], B.D. Mitchell[1], K. Tanner[1], R. Pakzy[1], U.S. Tantry[2], K.P. Bliden[2], P.A. Gurbel[2], A.R. Shuldiner[1].* 1) Division of Endocrinology, Diabetes and Nutrition, University of Maryland School of Medicine, Baltimore, MD; 2) Sinai Center for Thrombosis Research, Sinai Hospital of Baltimore, Baltimore, MD.

Anti-platelet therapy with clopidogrel prevents recurrent cardiovascular events in patients with coronary artery disease undergoing percutaneous coronary intervention (PCI). However, inter-individual variation in response to clopidogrel is widely recognized and has been attributed, in part, to genetic factors. The gene for carboxylesterase-1 (*CES1*), the primary enzyme responsible for metabolism of clopidogrel into inactive metabolites, is highly polymorphic. A relatively uncommon variant causing a glycine to glutamic acid substitution at position 143 (G143E; rs71647871) has recently been identified and shown to encode a protein with decreased CES1 activity. We measured *ex-vivo* platelet aggregation before and after clopidogrel administration in 565 healthy Amish individuals from the Pharmacogenomics of Antiplatelet Intervention (PAPI) study and evaluated the impact of the *CES1* G143E variant on clopidogrel response. The decreased function 143E allele was significantly associated with greater clopidogrel response (P = 0.003); this allele had no effect on baseline measures of ADP-stimulated platelet aggregation (P = 0.27). These findings were extended by examining the relationship between *CES1* G143E genotype and ADP-stimulated platelet aggregation as well as cardiovascular outcomes in 227 patients undergoing PCI at Sinai Hospital in Baltimore. Similar to the PAPI study, there was a considerable difference in post-clopidogrel maximal ADP-stimulated platelet aggregation between *CES1* 143GG homozygotes and 143E carriers (36.7% vs. 15.7% respectively, P = 0.07). Patients who carried the *CES1* E-allele (n = 3) tended to be less likely to experience a cardiovascular event or death compared to 143GG homozygotes (n = 224) at 1 year of follow-up (0.0% vs. 13.7% respectively). Taken together, the *CES1* G143E polymorphism may be an important determinant of clopidogrel response - improved cardiovascular outcomes and/or increased bleeding events - although further evaluations in larger populations are warranted.

1408F

Influence of NR1I3 nuclear receptor gene polymorphisms on the lipid-lowering efficacy and safety of statin therapy. *L.O. Lima[1,2], M.H. Hutz[1], C.R. Van der Sand[3], L.C. Van der Sand[3], M.E.W. Ferreira[3], R.C. Pires[3], S. Almeida[2, 4], M. Fiegenbaum[2,4,5].* 1) Departamento de Genética, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; 2) Laboratório de Biologia Molecular, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS, Brazil; 3) Centro de Diagnóstico Cardiológico - CDC, Porto Alegre, RS, Brazil; 4) Departamento de Ciências Básicas da Saúde, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS, Brazil; 5) Laboratório de Biologia Molecular, Centro Universitário Metodista do IPA, Porto Alegre, RS, Brazil.

HMG-CoA reductase inhibitors (statins) are the most effective and prescribed drug for dyslipidemia treatment and are generally well tolerated. Nevertheless, interindividual variability has been seen in relation to lipid-lowering efficacy and adverse effects occurrence. Constitutive androstane receptor (CAR) is a transcription factor involved in xenobiotic metabolism pathways by regulating the expression of drug-metabolizing enzymes (phase I and II) and transporters, and statins have been described as target of xenobiotic response mediated by this receptor. The aim of the present study was to investigate the association of genetic variants in the CAR gene, NR1I3, with lipid-lowering efficacy and safety of statin therapy. The sample consisted of 240 Brazilian hypercholesterolemic patients of European descent on simvastatin or atorvastatin therapy. For efficacy analyses these patients had their lipid and lipoprotein concentrations measured at baseline and after approximately 6 months (5.97 ± 2.44 months) of treatment. The safety analyses were carried out by genotype and allele frequencies comparisons between 98 patients who remained on the same dose for more than a year (37.70 ± 23.12 months) without presenting adverse drug reactions (non-ADR group) and 30 patients who developed adverse drug reactions (myalgia/ elevation of creatinine kinase/ alteration of hepatic function; ADR group). The polymorphisms were genotyped by TaqMan 5' allelic discrimination assays. When a recessive model was tested the NR1I3 rs2501873 polymorphism was associated with statin efficacy. G allele carriers showed a greater LDL-C reduction than A/A homozygotes (-37.63 ± 16.79% versus -29.82 ± 21.66%; P=0.026). The NR1I3 rs2307424 polymorphism was associated with side effects. All 30 individuals that presented an adverse reaction were carriers of a C allele (T/T versus C carriers; P=0.0066). The total absence of T/T homozygotes observed in the ADR group suggest that this genotype might confer a protection against statin ADRs. To our knowledge, this is the first study describing the possible influence of NR1I3 rs2307424 and rs2501873 polymorphisms on statin pharmacogenetics; therefore additional studies in independent and larger samples are warranted to confirm these results.

1409F

Common and rare variants in the POMC pathway markedly influence antipsychotic drug-induced weight gain: Discovery GWAS and subsequent replication data. *A.K. Malhotra[1,2], C. U. Correll[1,2], N. I. Chowdhury[3], D. J. Müller[3], P. K. Gregersen[2], A. T. Lee[2], A. K. Tiwari[3], J. M. Kane[1,2], J. A. Lieberman[4], H. Y. Meltzer[5], T. Lencz[1,2], J. L. Kennedy[3].* 1) The Zucker Hillside Hospital, Glen Oaks, NY; 2) The Feinstein Institute for Medical Research, Manhasset, NY; 3) Centre for Addiction and Mental Health, Toronto, CA; 4) New York Psychiatric Institute/Columbia University, New York, NY; 5) Vanderbilt University Medical Center, Nashville, TN.

Introduction: Weight gain and related metabolic abnormalities are a significant side effect associated with antipsychotic drug treatment. Treatment studies comprised of chronic patients may underestimate the severity of this side effect, as prior treatments may have caused weight gain and obscure the true weight liability of the current drug treatment. In a recent study (Correll et al. 2009, JAMA), we showed that pediatric patients without prior exposure to antipsychotic medications experienced marked weight gain at 12 weeks of treatment with each of the study drugs; the weight gain was far greater than reported for previously treated patients and the weight change was highly variable. Therefore, we have completed the first genome-wide association study of antipsychotic induced weight gain in previously untreated patients, **Methods:** We comprehensively characterized a discovery cohort of antipsychotic-naïve pediatric patients undergoing initial clinical treatment with the second generation antipsychotic drugs. Subjects were confirmed to be receiving antipsychotic drug by plasma blood levels, and were weighed at baseline, 4, 8 and 12 weeks of treatment. DNA was collected via blood sample and genotyping conducted with the Illumina 1M OmniQuad platform. Regression analysis of weight at 12 weeks of treatment versus baseline was conducted using both additive and recessive models. Subsequently, we assessed two additional cohorts of subjects treated with second generation agents and genotyped SNPs implicated in the discovery cohort, as well as conducted a rare variant analysis of our discovery cohort focused on missense SNPs. **Results:** QTL analysis revealed a region of the genome near the melanocortin 4 receptor gene (MC4R) that significantly associated with antipsychotic induced weight gain. SNPs in this region were also associated with weight gain in the two additional cohorts, with the same allele relationships and effect sizes observed across all three cohorts. Rare variant analysis also revealed a relationship between an amino acid substitution in the PCSK1 gene, also located in the proopiomelanocortin (POMC) pathway, and significant weight gain. **Conclusions:** Our data provide convergent evidence that genes in the POMC pathway are associated with antipsychotic-induced weight gain. These results have implications for gene x environment studies, as well as potential clinical impact for patients beginning antipsychotic drug treatment.

1410F

Multi-ethnic Cytochrome-P450 Copy Number Profiling Identifies Mechanism of Copy Number Variation Formation and Novel Pharmacogenetic Alleles. *S. Martis[1], H. Mei[1], R. Vijzelaar[2], L. Edelmann[1], R.J. Desnick[1], S.A. Scott[1].* 1) Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) MRC Holland, Willem Schoutenstraat 6, Amsterdam, The Netherlands.

The polymorphic cytochrome-P450 (CYP450) system is a superfamily of over 50 hemoproteins involved in drug metabolism and bioactivation. Inherited genetic variation among CYP450 genes contributes to disease susceptibility and interindividual differences in drug response. Copy number variants (CNV) can influence CYP450 activity, particularly for CYP2D6; however, little is known about the role of CNVs in other CYP450 family members. To determine if other CYP450 genes have common deletion or duplication alleles, multiplex ligation-dependent probe amplification (MLPA) that interrogated CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5 was performed on over 100 DNA samples each from healthy African-American, Asian, Caucasian, Hispanic, and Ashkenazi Jewish (AJ) individuals. Among the CYP450 genes studied, CNVs were identified in CYP2A6, CYP2B6, CYP2D6, and CYP2E1. The total deletion/duplication allele frequencies for these four genes among African-Americans, Asians, Caucasians, Hispanics, and AJs were 20%, 10%, 8.7%, 32%, and 18%, respectively, and all detected CNVs were confirmed by quantitative TaqMan copy number assays. To determine the breakpoint regions of these alleles, a high resolution oligonucleotide microarray (average probe spacing of ~270 bp) was designed that interrogated 32 core pharmacogenetic genes, including all those analyzed by MLPA, and array-based comparative genomic hybridization (aCGH) was performed on 46 selected samples. aCGH analyses localized the CYP2A6 CNV breakpoints to a cluster of neighboring repetitive Alu and LINE elements present in two directly orientated low copy repeats (LCRs; >90% similarity) resulting in full gene deletion and duplication. Interestingly, the CYP2B6 CNV breakpoint occurred in an intron 4 Alu region and in another directly orientated LCR (>90% similarity) that encompassed the CYP2B7P1 pseudogene, resulting in the CYP2B6*29 partial deletion allele and the reciprocal, and novel, CYP2B6-CYP2B7P1 duplication fusion allele (designated CYP2B6*30). CYP2E1 CNV breakpoints were also localized to two directly orientated LCRs (>98% similarity); however, only CYP2E1 full gene duplication was detected in the studied populations (designated CYP2E1x2). These data identify two novel CYP450 CNV alleles and indicate that common CYP450 CNV formation is likely mediated by nonallelic homologous recombination resulting in both full gene and gene-fusion copy number imbalances.

1411F

Genetic variability associated with antiretroviral therapy-related adverse effects in HIV-infected individuals. *V.S. Mattevi[1,2,3], A.S. Gasparotto[2], J.R. Trinca[2], M.L. Kayser[3], R.K. Lazzaretti[4], R. Kuhmmer[4], S. de Almeida[1,2], J.P. Ribeiro[4], E. Sprinz[4].* 1) Health Basic Sciences, UFCSPA, Porto Alegre, RS, Brazil; 2) Health Sciences Graduate Program, UFCSPA, Porto Alegre, RS, Brazil; 3) Pathology Graduate Program, UFCSPA, Porto Alegre, RS, Brazil; 4) Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil.

A significant improvement in the survival of human immunodeficiency virus (HIV)-infected patients has been observed since the introduction of highly active antiretroviral therapy (HAART). However, the development of adverse events may compromise adherence to HAART. Lipodystrophy (LD) stands out among the most important HAART-related adverse effects, being characterized by abnormal body fat redistribution, dyslipidemia, insulin resistance and metabolic syndrome. We investigated the association of selected polymorphisms in candidate genes involved in the pharmacokinetics and pharmacodynamics of HAART and the occurrence of the aforementioned adverse effects in HIV-infected individuals. Patients on HAART (n = 614) for, at least, one year, were recruited in three reference treatment services from the south of Brazil. Twenty-one polymorphisms in 15 candidate genes were genotyped through real-time polymerase chain reaction (PCR) using the TaqMan® methodology or conventional PCR-based methods. Poisson regression models with robust variance were used to assess the predictor variables for the development of categorical outcomes. Mean biochemical and anthropometric phenotypes related to adverse effects were adjusted for covariables and compared among genotypes through general linear models. The polymorphisms analyzed were located in the following candidate genes: SREBF1 (rs2297508), TNF (rs361525), PPARG (rs1801282), APOB (rs17240441 and rs693), UCP1 (rs1800592), UCP2 (+3474 45bp ins/del), UCP3 (rs1800849), ESR1 (rs2234693, rs1801132, rs7757956, rs2813544), ESR2 (rs3020450, rs7154455, rs4986938), CYP3A4 (rs2740574), CYP3A5 (rs776746), CYP2B6 (rs3745274), CYP2C19 (rs4244285), MDR1 (rs1045642), MRP4 (rs3742106). CYP3A4 and CYP3A5 variants were significantly associated with the LD phenotype, while the SREBF1 single nucleotide polymorphism (SNP) was associated with the lipohypertrophy subtype in euro-descendants, and one ESR2 SNP (rs3020450) was associated with lipoatrophy. Regarding the LD-related phenotypes, the TNF variant was associated with glucose levels and metabolic syndrome, while the two APOB variants were associated with lipid levels, and one ESR1 SNP (rs2813544) was associated with body mass index and subcutaneous fat in the female gender only. In conclusion, lipoatrophy and lipohypertrophy seem to be different entities from the genetics point of view. Our findings suggest that pharmacogenomics can give a better insight to the understanding of LD etiology.

1412F

Pharmacogenetic predictors of maintenance dose of Coumarinic oral-anticoagulants in patients with thromboembolic disorders in North Indian population. *B. Mittal[1], S.S. Rathore[1], S.K. Agarwal[2], S. Pande[2], S.K. Singh[3], T. Mittal[1].* 1) Dept Med Gen, Sanjay Gandhi Med Inst, Lucknow, UP, India; 2) Dept CVTS, Sanjay Gandhi Med Inst, Lucknow, UP, India; 3) Dept CTVS, CSMMU, Lucknow, India.

Coumarinic oral-anticoagulants (COAs) are the most commonly prescribed treatment option for thromboembolic events. However, their use requires close monitoring because of narrow therapeutic range and large inter-individual variations in drug response. Pharmacogenetic advancements in recent times have confirmed the role of genetic polymorphisms in VKORC1 and CYP2C9 in the dose requirements for COAs. The genotypic frequencies of VKORC1-1639 G>A, CYP2C9*2 and CYP2C9*3 polymorphisms in Indians are quite different from the HapMap populations. Therefore, we aimed at determining the relationship between these polymorphisms and weight normalised maintenance daily doses of oral anticoagulants for therapeutically stable INR in North Indians. We enrolled 206 patients taking acenocoumarol after heart valve replacement surgery (who had attained stable INR range between 2 and 3.5) and genotyped three polymorphisms. Mean daily maintenance drug doses were calculated for each genotype for the three polymorphisms under study. The patients with VKORC1 -1639 wild type (GG), heterozygous (GA) and variant (AA) genotypes showed a gradually decreasing requirement of mean daily maintenance drug dose (0.062±.023 mg/kg, 0.047±.023 mg/kg and .024±.010 mg/kg respectively). Patients with CYP2C9*1 (wild type) genotype needed higher mean daily maintenance drug dose (0.056±.023 mg/kg) than CYP2C9*2 and CYP2C9*3 carriers (0.049±0.019 mg/kg and 0.047±0.020 mg/kg respectively). These results are in accordance with the fact that promoter polymorphism affects the expression of VKORC enzyme and exonic polymorphisms influence the activity of the CYP2C9 enzyme. Based on these findings, we have developed a population specific pharmacogenetic dosing algorithm, including the clinical as well as genetic factors, by stepwise linear regression and found it to be more accurate in predicting doses than the published algorithms. Acknowledgement: Financial support from DBT (India).

1413F

Pharmacogenetic analysis of drug disposition genes in Azorean healthy population. *L. Mota-Vieira[1,2], S. Bulhoes[1,2], C. C. Branco[1,2], A. M. Vicente[2,3], R. Cabral[1,2], T. Pereirinha[1].* 1) Molecular Genetics & Pathology Unit of the Hospital of Divino Espirito Santo of Ponta Delgada, EPE - Azores Islands, Portugal; 2) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 3) INSA, Lisbon, Portugal.

Inter-individual and -ethnic variability of drug disposition genes can cause unexpected outcomes such as adverse reactions and toxicity. Therefore, the knowledge of pharmacologically relevant allele, genotype and/or haplotype distribution within specific populations is important for pharmacogenetics. Here, we evaluate in Azoreans, an admixed population, 17 polymorphisms in genes coding for 5 drug metabolizing enzymes (CYP2C19, CYP2D6, NAT2, TPMT and UGT1A1) and 1 transporter (ABCB1). Genotyping of 170 blood donors was performed by Taqman real-time PCR and fragment analysis by capillary electrophoresis. For CYP2C19, the metabolizing enzyme of clopidrogel activation and proton pump inhibitors, we found a lower incidence of poor metabolizers (1.2%). Concerning CYP2D6, an enzyme involved in about 25% of drugs biotransformation (eg. tamoxifen), the data showed 2 groups: carriers of at least 1 loss-of-function allele (11.2%), where, individuals are at risk for decreased tamoxifen response (postmenopausal early breast cancer treatment); and carriers of 2 functional alleles (30.6%), including gene duplication, which are likely to respond to tamoxifen. These results validate the need to genotype individuals before treatment. Regarding the NAT2, an enzyme involved in drug acetylation, 64.1% have slow acetylator phenotype, thus these individuals have increased risk for adverse effects if treated with isoniazid and hydralazine. The genotyping of TPMT, an enzyme of thiopurines metabolism used as anti-neoplasic or immunosuppressive drugs, identified 2 groups that require thiopurine pharmacogenetic based dosing prior treatment: high toxicity (4.7%), having 2 nonfunctional alleles; and moderate toxicity (2.4%) with 1 nonfunctional allele. The UGT1A1 analysis revealed that Azoreans have a high frequency of *28/*28 genotype (10.6%), resulting in reduced enzymatic activity and increased toxicity of irinotecan, a drug used to treat colorectal cancer. Finally, 25.3% of Azoreans are homozygous for 3435T (ABCB1), leading to a reduced expression and function of P-glycoprotein. Individuals with this genotype have been correlated with decreased bioavailability of digoxin, a P-gp substrate. Currently, we are analyzing haplotype and linkage disequilibrium of other pharmacogenes, in order to deeper understand the clinical impact of relevant pharmacogenetic information in Azorean population. (lmotavieira@hdes.pt; Funded by the Azores Government: M316/F/161/2009 and M316/F/425/2009).

1414F

A pharmacogenomic screen of common variation in candidate genes reveals moderators of antipsychotic-induced weight gain in the RUPP Autism samples. *E.L. Nurmi, S.L. Spilman, J.T. McCracken, The RUPP Autism Network.* Department of Psychiatry, University of California at Los Angeles, Los Angeles, CA.

Objective: Weight gain is a common adverse event resulting from antipsychotic exposure that carries significant morbidity and treatment complication. In order to dissect pharmacogenomic influences on antipsychotic-induced weight gain, we queried common variation and functional elements in key neurotransmitter receptor (DRD1, 2, 4, 5, ADRA2A, HTR2C, HRH1) and energy balance (FTO, MC4R, leptin, CNR1, FAAH) genes for association with weight gain in the two NIMH RUPP Risperidone RCT childhood autism samples. **Methods:** Gene variants were examined for association with weight gain during the first 8 weeks of similar risperidone exposure per protocol in a sample of 225 autistic children from the RUPP (2002) and RUPP-PI (2009) Risperidone studies. **Results:** Two independent gene variants in adrenergic receptor ADRA2A ($p<0.0001$ and $p=0.0002$) and single SNPs in dopamine D1, D2, and D4 ($p<0.0001$) as well as D5 ($p=0.0007$) receptor genes were associated with greater weight gain. While no individual SNPs in the serotonin receptor HTR2C reached significance, a protective haplotype explained 16.6% of the variance in weight gain in boys. A functional promoter variant and a synonymous SNP in the cannabinoid receptor 1 (CNR1) gene conferred independent risks for weight gain ($p<0.0001$) consistent with published results in adults. There was no evidence for association with a reported functional variant in the endocannabinoid metabolic enzyme FAAH. One leptin gene variant reached significance ($p=0.0001$) in our dataset. An additional leptin marker, BMI-associated SNPs in FTO and melanocortin 4 receptor (MC4R), and markers in histamine receptor HRH1 (the common target of antipsychotics with the greatest weight gain burden) showed only trends toward association. All reported results remained significant after correcting for multiple comparisons. **Conclusions:** These data support a greater genetic contribution of monoamine receptor and energy balance candidates to antipsychotic-related weight gain in children than prior adult studies suggest. Effects are robust enough to be detected after only 8 weeks of exposure. The variance explained by individual SNPs is modest, highlighting multifactorial contributions that likely include other unexamined factors, gene variants, and gene-gene and gene-environment interactions. Pharmacogenomics holds considerable promise in explaining individual variability in treatment effects and may help guide clinical intervention and drug development.

1415F

HLA-A*3101 **allele as a genetic risk factor for carbamazepine-induced cutaneous adverse drug reactions in Japanese population.** *T. Ozeki[1], T. Mushiroda[1], A. Yowang[1], A. Takahashi[2], M. Kubo[3], Y. Shirakata[4], Z. Ikezawa[5], M. Iijima[6], T. Shiohara[7], K. Hashimoto[4], N. Kamatani[1], Y. Nakamura[1,8].* 1) Research Group for Pharmacogenomics, RIKEN Center for Genomic Medicine, Yokohama, Kanagawa, Japan; 2) Research Group for Medical Informatics, RIKEN Center for Genomic Medicine, Yokohama, Kanagawa, Japan; 3) Research Group for Genotyping, RIKEN Center for Genomic Medicine, Yokohama, Kanagawa, Japan; 4) Department of Dermatology, Ehime University Graduate School of Medicine, Ehime, Japan; 5) Department of Dermatology, Yokohama City University Graduate School of Medicine, Kanagawa, Japan; 6) Department of Dermatology, Showa University School of Medicine, Tokyo, Japan; 7) Department of Dermatology, Kyorin University School of Medicine, Tokyo, Japan; 8) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

An anticonvulsant, carbamazepine (CBZ), is known to show incidences of cutaneous adverse drug reactions (cADRs) including Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DIHS). To identify a gene(s) susceptible to CBZ-induced cADRs, we conducted a genome-wide association study (GWAS) in 53 subjects with the CBZ-induced cADRs including SJS, TEN and DIHS, and 882 subjects of a general population in Japanese. Among the SNPs analyzed in the GWAS, 12 SNPs showed significant association with CBZ-induced cADRs, and rs1633021 showed the smallest *P*-value for association with CBZ-induced cADRs ($P = 1.18 \times 10^{-13}$). These SNPs were located within a 430-kb linkage disequilibrium (LD) block on chromosome 6p21.33 including the *HLA-A* locus. Thus, we genotyped the individual *HLA-A* alleles in 61 cases and 376 patients who showed no cADRs by administration of CBZ (CBZ-tolerant controls) and found that *HLA-A*3101* was present in 60.7%; (37/61) of the patients with CBZ-induced cADRs, but in only 12.5%; (47/376) of the CBZ-tolerant controls (odds ratio (OR) = 10.8, 95% confidence interval (CI) = 5.9-19.6, $P = 3.64 \times 10^{-15}$). To validate the significant associations of *HLA-A*3101*, we performed a replication study using an independent Japanese case-control cohort which consisted of 16 CBZ-induced cADR cases and 44 CBZ-tolerant controls. The association of HLA-A*3101 was replicated in the second cohort ($P = 1.53 \times 10^{-2}$, OR = 9.5, 95% CI of 1.5-24.5; combined-analysis $P = 1.09 \times 10^{-16}$, OR = 9.5, 95% CI of 5.6-16.3), implying that this allele has the 58.4% sensitivity and 87.1% specificity when we apply *HLA-A*3101* as a risk predictor for CBZ-induced cADRs. We further analyzed the association of *HLA-A*3101* according to the type of cADR using the combined cohorts. *HLA-A*3101* showed significant associations with DIHS ($P = 2.06 \times 10^{-9}$, OR = 9.5, 95% CI of 4.6-19.5) and SJS/TEN ($P = 2.35 \times 10^{-4}$, OR = 33.9, 95% CI of 3.9-295.6) as well as other cADRs ($P = 4.74 \times 10^{-8}$, OR = 8.0, 95% CI of 3.9-16.6), respectively. Although DIHS is clinically distinguished from SJS and TEN, our data presented here have indicated that they share a common genetic factor as well as a common pathophysiological mechanism. Our findings should provide useful information for making a decision of individualized medication of anticonvulsants.

1416F

Effect of PON1 Q192R genetic polymorphism on cardiovascular events in the CURE trial. *G. Pare[1, 2], S. Ross[1, 2], S. Mehta[1, 2], S. Yusuf[1, 2], S. Anand[1, 2], K. Fox[3], J.W. Eikelboom[1, 2].* 1) McMaster University, Hamilton, ON, Canada; 2) Population Health Research Institute, Hamilton, ON, Canada; 3) 4. Edinburgh University, Edinburgh, Scotland, UK.

A recent report suggested that carriers of the Q allele of the PON1 Q192R polymorphism had decreased biotransformation of clopidogrel into its active metabolite and decreased efficacy of clopidogrel in preventing cardiovascular events. Furthermore, PON1 has been reported to have a central role in the antioxidant function of HDL and the Q192R polymorphism has been previously associated with cardiovascular risk in patients not treated with clopidogrel. Patients from the CURE randomized trial that demonstrated benefits of clopidogrel versus placebo in preventing CV events in acute coronary syndromes (ACS) were genotyped for the PON1 Q192R polymorphism. Clopidogrel compared to placebo significantly reduced the primary efficacy outcome, irrespective of PON1 Q192R genotype (P=0.07 for heterogeneity). No association was observed between the Q192R polymorphism and cardiovascular events in the overall sample (HR= 1.09 per allele; 95%CI 0.95-1.24; p=0.23). However, an association was observed between the Q allele and increased cardiovascular events in the placebo group (HR=1.23 per allele; 95%CI 1.03-1.47; p=0.03), but not in the clopidogrel group (HR= 0.93 per allele; 95%CI 0.76-1.13; p=0.46). In conclusion, our study shows that PON1 Q192R genotype do not modify the efficacy and safety of clopidogrel in ACS patients. Further studies will be needed to confirm or refute the association of the Q allele with adverse cardiovascular events independently of clopidogrel in secondary prevention patients.

1417F

MDR1 variation is associated with adverse drug reactions of methylphenidate in Korean children and adolescents with ADHD. *HS. Park[1], SW. Kim[1], SH. Lee[1], KH. Kim[1], MG. Lee[1,2], HJ. Hong[3], KH. Yook[4], JH. Lee[1,2,5].* 1) Department of Pharmacology, Brain Korea 21 Project for Medical Science, College of Medicine, Yonsei University, Seoul, Korea; 2) Research Center for Human Natural Defense System, College of Medicine, Yonsei University, Seoul, Korea; 3) Department of Neuropsychiatry, College of Medicine, Hallym University, Anyang, Korea; 4) Department of Psychiatry, School of Medicine, CHA University, Seoungnam, Korea; 5) Corresponding Author.

Methylphenidate (MPH) is the drug of choice for treatment of patients with attention-deficit hyperactivity disorder (ADHD). MPH is a substrate of multidrug resistant protein 1 (MDR1, ABCB1, p-gp), which plays an important role in the clearance of psychotropic drugs and their metabolites from brain tissues. Therefore, genetic variations in the MDR1 gene may affect individual drug responses to MPH. In this study, we analyzed the adverse drug reactions (ADRs) of MPH in 134 Korean ADHD patients who completed a 4-week trial of osmotic-release oral system-MPH. The adverse effects of MPH were evaluated using the Barkley Stimulant Side Effects Rating Scale. From the association study between MDR1 polymorphisms and adverse effects of MPH, one nonsynonymous polymorphism showed a strong association with MPH-related adverse effects (P=0.008). Logistic regression analysis with multiple clinical variables indicated that this variation is an independent determinant of adverse effects attributed to MPH. In a functional study using adenosine triphosphatase assays, the variation markedly reduced MPH transport across the cell membrane. This study is the first to demonstrate that the single nucleotide polymorphism in the MDR1 gene is associated with ADRs to MPH.

1418F

Genetic determinants of the busulfan pharmacokinetics. *M.A. Rezgui[1,4], M. Ansari[1,5], Y. Théoret[1,2,4], M. Duval[1,3], M.F. Vachon[1], H. Bittencourt[1,3], M. Krajinovic[1,2,3,4].* 1) Charles-Bruneau Cancer Center, CHU Sainte-Justine Research Center, Montreal, QC; 2) Clinical Pharmacology unit, CHU Sainte-Justine, Montréal, QC; 3) Department of Pediatrics, Faculty of medicine, University of Montreal, Montreal, QC; 4) Department of Pharmacology, Faculty of medicine, University of Montreal, Montreal, QC; 5) Department of Pediatrics, Geneva University Hospital.

Busulfan (BU) is a key compound in conditioning myeloablative regimens for children undergoing hematopoietic stem cell transplantation (HSCT). There are wide interindividual differences in BU pharmacokinetics, which increase the risk of veno-occlusive disease, graft rejection and disease relapse. As BU is mainly metabolized by glutathione S-transferase (GST), it is possible that functional polymorphisms in GST genes may contribute to the variability in BU pharmacokinetics. Our recent pilot study interrogating the polymorphisms in GST gene and pharmacokinetics after the first dose of intravenous BU showed a significant association between GSTM1 null genotype and higher drug exposure. In order to replicate and validate obtained pharmacogenetic finding we carried out a prospective study which included~70 children who underwent HSCT at the CHU Sainte-Justine, Montreal. Blood samples were collected immediately before the first BU administration and at 15, 30, 60, 120, 180 and 240 min after drug administration. Plasma BU was determined using a modified high-performance liquid chromatography assay. All patients had individualized dosing based on pharmacokinetics after the first dose of intravenous BU. The genotypes in GSTA1 (C-69T, A-513G, G-631T, C-1142G), GSTM1 (deletion) and GSTP1 gene (A1578G, C2293T) were obtained by PCR followed by allele specific oligonucleotide hybridization or gel electrophoresis. Genotypes were dichotomized according to the presence or absence of minor allele or presence or absence of GSTM1 deletion (GSTM1 null genotype vs. non-null genotype). The distribution of pharmacokinetic parameters (AUC, Cmax, Css and clearance) obtained after the first BU dose was compared for patients with or without the given genotype using Student t-test. Results seem to successfully replicate those obtained in retrospective study. GSTM1-null individuals had higher drug exposure (P(Cmax)=0.03; P(AUC)=0.05; P(Css)=0.04) and lower clearance (P(CL)=0.05). No association was found between BU exposure and major GSTA1 or GSTP1 gene variants. GSTM1 polymorphism seems to modify BU pharmacokinetics after intravenous drug administration, as shown in two independent patients cohorts opening the possibility to tailor first Bu dose according to patient genetics.

1419F

Polymorphisms in DNA Repair Genes, chemotherapy and survival in Bladder Cancer. *A. Russo[1,5], S. Guarrera[1], F. Ricceri[1,5], C. Sacerdote[1,3], S. Polidoro[1], R. Critelli[1,5], A. Allione[1], P. Gontero[2], P. Destefanis[7], G. Cucchiarale[4], P. Vineis[1,6], G. Matullo[1,5].* 1) Human Genetics Foundation, Turin, Turin, Italy; 2) Urologia 1, S. Giovanni Battista Hospital, Turin, Italy; 3) Epidemiology of Cancer Unit, S. Giovanni Battista Hospital, Turin, Italy; 4) Unità operativa di Urologia, Clinica Cellini, Turin, Italy; 5) Department of Genetics, Biology and Biochemistry, University of Turin, Italy; 6) Imperial College, London, UK; 7) Urologia 2, S. Giovanni Battista Hospital, Turin, Italy.

Bladder cancer (BC) survival is highly influenced by environmental and susceptibility genetic factors. Evidence of the important role played by DNA repair systems in the survival after cancer therapy has been reported. We conducted a hospital-based case-control study. Cases are incident BC patients, males, aged 40 to 75 years. We followed up BC patients for death and recurrences. In the cohort of BC patients, we genotyped 36 Single Nucleotide Polymorphisms (SNPs) in 10 DNA repair genes to assess the relationship between DNA repair genes SNPs and survival after treatment in BC patients. We analyzed survival on BC patients using a Cox proportional-hazards regression statistics for codominant and per-allele models, adjusted for age, stage and grading and stratified by therapy (chemotherapy vs no or other therapies). Chemotherapy group include patients treated with systemic and local chemotherapy with different therapeutic plans (Carboplatinum doublets, Gencitabin, Epirubicin, etc). We identified 456 BC patients which were followed-up for mortality (median follow-up 62 months). In multivariate analysis both stage and grade predicted the outcome in a statistically significant way. The variant alleles of two SNPs in XRCC1 gene (rs2854509 and rs3213255) were associated to a significant lower risk of death whereas one SNP in ERCC2 gene (rs171140) showed a significant higher risk of death compared to the wild type only in the group treated with chemotherapy. Haplotype analyses confirmed the results. Our findings suggest that DNA damage levels induced by chemotherapy may be modulated by individual DNA repair activity, resulting in a different response to chemotherapy and, consequentially, in different survival rates.

1420F

Multi-ethnic Distribution of Clinically Relevant CYP2C Genotypes and Haplotypes. *S.A. Scott[1], I. Peter[1], J.S. Hulot[2], R. Kornreich[1], R.J. Desnick[1], S. Martis[1].* 1) Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY; 2) Cardiovascular Research Center, Mount Sinai School of Medicine, New York, NY.

The human cytochrome P450-2C (CYP2C) enzyme family, encoded by CYP2C19, CYP2C9 and CYP2C8 at 10q23.33, is responsible for the metabolism of ~25% of clinically used drugs. Although many CYP2C variant alleles have been identified, their frequencies in many populations remain unknown. Moreover, linkage disequilibrium (LD) between the common CYP2C19*17 and *2 gain- and loss-of-function alleles, respectively, and other known functional CYP2C variants has not been adequately studied in different ethnicities. Thus, to determine the haplotype structure of the CYP2C locus, 35 functional CYP2C19, CYP2C9, and CYP2C8 variant alleles were genotyped in up to 250 DNA samples each from healthy African-American (AA), Asian, Caucasian, Hispanic, and Ashkenazi Jewish (AJ) individuals. Allele and genotype frequencies, determined for each variant, differed significantly between racial and ethnic groups. Previously understudied variant alleles were detected at low frequencies (e.g. CYP2C19*6, *9, *13, *15; CYP2C8*13, *14) and the recently identified CYP2C19*4B allele, defined by c.-806C>T (*17) and c.1A>G (*4) in cis, was confirmed by cloning and sequencing in the Caucasian (0.4%), Hispanic (0.4%), and AJ (2.0%) populations. This allele was found on a haplotype with wild-type CYP2C9 and CYP2C8 (i.e. 2C19*4B-2C9*1-2C8*1). In total, 15 different CYP2C haplotypes with frequencies greater than 1% were inferred using 13 informative alleles and the two most common variant haplotypes were 2C19*2-2C9*1-2C8*1 (12-27%) and 2C19*17-2C9*1-2C8*1 (5-17%). Importantly, a 2C19*17-2C9*1-2C8*2 haplotype was also identified among AAs (7.8%), Hispanics (2.9%) and AJs (1.0%), indicating that CYP2C19*17 does not always tag a CYP2C haplotype that encodes efficient CYP2C-substrate metabolism as previously suggested. Moreover, a haplotype containing two CYP2C loss-of-function alleles (2C19*1-2C9*2-2C8*3) was identified in all populations except AAs. Unique ethnic-specific haplotypes were also detected, including 2C19*3-2C9*1-2C8*1 among Asians, and 2C19*2-2C9*1-2C8*4 and 2C19*17-2C9*1-2C8*4 among AJs. These data highlight that, despite largely acting as independent loci, CYP2C19*17 and *2 can also be found in LD with other CYP2C loss-of-function alleles that significantly modify the metabolizer phenotypes. These results have important implications for pharmacogenetic association studies involving the CYP2C locus and are clinically relevant when administering CYP2C-substrate medications.

1421F

Preclinical evaluation of relapse-predisposing variations in DHFR and TS genes. *B. Sharif-Askari[1,3], F. Fontaine[1], E. Haddad[1], D. Sinnett[1,2], M. Krajinovic[1,2,3].* 1) Research Center, Centre Hospitalier Universitaire Sainte-Justine, Montreal, QC; 2) Department of Pediatrics, University of Montreal, Montreal, QC; 3) Department of Pharmacology, University of Montreal, Montreal, QC.

Acute lymphoblastic leukemia (ALL) is the most frequent malignancy of childhood. It is the principal cause of cancer-related mortality in children due to a persistent group of patients who does not respond to standard anti-cancer treatment. Dihydrofolate reductase (DHFR) and tymidylate syntase (TS) are major targets of methotrexate (MTX), a crucial component of ALL treatment. Our pharmacogenetic study interrogating association between genetic variations of MTX action pathway and ALL outcome indicated a higher risk of relapse in patients who are homozygous for triple repeat (3R) allele of TS gene and/or have *1b haplotype defined by particular allelic combination of polymorphisms in the promoter of DHFR gene. Both variations were associated with an increase in respective gene expression, suggesting that lower sensitivity to MTX associated with these variations might be a cause of higher risk of relapse. To verify this and examine if any increase in MTX dose may overcome genotype-associated MTX resistance (and provide thus basis for individually tailored therapy), we recently initiated preclinical study which uses appropriate in vitro and animal models (NSG immuno-compromised mice injected with patients lymphoblasts) to address above questions. The preliminary results of this study will be presented. We are using lymphoblastoid CEPH cell lines to correlate in vitro sensitivity to MTX with relapse-predisposing genotypes in DFHR and TS genes. DNA from 94 lymphoblastoid cell lines was extracted for genotyping and cell viability test in response to MTX challenge was performed for each of them. Results indicate that 57 cell lines (60.6%) are sensitive to MTX, (with IC 50 ranging from 0.1to10 μM) and 22 (23.4%) are resistant to treatment with MTX (IC50 more than 100 μM), whereas remaining had intermediate phenotype. In the next step we will compare sensitivity status of each cell line with the results of TS 3R3R and DHFR *1b genotyping currently performed in cell lines. NSG mice injected with patient's leukemia cells, showed the expansion of leukemia cells, about four weeks after receiving patient's cells, MTX treated group showed significant reduction of leukemia cells compared to PBS control group, one week after treatment with MTX. We are currently proceeding with NSG mice experiments including patients having either TS or DHFR relapse predisposing genotypes.

1422F

Transcription factor protein levels correlate with chemotherapeutic susceptibility in lymphoblastoid cell lines. *A.L. Stark[1], R.J. Hause, Jr.[2], L. Gorsic[3], N. Antao[3], K.P. White[1,4,5], R.B. Jones[4,5], M.E. Dolan[3].* 1) Dept Human Gen, Univ Chicago, Chicago, IL; 2) Committee on Genetics, Genomics, and System Biology, Univ Chicago, Chicago, IL; 3) Section of Hematology/Oncology, Department of Medicine, Univ Chicago, Chicago, IL; 4) Ben May Department of Cancer Research, Univ Chicago, Chicago, IL; 5) Institute for Genomics and Systems Biology, Univ Chicago, Chicago, IL.

A substantial number of cancer patients undergo ineffective chemotherapy while enduring toxic side effects. Pharmacogenomics aims to develop predictive markers for patients at greatest risk of experiencing chemotherapeutic related toxicities and/or nonresponse to avoid this predicament. Our lab has developed models that employ HapMap lymphoblastoid cell lines (LCLs) to identify chemotherapeutic susceptibility genetic variants by evaluating performing GWAS with HapMap SNPs and chemotherapeutic-induced cytotoxicity. These LCLs have also been used to study correlations between mRNA expression and drug susceptibility. To expand our model into the proteome, we used newly developed micro-western arrays and reverse phase lysate arrays to assay protein levels for over 400 transcription factors and core signaling proteins in 68 unrelated Yoruba derived LCLs. Protein levels were quantified from three different thaws for each cell line providing a robust baseline measurement. We then correlated each protein level against 13 different phenotypes from 8 chemotherapeutic agents (etoposide, daunorubicin, cisplatin, carboplatin, cytarabine, capecitabine, pemetrexed, and paclitaxel). We identified 17 protein-drug relationships with an r^2 greater than 0.10 ($p<0.007$, $q<0.20$). Among these top relationships, only one quarter would have been identified through mRNA correlation with chemotherapeutic induced cytotoxicity ($p<0.05$). The highest correlation between protein level and cisplatin- and carboplatin- induced cytotoxicity was TUB with an r^2 correlation of 0.13 for both phenotypes. TUB protein levels showed no significant ($p<0.01$) correlation with any other drug phenotype. TULP1, TUB's binding partner was also significantly correlated with carboplatin-induced cytotoxicity ($r^2=0.09$). We also detected two different isoforms of STAT3 that are highly correlated with drug-induced apoptosis for cisplatin, cytarabine, and paclitaxel. In addition, we performed genomewide association studies and identified SNPs that associate with drug phenotypes that also predict protein level. We identified 42 SNPs that were associated with drug phenotype and protein level at $p<0.0001$. To our knowledge, these protein levels represent the largest dataset of its type. This allows pharmacogenomic discovery work to move from the transcriptome to the proteome. A better understanding of the variation in the proteome can aid in the discovery of genetic predictors of chemotherapy toxicity and response.

1423F

Unprogrammed presentation number

1424F

Genealogical analysis of intolerance to statins in the Saguenay-Lac-Saint-Jean population. *M. Tremblay[1], T. Bouhali[2], D. Gaudet[2], D. Brisson[2].* 1) Université du Québec à Chicoutimi, 555 boul. de l'Université, Chicoutimi, Qc, Canada, G7H 2B1; 2) ECOGENE-21 (Université de Montréal), Centre hospitalier affilié universitaire régional (CAUR) de Chicoutimi, 305 Rue St-Vallier, Chicoutimi, Qc, Canada, G7H 5H6.

Purpose: Statins are the most widely prescribed class of cholesterol-lowering drugs worldwide. Familial hypercholesterolemia (FH) patients are among those receiving the highest daily doses. Although well tolerated, statins can be associated with muscular and non-muscular side effects that affect compliance to drug treatment. Type of statins, age, gender and daily dose are factors associated with muscular side effects. Moreover, family aggregation of intolerance to statins is frequently observed, suggesting the involvement of gene factor transmission. The objective of the present study was to measure and compare various genealogical parameters that could shed some light on the origins and diffusion of intolerance to statins using FH as a model. **Data and methods**: Analysis was performed on a total of 224 genealogies from 112 FH subjects carrying either the D15kb (n=28) or W66G (n=84) mutations and 112 non-FH controls. For each mutation, carriers were split into two equal groups according to their tolerance to statins. Genealogical material was obtained from the BALSAC population register. Number of ancestors, geographical origins and genetic contribution of founders, identification of common and specific ancestors, inbreeding coefficients and kinship coefficients amongst and between subjects and controls were calculated using the S-Plus based GENLIB software package. **Results**: For both mutations, repeated occurrences of the same ancestors are more frequent among the carriers' genealogies than among the controls', but no important difference was observed between tolerant and non-tolerant subjects. Kinship coefficients are also significantly higher among carriers than among controls, with no difference according to tolerance to statins. However, inbreeding coefficients are slightly lower among intolerant subjects, which suggests different patterns of demographic behavior among their ancestors. Although analysis of common and specific ancestors helped to identify the founders who most probably introduced each mutation in the SLSJ population, no distinction could be made between tolerant and intolerant subjects. These findings suggest that if family aggregation of intolerance to statins is influenced by genetic factors, these are far from being the unique modulators. **Funding**: This study was supported by the ECOGENE-21 project (CIHR TEAM grant # CTP-82941) and by the Social Sciences and Humanities Research Council of Canada (grant # 410-2009-2292).

1425F

Genetic variation database of anticancer pharmacogenomic biomarkers using cross species sequence analysis. *L. Wang, J. Liu, D. Chen.* School of Medicine, University of California, Irvine, Irvine, CA.

Interindividual variability in the response to pharmacologic agents is well documented. Genetic differences is one significant component contributing to such variability. With the rapidly advancement in cancer treatment using targeted therapy, several biomarkers are known to be associated with drug response variability. Genetic variations in these biomarkers are known to cause either pharmacokinetic effect variability or pharmacodynamic effect variability. These biomarkers includes TPMT, UGT1A1, EGFR, KRAS, ABL, KIT, ERBB2 and Estrogen receptor. It is estimated these biomarkers contribute a significant portion of pharmacologic variability. Our goal is to design a comprehensive database containing all the genes in the associated biochemical pathways of these known biomarker causing cancer drug variable responses. Together, over 470 genes are found by multiple query approach. In addition, corresponding orthologous gene from 13 mammalian genomes that completely sequences are identified. We then conducted sequence comparisons at both the DNA and protein level of each of the 470 human genes with it's corresponding orthologous gene sequence. Nucleotide positions that are conserved across species and, if mutated would likely result in functional changes are identified. Our attempt is to build a database which enable targeted mutational analysis on nucleotide acid position that is most likely contributing to drug response variability currently not explained by the known drug biomarkers.

1426F

Pharmacogenomics Knowledge Base (PharmGKB): Personal genome annotation. *M. Whirl-Carrillo[1], L. Gong[1], M. Gong[1], J.M. Hebert[1], F. Liu[1], E.M. McDonagh[1], K. Sangkuhl[1], C.F. Thorn[1], R. Whaley[1], M. Woon[1], R.B. Altman[1,2], T.E. Klein[1].* 1) Genetics, Stanford University, Stanford, CA; 2) Bioengineering, Stanford University, Stanford, CA.

The Pharmacogenomics Knowledge Base (PharmGKB: http://www.pharmgkb.org) is an online resource devoted to pharmacogenomic knowledge acquisition and clinical applications of this knowledge. One potential application is personal genome annotation. In the age of Direct-to-Consumer genomics, genome-wide genotyping is readily available and the number of people in possession of their own genomic information is growing. Customers typically purchase these products because they are interested in phenotypes associated with their genotypes and DTC companies often provide some interpretation services for their customers. PharmGKB is well-positioned to use its specialized pharmacogenomics knowledge to annotate DTC genotypes and next-generation genome sequences. PharmGKB has previously used its manually curated database of drug-genetic variant associations to annotate a sequenced human genome (Lancet, 2010 May 1;375(9275):1525-35). Over 1600 variant annotations from PharmGKB were screened for quality of evidence and an MD determined the clinical relevance of the annotations. This process was entirely manual and required a substantial amount of personnel resources. Recently, PharmGKB systematically revisited its drug-variant associations and streamlined its genome annotation process. We updated all drug-variant annotations with more specific information and statistics such as study size, ethnicity and association p-value. We aggregated annotations from multiple literature sources for each drug-variant combination and created one “clinical annotation” for each combination based on ethnicity. We created a text summary for each possible genotype and assigned an evidence score based on standardized criteria to each clinical annotation. Finally, we implemented an automated pipeline to take an uploaded file of genotypes defined by dbSNP rs IDs and generate a report of personalized clinical annotations. We analyzed 23andMe genotyping results from four unrelated individuals using this system. We were able to quickly and automatically assign pharmacogenomic interpretations based on each individual's genotype. This system can be used routinely for pharmacogenomic genome annotation.

1427F

Association of CYP3A4 and CYP2C19 genotypes with treatment-related acid peptic disease. *LE. Wong-Ley[1,2], MA. Aguilar-Rodríguez[2], A. Flores García[2], P. Aguiar García[2], M. Pérez-Nuño[2], A. Puentes[1], E. Higareda-Almaraz[3].* 1) Dept Gen, SSN, Tepic, Mexico; 2) Dept Gen, UAN, Tepic, Mexico; 3) Dept Gen, IMSS, Tepic, Mexico.

The usage of omeprazol as a medical treatment for acid peptic disease and its main complication, ulcer disease, it is effective because it modifies the acid/pepsin homeostasis and it plays a very important role at the beginning and progression of the ulcers, the main cause of mobility in our country for about four decades Some differences have been reported in the response to the treatment with omeprazol related with ethnic origin and there are differences in the frequencies of the polymorphisms that are studied, that are why we considered a probable association between this polymorphisms and the response to the treatment. The objective the present study is to know the participation of CYP3A4 and CYP2C19 genes in the variation of omeprazol dose in Mexican population. They were included in the study 73 patients that were receiving omeprazol as part of the acid peptic disease treatment. All patients signed an informed consent and a questionnaire was answered. DNA was obtained from lymphocytes and followed the identification by PCR and enzymatic digestion of *1B of CYP3A4 and *2 of CYP2C19 variants. At of Student test was applied to determine doses differences between genotype groups. The genotype and allelic frequencies compared from the two referred polymorphisms between sensible and resistant groups without estimating differences that can refer to association among them and the clinical response to the treatment with omeprazol. When making the analysis of the polymorphisms of CYP3A4 and CYP2C19, between the reference group and patients with omeprazol treatment, we did not find significant statistical differences between the genotype and allelic frequencies. The genotype and allelic frequencies of the *1B of CYP3A4 and *2 of CYP2C19 polymorphisms in the patients with acid peptic disease in the reference population, are similar to the ones of the general population. As well as the distribution of the CYP3A4*1B and CYP2C19*2 polymorphisms, the reference population agreed with the predictions of the Hardy-Weinberg Law. No main variables play an important role in the dosing of omeprazol in the studied population.

1428F

Molecular study of SLC19A1 sequence variations in Iranian patients affected with psoriasis. *A. YASARI MAZANDARANI, S. MATOO, A. TAVAKOLI TAMEH, M. MAHDAVI, B. SEDAGHATI KHAYAT, N. HATAMNEJADIAN, SH. ABADPOUR, A. EBRAHIMI*. Dr. Ahmad Ebrahimi Molecular Genetics , PhD Parseh Medical Genetics Counselling Center, Floor 7, No.75, Royan Alley,Keshavarz Bolv. Tehran, Iran Tel-Fax: +98 21 88966579,88996889 E-mail: ae35m@yahoo.com.

Introduction: Psoriasis is a chronic autoimmune skin disease with polygenic inheritance with five main types including; Plaque, Guttate, Inverse, Pustular and Erythrodermic. Methotrexate (MTX) is a first-line systemic therapy for psoriasis. The use of MTX is limited by individual variability in response. It is transported into the cell via the solute carrier family 19, member1 (SLC19A1). This evidence suggests that SLC19A1 genetic variations may affect the response to mtx in psoriasis patients. Material and method: To assess if SLC19A1single nucleotide polymorphisms in order to evaluate treatment outcomes in patients with psoriasis we clinically screened affected patients and selected 100 patients with psoriasis who had been treated with methotrexate. Results: All coding regions were amplified using intronic primers and the PCR product was analyzed by direct sequencing We detect some sequence variations which show significant difference between case control groups. Also we detect some sequence variations were suspected as a new mutation that needs to be analyzed by protein assay methods. Key words: Psoriasis, SLC19A1, PCR, Sequencing.

1429F

CYP2C9*8 and Warfarin Dose Requirements in African-Americans. *C. King[1], C. Eby[1,2], P. Lenzini[1], R. Porche-Sorbet[2], E. Do[1], G. Moskowitz[1], P. Ridker[3], S. Scott[4], R. Desnick[4], B. Gage[1].* 1) Department of Internal Medicine, Washington Univeristy School of Medicine, St Louis, Missouri; 2) Department of Pathology & Immunology, Washington Univeristy School of Medicine, St Louis, Missouri; 3) Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts; 4) Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, New York.

Introduction: Warfarin initiation is associated with a high rate of adverse events, especially in African-American populations. While CYP2C9*2 and *3 and VKORC1 -1639 G>A single-nucleotide polymorphisms (SNPs) affect with warfarin dosing, they explain less than 8% of the variation in warfarin dose requirements in African-Americans. The goal of our study was to quantify the contribution of additional SNPs to warfarin dosing in African-Americans. **Methods:** We genotyped CYP2C9*8 (rs7900194), CYP2C9*11 (rs28371685), and VKORC1 D36Y (rs61742245) in 213 African-American patients whose therapeutic warfarin dose was known. For each SNP, we performed a regression of the residual dosing error from a validated pharmacogenetic dosing algorithm (i.e. ln(observed therapeutic dose - pharmacogenetic predicted therapeutic dose) onto the number of variant alleles. **Results:** CYP2C9*8 was a significant (p= 0.0008) predictor of residual dosing error and was associated with a 22.9% reduction in warfarin dose (95% CI: 10.5%-33.7%) per A allele. Among the 206 patients with complete information, 2 were homozygous for the *8 allele, both of whom had an elevated INR (> 4.0) within the first week of starting warfarin therapy. Thirteen percent (N = 26) were heterozygous. Patients with the CYP2C9*11 variant (N = 7) averaged a 10.6% lower therapeutic dose than predicted, but this reduction was not significant (P = 0.49) possibly due to the low frequency of the SNP. VKORC1 D36Y was not present in our population. **Conclusions:** CYP2C9*8 is a significant predictor of warfarin dose in African-American patients. Including CYP2C9*8 in a panel of SNPs may improve warfarin dosing accuracy in African-Americans and reduce the risk of bleeding during warfarin initiation.

1430F

Association of -354GG genotype of the *GGH* gene and bone marrow toxicity in rheumatoid arthritis patients treated with methotrexate. *L. Lukovic[1], B. Jekic[1], T. Damnjanovic[1], V. Milic[2], V. Bunjevacki[1], N. Maksimovic[1], J. Milasin[3], I. Novakovic[1], B. Popovic[3], N. Damjanov[2], G. Radunovic[2], N. Pejnovic[2], M. Krajinovic[4].* 1) Institute of Human Genetics, Faculty of Medicine, University of Belgrade, 26, Visegradska Str., 11000 Belgrade, Serbia; 2) Institute of Rheumatology, Faculty of Medicine, University of Belgrade, 69, Resavska Str., 11000 Belgrade, Serbia; 3) 3Institute of Biology and Human Genetics, Faculty of Dentistry, University of Belgrade, 1, Dr Subotica Str., 11000 Belgrade, Serbia; 4) Centre de Recherche, CHU Sainte-Justine, 3175 Chemin de la Côte-Ste-Catherine, Montréal, QC, H3T 1C5 Canada.

Due to its great interpatients variability in efficacy and toxicity in the therapy of rheumatoid arthritis (RA), methotrexate (MTX) is an attractive target for pharmacogenetic testings. In a group of 184 RA patients treated with MTX we have investigated whether 5 polymorphisms in 3 genes involved in MTX action [gamma-glutamyl hydrolase (GGH), cyclin D1 (CCND1) and thymidylate synthase (TYMS)] modulate MTX efficacy and/or have impact on drug adverse effects. The efficacy of MTX therapy has been estimated through the disease activity score in 28 joints (DAS28) based on EULAR criteria and relative DAS28 values (rDAS28). Among 184 RA patients 89 (48.4%) were classified as responders (good/moderate response)and 95(51.6%)as non-responders (poor response) according to EULAR criteria. rDAS28 values ranged from -0.02 to 0.80 (mean value 0.32±0.18). Fifty three (28.8%) patients experienced adverse drug events (ADEs) (hepatotoxicity, vomitus, cough and bone marrow toxicity). Genotypes of the selected polymorphisms in *GGH* gene (-354G>T and 452C>T) and *CCND1* (870 A>G) were obtained by PCR-RFLP. Genotypes of the 2R/3R tandem repeat polymorphism in the *TYMS* gene were analyzed by PCR and gele electrophoresis, whereas the SNP in the second repeat of 3R allele was detected by PCR-RFLP method. Obtained genotypes were then investigated for an association with the efficacy and toxicity of MTX. No significant associations of analyzed genetic variants with MTX efficacy and overall toxicity were demonstrated. Interestingly, when analysis were performed according to the type of ADE, an association was found between *GGH* -354G>T polymorphism and bone marrow toxicity (p=0.01). All patients with bone marrow toxicity were homozygous for -354G allele of the *GGH* gene (p=0.003, when individuals with GG genotype were compared to the carriers of T allele). Our data indicate strong association between MTX bone marrow toxicity and -354GG genotype of the *GGH* gene.

1431F

Impact of genetic polymorphism of xenobiotics detoxification on bronchial asthma phenotypic features in children. *Y. Alimova[1], L. Jelenina[1], A. Galustyan[1], A. Glotov[2].* 1) Saint-Petersburg State Pediatric Medical Academy, Saint-Petersburg, Russian Federation; 2) Research Institute of Obstetric and Gynecology under the RAMN, Saint-Petersburg, Russian Federation.

Bronchial asthma(BA)formation is genetically influenced by both “main” genes and “candidate” genes. The goal of this research is to study peculiarities of bronchial asthma genotypes in children depending on provoking factors and on polymorphism of “candidate” genes, namely genes I and II of xenobiotics detoxification phase. Research methods: Bronchial asthma was diagnosed in 81 children. The multiform variants of genes CYP2D6, CYP2C19,CYP2C9,CYP1A1 and GSTT1,GSTM were studied with the help of the PCR method in the prenatal diagnostics laboratory.Results In order to assess the contribution of several polymorphous genes to BA clinical course, it is possible to use the scoring system where each sign is coded with points (A.Glotov, 2009). The points were counted in the following way: “wild type” homozygotes were labeled as number 1, heterozygotes had number 2, and “mutant allele homozygotes were labeled as number 3. Then the points were summarized for genotypes of xenobiotics intoxication system for each patient, with different phenotypic variants of BA. On the grounds of clinical features, two phenotypes of bronchial asthma were found in children. The first phenotype (37 children) was bronchial asthma in combination with allergic rhinitis and atopic dermatitis (atopic march-AM); the second phenotype (44 children) was found in children who only had respiratory tract lesions (atopic bronchial asthma-ABA). Results 1.For ABA patients, the average count for “adverse alleles” was 3.4 points. 2.For children with BA in the atopic march structure, the average count for “adverse alleles” was 4.2 points, which is reliably higher than the count in the first group (p=0.005). 3.In the ABA group, 35%; of the children had a combination of more than four “adverse alleles”, whereas the number of child patients in the atopic march BA group was twice as high (65%;,p<0.05). 4.65%; of the patients with ABA had less than three “adverse alleles”. The number of child patients in the atopic march BA group was twice as low (35%;;, p<0.05). Conclusion: The research has established a higher frequency of gene defects in the xenobiotics detoxification system in patients with atopic march compared to patients with ABA. The phase I and phase II genetic defects of the xenobiotics detoxification system found in this research are likely to stipulate phenotypic differences in clinical presentation of BA; besides, they are an aggravating factor in the clinical course of the disease.

1432F

Polymorphisms in IL4R Gene are Associated with Asthma Exacerbation. *L. Huang, B. Koshy, M. Mosteller, W. Anderson.* GSK, Durham, NC.

Asthma is a heterogeneous disease with variable signs and symptoms among patients. Asthma exacerbations are defined as acute episodic events that require urgent action on the part of the patient and physician to prevent a serious outcome, such as hospitalization, intubation for respiratory failure or death from asthma. Although a number of clinical and environmental factors have been associated with recurrent asthma exacerbations, currently there is no way of accurately predicting those at risk of having a serious asthma exacerbation. This study sought to identify genetic marker(s) that are associated with asthma exacerbations and define a subgroup of asthmatics with increased risk for asthma exacerbation. Fifty-one genetic polymorphisms in 18 candidate genes were evaluated in asthmatics who participated in Phase IV clinical trials ADA109055/ADA109057 (clinicaltrials.gov No.: NCT00452699/NCT00452348) or SFA103153 (NCT00102765) and provided consent for pharmacogenetic analysis. Majority of asthmatics in ADA109055/ADA109057 studies are whites while SFA103153 is a study of asthmatic subjects of African descent. Association analyses between each of the markers and asthma exacerbation presence/absence status were performed within each of studies. A meta-analysis was applied to estimate and assess the genetic effect across studies. Based on the meta-analysis results, several coding variants from interleukin-4 receptor (IL4R) were identified as being associated with asthma exacerbation, including rs1805011 (E400A; P=0.0016), rs1801275 (Q576R; P=0.0021), rs2234898 (L414L; P= 0.0028), rs2234900 (L433L; P=0.0041) and rs1805015 (S503P; P=0.0047). All five SNPs were in strong LD, which may represent a single underlying association. Allele frequencies showed significant differences between whites and African American, such that the minor alleles for E400A and Q576R in whites were more frequent alleles in the African American. The C allele at E400A is a protective allele for asthma exacerbation, with odds ratio equal to 0.52 in ADA109055/ADA109057 studies and 0.53 in SFA103153 study when using an additive model. Genetic admixture measures were analyzed to better understand the differences in genetic association across different study/race groups. These findings add to our understanding of the genetics of asthma exacerbation and contribute information that may lead to the identification of a subgroup of asthmatics with increased risk for asthma exacerbation.

1433F

Molecular mechanisms underlying glucocorticoid response eQTLs. *F. Luca[1], J.C. Maranville[1], A.L. Richards[1], D.B. Witonsky[1], M. Stephens[1,2], A. Di Rienzo[1].* 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Department of Statistics, University of Chicago, Chicago, IL.

Despite the functional relevance of eQTLs, there is a general lack of understanding of the molecular mechanisms by which they affect gene expression levels. Intervention experiments informed by data on the molecular biology of the altered pathway represent an ideal setting to dissect the molecular mechanisms underlying eQTLs that interact with treatment condition (gene-by-environment interactions). Because glucocorticoids (GCs) are steroid hormones that mediate the response to stress and are also widely used as pharmacological agents, GC treatment is an intervention of clear biomedical and evolutionary interest. In a recent study, we have identified cis eQTLs that interact with GC treatment in lymphoblastoid cell lines (LCLs). Using a novel Bayesian approach, we discriminated between genetic variants that affect gene expression equally or differently in the presence/absence of GCs. GC action is largely mediated by the GC receptor (GR), which interacts with other transcription factors to regulate gene expression of direct targets. By leveraging the molecular biology of the response to GC, we attempt to characterize the molecular mechanisms of these distinct classes of interaction eQTLs. To this end, we performed ChIP-seq experiments for the GR and its interacting transcription factor NF2B in Yoruba (YRI) LCLs treated with GC or with vehicle control. We identified 5,668 GR binding regions (FDR<15%). A large fraction of differentially expressed genes are direct GR targets (42% of them have a GR binding site within 100Kb). We also identified 558 and 657 NF2B binding sites (in the absence and presence of Dex, respectively, FDR<15%). To identify functional polymorphisms within each interaction eQTL, we used the 1000 Genomes data to perform cis-eQTL mapping in the same 58 YRI individuals previously analyzed. By overlying the eQTL mapping on the ChIP-seq data, we showed that genetic variants that affect gene expression only in the presence of GC (GC-only eQTLs) are 2.6-fold more likely to occur in GR binding sites, than genetic variants that affect gene expression only in the absence of GC (Control-only eQTLs) (p=0.04). We also observed an opposite trend, with Control-only eQTLs being 1.5-fold more likely to occur near NF2B binding sites. Overall, our results indicate that the different classes of eQTLs correspond to distinct molecular mechanisms of regulation of gene expression by the GR and its interacting transcription factors.

1434F

Hindlimb Skeletal Muscle Function and Femoral Strength in +/*G610C* Mice With and Without Impact Exercise. *S.M. Carleton[1], B.A. Gentry[2], J.A. Ferreira[3], D.J. Salamango[1], A.M. Williams[1], A.D. Kettle[4], M.G. McCray[1], M. Brown[3], C.L. Phillips[1].* 1) Biochemistry, University of Missouri, Columbia, MO; 2) Veterinary Pathobiology, University of Missouri, Columbia, MO; 3) Biomedical Sciences and Physical Therapy Program, University of Missouri, Columbia, MO; 4) Biological Sciences, University of Missouri, Columbia, MO.

Osteogenesis imperfecta (OI) is a genetically and clinically heterogeneous disease characterized by impaired biomechanical properties of type I collagen-containing tissues such as bone. Greater than 85% of OI-causing mutations are in one of the two type I procollagen genes, pro(1(I) or pro(2(I). Recently, 64 individuals were identified who all carry the same glycine to cysteine substitution at position 610 of the (2(I) chain, causing mild OI type I/IV with reduced bone mineral density (BMD) and an increased number of fractures compared to non-affected family members. Heterozygote *G610C OI* mice (+/*G610C*) model this population in both genotype and phenotype, exhibiting reduced BMD and femoral biomechanical strength. OI type I children are reported to have significantly reduced exercise capacity and muscle force as compared to their healthy peers. It is unclear if the muscle weakness is due to reduced physical activity and/or an inherent muscle pathology. Bone is inherently mechanosensitive and the largest physiological loads bones typically experience are from muscles, with bone strength directly proportional to muscle mass. Physically active children accrue 10-40% more bone than inactive children and the lack of physical activity in OI children during the critical pubertal growth spurt may result in even poorer bone health as adults. Therefore, we sought to determine if +/*G610C* mice have 1) a muscle pathology, 2) altered activity levels, and 3) the ability to participate in and respond to weight-bearing treadmill exercise. +/*G610C* mice did not exhibit a gross muscle pathology (histomorphometric evaluation and contractile generating force analyses) or altered activity levels (open field monitors). +/*G610C* mice were able to participate in and complete an eight week treadmill regimen (10 m/min, 30 min/day, 5 days/week). Interestingly, male and female +/*G610C* femora responded differently to treadmill exercise, with +/*G610C* and wildtype exhibiting similar responses to this exercise regimen, though neither gender showed remarkable gains in bone strength. However, the relatively weak intensity and short duration of the exercise protocol may be insufficient to elicit significant changes in bone strength. These results indicate that despite an impaired musculoskeletal system therapeutic inclusion of moderate impact exercise may be beneficial to human patients with mild OI.

1435F

Induction *in vitro* of mesenchymal stem cells to chondrogenic phenotype by combined adenoviral mediated-gene transfer. *I. Garza-Veloz[1], V. Romero-Alvarez[2], M.L. Martinez-Fierro[3], A. Hernandez-Hurtado[1], J. Alcaraz[4], J.B. Kouri-Flores[5], E. Alvarez-Lozano[6], H.G. Martinez-Rodriguez[1], M.A. Guzman-Garcia[7], M.A.L Hernandez-Rodriguez[8], R. Ortiz-Lopez[1,9], A. Rojas-Martinez[1,9].* 1) Biochemistry and Molecular Medicine Department, Universidad Autonoma de Nuevo Leon, Monterrey, Nuevo Leon, Mexico; 2) Morphology Department, Universidad Autonoma de Nuevo Leon, Monterrey, Nuevo Leon, Mexico; 3) Molecular Medicine Laboratory, Unidad Academica de Medicina Humana y Ciencias de la Salud, Universidad Autonoma de Zacatecas, Zacatecas, Mexico; 4) Unitat de Biofísica i Bioenginyeria, Universidad de Barcelona, Barcelona, Spain; 5) Infectomica y Patogenesis Molecular, Centro de Investigacion y de estudios avanzados del I.P.N., Cd. Mexico, D.F., Mexico; 6) Banco de Hueso y Tejidos, Hospital Universitario, UANL. Monterrey, Nuevo Leon, Mexico; 7) Facultad de Medicina Veterinaria, Universidad Autonoma de Nuevo Leon. Monterrey, Nuevo Leon, Mexico; 8) Facultad de Ingeniería Mecanica y Electrica, Universidad Autonoma de Nuevo Leon. Monterrey, Nuevo Leon, Mexico; 9) Centro de Investigacion y Desarrollo en Ciencias de la Salud, Universidad Autonoma de Nuevo Leon, Monterrey, Nuevo Leon, Mexico.

INTRODUCTION. Due articular cartilage has a poor regeneration capacity, numerous cell-based approaches to therapy are currently being explored. Following stimulation with growth factors adipose-derived mesenchymal stem cells (MSCs) have the capacity to differentiate into connective tissues such as cartilage. Although MSCs are potentially useful as alternative cell source to chondrocytes, methods to effectively stimulate proliferation and subsequent chondrogenic differentiation of MSCs are needed to further development of tissue engineering approaches for cartilage repair or regeneration for the treatment of joint injury or osteoarthritis. In this study, the chondrogenesis of adipose-derived MSCs *in vitro* following genetic modification with adenoviral vectors encoding chondrogenic growth factors was investigated. METHODS. Adenoviral vectors carrying the cDNAs for human TGF-B1, IGF-1, FGF-2 and SOX9 were constructed. MSCs were isolated from adipose tissue of young adult Ovis aries. Confluent monolayer cultures of MSCs were individually infected with 100 MOIs of each recombinant adenovirus (Ad.IGF-1, Ad.TGF-B1, Ad.FGF-2 and Ad.SOX9), or in combination (Ad.IGF-1/Ad.TGF-B1, Ad.IGF-1/Ad.FGF2, Ad.IGF-1/Ad.TGF-B1/Ad.SOX9 and Ad.IGF-1/Ad.FGF2/Ad.SOX9), and cultured for 3, 7, 14, 21 and 28 days. Total RNA was isolated, and retrotranscribed to cDNA. Quantitative RT-PCR was used to evaluate the expression of cartilage-specific genes at each time. The evaluation of matrix proteoglycan, and collagen was done using toluidine blue, safranine-O and Fast green dyes. RESULTS. Levels of transgene expression were initially high at day 3, and declined thereafter. We found that co-expression of IGF-1 and TGF-B1, resulted in higher expression levels of aggrecan, proteoglycan, cartilage-matrix, biglycan, II and X collagen, and induced chondrogenesis of MSCs as evidenced by stronger staining for proteoglycans and collagen type II and X, better than either individual or combined transgene. CONCLUSIONS. Our findings indicate that gene-induced chondrogenesis of MSCs using multiple genes that act synergistically may enable the use of adenoviral mediated-gene transfer *in vivo* for the development of cell-based therapies for cartilage repair.

1436F

Hypophosphatasia (HPP): Affected infants and children benefit significantly from ENB-0040 treatment despite high allelic variability. *C. Greenberg[1], M.P. Whyte[2], E. Mornet[3], J.C. Hyland[4], L. Ala-Kokko[4], S. Mumm[2], A. Mhanni[1], K. Madson[2], N.C. Kreher[5], H. Landy[5].* 1) Dept Pediatrics & Child Hlth, Children's Hospital, Winnipeg, MB, Canada; 2) Shriner's Hospital for Children, Center for Metabolic Bone Disease and Molecular Research, St. Louis, MO, USA; 3) Laboratoire SESEP, Centre Hospitalier de Versailles, France; 4) Connective Tissue Gene Tests, Allentown, PA, USA; 5) Enobia Pharma, Cambridge, MA, USA.

HPP is the heritable disorder featuring impaired skeletal mineralization due to inactivating mutations within the gene that encodes the tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP). HPP is transmitted in an AR or AD pattern; AR HPP is generally more severe. More than 240 mutations in TNSALP have been reported (SESEP laboratory, http: www.sesep.uvsq.fr/03_hypo_mutations.php). We present genotypes and corresponding responses to therapy of infants and children receiving ENB-0040, an investigational, subcutaneous, bone-targeted enzyme replacement therapy. Methods: Mutation analysis of TNSALP was performed by PCR amplification of exons 1 — 12 and their flanking intronic regions. PCR products were sequenced using dye terminator methodology and migrated onto a capillary electrophoresis system. Mutations were confirmed by sequencing two independent PCR products and, if possible, inheritance and allelism were checked by sequencing parental DNA. Using a bioinformatic tool combining site-directed mutagenesis and predictive tools Polyphen, SIFT, and Pmut, a severity score was assigned to each mutation. Scores ranged from 0.4 -1.08 (benign — most severe); <0.75 is “moderate,” and >0.75 “severe”. Results: Of 31 patients, 26 were compound heterozygotes, 3 homozygotes (2 with known perinatal lethal “Mennonite” mutation), 1 AD, and 1 had no identifiable mutation in the coding regions of TNSALP. Using severity scores, most infants had 2 “severe” mutations whereas children were primarily compound heterozygotes for 1 “severe” and 1 “moderate” allele. Missense mutations represented the majority of defects; 4 were small deletions. Common mutations were c.571G>A (n=11), c. 1001G>A (n=7), and c.881A>C (n=5); 7 mutations have not been described (c.875_888delCAGGGGAinsT, c.997+3A>G, c.1881_1182delCT, c.252G>C, c.1010A>G, c.1077C>G, c.658G>A). Patients have received ENB-0040 for／ 6 months; 6 for nearly 2 years. All, including the 2 with the lethal Mennonite genotype, have improvements in the radiographic appearance of rickets, respiratory status, and/or functional status (Greenberg et al and Whyte et al, ASHG 2010). Conclusions: We confirm that high allelic heterogeneity in TNSALP underlies the remarkable clinical heterogeneity of HPP, and add 7 new mutations to the many reported. Whatever the genotype and clinical phenotype identified, all infants and children treated with ENB-0040 have experienced significant clinical improvement.

1437F

Parental Myostatin Status Affects Femoral Polar Moment of Area and Biomechanical Strength in Four Month Old Wildtype Male and Female Offspring. *C.L. Phillips[1], S.M. Carleton[1], M. Brown[2].* 1) Dept Biochem/Child Hlth, Univ Missouri, Columbia, MO; 2) Biomedical Sciences and Physical Therapy Program, Unic Missouri, Columbia, MO.

Osteogenesis imperfecta (OI) is a heritable connective tissue disorder in which mutations in type I procollagen genes result in bone deformity and fragility. The OI model mice (*oim/oim*) are homozygous for a null mutation in the COL1A2 gene of type I collagen and have significantly reduced bone biomechanical integrity and bone mineral density as well as altered bone mineral composition. Heterozygous mice (+/*oim*) have a phenotype intermediate to homozygous (*oim/oim*) and wildtype (wt) mice. Bone is inherently mechanosensitive, responding and adapting to its mechanical environment. Bone formation occurs in response to high mechanical loads, often changing its geometry to strengthen the skeleton. The largest physiological loads bones typically experience are from muscles with bone strength directly proportional to muscle mass. Myostatin (*mstn*) is a member of the transforming growth factor-) (TGF-)) superfamily and is a negative regulator of skeletal muscle growth. When the myostatin protein is missing the result is uncontrolled muscle growth with a concomitant increase in bone strength. Myostatin deficient mice (+/*mstn*) have been shown to have increased muscle mass and bone strength compared to wt mice. We hypothesized that a reduction in the amount of myostatin protein would potentially ameliorate the bone phenotype of heterozygous *oim* (+/*oim*) mice. A pilot study demonstrated the reduction in bone strength seen in male and female +/*oim* animals was partially rescued when those mice were also heterozygote for myostatin deficiency (+/*mstn*). However, a parental effect of myostatin deficiency was also seen. At four months of age, femora from wt male and female offspring had increased polar moment of area while wt female offspring had increased torsional loading to failure when both parents were +/*mstn* as compared to wt male and female offspring of +/*oim* parents. The tensile strength of the bone material was not significantly different in femora from either wt male or female offspring regardless of parental genotype, suggesting a potential geometric adaptation influencing bone integrity. Taken together, these data indicate that absence of parental myostatin influences bone parameters in the wt offspring and these changes appear persistent through adulthood.

1438F

Enzyme replacement therapy for children with hypophosphatasia (HPP): Dual-energy x-ray absorptiometry (DXA) improvement after 6 months of treatment with ENB-0040. *M.P. Whyte[1,2], F. Zhang[1], K.L. Madson[1], D. Wenkert[1], C.R. Greenberg[3], W.H. McAlister[4], A.L. Reeves[1], K.E. Mack[1], A.G. Yakimoski[3], A. Mhanni[3], A.M. Skrinar[5], H. Landy[5].* 1) Center for Metabolic Bone Disease and Molecular Research, Shriners Hospital for Children, St. Louis, MO, United States, 63131; 2) Division of Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, MO, United States, 63110; 3) Department Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2; 4) Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, MO, United States, 63110; 5) Enobia Pharma, Montreal, Quebec, Canada, H1W 4A4.

Background: HPP is the inborn-error-of-metabolism caused by inactivating mutation(s) within the gene that encodes the tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP). Severity ranges from stillbirth due to profound skeletal hypomineralization to adult fractures from osteomalacia. There is no established medical treatment. ENB-0040 is a bone-targeted, recombinant, TNSALP protein being studied in HPP. **Objective**: Assess changes in bone mineral density (BMD) after 6 months of ENB-0040 delivered subcutaneously (SC) in children with rickets from HPP. **Design/Methods**: This was a 6-mo, multi-center, open-label, historical control study of 13 children (ages 5-12 yr) given ENB-0040 (2 or 3 mg/kg SC 3X weekly). Controls were 16 children with HPP of similar age and clinical characteristics whose DXA data (20 mo interval) were retrieved from a natural history database. Hologic QDR4500A lumbar spine (L_1-L_4) and total hip BMD were assessed for all patients. BMD Z-scores were calculated based upon patient chronologic age (CA), and with equations to correct for stature using height-age (HA). BMD Z-score changes were expressed per mo for both groups to correct for the different DXA intervals. **Results**: DXA data were analyzed for 11 patients (one had withdrawn for elective surgery and another had started growth hormone). The baseline CA spine and hip z-scores for the ENB-0040 treated group ranged from -3.8 to +0.1 and from -4.8 to -2.2, respectively. The historical control CA baseline spine z-score range was -3.3 to -0.2 and hip -3.7 to -0.6. With treatment, the average 6 mo increase in CA spine BMD z-score was 0.74 and HA 0.86 compared to the control 20 mo CA change of 0.12 and HA 0.20. Similarly, the treatment 6 mo CA hip BMD z-score change was 1.41 and HA 1.55 compared to 20 mo control CA change of -0.09 and HA -0.24. Although the ENB-0040 six mo response was impressive, we also normalized the treatment and control data to a per mo interval for assessing statistical significance. The spine z-score change/mo for treatment vs control CA was 0.13 vs 0.0124 (p=0.0054), and for HA 0.16 vs 0.0179 (p=0.0044). The hip z-score change/mo for ENB-0040 vs control CA was 0.26 vs -0.0042 (p=0.0024) and for HA 0.28 vs -0.0038 (p=0.0014). **Conclusion**: Six months of ENB-0040 enzyme replacement therapy in children with HPP resulted in substantial increases in spine and hip BMD z-scores, whether adjusted for age or height, consistent with healing of their skeletal disease.

1439F

Studies on recombinant adeno-associated virus vector co-expressing hVEGF165 and hBMP-7 genes combined with BMSCs promoting steroid-induced avascular necrosis of the femoral head repair. *C. Zhang[1], K. Wang[1], M. Li[2], X. Dang[1], Z. Shi[1].* 1) Department of Orthopedic Surgery, the Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710004, China; 2) Department of Ultrasound, the Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710004, China.

Therapy for steroid-induced avascular necrosis of the femoral head (SANFH) diseases remains a considerable clinical challenge despite advances in surgical techniques. Vascular endothelial growth factor (VEGF)and bone morphogenetic protein(BMP)play important roles in angiogenesis and osteogenesis. Combining these two factors may be a promising therapeutic strategy for SANFH. To establish a more efficient gene transfer method, we investigated the therapeutic potential of adeno-associated virus (AAV)-mediated expression of VEGF and BMP (rAAV-hVEGF$_{165}$-internal ribosome entry site (IRES)-hBMP-7). Rabbit bone marrow-derived mesenchymal stem cells (BMSCs) were isolated and purified by density gradient centrifugation combined with attachment culture methods. The best MOI of BMSCs transfected with rAAV was detected by fluorescent cell counting, and cell viability was determined by MTT assay. Expression of the genes of interest was detected by GFP gene expression, RT-PCR assay and ELISA assay. The biological activities of VEGF and BMP were detected by angiogenic and osteogenic assays. After receiving an established inductive protocol for inducing SANFH rabbit's model, BMSCs modified with rAAV-hVEGF$_{165}$- IRES-hBMP-7 virus were injected into cavitas medullaris by core decompression operation. Before and after injection, MRI was performed on proximal femur for osteonecrosis detection. 12 weeks post injection, the reconstructions of bone tissue were detected by X ray and micro-CT and the reconstruction of vascular was detected by bone scintigraphy assessment. We detected that the best MOI of BMSCs transfected with rAAV was 5×10^4 v.g./cell. By comparing the cell growth curves, there was no significant difference of cell viability when rAAV vectors transfection. Expressions of the GFP, VEGF$_{165}$, and BMP7 genes were detected 1 day post-transfection, peaked 14 days post-transfection and sustained over one month. Recombinant adeno-associated viral vectors co-expressing the hVEGF165 and hBMP7 genes showed efficient gene expression ability. VEGF and BMP proteins secreted from BMSCs transfected with rAAV-hVEGF$_{165}$-IRES-hBMP7 enhanced angiogenesis and osteogenesis and have efficient biological activity in vitro. In vivo, rAAV-hVEGF$_{165}$-IRES-hBMP7 combined with BMSCs efficiently reconstructed bone formation and enhanced neovascularlaration. All these indicated that rAAV-hVEGF$_{165}$-IRES-hBMP7 virus vector may be a new therapeutic technique for the treatment of SANFH.

1440F

Enriched rearing improves behavioral responses of an animal model for CNV-based autism-like traits. *M. Heney[1], C. Spencer[1], W. Gu[1], R. Paylor[1], J. Lupski[1,2,3].* 1) Molec & Human Gen, Baylor College Med, Houston, TX; 2) Department of Pediatrics, Baylor College Med, Houston, TX; 3) Texas Children's Hospital, Houston, TX.

Autism and autism spectrum disorder (ASD) are common neuropsychiatric conditions diagnosed by the presence of specific behavioural characteristics, including impairment in social interactions and communication, and stereotypic or inflexible behaviour. Many mouse models for the disorder have been generated by knocking-out candidate genes, however, these genes are only causative for a very small portion of autism. In contrast, chromosomal abnormalities are thought to account for 10-20% of autism cases. In particular, many microdeletion/ microduplication syndromes are associated with specific autistic-like, abnormal behavioural phenotypes. Potocki-Lupski syndrome (PTLS; MIM #610883) is associated with a microduplication on chromosome 17p11.2, and it is characterized by neurobehavioral and congenital abnormalities as well as developmental delay. Previous studies characterizing the cognitive and behavioural phenotypes of PTLS patients determined that ~70-90% of the patients studied tested positive for autism or ASD. We previously generated a mouse model for PTLS (Dp(11)17/+) that harbors a duplication of a ~2 Mb region syntenic to the human PTLS region. Utilizing this mouse model, behavioral phenotypes were studied with a battery of experiments that have been established for the study of core and associated autistic-like phenotypes, including tests for social abnormalities, ultrasonic vocalizations, perseverative and stereotypic behaviors, anxiety, learning & memory deficits, and motor defects. Alterations were identified in both core and associated ASD-like traits. In addition, we have shown that rearing mice in an enriched environment altered and rescued some of the neurobehavioral phenotypes identified, suggesting a role for gene-environment interaction in the determination of phenotypic severity. The mouse model described here parallels both phenotypic and genotypic (CNV) aspects of autism in humans, making it an interesting model for the study of CNV-based autism and ASD.

1441F

Substrate reduction therapy in patients with Juvenile-Onset Batten disease due to CLN1 mutations using Cysteamine Bitartrate: A pilot study. *M. Velinov[1], M. Gavin[2], G.Y. Wen[3].* 1) Human Genetics, NYS Institute for Basic Research in Developmental Disabilities, Staten island, NY; 2) George Jervis Clinic,NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY; 3) Developmental Neurobiology, NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY.

Neuronal Ceroid Lipofuscinoses (NCL) are a group of inherited neurodegenerative storage disorders. Mutations of at least 10 different genes are responsible for the various NCL forms. Mutations in the gene CLN1 typically result in severe, infantile-onset form of NCL. CLN1 encodes the enzyme palmitoyl protein thioesterase (PPT) that participate in the lysosomal degradation of acetylated proteins. Cysteamine bitartrate (Cystagon), a lysosomotropic agent used in the treatment of cystinosis, has been previously shown to reduce the storage material in PPT deficient cells. We report the results of a 7 year, open label substrate-reduction therapy trial using Cystagon in four patients with atypical, juvenile-onset NCL due to milder CLN1 mutations. Cystagon was given in gradually increasing doses with the goal to achieve and maintain a dose of 40-50 mg per kg body weight when possible. Five untreated patients with the same CLN1 mutations, three of whom were participants' siblings were included as controls. Parental questionnaire along with physician evaluations were used to monitor the disease course during the treatment. In addition, patients' peripheral lymphocytes were studied for lysosomal storage inclusions using electron microscopy. The Cystagon treatment resulted in substantial decrease in the storage material in peripheral lymphocytes. This storage reduction paralleled the Cystagon dose. No severe side effects of the treatment were noted except for allergic rash in one of the patient that required dose reduction. A minor slowing of the disease progression compared to the controls was observed in three out of the four treated patients. The parents of two of the treated patients reported improvements in some of the disease symptoms after the start of treatment. In conclusion, we observed significant storage reduction and possible minor slowing of the disease progression in the treated patients. We conclude that the amount of storage material associated with PTT deficiency does not directly parallel the disease severity and progression. The Cystagone therapy was not successful in reversing the disease course but resulted in some minor slowing of the progression of the disease and a slight improvement of symptoms in some of the patients. It is possible that better outcomes may be achieved if the Cystagone treatment is started earlier in the disease course, and/or if it is applied in combination with other therapeutic agents.

1442F

***Peewee*, a Novel Mutant Allele of Natriuretic Peptide Receptor 2 with Skeletal Dysplasia and Female Infertility.** *K.A. Geister, M.L. Brinkmeier, S.A. Camper.* University of Michigan, Ann Arbor, MI 48109-5618 USA.

Skeletal dysplasias are an important contributor to short stature, occurring 1/5000 births. Half of the known subtypes can be explained by mutations in about 140 genes. There are no current treatments other than controversial leg lengthening surgeries. Mouse models of human skeletal dysplasias provide a tool for understanding the mechanisms of disease pathology and for exploring therapies. The *peewee* mouse is a novel spontaneous mutant allele of *Npr2*, the gene that encodes natriuretic peptide receptor 2 (NPR2), and serves as a model for a human form of skeletal dysplasia known as Acromesomelic Dysplasia Maroteaux Type (AMDM). Both the human and mouse forms of this condition are autosomal recessive. *Peewee* mutants exhibit severe disproportionate growth insufficiency and reduction in the hypertrophic zone of the epiphyseal growth plate. NPR2 is a guanylyl cyclase that is activated by C-type natriuretic peptide (CNP) to regulate the MEK/ERK axis of the MAP kinase signaling pathway in chondrocytes. NPR2 activation inhibits fibroblast growth factor signaling by inhibiting activation of MEK1/2 by inhibiting the action of RAF1, resulting in balanced regulation of bone growth. We treated fetal tibiae from normal and *peewee* mutants with U0126, a MEK1/2 inhibitor, in culture and rescued the deficiency in bone growth. Additionally, *Npr2* knock-out mutants have reduced levels of circulating IGF-1, which implicates the growth hormone axis as a contributor to the growth defect present in *peewee* mutants. We are currently assessing the role of *Npr2* in regulating the growth hormone axis, and are considering this pathway as a potential avenue for therapeutic intervention. *Peewee* females are infertile. *Npr2* is expressed in multiple tissues of the hypothalamic-pituitary-gonadal axis, including the brain, pituitary, uterus, and ovary. *Npr2* is necessary for maintenance of meiotic arrest in oocytes, which explains the infertility of *peewee* females. We are exploring the role of NPR2 in other parts of the hypothalamic-pituitary-gonadal axis. In summary, we present a mouse model of human skeletal dysplasia that is responsive to pharmacotherapy in culture.

1443F

The Soluble Myostatin Inhibitor Act2b(fc) Prevents Only Certain Forms of Acquired Muscle Atrophy. *E.M. MacDonald[1], S.J. Lee[2], R.D. Cohn[1,3].* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Department of Molecular Biology and Genetics, Johns Hopkins University, Baltimore, MD; 3) Departments of Pediatrics and Neurology, Johns Hopkins University, Baltimore, MD.

Myostatin is member of the TGF) signaling family that acts as a natural negative regulator of skeletal muscle mass via binding to the Act2b receptor on the cell surface of the myofibers. It has been shown that administration of a soluble form of the Act2b receptor causes hypertrophy of skeletal muscle in normal adult mice. Given the dramatic effect on muscle growth, inhibition of the myostatin signaling cascade has been explored as a therapeutic option for various inherited forms of skeletal muscle disorders. Here, we investigated whether administration of the soluble Act2b receptor is beneficial for combating acquired forms of muscle atrophy in models of hindlimb immobilization, denervation and steroid-induced myopathy. Each set of experiments lasted for three weeks and consisted of three groups of mice; controls, atrophy with placebo, and atrophy treated with soluble Act2b receptor. In both the immobilization and steroid myopathy models, administration of the soluble Act2b receptor prevented skeletal muscle atrophy based on muscle mass measurements (p-value = 4.1x10-3 and 1x10-7 respectively). Indeed, skeletal muscle from the Act2b treated group was either the same size or hypertrophic when compared to muscles from the control group (p-value = 0.883 and 1.2x10-4). In contrast to these first two models, the denervation model showed no difference between the skeletal muscle from the soluble Act2b receptor and placebo treated groups (p-value = 0.998). Skeletal muscle from both the soluble Act2b receptor and placebo treated mice were significantly smaller than the control mice (p-value = 2.7x10-7 and 1.4x10-10). Together, our data demonstrate that the soluble Act2b receptor provides a promising therapeutic avenue for preventing skeletal muscle atrophy in immobilization and steroid-induced myopathy. However, our data also indicates that when the muscle is denervated, administration of the soluble Act2b receptor does not protect against muscle atrophy development. This suggests that an intact nerve-skeletal muscle conduction system is essential for the inhibition to be effective in preserving skeletal muscle mass. Ongoing studies will explore the interaction between electrical stimulation of the muscle and the cellular signaling pathways associated with myostatin inhibition.

1444F

Characterization of a polyalanine antibody for the diagnosis of oculopharyngeal muscular dystrophy and other polyalanine related diseases. *S.J. Stochmanski[1], F. Blondeau[2], P.A. Dion[1], P.S. McPherson[2], G.A. Rouleau[1].* 1) Centre of Excellence in Neuroscience of the Université de Montréal (CENUM), CHUM Research Centre, Montréal, QC, Canada; 2) Dept. of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montréal, QC, Canada.

Nine severe human diseases have so far been associated with expansions of polyalanine (polyala) tracts. Among them, oculopharyngeal muscular dystrophy (OPMD) is a late onset autosomal dominant disorder characterized by the presence of intranuclear inclusions (INI) in skeletal muscle fibers. We previously have identified the OPMD causative mutation as a small expansion of a GCG repeat encoding for polyala in the *PABPN1* gene. Wild-type *PABPN1* has a $(GCG)_6$ repeat that encodes for the first six alanine residues within a stretch of ten alanines whereas patients with OPMD have modestly expanded and stable alleles (GCG_{8-13}), the same being true for the other polyala diseases. Data from OPMD suggest that polyala-induced toxicity is very sensitive to the dosage and length of the alanine repeat.

Here we report the characterization of a polyclonal antibody that selectively recognizes alanine expansions of 10 or more in the protein PABPN1 implicated in OPMD. To produce the antibody, an antigen containing a 19-mer peptide comprising 18 alanine residues followed by one glycine residue was generated. Two rabbits were immunized with the fusion protein and the resulting serum (final bleed after 3 boost injections) was affinity purified.

This antibody will serve to pinpoint and characterize the sub-cellular localization of proteins containing these polymers, as well as be used to screen other “orphan” neurodegenerative or developmental diseases for the presence of expanded alanine tracts, which may help uncover new polyala diseases.

1445F

Ex vivo adenoviral-mediated gene therapy: Phosphatase inhibitors improve gene transduction. *M. Samson[1, 2], A-M. Bessette[1, 2], M. Drouin[1], M-P. Cayer[1], D. Jung[1, 2].* 1) Research and development, Hema-quebec, Quebec, Canada; 2) Department of Microbiology and Biochemistry, Laval University, Quebec, Canada.

Intravenous immunoglobulin (IVIG) contains the pooled immunoglobulin G (IgG) from the plasma of approximately a thousand or more blood donors. From year to year, several studies are reporting new utilizations of IVIG and this is resulting in a continuous increase in their utilization, raising the possibility of product shortages and increasing costs. In order to exploit terminally differentiated normal B lymphocytes for the production of IVIg in vitro, we have undertaken to determine genes involved in the control of proliferation and differentiation of human peripheral blood B lymphocytes. However, the functional characterization of these genes is limited by the failure to efficiently introduce genes in B lymphocytes and haematopoietic progenitor cells. Adenoviral vectors (Ad) are an attractive alternative since they can efficiently transduce both proliferating and quiescent cells and achieve transgene expression within hours post-infection. Moreover, Ad do not integrate their genome within the host cell chromosomes, thus leaving the genome of the host cell intact. However, we have demonstrated that primary B cells were poorly transduced with Ad5/F35, a chimeric vector that utilizes the ubiquitous CD46 molecule as receptor. We have previously showed that treatment of primary human B lymphocytes with phosphatase inhibitors prior to adenoviral infection increases substantially transduction efficiency. Phophastase inhibitors are well-known for their effects on endocytosis and intracellular trafficking, especially those that are specific for PP1 and PP2A. Adenoviral transduction efficiency is unaffected by inhibition of PP1 by tautomycin, whereas inhibition of PP2A with cantharidic acid or both PP1 and PP2A with okadaic acid substantially increases transduction efficiency. In order to better understand the effects of PP2A inhibitor on the transduction efficiency of adenoviral vectors, we followed a CY3 labeled Ad5/F35 in normal human peripheral blood B lymphocytes treated with cantharidic acid, throughout endocytosis. We show that specific PP2A inhibitor, cantharidic acid, alters intracellular trafficking of Ad5/F35.

1446F

Derivation of Insulin Producing Cells from Mesenchymal Stem Cells. *S. Talebi[1], A. Aleyasin[1], M. Soleimani[2].* 1) medical genetic, natinal institute of genetic engineering and biote, tehran, tehran, Iran; 2) medical department, tarbiat modares university.

The Pancreatic cells transplantation is a promising approach for treatment of type1 diabetes that caused by insulin deficiency; however, lack of suitable donors limits the application. Differentiated mesenchymal stem cells (MSCs) can be therapeutic source for the cure of type 1 diabetes. The aim of current experiment is to explore the possibility of derivation of insulin producing cells from bone marrow MSCs by overexpression of pdx1. In this study rat MSCs were isolated and identified by flow cytometry. Then, MSCs were transduced by lentiviruses harboring pdx1. The appropriate expression of exogenous pdx1 was confirmed in level of mRNA and protein using RT-PCR and immunofluorescent analyses. In addition to ectopic expression of pdx1, differentiating medium containing nicotinamid and betamercaptoethanol used to efficient differentiation of MSCs into beta cells. Then, the expression of islet specific markers was investigated by quantitative RT-PCR. The immunoflurescent showed a nuclear localization of pdx1. Pdx1-expressing MSCs transcribed specific pancreatic endocrine markers such as endogenous Pdx1, Ngn3, Glucagon and insulin regardless to using Nico/betaME, but the quantitative RT-PCR showed a high increase in all markers except insulin, in Nico/betaME treated cells compared to non-treated cells. The beta cells derived from MSCs also expressed glucokinase and Glut2 indicating these cells have the glucose sensing ability. In contrast, treated MSCs didn't express P48; it implies these cells have been committed to endocrine linage. Also, Pdx1+ MSCs were transplanted into diabetic rats, euglycemia can be obtained within one week. In conclusion, MSCs as an available resource can have impacted in cell based gene therapy of type1 diabetes.

1447F

Randomized, Placebo-Controlled, Cross-Over Trial of Simvastatin in Smith-Lemli-Opitz Syndrome. *C.A. Wassif[1], S.K. Conley[1], H. Goodwin[1], S.E. Sparks[2], E. Tierney[3], F.D. Porter[1].* 1) Section on Molecular Dysmorphology, Program in Developmental Endocrinology and Genetics, Eunice Kennedy Shiver National Institute of Child Health and Human Development,NIH, DHSS. Bethesda, MD; 2) Clinical Genetics, Dept. of Pediatrics Levine Children's Hospital at Carolinas Medical Center PO Box 32861, Charlotte, NC; 3) Department of Psychiatry, Kennedy Krieger Institute, Baltimore, MD,.

Smith-Lemli-Opitz syndrome (SLOS) is a malformation syndrome with behavioral and cognitive impairment caused by mutation of the 7-dehydrocholesterol reductase gene (*DHCR7*). Mutations of DHCR7 result in impaired cholesterol synthesis and accumulation of dehydrocholesterol (DHC). Treatment of SLOS involves dietary cholesterol supplementation. However, cholesterol supplementation fails to normalize DHC levels, and does not treat the central nervous system (CNS)manifestations as cholesterol does not cross the blood-brain-barrier (BBB). Simvastatin inhibits the rate limiting step in cholesterol synthesis, the conversion of HMGCoA to mevalonate. Simvastatin also induces expression of SREBP2 regulated genes including DHCR7. We hypothesized that increased expression of hypomorphic DHCR7 alleles would improve cholesterol synthesis in SLOS. In vitro and in vivo preclinical studies and a small case series showing a paradoxical increase in cholesterol were consistent with this hypothesis. Because simvastatin crosses the BBB, we also postulated that it would improve sterol biochemistry in the brain and thus impact behavior. To test the safety and efficacy of simvastatin in SLOS we performed a placebo-controlled, cross-over trial in SLOS patients with significant (>10% normal) residual DHCR7 activity. Each phase was 12 months and patients were continued on dietary cholesterol supplementation (150 mg/kg/d). We enrolled 23 patients and 18 patients completed both phases of the study. The primary outcome measure was improvement of the serum DHC/cholesterol ratio. Secondary outcome measures were improvement of the cerebral spinal fluid (CSF) DHC/cholesterol ratio and the irritability subscale of the Aberrant Behavioral Checklist (ABC). We did not replicate the finding of a paradoxical increase in cholesterol levels. The average serum cholesterol level showed a mild decrease from 132 to 113 mg/dl ($p<0.01$). However, in serum we observed a significant reduction ($p<0.001$) in DHC and a significant improvement ($p<0.005$) in the total DHC/cholesterol ratio. CSF cholesterol values remained constant but there was a trend ($p=0.06$) toward an improved DHC/cholesterol ratio. No significant changes in behavior were detected by the ABC. These data suggest that simvastatin can be used to alter sterol levels in SLOS; however, an increased response in the CNS may be needed to impact behavior.

1448F

Treating inflammatory aspects of Gaucher disease: Do different ERT choices matter? *O. Goker-Alpan, S. Kasmani, A. Farwah, T. Taber, O. Alpan.* Lysosomal Disorders Research and Treatment Unit, Center for Clinical Trials, Springfield, VA.

The standard of care for Gaucher disease (GD), the inherited deficiency lysosomal enzyme glucocerebrosidase is enzyme replacement therapy (ERT). Imiglucerase is highly effective in reversing the visceral and hematologic manifestations. However, GD is still regarded an inflammatory condition, as the response to therapy is variable. Velaglucerase has a specific activity comparable to imiglucerase, but different patterns of glycosylation, displaying high-mannose type glycans. In this study, we followed the therapeutic response among patients with type 1 GD, who switched to or initially treated with velaglucerase by following macrophage activation (chitotriosidase) and inflammatory markers Methods and subjects: 6 (5F:1M) patients with type 1 GD were treated with commercial velaglucerase alpha for an average duration of one year. Switch patients were kept at the same dose, whereas naïve patients received 60 IU/kg EOW. All four splenectomized patients, among whom two were naïve, had significant disease-load, and inflammatory aspects of disease, such as systemic inflammation with increased ESR, inflammatory bowel disease, skeletal and pulmonary involvement. Results: Chitotriosidase levels while on imiglucerease were between 54 to1100, and the levels in naïve patients were 737 and 7366 nmoles/hr/ml. At the end of one year on velaglucerase alpha, the decrease in chitotriosidase ranged 15 to 90 %, in all but one. The levels did not correlate with organ volumes. In one patient, persistently increased ESR levels were normalized. Conclusions: Chitotriosidase is a marker of activation of the macrophage alternate pathway of the immune system, and the decrease may reflect better internalization into human macrophages. For GD1, there is now a choice for ERT, which should be individualized to each patient.

1449F

Treatment with galsulfase results in improved endurance in a MPS VI patient with history of bone marrow transplantation in early childhood. *K. Kim[1,2], B. Burton[1,2].* 1) Division of Genetics, Children's Memorial Hospital, Chicago, IL; 2) Department of Pediatrics, Northwestern University, Feinberg School of Medicine, Chicago, IL.

We report our experience with weekly galsulfase treatment in a 16 year old boy with mucopolysaccharidosis type VI who had previously undergone bone marrow transplantation (BMT) from an unrelated donor at 26 months of age. He had complete engraftment of donor cells with minimal post-transplantation complications. Despite successful BMT, he developed multiple complications associated with MPS, including short stature, bilateral genu valgum, degenerative hip disease, carpal tunnel syndrome, corneal clouding, cervical cord compression, mitral and aortic valve disease, and mild restrictive airway disease. At his initial evaluation at our center in 2009, measurement of arylsulfatase B enzyme activity in leukocytes was normal at 111.1 nmoles/mg/hr (normal:40 - 150). Urine glycosaminoglycan (GAG) level was normal at 54.5 mg GAG/g creat (normal:0 - 60). At the patient's one year follow up visit, he complained of decreased endurance and expressed interest in a trial of galsulfase treatment. We discussed the potential risks associated with galsulfase treatment and unknown benefit of ERT for BMT patients with normal leukocyte enzyme activity and urine GAG level. The patient elected to proceed and began weekly infusions of galsulfase at the recommended dose of 1 mg/kg. Pulmonary function tests and 6 minute walk tests were obtained prior to start of ERT and again after 7 months of galsulfase treatment. After 7 months of treatment, there were no significant changes on physical examination. PFT's showed worsening of the restrictive airway disease. Despite this, the patient had an increase in the distance walked in 6 minutes of 450 feet. His mother also reported a decrease in respiratory infections and congestion since initiating ERT. The patient remains on weekly home based infusions of galsulfase. No infusion related reactions have been observed. Urine GAG levels remain normal at 12.63 ug GAG/mg creat at start of ERT and 16.69 ug GAG/mg creat after 7 months of treatment (normal:0 - 48.68). The benefits of galsulfase infusions in MPS VI patients with prior BMT/HSCT remain unclear. Given our patient's experience, the possibility remains that successfully transplanted patients could derive additional benefit from ERT. Long-term follow up and study of additional treated patients is needed before any definite conclusions can be drawn.

1450F

Galsulfase enzyme replacement therapy improves urine GAG excretion and clinical course in Maroteaux-Lamy syndrome (MPS type VI) after donor-engrafted bone marrow transplant. *M.L. Raff.* Genomics Institute, MultiCare Health System, Tacoma, WA.

Maroteaux-Lamy syndrome, mucopolysaccharidosis type VI, is an autosomal recessive lysosomal storage disorder (LSD) due to deficient arylsulfatase B activity. Therapies include hematopoietic stem cell transplantation (HSCT) and enzyme replacement therapy (ERT) with intravenous recombinant enzyme, galsulfase. ERT with recombinant galsulfase was approved in the US in 2005 after its efficacy and safety were demonstrated in clinical trials. This paper describes a young girl with a rapidly progressing form of Maroteaux-Lamy syndrome who received HSCT at age 23 months, prior to the availability of galsulfase ERT. She presented prior to one year of age with pectus carinatum, mild motor delay, and short stature. Urine dermatan sulfate was elevated and WBC arylsulfatase B was greatly reduced. She had an HLA-matched related donor bone marrow transplant with successful engraftment. 100 days post-transplant, her urine GAGs were moderately elevated at 86-125 μg/mg creatinine (normal range < 60). Galsulfase ERT was initiated 2 years post-bone marrow transplant. After > 1 year of weekly galsulfase ERT, urine GAGs decreased to 56-75 μg/mg creatinine. Growth velocity remained reduced after both transplant and after ERT initiation, but physical endurance and subjective quality of life improved at and > 1 year post-ERT initiation. This suggests that ERT therapy may provide therapeutic benefit even after bone marrow/HSCT in patients with MPS disorders.

1451F

Lithium chloride protects against the toxicity caused by oculopharyngeal muscular dystrophy. *A. Abu-Baker, R. Gaudet, J. Laganiere, P. Dion, G. Rouleau.* Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada.

Oculopharyngeal muscular dystrophy (OPMD) is an adult-onset disorder characterized by progressive eyelid drooping, swallowing difficulties and proximal limb weakness. OPMD is caused by a small expansion of a short polyalanine tract in the poly (A) binding protein nuclear 1 protein (PABPN1). No medical treatment is available for OPMD. Here, we demonstrate for the first time that lithium chloride (LiCL) can protect against the toxicity caused by mutant PABPN1 protein in our established OPMD cell models. The enhancement of cell survival after LiCl treatment was measured at different days post treatment using live cell imaging and FACS methods. We also show that these protective effects can be partly accounted for by LiCl acting through the Wnt/beta-catenin pathway, as elevated level of beta-catenin protein was detected. Our results suggest that LiCl should be considered as a new therapeutic intervention for OPMD.

1452F

Balloon delivery of helper-dependent adenoviral vector results in sustained FIX expression in rhesus macaques. *N. Brunetti-Pierri[1,2], A. Liou[3], P. Patel[3], D. Palmer[4], N. Grove[4], M. Finegold[5], A. Beaudet[4], C. Mullins[3], P. Ng[4].* 1) Telethon Institute of Genetics and Medicine, Naples, Italy; 2) Dept. Pediatrics, Federico II University of Naples, Italy; 3) Dept. Cardiology, Baylor College of Medicine, Houston, TX; 4) Dept. Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 5) Dept. Pathology, Baylor College of Medicine, Houston, TX.

Hemophilia B is an excellent candidate for gene therapy because low levels (< 1%) of factor IX (FIX) result in clinically significant improvement of the bleeding diathesis. Helper-dependent adenoviral (HDAd) vectors hold tremendous potential because they mediate long-term transgene expression without chronic toxicity. To determine the potential for HDAd-mediated hemophilia B gene therapy, we administered an HDAd expressing the human FIX (hFIX) into rhesus macaques, pre-treated with recombinant hFIX, through a balloon occlusion catheter-based delivery method designed to achieve high efficiency of hepatocyte transduction by clinically relevant vector doses. In this approach a balloon catheter is percutaneously positioned in the inferior vena cava to occlude hepatic venous outflow and various doses of an HDAd expressing hFIX under the control of a liver-specific promoter were injected directly into the liver via a hepatic artery catheter. Animals injected with 1x10e12 and 1x10e11 vp/kg exhibited therapeutic levels of hFIX (>5% of normal FIX activity) long-term for at least 1.5-2 years. Sub-therapeutic levels corresponding to ~0.1-0.5% were achieved in the animals injected with 3x10e10 vp/kg and 1x10e10 vp/kg. Therefore, the minimum therapeutic dose appears to be between 3x10e10 vp/kg and 1x10e11 vp/kg. HDAd administration at all doses tested resulted in negligible hepatotoxicity. The development of neutralizing anti-hFIX antibodies was observed in two out of the six rhesus macaques which received the lowest vector doses of 3x10e10 vp/kg and 1x10e10 vp/kg. In one animal inhibitor antibodies were detected at low titer after infusions of recombinant FIX protein and prior to vector injection. In conclusion, these results support a potential clinical trial for hemophilia B gene therapy with HDAd vectors.

1453F

Cysteine quantity correction in CADASIL using antisense mediated exon-skipping. *J.G. Dauwerse[1,2], S.A.J Lesnik Oberstein[1], D.J.M Peters[2], M.H. Breuning[1], G.J.B van Ommen[2], A.M. Aartsma-Rus[2].* 1) Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands; 2) Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands.

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most prevalent type of hereditary vascular dementia and is recognized as an important cause of stroke and dementia in the young. CADASIL is caused by characteristic missense mutations of the *NOTCH3* gene which lead to an uneven number of cysteine residues in the protein's ectodomain. This causes misfolding of the protein which leads to aggregation of NOTCH3 at the surface of vascular smooth muscle cells, a key factor in CADASIL pathophysiology We are currently testing a novel application for antisense-mediated protein modulation, named `cysteine quantity correction', as a treatment strategy for CADASIL. In this approach we use antisense mediated exon-skipping to intervene in protein synthesis at the pre-mRNA level. By inducing the skipping of specific exons, we re-establish an even number of cysteine residues in the NOTCH3 protein's ectodomain. Our hypothesis is that by this modulation the protein maintains function, but loses toxicity. With this approach, we can target ca. 70% of CADASIL related mutations using the same set of antisense oligonucleotides. Technically, we are able to induce the desired exon-skipping in our CADASIL cell models as shown by RT-PCR and sequencing analysis. Currently, we are evaluating whether the induced cysteine quantity correction reduces CADASIL associated NOTCH3 accumulation *in vitro*, using Western blot and immunocytofluorescence. Our hypothesis and preliminary, but promising, *in vitro* results will be presented.

1454F

Systemic administration of AAV9 gene therapy rescues a Menkes disease mouse model. *A. Donsante, M. Haddad, S. Kaler.* Unit on Human Copper Metabolism,NICHD, NIH, Bethesda, MD.

Menkes disease is a lethal infantile neurodegenerative disorder of copper metabolism caused by mutations in a P-type ATPase, ATP7A. Currently, treatments for this devastating disorder of copper metabolism are limited. Therefore, exploring the utilization of gene transfer for this disorder is well justified. In an animal model that recapitulates the Menkes disease phenotype, the *mottled-brindled* (*mo-br*) mouse, we previously reported promising improvement of several parameters, as well as a substantial increase in life span, using combination brain-directed AAV5 gene transfer plus copper. Systemic viral therapy in Menkes disease would enable less invasive delivery and may provide substantial improvement of the non-neurological components of this disorder including renal, pulmonary, and musculoskeletal effects. To refine prospects for clinical translation of gene therapy for this disorder, we evaluated transduction efficiency and preliminary therapeutic effectiveness of AAV serotype 9, which has a unique capacity to cross the blood-brain barrier, and thus may be administered systemically. We treated mo-br mice with $5x10^{11}$ viral particles of AAV9 carrying a human ATP7A cDNA delivered by intraperitoneal injection on day 2 of life and 50 ng copper chloride to the lateral ventricles on day 3 (similar to our previous study). Over 70% of treated *mo-br* mice survived to weaning and median life span doubled. Western blot analysis indicated high ATP7A transgene expression in skeletal and cardiac muscle. AAV9-ATP7A transduction of liver, kidney, heart, lung, intestine, cerebral cortex and choroid plexus was confirmed by quantitative PCR. Measurements of plasma catecholamines at 28 days of age suggested normal neurochemical ratios, consistent with corrected deficiency of dopamine-) -hydroxylase activity, a copper-dependent enzyme. Our findings provide the first definitive evidence that systemic administration of AAV9 gene therapy may have clinical utility for treatment of Menkes disease.

1455F

Adeno-associated Viral Vector 2/8 (AAV2/8) Rescues Retinal Degeneration in a Mouse Model of Retinitis Pigmentosa. *K.J. Wert[1,2,3], S.H. Tsang[1,3,4].* 1) Department of Ophthalmology, Columbia University Medical Center, New York, NY; 2) Institute of Human Nutrition, Columbia University Medical Center, New York, NY; 3) Bernard & Shirlee Brown Glaucoma Laboratory, Columbia University Medical Center, New York, NY; 4) Department of Pathology and Cell Biology, College of Physicians & Surgeons, Columbia University, New York, NY.

BACKGROUND: Approximately 36,000 cases of simplex and familial retinitis pigmentosa (RP) worldwide are caused by mutant phosphodiesterase (PDE6). In spite of this high prevalence, the interplay between defective PDE metabolism and RP pathogenesis remains poorly understood. There is, however, a mouse model for this form of RP: *Pde6(* ^{nmf363}*/Pde6(* ^{nmf363}. In this model, defects in the PDE6(subunit results in the loss of photoreceptors and visual function. We hypothesize that an AAV2/8 vector delivery that increases PDE6(production will improve retinal function and survival. **METHODS:** We tested an AAV2/8 vector for PDE6(driven by an opsin promoter: *Opsin::PDE6(* AAV2/8. We injected one microliter subretinally into the right eye of *Pde6(* ^{nmf363}*/Pde6(* ^{nmf363} mice at P5 and the left eye was injected subretinally with one microliter of saline or *CMV::EGFP* AAV2/8 and used as a control. The number and morphology of photoreceptor cells of the *Opsin::PDE6(* AAV2/8 injected eyes were compared to control eyes. Electroretinograms (ERGs) were performed weekly beginning at P28 to assess global retinal function in transduced and control eyes, using an Espion simulator. **RESULTS:** The *Opsin::Pde6(* AAV2/8 vector increased survival of photoreceptor cells and retinal function in the *Pde6(* ^{nmf363}*/Pde6(* ^{nmf363} mutants. At P36, the mutant retinae transduced with *CMV::EGFP* AAV2/8 exhibited only a single row of photoreceptor cells. In contrast, *Opsin::PDE6(* AAV2/8 transduced eyes showed an increase in the number of photoreceptor cells. ERGs performed weekly beginning at P28 showed higher b-wave amplitudes in *Opsin::PDE6(* AAV2/8 injected eyes compared to control eyes throughout the study period. **CONCLUSIONS:** Increased photoreceptor survival and function in a phosphodiesterase mouse model of RP can be induced by an AAV2/8 mediated increase in the PDE6(enzyme. This strategy tested in mice shows that increasing PDE6(production through gene therapy may be a novel approach for treating patients with RP occasioned by PDE6 deficiency.

1456F

Antisense RNA/Ethylene-bridged nucleic acid chimera induces exon 45 skipping in cultured myocytes from DMD patients with 6 different deletion mutations. *M. Yagi[1], T. Lee[1], H. Awano[1], Y. Takeshima[1], M. Matsuo[2].* 1) Dept Pediatrics, Kobe Univ Graduate Sch Med, Hyogo, Japan; 2) Dept Medical Rehabilitation, Kobe Gakuin Univ, Hyogo, Japan.

Duchenne muscular dystrophy (DMD) is the most common muscle wasting disease, with patients succumbing in their twenties and caused by mutations in the *dystrophin* gene. Antisense oligonucleotides that induce exon skipping is now attracting much attention to express internally deleted-dystrophin in DMD [1]. In our previous study, we identified antisense RNA/2'-*O*, 4'-*C*-ethylene-bridged nucleic acid (ENA) chimera (AO85) that could induce exon 45 skipping efficiently. The skipping of exon 45 would theoretically be applicable to 9 % of DMD patients [2]. Here, we investigated efficiencies of exon 45 skipping and dystrophin expression in DMD patient-derived myocytes carrying different types of deletion mutations. We examined the ability of AO85 to induce exon 45 skipping and dystrophin expression in ten DMD myocytes carrying six different deletion mutations, as follows: exon 44, 46-47, 46-48, 46-49, 46-51, or 46-53. In each case, the exon 45 skipping was induced by AO85 as expected, however, the skipping efficiency was different from patients to patients. In myocytes with exon 46-48, 46-49, 46-51, or 46-53 deletion more than 50% of the dystrophin mRNA showed exon 45 skipping, while less than 20% in myocytes carrying exon 44 or 46-47 deletion. Western blot analysis showed clear visible signal for dystrophin in three myocytes with exon 44, 46-49, or 46-51 deletion. It was remarkable that myocytes from one DMD patient with exon 44 deletion showed low exon 45 skipping efficiency but expressed dystrophin detectable by the Western blotting. It needs further study to clarify the mechanism that provides discrepancy between exon skipping and dystrophin expression. Results of our in vitro study indicated that exon 45 skipping with AO85 is applicable to the DMD treatment. <Reference> 1.Takeshima et al. Optimizing RNA/ENA chimeric antisenseoligonucleotides using in vitro splicing. In Methods in Molecular Biology: Exon skipping. A. Aartsma-Rus, editor. (in press) 2. Takeshima et al. Mutation spectrum of the dystrophin gene in 422 Duchenne/Becker muscular dystrophy cases from one Japanese referral center. J Hum Genet. 55:379-388 (2010).

1457F

Changes in Biomarkers in patients with type 1 Gaucher Disease (GD1) transitioned from imiglucerase to velaglucerase alfa: cumulative 2-year results from the phase II/III trial TKT 034 and extension. *A. Zimran[1], GM. Pastores[2], A. Tylki-Szymanska[3], D. Hughes[4], D. Elstein[5], R. Mardach[6], C. Eng[7], L. Smith[8], M. Heisel-Kurth[9], J. Charrow[10], P. Harmatz[11], P. Fernhoff[12], W. Rhead[13], N. Longo[14], P. Giraldo[15], D. Zahrieh[16], E. Crombez[17], G. Grabowski[18].* 1) Ari Zimran, MD, Shaare Zedek Medical Center, Jerusalem, Israel; 2) GM. Pastores, New York University School of Medicine, New York, NY USA; 3) A. Tylki-Szymanska, Children's Memorial Health Institute, Warsaw, Poland; 4) D Hughes, Royal Free Hospital, UCL Medical School, London, UK; 5) D Elstein,Shaare Zedek Medical Center, Jerusalem, Israe; 6) R Mardach, Kaiser Permanente, Los Angeles, CA, USA; 7) C Eng, Baylor College of Medicine, Houston, TX; 8) L Smith, Children's Mercy Hospital, Kansas City, MO, USA; 9) M Heisel-Kurth, Children's Hospital and Clinic of Minnesota, Minneapolis, MN, USA; 10) J Charrow, Children's Memorial Hospital, Chicago, IL, USA; 11) P Harmatz, Children's Hospital Oakland, Oakland, CA, USA; 12) P Fernhoff,Emory University, Decatur, GA, USA; 13) W Rhead, Children's Hospital of Wisconsin, Milwaukee, WI, USA; 14) N Longo, University of Utah, Salt Lake City, UT, USA; 15) P Giraldo, CIBERER and University Hospital Miguel Servet, Zaragoza, Spain; 16) D Zahrieh, Shire Human Genetic Therapies, Lexington, MA, USA; 17) E Crombez, Shire Human Genetic Therapies, Lexington, MA, USA; 18) G Grabowski, Cincinnati Children's Hosp Med Cent, Cincinnati, OH, US.

Statement of purpose: To measure changes in plasma biomarkers, chitotriosidase and CCL18, in patients with type 1Gaucher Disease (GD1) transitioned from imiglucerase to velaglucerase alfa in a phase II/III trial and its extension. Methods: In trial TKT034, GD1 patients aged ⁄ 2 years and clinically stable on imiglucerase switched to velaglucerase alfa for 12 months (same U/kg [15-60] every other week as prior imiglucerase treatment), after which they could continue in the ongoing extension HGT-GCB-044. Results: All 38/40 treated patients completing TKT034 enrolled in HGT-GCB-044. At TKT034 baseline, 47% were male, the median age was 35 years (range 9-71; 24% <18), 8% were splenectomized, and the prior range for imiglucerase exposure was 22-192 months (median 65). The median (range) CCL18 level was 325 ng/mL (49-1582) and for chitotriosidase activity was 3363 nmol/mL/h (108-30785). One patient was chitotriosidase deficient and was excluded from the analysis of chitotriosidase. After 24 months' velaglucerase alfa, the mean (95% CI) change in CCL18 level was -50.9% (-55.5, -46.3) and for chitotriosidase activity -34.5% (-42.2, -26.9), while hemoglobin concentration, platelet count, liver and splenic volumes remained within prespecified clinically significant bounds of stability. Conclusions: GD1 patients switching from long-term imiglucerase maintained key clinical parameters through 2 years of velaglucerase alfa while biomarkers of glucosylceramide burden improved. Further studies with active control groups that remained on imiglucerase are recommended to determine whether the normalization of biomarkers is similar and clinically significant between patients who switch to velaglucerase alfa versus patients who do not.

1458F

Abnormal autophagy in mucopolysaccharidosis VII can be rescued by enzyme replacement therapy. *R. Datta[1], J. Grubb[2], A. Waheed[2], J. Ryerse[3], W. Sly[2].* 1) Department of Biological Sciences, Indian Institute of Science Education and Research-Kolkata, Mohanpur campus, West Bengal, INDIA; 2) Edward A. Doisy department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, 1100 South Grand Blvd. Saint Louis, MO 63104, USA; 3) Department of Pathology, Saint Louis University School of Medicine, 1100 South Grand Blvd. Saint Louis, MO 63104, USA.

Mucopolysaccharidosis VII (MPSVII) is an autosomal recessive lysosomal storage disorder caused by the deficiency of) -glucuronidase () -GUS), a lysosomal enzyme required for degradation of glycosaminoglycans (GAGs). Accumulation of undegraded or partially degraded GAGs in the lysosomes results in cellular and multiple organ dysfunctions leading to premature death in most cases. To better understand the pathogenesis of this disease we used MPSVII patient's fibroblasts and mouse model () -GUS knockout) to study the status of autophagy, a vesicular transport system by which cellular debris are targeted to the lysosome for degradation and reuse. We report here that in MPSVII patient's fibroblasts as well as in the brain of MPSVII mice there is an abnormal accumulation of autophagosomes as evidenced by the elevated LC3II levels. Electron microscopic evaluation of the MPSVII mice brain sections also showed increased abundance of autophagosomes. Co-localization studies with LC3II positive vesicles and a neuronal marker suggest that the autophagosomes primarily accumulate in the neurons. Our data also show massive increase in the levels of polyubiquitinated proteins (autophagic cargo) in the MPSVII fibroblasts as compared to the normal fibroblasts, suggesting impaired clearance of autophagosomes rather than increased formation. Nutrient acquisition through induction of autophagy is a well established mechanism of survival during starvation. That MPSVII fibroblasts are highly sensitive to serum starvation confirms that autophagy is indeed defective in this disease. Treatment of MPSVII fibroblasts with purified) -GUS (8000 units/ml for 72 hrs) completely reversed the autophagy defect. More importantly, intraperitoneal injection of the MPSVII mice with periodate modified) -GUS (2mg/kg, bi-weekly) not only restored normalcy in terms of brain pathology but also significantly reduced the LC3II levels in the brain. Taken together our data establishes MPSVII as an autophagy disorder and suggests that abnormal autophagy can be a useful biomarker for testing the efficacy of treatment of MPSVII patients.

1459F

The Pharmacological Chaperone AT2220 Increases the Muscle Uptake of Recombinant Human Acid (-Glucosidase Resulting in Greater Glycogen Reduction in a Mouse Model of Pompe Disease. *J. Feng, R. Soska, L. Pellegrino, M. Frascella, Y. Lun, J. Flanagan, D. Guillen, D. Lockhart, K. Valenzano, R. Khanna.* Amicus Therapeutics, 6 Cedar Brook Drive, Cranbury, NJ. 08512.

Pompe disease is an inherited metabolic disorder caused by a deficiency in acid (-glucosidase (GAA) activity, which impairs lysosomal glycogen catabolism and results in progressive skeletal muscle weakness, reduced cardiac function, and respiratory insufficiency. Recombinant human GAA (rhGAA; Myozyme®, Genzyme Corp.) given as a bi-weekly infusion is an approved enzyme replacement therapy (ERT) for Pompe disease. While somewhat effective, rhGAA has a short circulating half-life, low tissue uptake, and can elicit immune responses that adversely affect tolerability and efficacy. AT2220 (1-deoxynojirimycin-HCl) is a small molecule pharmacological chaperone that binds endogenous GAA in cells and tissues, resulting in increased lysosomal GAA levels and activity. We hypothesized that AT2220 might also interact with exogenous rhGAA to improve its pharmacological properties. In human plasma, AT2220 co-incubation increased the stability and prevented denaturation of rhGAA at neutral pH and 37°C for up to 24 hours. In cultured rat myoblasts, AT2220 co-incubation also increased the cellular uptake of rhGAA by more than 3-fold. In rats, a single intravenous bolus administration of rhGAA 30 minutes after a single oral administration of AT2220 resulted in a dose-dependent increase of up to 2-fold in the circulating half life of rhGAA, indicating that AT2220 can bind and stabilize rhGAA in vivo. A similar effect of AT2220 was seen on the circulating half-life when rhGAA was administered by 1-hour infusion. In GAA KO mice, oral administration of AT2220 resulted in up to 2.5-fold greater tissue uptake and glycogen reduction compared to administration of rhGAA alone. Collectively, these results indicate that AT2220 increases the stability of rhGAA, increase enzyme activity and substrate turnover in muscle when administered orally prior to rhGAA IV dosing. Based on these findings, a Phase 2a study with AT2220 co-administration with rhGAA has been initiated.

1460F

Oral Migalastat HCL (AT1001/GR181314A) is an Investigational Therapy Evaluated in Females with Fabry Disease. *P. Fernhoff[1], R. Giugliani[2], K. Nicholls[3], D.P. Germain[4], S. Waldek[5], D.G. Bichet[6], V. Sniukiene[7], P. Boudes[7].* 1) Div Med Gen/Dept Human Gen, Emory Univ Sch Med, Decatur, GA. 30033 United States; 2) Department of Genetics, UFRGS Director,WHO CC Develop Med Genet Serv Lat America Medical Genetics Service Hospital de Clínicas de Porto Alegre Rua Ramiro Barcelos 2350 90035-903 - Porto Alegre - RS - Brazil; 3) Department of Nephrology The Royal Melbourne Hospital Parkville, Victoria 3050; 4) Department of Medical Genetics, Hôpital Raymond Poincaré, University of Versailles, Saint Quentin en Yvelines, Garches, France; 5) Willink Biochemical Genetics Unit Genetic Medicine, 6th Floor, Pod 1, St Mary's Hospital, Oxford Road, Manchester, M13 9WL; 6) Department of Medicine Université de Montréal Hôpital du Sacré-Coeur de Montréal 5400 Blvd. Gouin Ouest Centre de Recherches Montreal, Quebec H4J 1C5 Canada; 7) Amicus Therapeutics 6 Cedar Brook Drive Cranbury, New Jersey 08512.

Background: Fabry disease (FD) is an X-linked lysosomal storage disorder characterized by deficiency of alpha-galactosidase A ((- Gal A) with accumulation of globotriaosylceramide (GL-3) in tissues and body fluids, leading to progressive, multi-organ dysfunction. Heterozygote females develop signs and symptoms generally later than men and may require treatment. Objective: A phase II study to evaluate safety and tolerability of migalastat, an orally administrated pharmacological chaperone, in symptomatic FD females. Methods: Migalastat was administered at 50, 100 or 250 mg once every other day for 48 weeks. Results: Nine females (36 to 62 years old) with baseline leukocyte (- Gal A activity ranging from 15% to 114 % of normal were enrolled. Five of nine had GLA mutations characterized as responsive to migalastat using an in vitro assay. Medical history and baseline findings were significant: five females had left ventricular hypertrophy, five had Stage 2 chronic kidney disease and two experienced transient ischemic attack prior to treatment. Seven of nine had elevated urine GL-3 (uGL-3). Kidney biopsy demonstrated GL-3 inclusions in multiple cell types predominantly in podocytes. On treatment all females (N=5) with responsive GLA mutations demonstrated an increase in leukocyte (-Gal A with mean increase at Week 48 of 61% and mean decline in uGL-3 of 46 %; four of them had a decline in interstitial capillary GL-3 (mean 56%). Migalastat was generally safe and well- tolerated. All subjects completed treatment; no treatment-related serious adverse events and no treatment-limiting toxicities were identified. Conclusion: Data suggest that migalastat is safe and tolerable investigational therapy with emerging evidence of efficacy in symptomatic FD females with amenable GLA mutations.

1461F

Improvements in Skeletal Manifestations in Gaucher Disease Type 1 Patients After 3 Years of Treatment with Oral Eliglustat During a Phase 2 Trial. *R.S. Kamath[1], E. Lukina[2], N. Watman[3], M. Dragosky[4], M. Iastrebner[4], G. Pastores[5], E. Avila Arreguin[6], M. Phillips[7], H. Rosenbaum[8], E. Sysoeva[2], R. Aguzzi[9], A.C. Puga[9], M.J. Peterschmitt[9], D.I. Rosenthal[1].* 1) Massachusetts General Hospital, Boston, MA; 2) National Research Center for Haematology, Moscow, Russia; 3) Hospital Ramos Mejia, Buenos Aires, Argentina; 4) Instituto Argentino de Diagnostico y Tratamiento, Buenos Aires, Argentina; 5) New York University, New York, USA;; 6) Instituto Mexicano del Seguro Social, Hospital de Especialidades, Col. La Raza, Mexico; 7) Sha'are Zedek Medical Center, Jerusalem, Israel; 8) Rambam Medical Center, Haifa, Israel; 9) Genzyme Corporation, Cambridge, MA, USA.

Background: In Gaucher disease type 1 (GD1), the deficiency of lysosomal acid) -glucosidase leads to the accumulation of glucosylceramide. Skeletal complications are a major cause of morbidity and include bone marrow infiltration, osteopenia/osteoporosis, lytic lesions, fractures, avascular necrosis and bone pain. Eliglustat, a novel, oral inhibitor of glucosylceramide synthase, is under investigation for the treatment of GD1. **Aim:** Report skeletal changes after 3 years of eliglustat therapy. **Methods:** Phase 2 study entry criteria required that patients be untreated, have no new pathologic bone involvement or bone crises within the preceding 12 months, and not be using bisphosphonates. Changes from baseline were reported for centrally reviewed skeletal x-rays, dual-energy x-ray absorptiometry (DXA) and MRI assessments. **Results:** Of 26 enrolled patients, 19 completed 3 years of treatment. In 15 patients with evaluable DXA results at baseline and 1, 2 and 3 years, mean lumbar spine BMD increased by 0.6±0.69 Z-score (baseline -1.28) with greatest increases in osteoporotic patients. Mean femur BMD (T- and Z-score) remained normal through 3 years. Femur dark marrow on MRI reflecting bone marrow infiltration by Gaucher cells was reduced (56%, 10/18) or stable (44%, 8/18) in 18 patients with findings at baseline. No bone crises or reductions in mobility occurred. On baseline radiographs, no patients had fractures, 8/19 (42%) had femoral lytic lesions and 7/19 (37%) had bone infarcts. After 3 years, the lumbar spine and femurs showed no new lytic lesions, bone infarcts, fractures or areas of osteonecrosis and no worsening of pre-existing lytic lesions or bone infarcts; 1 patient had worsening of asymptomatic osteonecrosis after 1 year that was noted retrospectively at baseline. Eliglustat was well-tolerated. Most adverse events (AEs) were mild and unrelated to treatment; the most common were viral infections (6 pts); urinary and upper respiratory tract infections (4 pts each); and headache, increased blood pressure, abdominal pain, diarrhea (3 pts each). Eight drug-related AEs, all mild, occurred in 6 patients. **Conclusions:** During 3 years of eliglustat treatment, radiologic monitoring showed improvement or stabilization of GD1 bony manifestations with no noted safety-related trends. Results suggest that eliglustat may be a promising treatment for skeletal complications of GD1. Ongoing Phase 3 studies will provide more information on the bone effects of eliglustat.

1462F

The Pharmacological Chaperone AT1001 Increases the Tissue Uptake of Agalsidase Alfa Resulting in Greater Substrate Reduction in a Mouse Model of Fabry Disease. *L. Pellegrino, J. Feng, M. Frascella, R. Soska, J.J. Flanagan, D. Guillen, D.J. Lockhart, K.J. Valenzano, R. Khanna.* Amicus Therapeutics, Cranbury, NJ.

Fabry disease is an X-linked lysosomal storage disorder caused by mutations in the gene that encodes (-galactosidase A ((-Gal A), and is characterized by pathological accumulation of globotriaosylceramide (GL-3). Regular infusion of recombinant human (-Gal A (rh(-Gal A), termed enzyme replacement therapy (ERT), is the primary treatment for Fabry disease. However, rh(-Gal A has low physical stability (especially at neutral pH and body temperature), a short circulating half-life, and variable access and uptake across different tissues. The iminosugar AT1001 (GR181413A, deoxygalactonojirimycin; migalastat hydrochloride) is a pharmacological chaperone that selectively binds endogenous (-Gal A (wild-type and mutant forms), increasing physical stability, lysosomal trafficking, and total cellular activity. We hypothesized that AT1001 might also improve the pharmacological properties of exogenous rh(-Gal A. Hence, in this study we investigated the effects of AT1001 on agalsidase alfa (Replagal™, Shire Human Genetic Therapies, Inc.) in vitro and in vivo. Binding of AT1001 increased the physical stability of agalsidase alfa, preventing denaturation and loss of activity at neutral pH and 37°. In rats, oral administration of AT1001 in combination with intravenous administration of agalsidase alfa dose-dependently increased the circulating half-life of the enzyme by up to 3-fold. In GLA knockout mice, AT1001 co-administration increased the uptake of agalsidase alfa into skin, heart, and kidney up to 4-fold more than seen following administration of agalsidase alfa alone; these higher levels were also sustained for longer periods of time. In addition, AT1001 co-administration resulted in up to 20% greater GL-3 reduction in tissues compared to administration of agalsidase alfa alone. Collectively, these results indicate that AT1001 increases the stability of rh(-Gal A, translating to greater enzyme activity and substrate turnover in situ when administered orally prior to rh(-Gal A dosing. Based on these findings, a Phase 2a study of AT1001 in combination with agalsidase alfa has been initiated.

1463F

Eliglustat, an Investigational Oral Therapy for Gaucher Disease Type 1 (GD1): Phase 2 Results after 3 Years. *M.J. Peterschmitt[1], E. Lukina[2], N. Watman[3], M. Dragosky[4], M. Iastrebner[4], G. Pastores[5], E. Avila Arreguin[6], M. Phillips[7], H. Rosenbaum[8], E. Sysoeva[2], R. Aguzzi[1], L.H. Ross[1], A.C. Puga[1].* 1) Genzyme Corporation, Cambridge, MA, USA; 2) National Research Center for Haematology, Moscow, Russia; 3) Hospital Ramos Mejia, Buenos Aires, Argentina; 4) Instituto Argentino de Diagnostico y Tratamiento, Buenos Aires, Argentina; 5) New York University, New York, USA; 6) Instituto Mexicano del Seguro Social Hospital de Especialidades, Col. La Raza, Mexico; 7) Sha'are Zedek Medical Center, Jerusalem, Israel; 8) Rambam Medical Center, Haifa, Israel.

Background: Gaucher disease type 1 (GD1) is an inherited lysosomal storage disorder caused by deficient activity of acid) -glucosidase. This enzymatic deficiency leads to accumulation of undegraded glucosylceramide primarily in tissue macrophages and results in multisystemic manifestations, including thrombocytopenia, anemia, hepatosplenomegaly and skeletal complications. Eliglustat, a novel, potent and specific inhibitor of glucosylceramide synthase, is under development as an oral substrate reduction therapy (SRT) for GD1. **Aim**: To report long-term efficacy and safety results of eliglustat. **Methods**: This ongoing, open-label, uncontrolled, multicenter Phase 2 clinical trial enrolled 26 adults with GD1 not on treatment for the previous 12 months, who had splenomegaly with thrombocytopenia and/or anemia. Efficacy outcomes included changes from baseline in hemoglobin, platelets, spleen and liver volumes and bone mineral density (mean±SD); biomarker levels (median); and achievement of therapeutic goals for anemia, thrombocytopenia, splenomegaly and hepatomegaly (Pastores *Semin Hematol* 2004). **Results**: Nineteen patients completed 3 years of treatment; 7 patients discontinued the trial. After 3 years, hemoglobin increased by 2.6±1.39 g/dL (11.3±1.63 to 13.8±1.37); platelets increased by 91±65.9% (70,000±21,700 to 126,800±40,500/mm^3); and spleen and liver volumes (multiples of normal, MN) decreased by 61±12.2% (16.8±9.5 to 6.2±3.6 MN) and 29±15.8% (1.7±0.5 to 1.2±0.3 MN), respectively. Most patients met long-term therapeutic goals for hemoglobin (100%), spleen volume (100%), liver volume (89%) and platelets (63%); all patients met⁄3 therapeutic goals at 3 years. Plasma GL-1 and GM3 levels normalized; median chitotriosidase and CCL-18 decreased by 80% and 73%, respectively. Mean lumbar spine BMD increased by 0.6 Z-score (-1.3±1.0 to -1.7±1.1). Eliglustat was well tolerated. Most adverse events (AEs) were mild and unrelated to treatment. The most common AEs were viral infections (6 pts); urinary and upper respiratory tract infections (4 pts each); and headache, increased blood pressure, abdominal pain and diarrhea (3 pts each). Eight drug-related AEs, all mild, occurred in 6 patients. **Conclusions**: Eliglustat has shown promising efficacy and safety, with clinically meaningful hematologic, visceral, and bone improvements. Most patients met long-term therapeutic goals by 3 years. Eliglustat has been well tolerated. Three international Phase 3 studies are ongoing.

1464F

Continued improvement in a forty year old man with mucopolysaccharidosis type II after four years of enzyme replacement therapy. *F.J. Stewart[1], M. McCloskey[2], J.E. Wraith[3].* 1) Dept Medical Genetics, Belfast City Hosp, Belfast, United Kingdom; 2) Altnagelvin Hospital, Londonderry, UK; 3) Willink biochemical Genetics Unit, St Mary's Hospital, Manchester, UK.

Our patient was diagnosed with Mucopolysaccharidosis (MPS) Type II Hunter disease at the age of 12 years. MPS II is caused by a deficiency of iduronate sulphatase which is one of the lysosomal enzymes. Developmental outcome ranges from severe learning disability to normal intelligence as is seen in our patient. Our patient had short stature, characteristic facial features, joint contractures and marked hepatosplenomegaly. He developed worsening respiratory problems and his FEV1 dropped from 0.98 to 0.42 (17.6% predicted). At age 28 he was started on overnight CPAP as he had episodes of sleep apnoea with overnight O2 saturations falling to 61%. He started on idursulfase (Elaprase ®) with a weekly dose of 0.5mg/kg in June 2007 at the age of 36. He was gradually able to reduce his CPAP and within a year this was discontinued. His sats improved to 99% in room air. Overnight oximetry was normal. FEV1 improved to 0.71 (30% predicted).His hepatosplenomegaly has reduced and his waist is four inches smaller. He has much more energy and was able to cook Christmas dinner unaided. He was also able to travel to Vancouver which previously would have been unthinkable. His joint contractures are also improving slightly giving him better hand function. He has now been on treatment for 4 years and his respiratory function has not deteriorated. He remains off his CPAP. Overall his quality of life has continued to improve. Our patient is one of the older patietns with MPS II to have received enzyme replacement therapy. His case demonstrates that treatment of an older MPS II individual may lead to a significant improvement in their clinical condition and quality of life and that treatment should not be discounted on the grounds of age.

1465F

Quantitative evaluation of bones in murine MPS VII after replacement therapy using chemically modified enzyme. *S. Tomatsu[1], DJ. Rowan[1], JH. Grubb[3], B. Haupt[2], AM. Montaño[1], WS. Sly[3].* 1) Dept Pediatrics, Saint Louis Univ Doisy Res Ctr, St Louis, MO; 2) Edward A. Doisy Department of Biochemistry and Molecular Biology, Saint Louis University, St. Louis, Missouri; 3) Department of Pathology, Saint Louis University, St. Louis, Missouri.

Mucopolysaccharidosis (MPS) type VII is a lysosomal storage disease caused by deficiency of the lysosomal enzyme)-glucuronidase (GUS), which is involved in degradation of glycosaminoglycans (GAGs). GAG storage in the tissue of patients with MPS VII causes a wide spectrum of clinical manifestations that affect the central nervous system (CNS), bone, and viscera. Enzyme replacement therapy (ERT), effectively clears GAG storage in the viscera. However, clearance of storage material in CNS has been limited by the blood brain barrier. Nonetheless, recent studies showed that a chemically modified form of GUS (PerT-GUS) escaped clearance by mannose 6-phosphate and mannose receptors leading to its prolonged circulation and reduced CNS storage more effectively than native GUS. Although clearance of storage in bone is somewhat limited by the avascularity of the growth plate, prior work showed clinical and pathological improvement of skeletal disease in MPS VII mice treated from birth with weekly IV injections of native GUS. To evaluate the effectiveness of PerT-GUS in reducing the skeletal pathology, we treated MPS VII mice from birth with PerT-GUS and used micro-CT, X-rays and quantitative histopathological analysis for assessment of bones. Micro-CT and X-rays demonstrated marked improvements in bone lesions of legs, ribs, and spine of treated mice. Quantitative histopathological assay also showed moderate improvements in GAG storage and morphology of articular and epiphyseal chondrocytes. These findings indicate that PerT-GUS therapy from birth may significantly reduce disability caused by bone dysplasia in MPS VII in addition to addressing CNS storage.

1466F

Cell-based evaluation of small molecules for treatment of Pompe disease. *W. Westbroek[1], A.M. Gustafson[1], J.J. Marugan[2], J. Xiao[2], W. Zheng[2], A. Velayati[1], E. Goldin[1], E. Sidransky[1].* 1) Medical Genetics Branch/NHGRI/NIH. Bl35, 35Convent Drive, Bethesda, MD 20892; 2) NIH Chemical Genomics Center/NIH. 9800 Medical Center Drive, Rockville, MD 20850.

Glycogen storage disease II or Pompe disease is an autosomal recessive lysosomal storage disorder (LSD) caused by dysfunction of the lysosomal enzyme acid alpha-glucosidase (GAA). Mutations in GAA impair the breakdown of glycogen, causing storage of the substrate in enlarged lysosomes. Cardiac and skeletal muscles are severely affected, causing impaired breathing and mobility. More than 100 distinct disease-causing mutations in GAA have been identified, some of which retain enzymatic activity in vitro, but are not trafficked to the lysosome. Instead the misfolded GAA stays in the endoplasmic reticulum (ER) and eventually undergoes proteosome-mediated breakdown. Enzyme replacement therapy with Myozyme®, the only current treatment for Pompe disease, improves clinical outcome, but the treatment remains expensive, inconvenient and does not reverse all disease manifestations. It was postulated that small molecules which aid in protein folding, ER trafficking, and translocation of enzymes to lysosomes could provide an alternate therapeutic strategy. We identified several promising non-inhibitory compounds for GAA from a chemical compound library by performing distinct in vitro assays. For further biological evaluation of these GAA compounds, we developed an in vivo cell-based immune-fluorescence cytochemistry assay to assess chaperone capacity. Translocation of GAA to the lysosomes in Pompe cells was evaluated by measuring the extent of co-localization between GAA and Cathepsin-D, a lysosomal marker, using laser scanning confocal microscopy. Using a GAA-specific antibody, control fibroblasts showed specific GAA staining in the lysosomes, while fibroblasts from patients with Pompe disease lacked this signal with immune-fluorescence cytochemistry. However, after six days of treatment of Pompe fibroblasts by adding the GAA activator to the growth medium, the expression level and translocation of GAA to lysosomes was comparable to control cells. Such activators are particularly desirable chaperones, since enzyme function remains intact. These promising small molecules, identified using an in vivo cell-based assay, merit further evaluation as a potential new therapy for Pompe disease.

1467F

Current treatment and outcome of infantile cobalamin C disease. *N. Carrillo-Carrasco[1], J. Sloan[1], I. Manoli[1], N. Hauser[1], A. Gropman[5], C. O'Shea[3], R.D. Graf[3], W.M. Zein[2], P. Tanpaiboon[5], S.M. Paul[3], J. Snow[3], C. Wagner[6], S.H. Mudd[4], B.P. Brooks[2], C.P. Venditti[1].* 1) National Human Genome Research Institute, NIH, USA; 2) National Eye Institute, NIH, USA; 3) Clinical Research Center, NIH, USA; 4) National Mental Health Institute, NIH, USA; 5) Children's National Medical Center, USA; 6) Vanderbilt University Medical Center, USA.

Background: Cobalamin C disease (cblC), thought to be the most common inborn error of intracellular vitamin B12 metabolism, is caused by mutations in MMACHC and results in impaired synthesis of adenosyl- and methylcobalamin. Manifestations of this multisystemic disorder include impaired growth, neurologic and ophthalmologic abnormalities, and decreased lifespan. **Methods**: We evaluated 22 patients with infantile cblC disease (age range, 2 to 27 years) through NIH study 04-HG-0127 (clinicaltrials.gov: NCT00078078) to define their outcomes under current management strategies. **Results**: The median age of detection of patients diagnosed clinically was 2.6 months (range 0.6-24 months, n=16), compared to 0.3 months (n=3) by newborn screening. Common presentations included failure to thrive, encephalopathy, poor eye contact and nystagmus. All patients were treated after diagnosis, with considerable clinical improvement. We found that management was highly variable among patients. Protein restriction, although controversial, was seen in 41% of patients, with a median intake of 70% of their RDA. All patients had long-term complications despite treatment. Head circumference was the growth parameter most affected (median z-score -0.97; range -0.5 to -3.4). Seizures were reported in 55% and included refractory (14%; n=3), controlled (9%; n=2) and past history of seizures (32%; n=7); resolution coincided with lowering tHcy levels (n=4). The mean Vineland-II Adaptive Behavior Composite score was over 3 SD below normal (n=14, 52.9±18.87), but earlier age of initiation of treatment correlated with improved neurocognitive outcome (n=13, R=-0.755, p=0.003). Patients had progressive visual loss with a mean acuity of 20/500 (range 20/50 to 5/400). Fundoscopy revealed the characteristic findings of maculopathy, pigmentary retinopathy and/or optic pallor. Mean plasma MMA was 20 μM (range: 0.49-151 μM), tHcy 70.8 μM (22-134 μM) and methionine 19.5 μM (4-53 μM). Most patients had trough plasma vitamin B12 levels below 1,000,000 pg/ml, a level noted to be therapeutic in other studies. **Conclusion**: We performed a single center study to define the outcome of infantile cblC. Early diagnosis and treatment seem to improve neurocognitive outcome, but not the progression of eye disease. Current treatment strategies are variable and commonly suboptimal. This study also provides baseline information to evaluate the efficacy of newborn screening and novel therapeutic interventions.

1468F

Long-term effectiveness of helper-dependent adenoviral gene therapy in an hypomorphic mouse model of argininosuccinic aciduria. *P. Campeau[1], O. Shchelochkov[2], K. Guse[1], S.C. Sreenath Nagamani[1], Y. Chen[1], J. Zhang[1], M.H. Premkumar[3], T. Bertin[1], D. Palmer[1], N. Brunetti-Pierri[4], J. Marini[3], Q. Sun[1], W. O'Brien[1], N.S. Bryan[5], A. Erez[1], B.H. Lee[1,6].* 1) Molec & Human Gen, Baylor College Med, Houston, TX; 2) Division of Medical Genetics, Department of Pediatrics, University of Iowa Children's Hospital; 3) Department of Pediatrics, Baylor College of Medicine; 4) Telethon Institute of Genetics and Medicine, Napoli, Italy; 5) Institute of Molecular Medicine, UT Health Science Center; 6) Howard Hughes Medical Institute, Houston, TX.

Argininosuccinic aciduria (ASA) is an inborn error of the ureagenesis caused by mutations in the gene encoding argininosuccinate lyase (ASL). The enzyme is a homotetramer expressed in the liver where it participates in the detoxification of ammonia by converting argininosuccinate to arginine which is then converted to urea for excretion. Patients can present clinically with encephalopathy, seizures or lethargy caused by elevated plasma ammonia and are diagnosed biochemicaly by having increased citrulline and elevated ASA in their blood. Current treatments for ASA are limited to arginine supplementation, nitrogen scavenging medications and liver transplantation in rare cases. We have generated a hypomorphic model of argininosuccinic aciduria by inserting a neomycin resistance cassette in the mouse *Asl* locus. *Asl* hypomorphic mice die within the first month of life from multi-organ failure. In an attempt to rescue the mice, we delivered a helper-dependent adenovirus encoding *Asl* under a liver-specific promoter at 4 weeks of life. The gene therapy group showed dramatic improvement in their growth with normalization of Asl expression in the liver by 2 weeks which allowed survival past the critical period of weaning. Most mice survived more than 10 months, showing a correction of their blood and tissue amino acid levels. Gene therapy also corrected the citrulline flux in these mice providing additional evidence for functional correction of Asl. At 10 months of age, the mice displayed normal motor coordination and balance on the rota-rod test, and normal learning and memory on the cued and contextual fear conditioning test in spite of lower levels of Asl in the liver. These data highlight the age related different requirement for ASL in the neonatal versus long term adult correction and importantly demonstrate the potential of gene therapy for argininosuccinic aciduria.

1469F

A New Pharmacologic Approach for Cystinuria. *A. Sahota[1], M. Yang[1], S. Shikhel[1], M.R. Lewis[2], M.D. Ward[3], D.S. Goldfarb[4], J.A. Tischfield[1].* 1) Dept Gen, Rutgers Univ, Piscataway, NJ; 2) Faculty Med, Imperial Coll, London, UK; 3) Dept Chem, New York Univ, NY, NY; 4) NYU Langone Med Ctr, NY, NY.

Background: Slc3a1 knockout mice excrete cystine in the urine, but males from age 3 months also have cystine stones in the bladder and kidney. Current therapies for cystinuria are inadequate and little progress has been made in this area. Our recent in vitro studies have shown that cystine dimethyl ester (CDME), a structural analog of cystine, inhibits cystine crystallization by attaching to cystine crystal surfaces, thus blocking their further growth. CDME may provide a paradigm shift for the treatment of cystinuria. Methods: We gave CDME (200 µl of 1 mg/ml) or water only daily by gavage to 2-month old cystinuria male mice for 4 weeks to determine whether this compound reduces bladder stone formation. Following treatment, urine samples were analyzed for amino acids by HPLC. Animals were then sacrificed and bladders examined by micro computed tomography (micro CT). Analysis of urine and stone samples was carried out by LC-MS. Results: In the CDME group (n = 14), 7 mice had small stones and 7 had no stones. In the control group (n = 11), 6 mice had small-to-large stones and 5 had no stones. The number of stones per bladder in the CDME group ranged from 16-45 (total 181). The number of stones per bladder in the control group ranged from 1-19 (total 47). All stones in the CDME group were in the size range 0.5 to 2 mm except one, which was 3 mm. The control group had a wide variation in stone size, ranging from 0.5 to 2.0 mm (41 stones) to 9 mm (1 stone). In the CDME group, mean weight of the stones was 29.2 +/- 14.8 mg and in the control group it was 55.2 +/- 28.0 mg. The difference in stone burden in the bladder between the CDME and water groups was clearly evident by micro CT. There was no difference in cystine excretion between the CDME and water groups, suggesting that urine was saturated with cystine in both groups. Analysis by LC-MS of urine and stone extracts from the CDME group demonstrated the presence of cysteine methyl ester, suggesting that a metabolite of CDME, rather than CDME itself, was incorporated into the crystal structure. Conclusions: CDME-treated mice had a significantly smaller stone mass and more stones, but the stones were of a smaller size range compared with the water-treated mice. These studies strongly suggest that, as in vitro, CDME is inhibiting the growth of cystine crystals into large stones in vivo. CDME had no apparent side effects, suggesting that this compound may have potential for the treatment of cystinuria.

1470F

Restoration of impaired nitric oxide production in MELAS syndrome with citrulline and arginine supplementation. *A. El-Hattab[1], J.W. Hsu[2], L.J. Wong[1], W. Craigen[1], F. Jahoor[2], F. Scaglia[1].* 1) Dept Molec & Human Gen, Baylor Col Med, Houston, TX; 2) Dept Ped, Baylor Col Med, Houston, TX.

Background The mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is one of the most common mitochondrial disorders. The pathogenesis of stroke-like episodes remains unclear. However, it has been suggested that mitochondrial proliferation may result in endothelial dysfunction and decreased nitric oxide (NO) availability leading to cerebral ischemic events. This study aimed to assess NO production in adult subjects with MELAS syndrome and the effect of oral supplementation with the NO precursors arginine and citrulline. Methods Using a stable isotope infusion technique, we measured NO synthesis, amongst other related variables, in 10 control adult subjects and 7 adult subjects with MELAS syndrome before and after arginine or citrulline supplementation. Results Adult subjects with MELAS syndrome had lower NO synthesis rate and plasma arginine and citrulline concentrations, higher plasma arginine clearance rate, and lower citrulline flux and *de novo* arginine synthesis. In subjects with MELAS syndrome, arginine and, to a greater extent, citrulline supplementation increased the *de novo* arginine synthesis rate, the plasma concentrations and flux of arginine and citrulline, and NO production. Conclusions The lower NO production in subjects with MELAS syndrome is primarily due to decreased *de novo* arginine synthesis secondary to decreased citrulline availability. *De novo* arginine synthesis increased markedly with citrulline supplementation, explaining the superior efficacy of citrulline in increasing NO production. The improvement in NO production with arginine or citrulline supplementation supports their use in MELAS syndrome and suggests that citrulline may have a better therapeutic effect than arginine. These findings can have a broader relevance for other disorders where perturbations in NO metabolism occur.

1471F

Bezafibrate differentially impacts respiratory chain deficiencies resulting from various mtDNA mutations. *S. GOBIN-LIMBALLE[1], J. WONG[1], D. CHRETIEN[1], Z. ASSOULINE[1], V. SERRE[1], L. NONNEN-MACHER[2], S. MONNOT[1], A. ROTIG[1], P. DE LONLAY[1], AS. LEBRE[1], M. RIO[1], A. MUNNICH[1], J. STEFFANN[1], JP. BONNEFONT[1].* 1) Paris Descartes University, Inserm unit U781, and Genetics Department, Necker Hospital, Paris, France; 2) Metabolic Biochemistry unit, Necker Hospital, Paris, France.

Inherited disorders of mitochondrial oxidative phosphorylation result from mutations in nuclear or mitochondrial DNA (mtDNA) genes. mtDNA disorders have a maternal inheritance, a high transmission risk, and their clinical severity depends on the tissue distribution of the mutant load (heteroplasmy rate). To date, no therapeutic approach has revealed to be efficient to treat these disorders. Bezafibrate (BZF), an agonist of the PPAR transcription factors, might be a relevant candidate drug, based on its capacity to activate the NRF1-2/PGC1(pathway, a major regulator of mitochondrial biogenesis. A recent study reported an improvement of the respiratory chain (RC) function after BZF treatment of cybrids homoplasmic for mutations of mt.tRNA genes. There are however no available data regarding primary cell lines from patients with mutations of mtDNA genes encoding a RC subunit. We have analyzed the effect of BZF on RC functions, using fibroblasts from 2 controls and 5 patients with a mtDNA mutation affecting either complex I (CI, m.13514A>G in ND5) or complex V (CV, m.8993T>G or m.9185T>C in ATP6, NARP/Leigh syndromes). The two CI-deficient cell lines had 20% and 50% mutant loads, respectively, while the three CV-deficient lines were homoplasmic mutants. Our data indicate that BZF: 1/ does not modify the lactate level in culture media from control and mtDNA mutated cell lines as well, thus ruling out a hypothetical adverse effect of BZF in mtDNA mutated patients, 2/ stimulates CI enzyme activity in ND5-mutant cells and controls as well (+ 50 %), without impacting CV activity in ATP6-mutant cells, 3/ increases the mtDNA copy number in CI-mutant cells and controls as well (+150%), without affecting CV-mutant cells, 4/ mildly decreases the m.13514A>G heteroplasmic mutant loads (- 6 %), but has no effect on m.8993T>G / m.9185T>C homoplasmic mutant loads, 5/ increases a number of transcripts from nuclear (NRF1 and PGC1(transcription factors, POLG1 mtDNA polymerase, superoxide dismutase 2) but also mitochondrial genes (ATP6 and ND5 RC subunits), both in CI and CV-deficient lines. These data indicate that BZF i) has a variable impact on RC deficiencies secondary to a mtDNA mutation, depending on the mutation type and/or the mutant load, ii) is able to rescue in vitro a CI enzyme deficiency resulting from a mutation of a mtDNA-encoded CI subunit, and therefore, iii) might be a candidate drug for treating patients carrying such mutations at a heteroplasmic state.

1472F

Reduction of NADPH-oxidase activity ameliorates the cardiovascular phenotype in a mouse model of Williams-Beuren syndrome. *L.A. Perez-Jurado[1,2], M. Segura[1,2], V. Terrado[1,2], C. Sanchez-Rodriguez[3], M. Coustets[1], M. Menacho-Marquez[4], J. Nevado[2,5], X. Bustelo[4], U. Francke[6], V. Campuzano[1,2].* 1) Unitat de Genetica, DCEX, Universitat Pompeu Fabra, Barcelona, Spain; 2) Centro de Investigación Biomédica en Red de Enfermedades Raras, CIBERER, ISCIII, Spain; 3) Unidad de Investigación, Hospital Universitario de Getafe, Madrid, Spain; 4) Centro de Investigación del Cáncer e Instituto de Biología Molecular del cáncer de Salamanca (IMBCC). CSIC-Universidad de Salamanca, Salamanca, Spain; 5) INGEMM-Instituto de Genética Médica y Molecular / IdiPAZ, Madrid, Spain; 6) Department of Genetics, Stanford University School of Medicine, Stanford, CA. USA.

Williams-Beuren syndrome (WBS) is a developmental disorder with multisystemic manifestations caused by a segmental aneusomy of 1.55Mb at chromosomal band 7q11.23, which includes the elastin gene (*ELN*). A hallmark feature of WBS is a generalized arteriopathy due to elastin deficiency, presenting as stenoses of medium and large size arteries and leading to hypertension and other cardiovascular complications. Currently, there is not enough data available to recommend specific drug therapy for the hypertension in WBS. Deletion of a functional *NCF1* gene copy has been shown to protect a proportion of WBS patients against hypertension, likely through reduced NADPH-oxidase (NOX) mediated oxidative stress. We studied a mouse model for WBS cardiovascular disease, carrying a 0.67Mb heterozygous deletion including Eln (DD). DD mice presented with a generalized arteriopathy, hypertension and cardiac hypertrophy, associated with elevated angiotensin II, oxidative stress parameters, and *Ncf1* expression. We then crossed DD mice with an Ncf1 mutant, to generate double heterozygous mutants (DD/*Ncf1*-), mimicking the genotype of WBS individuals with lower cardiovascular risk. In addition, DD mice were also treated (groups of prenatal and postnatal onset) with either angiotensin II type 1 receptor blocker (losartan) or NOX inhibitor (apocynin), in an attempt to reduce NOX activity. DD/*Ncf1*- and all groups of treated DD mice revealed almost complete control of the hormonal and biochemical parameters, with normalized blood pressure and improved cardiovascular histology with respect to the untreated DD animals. Our data provide strong evidence for implication of the redox system in the cardiovascular pathophysiology of a mouse model of WBS. The phenotype of these mice can be ameliorated by either genetic or pharmacological intervention reducing NOX activity, likely through reduced angiotensin II-mediated oxidative stress. Therefore, anti-NOX therapy merits evaluation to prevent potentially serious cardiovascular complications of WBS, as well as in other cardiovascular disorders mediated by similar pathogenic mechanism.

1473F

Elevated cerebral spinal fluid Calbindin-D levels in Niemann-Pick Disease, type C and response to miglustat therapy. *N.M. Yanjanin[1], A. Remaley[2], M. Sampson[2], F.D. Porter[1].* 1) PDGEN, NICHD/NIH/DHHS, Bethesda, MD; 2) CCS, DLM, CC/NIH/DHHS, Bethesda, MD.

Niemann-Pick Disease, type C (NPC) is a rare, autosomal recessive lysosomal storage disorder characterized by impaired transport of glycosphingolipids and unesterified cholesterol. Intracellular accumulation of lipids results in neuroinflammation and microglial activation as well as induction of the autophagic pathway and loss of neurons. Clinical manifestations of NPC range from fetal ascites to adult-onset dementia, but the classic phenotype presents with childhood onset of progressive neurodegeneration. There is currently no FDA approved therapy. A randomized trial using miglustat, an inhibitor of glyscosphingolipid synthesis, showed improvement of neurological symptoms after one year but long-term efficacy has not been established. Protein biomarkers are a recent focus of rare disease research as surrogate endpoints to facilitate development of therapeutic interventions. Protein markers in cerebrospinal fluid (CSF) have been useful in diagnosing and describing neurodegenerative diseases, and provide insight regarding pathological mechanisms and disease status.

Calbindin-D is a vitamin D-dependent calcium binding protein localized predominately in cerebellar Purkinje cells, and is released into CSF as a result of cerebellar damage. Purkinje cell loss is a hallmark of NPC and histology of Npc1-/- cerebella shows progressive loss of Purkinje cells. Calbindin-D from CSF of 42 NPC1 patients enrolled in an observational study at the NIH and 30 pediatric controls was quantified using ELISA.

Initial analysis of Calbindin-D shows significant elevation in NPC1 patients ($p<0.0001$). In a cross-sectional analysis, Calbindin-D levels did not correlate with age or disease status, likely due to the large degree of inter-patient variation. Off label miglustat use in 19/42 (45%) of NPC1 patients resulted in lower Calbindin-D CSF values compared to those not taking the drug, but this was not statistically significant. However, in five patients evaluated prior to initiation of miglustat therapy and 6-15 months after therapy was started, we observed a significant decrease (mean = -33%) of Calbindin-D in the CSF after initiation of miglustat. These results help validate the use of Calbindin-D as a biomarker and support therapeutic efficacy of miglustat for treatment of NPC.

1474F

Recovery of peroxisome dysfunction by flavonoid compounds in fibroblasts from Zellweger spectrum patients having the common allele, PEX1-Gly843Asp. *G. MacLean[1], S. Steinberg[3], N. Braverman[1, 2].* 1) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Montreal Children's Hospital Research Institute, Montreal, Quebec, Canada; 3) Kennedy Krieger Institute, Department of Neurogenetics, Baltimore, MD, USA.

Zellweger spectrum disorder (ZSD) is due to defects in any one of 12 proteins, encoded by PEX genes, that are required for peroxisome biogenesis. ZSD features neurologic, hepatic and renal abnormalities; however, one common mutation, PEX1-p.Gly843Asp (G843D), confers a milder phenotype and represents 30% of all ZSD alleles. Thus, identifying treatments for this allele will benefit many patients. We recently showed that PEX1-G843D behaves as a misfolded protein amendable to improved function by chemical and pharmacological chaperones. In a small molecule screen, we reported functional recovery of peroxisomal matrix protein import by acacetin diacetate, a flavonoid that may bind to the ATP binding domain of PEX1-G843D to improve conformation. In order to develop a lead compound from this library `hit', we evaluated an additional 40 flavonoids using a patient fibroblast cell line hemizygous for PEX1-G843D and expressing the PTS1-GFP reporter, as described previously. Recovery of PTS1-GFP peroxisomal import was determined by visually scoring the number of cells with punctate vs. cytosolic GFP fluorescence. Eight flavonoids were identified as potential pharmacological chaperones and a dose response experiment showed that diosmetin (EC_{50}, 2.75 μM), acacetin diacetate (EC_{50}, 7.5 μM), acacetin (EC_{50}, 0.5 μM), apigenin (EC_{50}, 15 μM), and kaemferol (EC_{50}, 20 μM) were most effective, while tamarexitin, galangin, and chrysin showed little effect. These findings will aid in determining structure activity relationships, as knowledge of chemicals that are effective and ineffective will be used to determine the interactions that are important in developing a pharmacophore model. We further reasoned that recovery of Pex1p protein folding should improve PEX1p's interaction with PEX6p and localize to the peroxisomal membrane. Thus, we examined the subcellular localization of PEX1p after drug treatment. A PEX1-G843D hemizygote cell line was treated with various flavonoids and peroxisome enriched fractions were isolated by differential centrifugation to obtain the nuclear pellet, cytosolic fraction and peroxisome enriched fraction. Immunoblotting of these fractions showed that following treatment, PEX1p was increased in the peroxisome enriched fraction. We are continuing these experiments to analyze localization of PEX1p binding partners, PEX6p and PEX26p, and the receptor protein PEX5p. Blue Native PAGE will also be utilized to determine complex formation.

1475F

Histone deacetylase inhibitor suberoylanilide hydroxamic acid normalizes the levels of very long chain fatty acids in human skin fibroblasts from X-Adrenoleukodystrophy patients and downregulates the expression of proinflammatory cytokines in Abcd1/2 silenced mouse astrocytes. *J. SINGH, M. KHAN, I. SINGH.* PEDIATRICS, MEDICAL UNIVERSITY OF SOUTH CAROLINA, CHARLESTON, SC., USA.

X-Adrenoleukodystrophy (X-ALD) is a peroxisomal metabolic disorder, caused by mutations in the ABCD1 gene encoding the peroxisomal ABC transporter adrenoleukodystrophy protein (ALDP). The consistent metabolic abnormality in all forms of X-ALD is an inherited defect in the peroxisomal) -oxidation of very long chain fatty acids (VLCFA>C22:0) and the resultant pathognomic accumulation of VLCFA. In addition to the deficient degradation of VLCFA, elongation of very-long-chain-fatty acids (ELOVL) also contributes towards VLCFA cellular homeostasis. The accumulation of VLCFA leads to a neuroinflammatory disease process associated with demyelination of the cerebral white matter. The present study underlines the importance of a potent HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) in inducing the expression of ABCD2 (ALDRP), and normalizing the peroxisomal) -oxidation as well as the saturated and monounsaturated VLCFAs in cultured human skin fibroblasts of X-ALD patients. The expression of ELOVL1, the single elongase catalysing the synthesis of both saturated VLCFA (C26:0) and mono-unsaturated VLCFA (C26:1), was also reduced by SAHA treatment. In addition, using Abcd1/Abcd2-silenced mouse primary astrocytes we also examined the effects of SAHA in VLCFA-induced inflammatory response. SAHA treatment decreased the inflammatory response as expression of inducible nitric oxide synthase, inflammatory cytokine, and activation of NF-2B in Abcd1/Abcd2-silenced mouse primary astrocytes was reduced. The observations indicate that SAHA corrects both the metabolic disease of VLCFA as well as secondary inflammatory disease; therefore, it may be an ideal drug candidate to be tested for X-ALD therapy in humans.

1476F

Homocystinuria: Treatment with N-acetylcysteine. *B.M. GILFIX.* Medical Biochemistry, Royal Victoria Hospital, Montreal, Quebec, Canada.

Introduction: Homocsytinuria (MIM 236200) due to cystathionine) -synthase deficiency (CBS; EC 4.2.1.22) is the most common inborn error of sulfur amino acid metabolism. Treatment of CBS deficiency has traditionally been based on the supplying additional cofactor (vitamin B6), substrate restriction (low methionine diet) or activation of an alternative pathway for Hcy metabolism (betaine). Betaine is the current mainstay in the treatment of vitamin B6 unresponsive patients. Unfortunately, it is rather expensive at 233$ per 180 g (Cystadane®, Orphan Medical Inc.). Moreover, individuals treated with betaine may still fail to normalize their tHcy (< 15 µmol/L). Hultberg et al (1994) first demonstrated that oral N-acetylcysteine (NAC) reduced total plasma homocysteine in healthy subjects in a dose-dependent fashion; possibly by increasing renal excretion of tHcy. Based on this and similar observations, we sought to determine if oral N-acetylcysteine (NAC) would reduce plasma total homocysteine levels in subjects with homocystinuria. Methods: Five subjects (4 female; 1 male) with CBS deficiency were recruited. Subject C was vitamin B6 responsive. All were maintained on their usual medications during the course of the study. After 2 visits at one-month intervals to establish a baseline total plasma homocysteine (tHcy), the subjects received oral NAC (2 g p.o. BID) for 2 months. tHcy was measured twice during this period and once more after the NAC had been discontinued for 1 month. DNA from each individual was sent for sequencing to a commercial provider. Results: tHcy levels recorded at one month intervals: Pt. A: 20.5; 26.4; 20.1 (+NAC); 14.6 (+NAC); 28.7 Pt. B: 109.0; 160.0; 202.4 (+NAC); 113.9 (+NAC); 255.4 Pt. C: 209.0; 246.3; 158.5 (+NAC); 177.3 (+NAC); 154.5 Pt. D: 48.4; 88.9; 20.0 (+NAC); 21.3 (+NAC); 17.2 Pt. E: 236.0; 189.3; 156.4 (+NAC); 132.5 (+NAC); 134.6 Mutations Identified [†=not previously described]: Subject A (Eastern European) - c.833C>T (I278T); IVSII-2 A>C; Subject B (French Canadian) - c.313C>G (L105V)†; c.1676G>A (A222T); Subject C (Eastern European) - c.526G>A (E176K); c.1039G>A (G347S); Subject D (Eastern European) - c.430G>C (E144Q)† ; IVSII-2 A>C; Subject E (African & French Canadian) - c.919G>A (G307S); c.941G>C (V314A)†. Conclusions: NAC failed to produce a discernable decrease in tHcy levels. Three novel CBS mutations were identified. [Supported by the March of Dimes Foundation].

1477F

Disease-causing allele specific silencing against the *ALK2* mutants, *R206H* and *G356D*, in Fibrodysplasia Ossificans Progressiva. *H. Hohjoh[1], M. Takahashi[1], T. Katagiri[2], H. Furuya[3].* 1) NCNP, Natl Inst Neuroscience, Tokyo, Japan; 2) Division of Pathophysiology, Research Center for Genomic Medicine, Saitama Medical University, Hidaka-shi, Saitama, Japan; 3) Department of Neurology, Neuro-Muscular Center, National Omuta Hospital, Fukuoka, Japan.

Fibrodysplasia ossificans progressiva (FOP) is a rare autosomal dominant congenital disorder characterized by progressive heterotopic bone formation. Currently, there is no definitive treatment of FOP. The *activin-like kinase 2* (*ALK2*) gene, a bone morphogenetic protein (BMP) receptor gene, has been identified as the responsible gene for FOP, and a recurrent mutation, *617G>A (R206H)*, has been found in both familial and sporadic FOP cases of various ethnic groups. The mutant ALK2 receptors appear to gain function(s) as a constitutively-active BMP receptor, thereby triggering heterotopic bone formation without binding to BMPs in FOP. Chemical inhibitors of the BMP type I receptors are currently considered possible medicinal agents for FOP; but, they appear to affect not only pathogenic (mutant) ALK2 receptors but also normal ALK2 and also other BMP type I receptors. Thus, the risk of side effects of the inhibitors cannot be excluded. In this study, we show small interfering RNAs (siRNAs) conferring disease-causing allele specific RNA interference (RNAi) against the *617G>A* and *1067G>A ALK2* mutants found in FOP patients. Our designed siRNAs successfully inhibited the expression of the mutant *ALK2* alleles without affecting normal *ALK2* allele. Our current study suggest that the siRNAs presented here may be capable of becoming a novel therapeutic agent for FOP, and that disease-causing allele-specific RNAi by the siRNAs may pave the way for the achievement of radical treatment for FOP and/or for the relief of its severe symptoms.

1478F

Small interfering RNAs targeting oncogenic EGFR alleles as a possible cancer agent. *M. Takahashi[1], T. Chiyo[2], T. Okada[2], H. Hohjoh[1].* 1) Department of Molecular Pharmacology, NCNP, Kodaira, Tokyo, Japan; 2) Department of Molecular Therapy, NCNP, Kodaira, Tokyo, Japan.

Selective silencing of dominant oncogenic allele by RNA interference (RNAi) is a promising treatment for certain types of cancers possessing dominant mutations involving nucleotide changes in the coding regions of oncogenes. In the case of medical application of RNAi to cancer therapy, the ability of RNAi on allele discrimination is critical for avoidance of side-effects because many proto-oncogenes (namely, normal alleles) play important roles in normal cells and tissues. In this study, we show small interfering RNAs (siRNAs) conferring allele-specific RNAi (ASP-RNAi) against mutant *epidermal growth factor receptor* (*EGFR*) gene found in lung cancer and glioma patients. We designed and assessed siRNAs targeting two recurrent deletions in *EGFR* by using our in vitro assay system, and selected the most effective siRNAs against such *EGFR* mutations. Allele-specific silencing using the siRNAs in either chemo-sensitive or -resistance human lung cancer cells resulted in the significant inhibition of cell proliferation and induction of cell death. We further carried out *in vivo* studies using xenograft models of chemoresistant human lung cancer cells. The data indicated that a single injection of the siRNA conferring ASP-RNAi were able to remarkably inhibit the growth of xenograft tumors without altering toxicological parameters such as body weight and hematological indexes. When siRNAs targeting wild-type (normal) *EGFR* were used, significant alterations in several hematological parameters were detected, suggesting adverse effects of such siRNAs targeting both mutant and wild-type (normal) alleles. Our present data indicated that cancer therapy using ASP-RNAi against mutant *EGFR* could be a potential treatment with few adverse effects, even though cancers possess anti-cancer drug resistance.

1479F

Intrathecal Enzyme Replacement Therapy for Neurological Impairment in Mucopolysaccharidosis 1. *Z. Hadipour, F. Hadipour, P. Sarkhail, Y. Shafeghati.* Medical Genetics, Sarem Cell Research Center & Hospital, Tehran, Iran.

Bachground: MPS1 is an autosomal recessive disorder caused by deficient activity of the lysosomal enzyme alpha-L iduronidase, which leads to accumulation of heparan sulfate and dermatan sulfate, resulting in a progressive multisystem disease with respiratory, skeletal , and neurologic manifestations. Treatment for MPS1 consists of supportive care, symptom - based interventions, and enzyme - replacement therapy with Laronidase. Bone marrow and hematopoietic stem cell transplantation is the treatment of choice for patients suffering from MPS1 with no or minimal central nervous system manifestation. Case Report: A 50 months -old Iranian boy Who has phenotype of MPS1, coarse facial features, prominent forehead, corneal cloudy, sleep disturbance, hepatosplenomegaly, inguinal hernia, joint stiffness, and dysostosis multiplex congenital. He diagnosed MPS1 on the basis of clinical findings, an elevated urinary glycosaminoglycan level and low alpha- L- iduronidase activity in leukocytes. Mutation analysis revealed homozygous splice mutation in the IDUA gene. He has been started injection of aldurozyme intravenously every week from 26 months - old. To prevent neurological impairment before bone marrow transplantation, he receives intrathecal enzyme replacement of aldurozyme and samples of his CSF for testing monthly. He tolerates intrathecal ERT with no adverse events. When he is 50 months - old he receives bone marrow transplantation.

1480F

Gene and Stem Cell Therapy for Tay-Sachs and Sandhoff DiseaseS. *B.V. FEINERMAN[1], J. PAINO[2].* 1) RESEARCH, STEMCELLREGENMED, SARASOTA, FL; 2) SAN MARTIN COLLEGE OF MEDICINE, LIMA, PERU CLINICA SAN FELIPE HOSPITAL CONCEBIR HOSPITAL, LIMA, PERU.

Tay-Sachs and Sandhoff diseases have juvenile and infantile onsets. A normal child will start reversing development and be unable to turn over, crawl, sit, walk or talk. Later cognitive and intellectual development will be impaired. Previous treatments have involved giving medications to induce enzyme replacement; substrate reduction or correction of misfolded proteins. Six patients with Tay-Sachs and one child with Sandhoff disease were treated with a combination of gene and stem cell therapy. The children ranged in age from 19 months to eight years of age. Mutations of genes were deleted with specific shRNA. This was followed by retroviral transfection of the Hex A gene in patients with Tay-Sachs and Hex B gene in the patient with Sandhoff disease into neuron stem cells derived from umbilical cord blood. The transfected neuron stem cells were administered into the central nervous system and brain through the spinal canal. In addition brain derived neurotrophic factor, nerve growth factor, glial derived neurotrophic factor and neurotrophins 3 were given intrathecally. The procedure was well tolerated without any adverse effects. Two months following treatment 6 out of the 7 children showed improvment in loss of spasticity; ability to raise their arms and move their legs; 4 out of 7 lost their cherry red spots; 5 out of 7 appeared to have improved hearing; 3 out of 4 that had seizure activity appeared to have stopped; improved motion of head and neck 5 out of 7: improved swallowing in 3 out of 7. The patients came from a wide variety of locations in the USA, Brazil, Germany.

1481F

Gene therapy as a potential treatment of cardiomyopathy in propionic acidemia. *R.J. Chandler[1], N. Carrillo-Carrasco[1], S. Chandrasekaran[1], P.M. Zerfas[2], C.P. Venditti[1].* 1) Genetic and Molecular Biology Branch, National Human Genome Research Institute, National Institutes Health, Bethesda, Maryland; 2) Diagnostic and Research Office Branch, Office of the Director, National Institutes of Health, Bethesda, Maryland.

Propionic Acidemia (PA) is autosomal recessive metabolic disorder caused by a deficit in the enzymatic activity of propionyl-CoA carboxylase. Genetic defects in *PCCA* or *PCCB*, which code for propionyl-CoA carboxylase, result in PA. Patients with PA exhibit elevated levels of propionic acid and methylcitrate in their plasma and urine, and can present with potentially lethal metabolic decompensations, hyperammonemia, neurological complications, and a potentially lethal cardiomyopathy. These medical problems can arise and the cardiomypathy does progress in spite of current medical treatments. A therapy that stabilized the patients and/or addresses the progressive and often lethal cardiomypathy would be beneficial to patients. We have previously demonstrated the ability of rAAV8 gene delivery to rescue the murine model of PA from lethality and decrease the levels of disease related metabolites. In light of the cardiomyopathy observed in the human disease, the cardiac histology of the PA mouse model was examined to determine whether cardiac abnormalities were present. Oil red staining revealed an abnormal accumulation of lipid and electron micrographs (EMs) revealed gross abnormalities of the cardiac tissue in the PA mice. Specifically, the cardiac myofibers were poorly organized and reduced in number. However, cardiac EMs from the PA mice treated by rAAV8 gene delivery of the *PCCA* gene exhibited no abnormal histopathological changes. These results demonstrate the potential efficacy of rAAV8 gene delivery as an alternative treatment to transplantation for the potentially lethal cardiomyopathy observed in PA.

1482F

Dye laser photodynamic therapy for Bowen's disease in a patient with epidermodysplasia verruciformis. *K. Fukai[1], M. Sunohara[2], T. Ozawa[2], T. Harada[2], M. Ishii[1].* 1) Dept Dermatology, Osaka City Univ, Osaka, Japan; 2) Dept Plastic and Reconstructive Surgery, Osaka City Univ, Osaka, Japan.

Epidermodysplasia verruciformis (EV) is a rare heritable skin disease that results in unusual susceptibility to infection with specific types of human papillomavirus (HPV). Here we report a 53-year-old man with EV who developed Bowen's disease on his lower eyelid and the chest. Mutation analysis of EVER1 gene revealed homozygous splice acceptor site mutation (IVS8-2, A>T). In this patient, HPV3, HPV14 and HPV38 had been identified from the skin lesions. The Bowen's skin lesion on the left lower eye-led was treated by photodynamic therapy (PDT) using 5-aminolevulinic acid (ALA) and pulsed dye laser (PDL). After two rounds of the PDT treatment, the skin lesion disappeared and a skin biopsy confirmed the efficacy of the treatment. As the light source for PDT, we used a PDL set at the excitation wavelength (585 nm) for PpIX, which is the metabolite of ALA. The 585-nm PDL has been primarily used for the treatment of vascular lesions because it efficiently generates heat and thus damages tumor blood vessels. As HPV is sensitive to heat and warts have more prominent superficial blood vessel than healthy skin, PDL has already been used to treat common warts caused by HPV infection and the efficacy has been confirmed in multiple studies. Moreover, it has been reported that ALA combined with PDL was more effective for eliminating viral warts than either PDT or PDL alone.The fact that EV associated with Bowen's disease and warts are both caused by HPV infection could explain our finding that ALA-PDT with PDL was effective for treating Bowen's disease in the present patient. In other words, the effect of the PDL on tumor blood vessels and heat-sensitive HPV, in addition to the effect of PDT itself, could have contributed to the good response observed in this case, both histologically and cosmetically. This patient had Bowen's disease on the lower eyelid and was the first case to be successfully treated by ALA-PDT combined with PDL. This treatment was a less invasive and simpler method of achieving a satisfactory cosmetic outcome compared with other treatments. Patients with EV are highly susceptible to HPV infection and thus develop numerous and widespread lesions. Because they require repeated treatment, effective and minimally invasive therapy such as PDT described here could be beneficial for these patients.

1483F

Evaluation of the therapeutic potential of Ramipril in a murine model for Marfan syndrome. *B.L. Lima, G.R. Fernandes, L.V. Pereira.* Dept de Genética, Inst de Biociencias - USP, São Paulo, Brazil.

The Marfan syndrome (MFS) is an autosomal dominant disease of connective tissue, which affects 1 in 5,000 individuals. The main clinical manifestations include aneurysms and aortic disruption, excessive growth of bones, scoliosis and thoracic deformities. Mutations in the FBN1 gene, which encodes the fibrillin-1 protein, were genetically linked to the MFS, classifying this disease in the fibrilinopathies group. Studies with animals model for MFS demonstrated a strong negative correlation between overall levels of Fbn1 expression and the severity of the phenotypes. Fibrillin-1 alterations also lead to an increase in the activity of TGF in tissues, resulting in cardiovascular and pulmonary phenotypes. Thus, during the last years, therapeutic strategies, based on the use of drugs, have been studied in animal models and patients with MFS. Among these drugs we find the Losartan, an antagonist of AT1, which may restore the vascular and pulmonary phenotype in animal models. Clinical trials involving patients with MFS revealed that, even with the patients presenting improvements in vascular alterations, the results are far from those seen in animal models. The hypothesis is that the dose given to patients is not sufficient to reproduce the effects observed in animals, which received very high drug dose. During the year of 2010 our group evaluated the effect of Ramipril, an ACE inhibitor, on the phenotypic manifestations of SMF in the murine model mg$\Delta^{loxPneo}$. Treated animals showed an increase of approximately 35% in the FBN1 gene transcription, and a significant improvement in pulmonary and bone manifestation - the last has never been reported in other models for SMF treated with Losartan. Differently of studies involving Losartan, the treatment with Ramipril was realized with doses equivalent to those already used in humans. These results revealed that the restoration of normal levels of fibrillin-1 seem to have the potential to modulate the clinical severity of the disease, indicating new treatment possibilities.

1484F

BMN 111, a CNP analogue, promotes skeletal growth and rescues dwarfism in two transgenic mouse models of Fgfr3-related chondrodysplasia. *F. Lorget[1], N. Kaci[2], J. Peng[1], C. Benoist-Lasselin[2], F. Di Rocco[2], E. Mugniery[2], S. Bullens[1], S. Bunting[1], L. Tsuruda[1], C. O'Neill[1], A. Munnich[2], L. Legeai-Mallet[2].* 1) BioMarin Pharmaceutical Inc. 105 Digital Drive, Novato, California 94949 USA; 2) INSERM U781-University Paris Descartes-Necker Hospital - Enfants Malades, 75015 Paris, France.

Although Fgfr3-related chondrodysplasia genes were identified in 1994, no effective treatments are available to date and therapeutic interventions are limited to surgical procedures. The severity of Fgfr3-related dwarfisms ranges from mild (hypochondroplasia) to severe (achondroplasia) and lethal (thanatophoric dysplasia) depending on the point mutation. Patients with achondroplasia, the most severe form of dwarfism, display short stature with disproportionate shorter proximal bones (rhizomelia), narrow trunk, brachycephaly and spinal stenosis. C-type natriuretic peptide (CNP) is considered to be a potential therapeutic agent due to its inhibition of Fgfr3 downstream signaling. Targeted in vivo overexpression of a CNP transgene or continuous infusion of native CNP were shown to rescue the impaired bone growth in a mouse model of achondroplasia. To overcome the short half-life of native CNP, BioMarin developed a CNP analogue, BMN 111, with a longer half-life allowing daily subcutaneous administration. BMN 111 pharmacological activity was analyzed in two relevant mouse models of Fgfr3-related chondrodysplasia. In the $Fgfr3^{G380R}$ homozygous mouse model (Ach), the transgene expression is restricted to the chondrocytes while it is ubiquitously expressed in the $Fgfr3^{Y367C/+}$ heterozygous mouse model (TD). A more severe phenotype is observed with TD mice (shorter bones, pronounced prognathism, marked spinal stenosis and reduced lifespan). In a culture model using femurs isolated from TD embryos, BMN 111 co-incubation led to increased chondrocyte proliferation and differentiation, thus confirming BMN 111 mechanism of action. In the in vivo assessment of BMN 111 pharmacological activity, the Fgfr3 mice received daily subcutaneous BMN111 administrations from 6 days of age in the TD mice and three weeks of age in the Ach mice. The TD mice were treated for 10 days at dose levels up to 800 μg/kg. The Ach mice were treated for 5 weeks at dose levels up to 280 μg/kg. Overall, the treatment was well tolerated. A dose related increase in the axial and appendicular skeletons was measured in both models (~5-10% increase in the high dose groups). In addition, improvements in clinically relevant features were observed in the TD mice including rhizomelia, shape of the skull and spinal stenosis (normalization of the sagittal diameter of the atlas vertebra). Based on these data, BMN 111 is a potential therapeutic for chondroplastic disorders and warrants further development.

1485F

Modeling Machado-Joseph disease in C. elegans: genetic and pharmacologic rescue. *P. Maciel[1], A. Teixeira-Castro[1,2], A. Jalles[1], A. Miranda[1], C. Bessa[1], R. Morimoto[2].* 1) School of Health Sciences, University of Minho, Braga, Portugal; 2) Department of Molecular Biosciences, Northwestern University Institute for Neuroscience, Rice Institute for Biomedical Research, Northwestern University Evanston, IL 60208, USA.

The risk of developing neurodegenerative diseases increases with age. Although many of the molecular pathways regulating proteotoxic stress and longevity are well characterized, their contribution to disease susceptibility remains unclear. In this study, we describe a new Caenorhabditis elegans model of Machado-Joseph disease, also known as spinocerebellar ataxia type 3, a neurodegerative disease caused caused by expansion of a polyglutamine (polyQ) tract within the protein ataxin-3 (ATXN3). Pan-neuronal expression of mutant human ATXN3 leads to a polyQ-length dependent, neuron subtype-specific aggregation and neuronal dysfunction. Analysis of different neurons revealed a pattern of dorsal nerve cord and sensory neuron susceptibility to mutant ataxin-3 that was distinct from the aggregation and toxicity profiles of polyQ-alone proteins. This reveals that the sequences flanking the polyQ-stretch in ATXN3 have a dominant influence on cell-intrinsic neuronal factors that modulate polyQ-mediated pathogenesis. Aging influences the mutant ATXN3 aggregation and toxicity phenotypes in our model, which can be suppressed by the genetic or pharmacologic downregulation of the insulin/ insulin growth factor-1-like signaling pathway and activation of heat shock factor-1. We are currently using this model to test a library of 1,200 FDA-approved compounds in order to find pharmacological modulators of mutant ATXN3 aggregation and neuronal dysfunction. We have completed the toxicity screening and identified 618 compounds that were non-toxic for the worms. In our screening we are using as endpoints ataxin-3 aggregation, as quantified by an imaging software we developped, and motility assessment of transgenic worms. So far, we have found three promising small molecules that reduce the neurological phenotype of this model, and we are exploring the manner in which they may target cellular pathways linked to disease pathogenesis.

1486F

Truncal obesity in Cohen syndrome and its potential treatment. *H. Wang, A. Bright, L. Nye, B. Xin.* DDC Clinic for Special Needs Children, Middlefield, OH.

Cohen syndrome is a rare autosomal recessive disorder with less than 200 cases reported worldwide. The disease is characterized by facial dysmorphism, microcephaly, hypotonia, developmental delay, myopia and retinal dystrophy, neutropenia, and truncal obesity. Mutations in VPS13B (also known as COH1) gene on chromosome 8q22 are believed responsible for the syndrome. However, the pathogenesis and pathology of the condition is unclear, thus how to treat those signs and symptoms in this disease often becomes difficult. The truncal obesity, for example, as one of the most prominent health issues in patients with Cohen syndrome after they reach their teenage, is still poorly understood and its treatment remains challenging. The purpose of this study is to better understand the development and progress of the obesity, and to find effective measures of weight control. As a part of the comprehensive study, a questionnaire was distributed to the families of the confirmed Cohen patients registered in our National Cohen Syndrome Family Support Center with 54 surveys completed, 27 from Amish and 27 from non-Amish. The average patient age is 16.8 years (range from 1.5 to 45), with 43% male and 57% female. Preliminary data indicated that 46% of parents reported their affected child being overweight, with the average age of developing overweight at 12 years old (Amish 14.6 years old, non-Amish 9 years old). However, at the time truncal obesity developed, 52% of parents reported a fairly normal food intake in these children. Excessive eating was only noted in 20% of patients. Nonetheless, neither significant hypercholesterolemia nor hypertriglyceridemia was observed in this cohort. Various behavior problems such as anxiety and attention deficit and hyperactivity were noted by parents. A stimulant nethylphenidate was used in 5 patients who had attention deficit and hyperactivity, anxiety and obesity. We found significant improvements in the psychosocial behaviors in all 5 patients. The body mass index (BMI) in all five patients was also significantly improved with the treatment. Our preliminary results show that the treatment of psychosocial and behavioral problems with the stimulant may bring a favorable outcome for the truncal obesity in these patients.

1487F

Discovery Partnerships with Academia - A New Option for Moving Genetic Learnings to a Medicine. *R.A. Gibson[1], I. Uings[1], M.J. Bamford[1], J. Collins[2], P. Williams[1], D. Holmes[1].* 1) Academic DPU, GlaxoSmithKline, Stevenage, Herts, United Kingdom; 2) Academic DPU, GlaxoSmithKline, Research Triangle Park, North Carolina, USA.

The global academic Genetics community generates many innovative ideas for new therapeutic targets, but translating this innovation into medicines can be challenging and resource intensive. GlaxoSmithKline (GSK) has established Discovery Partnerships with Academia to provide a way to bring together the insight of the academic world with the complementary expertise and capabilities of GSK in joint, target-focused, early drug discovery projects to identify and develop potential medicines. Discovery Partnerships with Academia operates globally and cross-therapeutically, and is looking for partners who really want to be involved in developing a medicine. Suitable projects have a compelling therapeutic hypothesis, and are at a stage where partnership with GSK will make a key contribution to success. GSK applies its resources to these projects in many ways, including development of robust assays, lead identification (using conventional screening, rational design and encoded library technology), medicinal chemistry and preclinical development. This work is fully integrated with the activities ongoing in the academic groups. By aligning this expertise with the academic's deep understanding of disease biology, we ensure that the very best molecules are selected for clinical progression. An overview of the Discovery Partnership model will be presented, highlighting the opportunities that it offers academic researchers to progress their research towards creation of a medicine. Examples of how these partnerships work in practice will also be provided.